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ABA and ABC renewal of conditioned magazine approach are not impaired by dorsal hippocampus inactivation or lesions

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Abstract

Three experiments investigated the role of the dorsal hippocampus (DH) in renewal of conditioned and then extinguished magazine approach responding in rats. Experiments 1 and 2 found no effect of muscimol inactivation of the DH during testing on ABA and ABC renewal, respectively. However, subjects from these studies were subsequently found to be impaired on a delayed non-matching-to-place task following muscimol but not saline infusions. Experiment 3 found no effects of post-training excitotoxic lesions of the DH on ABA and ABC renewal. Lesioned subjects were, however, impaired on the delayed non-matching-to-place task compared to control subjects. These findings suggest that the DH may not play a similar role in Pavlovian extinction in appetitive learning tasks as has previously been reported in aversive learning.

Keywords

Renewal; Hippocampus; Appetitive learning; Occasion-setting; Extinction; Conditional learning

1. Introduction

Investigations into the extinction of classically conditioned responding (CR) generally support the idea that the originally learned association between the conditioned and unconditioned stimulus (i.e., the CS–US association) to a large degree survives this treatment (for a review see [1]). One mechanism to account for extinction has been proposed by Bouton [2,3] who suggested that the extinction context comes to modulate expression of an inhibitory CS–US association through an “occasion setting” process (e.g., [4,37]), which develops during extinction. This occasion-setting model nicely explains “the renewal effect”, which demonstrates that extinction learning is context specific. For example, Bouton and King [5] showed that rats fear conditioned in one context (A) but then extinguished in a second, physically distinct, context (B) recovered their fear reaction to a stimulus when post-extinction tests of fear were conducted back in the conditioning context. This is an example of ABA renewal. Additional studies by Bouton and his colleague [6] established that this recovery occurs even when the test stimulus is tested in a third context (C) in which no prior fear conditioning had taken place (ABC renewal). The basic principle is that renewal occurs

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whenever the test stimulus is tested outside of the physical context where extinction had taken place.

In this model, the extinction context is thought to “set the occasion” for expression of extinction learning by retrieving the inhibitory CS–US association acquired during extinction. When the extinguished CS is tested outside of the extinction context, the inhibitory association is not retrieved and renewal is observed due to the more robust activation of the intact excitatory CS–US association. However, while this model of extinction is generally accepted there are other explanations of extinction that do not posit mechanisms of such complexity. For instance, a summation-based account of the renewal effect more parsimoniously explains this phenomenon in some circumstances. By assuming that the CS maintains its excitatory properties because the extinction context itself acquires a direct inhibitory association during extinction (e.g., see [7]), the renewal effect can be explained without the inclusion of modulatory, occasion-setting processes. In other words, the context protects the CS from extinction by acquiring inhibition as a result of the omission of the US on extinction trials [8].

In this case, the extinction context is assumed to summate its direct inhibitory association with the stimulus’ own excitatory association to reduce responding when tests occur in the extinction context. Renewal arises when CS presentations occur in the absence of this inhibitory source (i.e., the extinction context). Therefore, when the stimulus is tested outside of the extinction context, because the CS has preserved excitatory properties, it produces the CR. In several early studies, Bouton and colleagues failed to obtain any direct evidence that the extinction context becomes inhibitory and this prompted the conclusion that the extinction context might be working through a separate modulatory (i.e., occasion setting) mechanism [5,9,10]. However, more recent empirical work has provided such evidence at least under some circumstances [11]. In addition, more recent theoretical work has provided an account for why evidence for direct inhibitory context-US associations might be difficult to observe [12]. Thus, it may be premature to completely rule out the possibility from these investigations that the extinction context may exert its effects through a direct inhibitory association as opposed to a modulatory occasion setting mechanism.

Studies conducted at the neural systems level of analysis have provided additional clues to how extinction in fear conditioning works that are relevant to the psychological models discussed above (e.g., [13]). While this literature points to the amygdala ([14]; for a review see [15]) and ventromedial prefrontal cortex [16] as being involved in extinction, Maren and colleagues [17,26,18] have also found that the dorsal hippocampus (DH) is especially important in the contextual control of extinction. For example, while inactivation of the DH impaired one form of renewal (i.e., ABC renewal), electrolytic lesions of this structure impaired another form of renewal (i.e. ABA renewal, but see [19]). The renewal designs used in many of these studies, however, did not allow a clear determination as to which of the two psychological mechanisms discussed above (i.e., occasion-setting or inhibitory context-CS summation) might have been impaired by the manipulation.

In a study conducted by Maren and Hobin [20], an occasion setting role for the DH was more clearly identified. In this experiment, two stimuli were fear-conditioned in one context (Ctx 1: CS1+, CS2+) and then each of these was extinguished in separate contexts (Ctx 2: CS1–, Ctx 3: CS2–). Finally, both stimuli were then tested in both of the extinction contexts (Ctx 2: CS1– vs CS2–, Ctx 3: CS1– vs CS2–). The reason why this is an especially clever design is that it equates for any direct inhibitory context-US associations that could be learned during the extinction phase. Thus, if this mechanism were responsible for depressing conditioned responding (i.e., fear CRs) during extinction, then it should not matter which stimulus was tested in their presence – both CS1 and CS2 should display low levels of fear

in both contexts. However, if the contexts work through an occasion setting mechanism, then each context should target specifically the inhibitory CS–US association that was learned in its presence. In other words, Context 2 should retrieve the inhibitory CS1–US link while Context 3 should retrieve the inhibitory CS2–US link. Retrieval of these links should be more difficult when the two CSs are tested outside of their extinction context, in spite of the fact that those contexts also signaled extinction of a different stimulus. Under these circumstances, Maren and Hobin [20] reported that more fear was displayed to the CSs when they were tested outside (than inside) their extinction contexts. Furthermore, this difference was eliminated by DH inactivation prior to the test session. The results more convincingly demonstrate that the DH plays an occasion setting role during extinction in fear conditioning.

The majority of studies investigating the neural mechanisms of extinction have focused on aversive learning paradigms. However, several studies suggest that parallel circuits may be involved in extinction learning in appetitive and aversive paradigms. Rhodes and Killcross, for example, demonstrated that spontaneous recovery [27] and renewal [36] are both enhanced by lesions of the infralimbic portion of the ventromedial prefrontal cortex, results that parallel those found by Quirk and his colleagues using a fear conditioning paradigm [16]. However, we are aware of no studies that have investigated the role of the DH in renewal in an appetitive task. A possible role for the hippocampus in some forms of appetitive occasion setting was suggested by Holland and his colleagues [21] and by Honey and his colleagues [22,23]. Thus, the present studies were designed to explore the effects of DH manipulations upon both ABA and ABC renewal in an appetitive Pavlovian magazine approach task.

We used renewal designs that more clearly isolate an occasion-setting mechanism in both of these tasks. We observed the impact of inactivating the DH prior to testing for ABA renewal of magazine approach in Experiment 1 or ABC renewal in Experiment 2. Experiment 3 evaluated the effects of post training excitotoxic lesions of the DH on ABA and ABC renewal. To study ABC renewal we used the same experimental design as Maren and Hobin [20] described above. Our ABA renewal task has not been commonly employed in the literature (but see [38]). In this task, two stimuli were each trained in distinct contexts (Ctx 1: CS1+, Ctx 2: CS2+) before each was extinguished in the other stimulus' training context (Ctx 1: CS2–, Ctx 2: CS1–). Then, each stimulus was tested in each of the two contexts (Ctx 1: CS1– vs CS2–, Ctx 2: CS1– vs CS2–). Greater renewal of responding is expected when the CSs are tested in their acquisition than their extinction context. As is true in the Maren and Hobin [20] ABC renewal design, this experimental design controls for the separate associative histories of the two contexts. Thus, renewal in this circumstance cannot easily be attributed to a summation-based associative mechanism, and points more strongly to control by an occasion setting mechanism.

2. Methods

2.1. Experimental subjects

A total of 92 experimentally naïve Long–Evans rats were used as subjects in the following studies. The subjects were bred at Brooklyn College (from rats of Charles River descent) and housed in standard clear-plastic tubs 10.5 in. × 19 in. × 8 in. with woodchip bedding within a colony room on a 14:10 light:dark schedule. Subjects had free access to water while in their home tubs and were maintained within a ± 5 g range of their target weights with supplemental feedings of home cage chow given after experimental sessions on a given day. The twelve males used as subjects in Experiment 1 weighed between 330 g and 420 g at the start of the study. Twenty-eight males were used in Experiment 2 with weights ranging between 366 g and 534 g at the start of the study. In the 3rd experiment, 26 males and 26

females were used as subjects. The weight range for females was from 189 g to 231 g, while males weighed between 246 g and 391 g at the start of the study. Each experiment was run in two separate replications.

2.2. Surgical procedures

Subjects in Experiments 1 and 2 received implantation of guide cannulas prior to any training. Subjects in Experiment 3 were given excitotoxic lesions following behavioral training, prior to the test phase.

2.2.1. Cannulations—Subjects in Experiments 1 and 2 were anaesthetized using a 0.1% body weight injection of sodium pentobarbital at a 50 mg/mL concentration resulting in a dose of 50 mg/kg. Subjects were treated with a 1.35% body weight injection of atropine sulfate concentrated at 0.4 mg/mL 10 min following injection of the sodium pentobarbital in order to aid respiratory function. The scalp was then shaved and the rat placed in a Stoelting stereotaxic apparatus for implantation of bilateral guide cannulas (Plastics One: 22 gauge, 10 mm long beyond pedestal with a 5 mm separation). Lubricant was placed on the cornea to maintain moisture and the head swabbed with a 10% ethanol solution. An incision was made over the midline of the skull and the fascia pushed aside to reveal bregma and lambda. Once bregma and lambda were confirmed to lie on the same horizontal plane, the unit was calibrated for placement over the dorsal hippocampus by touching both tips of the cannula unit onto bregma and recording the coordinates for each tip. Two holes were drilled over the dorsal hippocampus and an additional 4 holes were drilled around the unit for placement of jeweler's screws. The cannulas were placed at the following coordinates: 3.8 AP, 2.5 ML, 2.5 DV. The unit was lowered and then fixed into place using dental cement. Once the cement was dry the edges of the incision to the scalp were sutured and antibacterial ointment was applied to the wound. The guide cannulas were plugged with obturators (Plastics One: 24 gauge, projected 0.5 mm beyond tips of guide) and covered with a dust cap on top of the unit. Subjects were placed in a recovery area until locomotor activity was observed and then returned to their home tub for 1-week recovery with food freely available. Following surgery, obturators were changed every other day until the completion of the study.

2.2.2. Lesions—Subjects in Experiment 3 were anaesthetized and treated as described above. Once bregma and lambda were confirmed to lie on the same horizontal plane, the stereotaxic unit was calibrated by placing the tip of a 1 μ L Hamilton syringe onto bregma and recording the coordinates. Four holes were drilled over the dorsal hippocampus (1 on each side of the midline at each anterior-posterior coordinate), two holes at 3.8 mm posterior to bregma, 1.6 mm lateral to the midline. The other two holes were drilled 4.2 mm posterior to bregma and 2.6 mm lateral to the midline. The injector was lowered 3.3 mm below the surface of the skull for the anterior sites and 3.0 mm for posterior sites. Once at the appropriate depth .4 μ L of NMDA (20 mg/mL) was infused at each site at a rate of .1 μ L/min, the injector remained in place for another 4 min following each infusion. For sham surgeries no neurotoxin was infused during this time. For these surgeries the needle was simply lowered into place at the injection site, a procedure commonly employed in the literature, (e.g., see [24]). Additionally, it should be noted that the sham procedure used by Ji and Maren [18] consisted of lowering the electrode used to create lesions to the appropriate depth and did not deliver any stimulation, which is analogous to what was done in the current surgeries. The infusion was automated using a KD scientific pump. Once all infusions were made the wound to the scalp was sutured and bacitracin applied to the area. Subjects were placed in a recovery area until locomotor activity was evident and then returned to their home tub for 2-week recovery with food freely available.

2.3. Muscimol infusions

For Experiment 1, infusions into the dorsal hippocampus were done one hemisphere at a time. A 25 μ L Hamilton syringe attached to PE50 tubing was connected to a 28 g injector (Plastics One), which extended 1 mm beyond the tip of the guide cannula. The syringe was automated by a KD scientific pump (Model No.: 780310) dispensing 1 μ L of solution into each hippocampi at a rate of 0.32 μ L/min. Subjects were restrained during this process and remained connected to the infusion line for an additional 2 min following each infusion. Muscimol was concentrated at 1 mg/mL in physiological saline. Control subjects received the same treatment except that only the saline vehicle was infused. Order of infusion was counterbalanced with regard to cerebral hemisphere.

Infusion parameters for Experiment 2 were identical to Experiment 1, though the technique used was modified slightly. Infusions were done simultaneously in both hemispheres using bilateral injector units from Plastics One (the units were the same dimensions as those described above). Each side of the injector was attached via PE50 to a 5 μ L Hamilton syringe, the two syringes were operated manually. Infusions occurred over an approximately 1 min time window with the rat restrained for a further 2 min following the infusion.

2.4. Histology

Subjects were anaesthetized using the sodium pentobarbital and transcardially exsanguinated with 0.9% saline and perfused using a 10% formalin solution. When extracted, brains were cryoprotected in a 20% sucrose + 10% formalin solution and stored in 10% formalin until sectioning between 3 and 4 days later. Sections were cut 40 μ m thick and placed in distilled water for mounting onto 2% bovine gelatin coated standard microscope slides. The samples were stained with cresyl echt violet for nissl bodies using the standard protocol. Slides were held in xylene and then covered slipped using permount and let to dry. Sections were scored under an Optimus light microscope using Paxinos and Watson's [25] atlas of the rat brain as a reference.

2.5. Apparatus

For ABA renewal training, rats were trained in two contexts. Context One consisted of eight identical standard BRS Foringer conditioning chambers (30.5 cm $\times$ 24.0 cm $\times$ 25.0 cm) which were housed in individual light and sound attenuating shells. The two end walls of each chamber were made out of aluminum, while the side walls and ceiling were made from transparent Plexiglas. The floors consisted of 0.60 cm stainless steel rods spaced 2.0 cm apart. A recessed food magazine measuring 30 mm $\times$ 36 mm $\times$ 20 mm (length $\times$ width $\times$ depth) was located on one end wall of the chamber 12.0 mm above the grid floor. Positioned approximately 3 mm deep on the side walls of the food trough and 3 mm above the base of the magazine were an infrared emitter and detector which enabled the automatic recording of head entries inside the magazine. Head entries into the magazine were registered by the breaking of the infrared beam in the food trough.

Context Two consisted of another set of eight conditioning chambers in sound and light attenuating shells located in another room. The chambers that comprised Context Two were a different physical set of chambers in addition to being tailored to promote as distinct of a sensory experience as possible. Context Two was identical to Context One with the exception that stainless steel metal sheets were inserted into the chamber to alter its shape. One sheet completely covered the grid floor, and a second sheet was positioned diagonally (from the bottom of the wall opposite the food magazine towards the front portion of the ceiling) to give the chamber a trapezoidal shape. With the metal inserts in place, the dimensions of the chamber became 29.2 cm $\times$ 30.5 cm $\times$ 9.5 cm $\times$ 24.1 cm (base $\times$ diagonal side $\times$ ceiling $\times$ vertical side).

For the ABC renewal task an additional set of inserts (placed in the chambers described above) were used to produce a 3rd context. This set of inserts consisted of a full Plexiglas floor with 5 cm diameter holes drilled approximately 5 cm apart from one another. Additionally, a solid Plexiglas wall 15 in. in length was inserted so that the chamber was reduced to half of its total size resulting in a triangle shaped floor space instead of the normal rectangular shape. It should be noted that each of the three contexts were different physical chambers with the attributes described above introduced to make them more distinct from one another.

All of the conditioning boxes were equipped with a 7.6 cm diameter 8 Ω speaker (Radio Shack) mounted outside the magazine side of the chamber. This speaker delivered a computer generated 1500 Hz tone (T) amplified by a Radio Shack amplifier. The volume of the tone CS was 4 dB above the 78 dB background noise produced by fans mounted on the light and sound attenuated outer shells of the chambers for ventilation. The boxes also contained a 6 W light bulb (L) positioned at the base of the shell just behind the chamber. When activated these stimuli (T and L) remained on for 15 s. Both stimuli terminated with the delivery of two 45 mg food pellets (manufactured by Test Diet) delivered to the food magazine.

The T-maze used after the renewal tasks had metal bar floors (0.60 cm stainless steel rods spaced 2.0 cm apart) and opaque plastic walls with a clear ceiling. The walls of the maze were 6 in. high and the interior was 3½ in. wide. The main arm of the maze was 50 in. long. The upper length of the maze (i.e., the top of the T) was 67½ in. long, with food wells at each end. The start box at the base of the maze and each of the goal boxes at the top corners of the maze could be closed off by manually lowering an opaque plastic guillotine door. The maze was on a T-shaped wooden table raised approximately 4 ft from the floor. Room lighting was provided by overhead fluorescent lights.

2.6. Behavioral procedures

Subjects underwent behavioral training, consisting of preliminary magazine training, followed by excitatory training, extinction and ultimately testing. It should be noted that while for subjects in Experiments 1 and 2, surgery occurred prior to any training, those in Experiment 3 received surgery following extinction, but prior to the renewal test phase. Following the renewal test phase, subjects were then trained on a delayed non-matching-to-place task using a T-maze.

2.6.1. Magazine training—Subjects in Experiment 1 were placed in each context and had food pellets delivered to the food trough on a variable time 60 s schedule for 20 min. Subjects were trained in one context on day one and then the other context on day 2 (with context identity counterbalanced).

Subjects in Experiment 2 underwent two sessions of magazine training, with one session each day for 2 days. Both sessions were conducted in a single context (Context 1) using the parameters described above. For all subjects, Context 1 was the bare training chamber (i.e., no inserts were in place), while the identities of Contexts 2 and 3 were counterbalanced using the two sets of inserts described above. In our ABC renewal designs, magazine training was only conducted in Context 1. Because Contexts 2 and 3 were only used during extinction and testing, magazine training in these contexts was not deemed necessary. Additionally, conducting magazine training in each of the contexts would have resulted in much more exposure to the US than had been used in our ABA designs. However, this approach may have resulted in very low levels of responding during the extinction phase of the studies using this design (see Section 3).

For Experiment 3, those subjects trained on the ABA renewal task received magazine training as was described for Experiment 1, while those trained on the ABC renewal task received magazine training as was described for Experiment 2.

2.6.2. Acquisition—Following magazine training subjects in Experiment 1 were trained to associate two conditioned stimuli with food pellet reinforcers in two distinct contexts. Subjects received two sessions each day for 8 days, with one session occurring in each context and one stimulus being trained in each session. These sessions were separated by 30 min and lasted for 28 min. The order of these sessions, the identity of the contexts and the stimuli undergoing training in each location (T, L) were all counterbalanced. There were 8 trials in each session. Each trial consisted of a 15 s stimulus presentation that was immediately followed by the delivery of two food pellets. The average intertrial interval was 3 min with a range from 1 to 5 min. An additional two minutes were inserted at the end of the session following the last US delivery of each session (Table 1).

Subjects in Experiment 2 were trained, following magazine training, to associate the two stimuli (T and L) with food in a single context. Stimuli were trained in the same session over 8 days. During each 56 min session, each stimulus was presented eight times in a random order and terminated with the delivery of two food pellets to the magazine. The intertrial interval and range were the same as in Experiment 1 with an additional 4 min included after the final US presentation.

In Experiment 3, subjects trained on the ABA renewal task were given excitatory training as described for Experiment 1. Subjects trained on the ABC renewal task were trained as described for Experiment 2.

2.6.3. Extinction—For Experiment 1, extinction was run in a similar manner to acquisition except that the stimuli were presented in the alternative context and they were no longer followed by food. Specifically, if T was paired with food in Context 1 and L was paired with food in Context 2, then T was extinguished in Context 2 and L was extinguished in Context 1. The extinction phase lasted for 3 days. For Experiment 2, each stimulus was extinguished in a separate context (Contexts 2 and 3), the identities of which were counterbalanced. Each day, subjects underwent two extinction sessions, one in each extinction context with one stimulus undergoing extinction in each session. During these sessions the appropriate stimulus was presented eight times without reinforcement. The average intertrial interval and range during extinction were the same as during acquisition but the session duration was half as long as in acquisition. These daily sessions were separated by 30 min and run in a random order.

For Experiment 3, those subjects trained on the ABA renewal task were extinguished as described for Experiment 1, while those trained on the ABC task were extinguished as described for Experiment 2.

2.6.4. Renewal test phase—In Experiment 1, subjects underwent two test sessions, with one occurring in each context. Test 2 occurred 5 days after test 1 in order to promote some general level of response recovery. During each of these tests, each stimulus was presented without food four times with the average intertrial interval and range the same as in the training and extinction phases. The order of stimulus presentations on test 1 was T, L, L, T, L, T, T, L and the order was reversed for test 2. Magazine entries were monitored on each trial starting 15 s prior to CS onset until 20 s following CS offset. Animals were infused 15 min prior to these tests with either muscimol or its saline vehicle (see Section 2.3 above for details). Group assignments were made by matching for average responding to both stimuli on the last day of each preceding training phase (e.g., day 8 of acquisition and day 3 of

extinction). Six subjects were assigned to each infusion condition as a between subjects variable. Between tests, subjects were weighed and maintained at their target weights daily. Test 2 was conducted 5 days later but in the alternative context.

In Experiment 2, subjects underwent two tests, with one occurring in each of the two extinction contexts. The order of stimulus presentations on test 1 was T, L, T, L, L, T, L, T, L, T, L, T, L, T, L and this was reversed for test 2. Magazine approach was recorded as in Experiment 1, resulting in the Pre CS, CS and Post CS intervals. These tests were separated by 5 days. Additionally, group assignments for the test phase were made as described for Experiment 1. During each of these tests, each stimulus was presented without food eight times with the other session parameters, including intertrial interval, identical to the training phase. Animals were infused 15 min prior to these tests with either muscimol or its saline vehicle.

Subjects trained on the ABA task in Experiment 3 were tested as described for Experiment 1 (except for test sequence, see below), while for subjects trained on the ABC task, tests were conducted as described for Experiment 2. In contrast to Experiments 1 and 2, subjects in Experiment 3 did not undergo testing the day following extinction. Instead, they received stereotaxic surgery at this point. Following a 2-week recovery period from surgeries, rats received two tests separated by 5 days. The order of stimulus presentations (for both ABA and ABC tasks) on test 1 was T, L, T, L, L, T, L, T, L, T, L, T, L, T, L and this was reversed for test 2. Because animals had been lesioned, no infusions took place prior to tests in this experiment. Groups were matched using the same process as described for Experiments 1 and 2. In all cases, between tests, subjects were weighed daily and maintained at their target weights.

2.6.5. Delayed non-matching to place training—Following the second renewal test in Experiment 1, animals were given four sessions of exposure to the T-maze over 2 days, with two sessions on each day. During these sessions both arms of the maze held two food pellets each and were freely accessible. Rats were given a total of 10 min to explore the maze and retrieve the food rewards on day 1 and 5 min on day 2. If a rat picked up the food pellets before the allotted time had passed the animal was removed and returned home. If an animal failed to retrieve the food pellets they were removed and given another attempt 15 min later. The two daily sessions were separated by 2 h.

During training rats were transported to the maze room in their home tub with water freely available between trials. A single training trial consisted of two runs, one ‘sample’ run and one ‘choice’ run. On the sample run, one of the two arms was blocked at the junction while the available arm held a single food pellet. The side that was blocked off varied irregularly across trials. At the start of the trial, the rat was placed in the start box at the bottom of the T and released. Once the rat retrieved the sample food pellet they were removed from that corner of the maze and returned to the start box for the choice run. Released immediately, the animal then was given a choice between the two arms. The correct choice in this task was to choose the arm not visited during the forced sample run. This choice was reinforced with two food pellets. Choosing the incorrect arm resulted in no food, subjects simply remained in the incorrect corner for 10 s and were then brought back to the waiting area until the next trial. The intertrial interval was approximately 4 min and each rat received 10 trials daily. Testing began once the subjects reached the criterion of performing at 80% accuracy over two consecutive days.

Testing consisted of imposing varying delays between the sample and choice runs 15 min following infusions of either muscimol or its saline vehicle. Tests were done 1st using a 30 s interval, then using 60 s and finally 120 s. Each subject received 2 sessions at each of these

delays, one session followed saline infusions and the other followed muscimol infusions. The order of infusions for each delay was counterbalanced, however, the delays themselves were not. Sessions 1 and 2 used the 30 s delay, while sessions 3 and 4 used the 60 s delay and sessions 5 and 6 used the 120 s delay.

For Experiment 2, maze exposure, training and criteria were as described for Experiment 1 above. During the test phase, 60-s and 120-s delays were inserted between the sample and choice runs. Each subject received 2 sessions at each of these delays, one session followed saline infusions and the other followed muscimol infusions. Both the order of infusions for each delay and the delays were counterbalanced. There were 10 trials per session during tests.

For Experiment 3, initial training on the T-maze was the same as described above for Experiments 1 and 2. However, due to the nature of the neural manipulation used in this study, subjects were not trained to a specific acquisition criterion. They were simply given 4 sessions of zero delay training prior to the test session as previously described. During the test, a delay of 3 min was introduced between the sample and choice.

3. Results

3.1. Experiment 1: the effects of DH inactivation on ABA renewal on magazine approach

3.1.1. Acquisition and extinction—In all experiments reported here, magazine training was successful. By the end of this phase animals in each study had learned to retrieve food pellets from the magazine promptly following their delivery.

Data from the acquisition and extinction phases of the magazine approach task are presented in Fig. 1 for each day of training for Experiment 1. Preliminary analysis revealed that responding to the auditory stimulus was greater than to the visual stimulus during training $F(1,10) = 6.68, p < 0.05$, but this did not interact with either the group or training factors. Therefore, the data have been collapsed across this factor. Additionally, no significant effect of replication was found, so data were collapsed across this factor as well. In order to provide a measure of the conditioned effect of the stimuli, the data were expressed as CS–Pre CS difference scores. The training data were analyzed using a repeated measures ANOVA with an eight level within subjects factor of training day. Because preliminary analyses showed that group assignment did not interact with the factor of training day, the data were collapsed across this factor. The ANOVA yielded a significant main effect of training day $F(7, 70) = 7.93, p < 0.05$, indicating that as training progressed subjects came to respond more to the stimuli.

The extinction data were analyzed in the same manner as for the acquisition data. A significant main effect for training day confirmed that magazine approach was attenuated over the three days of extinction, $F(2, 20) = 19.94, p < 0.01$. A trial by trial inspection of these extinction data using a 2 (Interval; Pre and CS) $\times$ 24 (Trial) ANOVA revealed a main effect for Trial and Interval ($F(1, 11) = 40.21, F(23, 253) = 4.20$, both $ps < 0.01$) as well as an interaction between the Trial and Interval factors, $F(23, 253) = 3.56, p < 0.01$ (see Fig. 2). This analysis suggests that more responding was observed early in extinction during the CS interval compared to the Pre CS interval but that responding to the CS declined over trials and reached Pre CS levels late in extinction.

3.1.2. Renewal test phase—Data from the test phase for Experiment 1 are presented in Fig. 3 for the 12 subjects in this study (split into two equal groups of 6 vehicle and 6 muscimol subjects). Because no interactions with test session were found, the data were collapsed across this factor. Responding during the 15 s stimuli as well as the 20 s post

stimulus periods were converted into difference scores by subtracting from these values, responding during the 15 s baseline (pre stimulus) period. Responding during the 20 s period following CS offset was included in the analysis because food had normally been delivered at this point. Therefore, an increase in magazine responding following a CS compared to the baseline period would reflect anticipation of reinforcement. Because each CS was tested in each context over the two test sessions, responding is expressed as a function of test location. In other words, stimuli that were tested where they had undergone extinction (i.e., Cxt 1: CS2, Cxt 2: CS1) were averaged together and are referred to as ABB compared to ABA stimuli, which were tested in their acquisition contexts (i.e., Cxt 1: CS1, Cxt 2: CS2). The data in Fig. 3 suggests that subjects infused with the saline vehicle prior to the test sessions showed more responding when a CS was tested in its acquisition context than its extinction context. This renewal effect was evident during the stimuli as well as during the 20 s post stimulus period when the food US had normally been delivered. The animals infused with muscimol did not differ from the control group. These impressions were confirmed by a $2 \times 2 \times 2$ split plot ANOVA using recording interval (CS, Post CS) and stimulus condition (ABB, ABA) as within subject factors and infusion (Vehicle, Muscimol) as a between subject factor. This analysis revealed a significant main effect of stimulus, $F(1, 10) = 6.14$, $p = 0.033$, indicating that subjects responded more when a stimulus was presented in its acquisition context than in its extinction context. Furthermore, a significant effect of recording interval $F(1, 10) = 7.50$, $p < 0.05$ indicates that subjects responded more during the stimulus than during the 20 s post stimulus period. Importantly the renewal effect did not interact with the infusion factor, $F(1, 10) = 0.04$, $p > 0.05$, confirming that both groups equally exhibited the effect. Furthermore, the stimulus effect did not interact with interval, $F(1, 10) = 2.17$, $p > 0.05$.

An analysis on Pre CS responding revealed no significant effects. The average Pre CS rates for subjects infused with vehicle were 2.3, and 1.4 responses per minute for the Pre ABB and Pre ABA intervals respectively. For the subjects infused with muscimol, the average Pre CS rates were 5.0 and 2.7 for the Pre ABB and Pre ABA intervals respectively.

3.1.3. Delayed non-matching-to-place—Data from the T-maze task in Experiment 1 are presented in Fig. 4 in terms of mean percent correct following infusions of muscimol or its saline vehicle irrespective of delay condition. Because preliminary analyses found no interactions between infusion and delay length this factor was ignored in the analysis. Four subjects that participated in the renewal task were unable to contribute to the T-maze task. Two of these animals appeared to have loose cannulas while the other two exhibited poor health following the end of the renewal task. These animals were perfused in the time between the two tasks in order to preserve their brains for histological analysis. A 1-way ANOVA indicated that performance was significantly worse following infusions of muscimol than when testing occurred after saline infusions, $F(1, 7) = 7.63$, $p < 0.05$. A supplementary 2-way ANOVA found that the effect of Infusion did not interact with group assignment for the renewal task, $F(1, 6) = 0.36$, $p > 0.05$, indicating that animals in both infusion conditions displayed the delayed non-matching to place impairment.

3.1.4. Histology—The results of the histological analysis are presented in Fig. 5 for all subjects in Experiment 1. No subjects were excluded on the basis of inaccurate cannula placement. While four of the subjects did not contribute data to the delayed non-matching to place task, they are included in this figure because they did contribute data to the renewal test results. Fig. 5 shows the sites of infusion for all subjects in this study. Injection sites are indicated for subjects infused with muscimol during the renewal tests by white boxes and for subjects treated with muscimol during these tests with black boxes. These sites were identified at the base of the cannula tracks and their locations were determined by using a stereotaxic atlas as a reference. The infusion sites were mostly found to be along the dorsal

portions of the hippocampus, with some sites located slightly more ventral towards the anterior part of the hippocampus, however, well within the dorsal most layer of the structure. No subjects were excluded from the analysis due to inaccurate placement of the cannulas.

3.2. Experiment 2: the effects of DH inactivation on ABC renewal of magazine approach

3.2.1. Acquisition and extinction—Out of the 28 subjects that started the study only 19 were included in the magazine approach task (12 subjects were infused with vehicle while 7 received muscimol). Of those excluded, two subjects' cannulas became loose and fell off during training, two rats were excluded due to improper placement of the cannulas and five were excluded due to problems during the infusions preceding the renewal test phase (e.g., animals either became very sluggish or incapacitated as a result of infusions). Out of the 19 rats given renewal testing, three subjects did not contribute to the delayed non-matching to place task because these rats exhibited signs of poor health prior to or during the course of maze training. Of these three subjects, one had received muscimol during the renewal test phase while the other two served in the control group.

Magazine entry data from the acquisition and extinction phases are presented in Fig. 1. Preliminary analysis, once again, revealed more responding to the auditory stimulus compared to the visual cue, $F(1, 17) = 35.77, p < 0.01$, but because there were no interactions with group assignment the data were collapsed across this factor. Additionally, there was no significant effect of replication during training and extinction, therefore the data were collapsed across this factor. Responding to the stimuli increased over training sessions. During extinction responding was quickly attenuated and remained low throughout extinction. It should be noted that in this phase the stimuli were presented in novel contexts, and this could account for why responding was immediately suppressed on the first day of extinction. A significant main effect of training day confirmed the impression that conditioned magazine approach responding increased over training, $F(7, 126) = 29.20, p < 0.01$. By the first day of the extinction phase, responding during the stimuli was attenuated such that no significant effects were observed in the analysis of those data. Responding remained very low through out the extinction phase.

The extinction data were further explored on each trial to determine if some level of conditioned responding was evident at the very beginning of extinction training (see Fig. 2). Responding during the CS clearly exceeded Pre CS levels early in extinction, but this difference disappeared by the end of extinction. These data were analyzed with a Trial $\times$ Interval (CS, Pre) ANOVA, and the analysis revealed a significant effect of Interval and Trial ($F(1, 13) = 7.10, p = 0.019$; $F(23, 299) = 2.55, p < 0.001$), but no significant Trial $\times$ Interval interaction ($F(23, 299) = 1.40, p = 0.108$), indicating that while responding was generally higher during the CS, overall responding was attenuated across trials comparably, suggesting that extinction was more gradual than suggested by the blocked data in Fig. 1.

3.2.2. Renewal test phase—Thirteen subjects were infused with vehicle prior to tests, while 7 subjects received muscimol. Data from the Experiment 2 test phase are presented in the form of difference scores (CS–Pre CS) in Fig. 3(upper right panel) and are expressed as responding to a CS undergoing testing either in its extinction context (ABB) or outside of the extinction context (ABC). For example, when CS1 was tested in Context 2, this was coded as an ABB condition, but when CS1 was tested in Context 3 this was coded as an ABC condition. Subjects infused with saline prior to being tested showed more CRs when a CS was tested outside of compared to within its extinction context. This effect was seen during the presentation of the stimuli as well as during the 20 s period immediately following CS offset. Subjects who had their DH inactivated with muscimol prior to testing showed the same pattern of results as the control group. Preliminary analyses did not find a

significant effect of test (e.g., test session 1 vs test session 2) or a stimulus $\times$ test session interaction, therefore, the data are collapsed across this factor. Additionally, there were no significant effects of replication, and so the data were collapsed across this factor as well. A split plot ANOVA (Renewal (ABB, ABC) $\times$ Interval (CS, Post CS) $\times$ Infusion (Veh, Mus)) revealed a significant main effect of Renewal, $F(1, 18) = 4.51$, $p < 0.05$, which did not interact with any of the other factors. Finally, there was no significant main effect of Infusion despite a trend of overall reduced performance in the animals infused with muscimol.

3.2.3. Delayed non-matching-to-place—Data from the delayed non-matching to place task are presented in Fig. 4. Mean percent correct choice responding is shown as a function of delay (60 s vs 120 s) and infusion condition (saline or muscimol). The figure shows that subjects performed worse (i.e., with reduced accuracy) on muscimol than vehicle sessions. This was true for both the 60 s and 120 s delay tests. The data were analyzed using an Infusion $\times$ Delay repeated measures ANOVA and revealed that subjects performed worse following muscimol infusions compared to its saline vehicle regardless of the delay interpolated between sample and choice runs, $F(1, 15) = 22.08$, $p < 0.01$. A supplementary ANOVA with group assignment during the ABC renewal task as a factor, revealed that subjects in the vehicle and muscimol conditions during ABC renewal both showed the delayed non-matching to place impairment.

3.2.4. Histology—Histological results displaying the intracerebral injection sites for animals included in Experiment 2 can be found in Fig. 5. The injections sites were located and referenced as described above. Generally the injections occurred at the boundary of the DH and the cortex with very few sites being found ventrally beyond this area.

3.3. Experiment 3: the effects of post training DH lesions on ABA and ABC renewal of magazine approach

3.3.1. Acquisition and extinction—Out of the 52 animals that started the study a total of 8 were excluded from analysis. One of these rats died during the course of training, another was excluded due to aberrant responding and the remainder were excluded due to insufficient damage to the DH. A further three animals that were included in the renewal tasks were excluded from the delayed non-matching to place task, as they were perfused prior to completion of the maze task because these rats became sick and could not maintain their body weights.

Magazine response rate data from the acquisition and extinction phases are presented above in Fig. 1 for subjects trained on the ABA (lower left panel) and ABC (lower right panel) tasks. Because stimulus did not interact with surgical condition the data were collapsed across this factor. Additionally, no significant effects were found with gender as well as replication, therefore, the data were collapsed across these factors. Subjects in both the ABA and ABC training conditions showed increased CRs to the stimuli over the acquisition phase. Conditioned responding was attenuated during the extinction phase for subjects in all conditions as well. However, subjects trained on the ABC tasks showed a greater reduction in CRs on day 1 of extinction than the ABA trained subjects replicating the effect seen during extinction in Experiment 2.

The data for each training phase were analyzed using separate Task $\times$ Session repeated measures ANOVAs. The analysis of acquisition data revealed a significant main effect for session confirming the impression that as training proceeded subjects increased their responding during the stimuli, $F(7, 301) = 49.85$, $p < 0.05$. Session did not interact with the Task factor indicating that acquisition was the same for subjects in all conditions, $F(7, 301)$

$= 0.65$, $p > 0.05$. Data from the extinction phase were analyzed similarly, and revealed that responding during the stimuli was attenuated over the extinction phase, $F(2, 86) = 12.10$, $p < 0.05$. However, a significant Task $\times$ Session interaction confirmed the impression that for subjects trained on the ABC task, conditioned responding was more quickly reduced in the first extinction session than for subjects trained on the ABA task, $F(2, 86) = 6.85$, $p < 0.05$.

Trial by trial data from the extinction phase for subjects trained on the ABC task can be seen in Fig. 2. These data were further analyzed with a Trial $\times$ Interval (CS, Pre) ANOVA, which revealed a significant effect of Interval and Trial ($F(1, 27) = 5.41$, $p = 0.028$; $F(23, 621) = 3.30$, $p < 0.001$), as well as a significant Trial $\times$ Interval interaction, $F(23, 621) = 1.62$, $p = 0.035$. Follow up Least Significant Difference (LSD) effect tests indicated that responding during the Pre CS period was significantly lower than during the CS on trial 8, 95% CI Pre CS [1.322, 3.392], CS [3.424, 8.004] and trial 24, 95% CI Pre CS [0.009, 0.562], CS [1.063, 4.223]. These results indicate that overall responding generally decreased over trials, and that CS responding was modestly higher than Pre CS responding on at least some trials (see Fig. 1).

3.3.2. Renewal test phase—Magazine entry data from the test phase are presented in Fig. 3 in the lower left panel for the two groups trained on the ABA task (8 lesions and 11 shams) and the lower right panel for the groups trained with the ABC task (12 lesions and 13 shams). One outlier subject from the sham-operated group trained on the ABC task was excluded from the analyses because this subject responded beyond two standard deviations above the mean of the group. None of the statistical results to be reported changed when this subject's data were included. Preliminary analyses detected a main effect of Test Session. However, this did not interact with any other factor, therefore, the data were collapsed across this factor. Because replication and gender did not interact with Surgical Condition (Sham or Lesion) or Task (ABA or ABC renewal), the data were collapsed across these factors as well. Sham operated subjects trained on the ABA task showed more magazine CRs when stimuli were tested in their acquisition compared to extinction context (Fig. 3, lower left panel). This same pattern of responding was seen in the lesioned animals trained on the ABA task further suggesting that the DH is not involved in ABA renewal of appetitive learning. Similarly, lesioned subjects trained on the ABC task showed a similar pattern of responding, although, the overall magnitude of renewal appeared reduced compared to ABA renewal (Fig. 3, lower panels). More CRs were observed for these subjects when stimuli were tested outside of compared to within their extinction context.

Test data were analyzed using a stimulus $\times$ recording interval $\times$ surgical condition $\times$ task split plot ANOVA. This analysis revealed a significant main effect of stimulus, $F(1, 40) = 13.56$, $p = 0.001$, which did not interact with task or surgical condition. This effect indicates that subjects in all surgical conditions showed an increase in magazine approach CRs when extinguished stimuli were tested outside of their extinction contexts. A significant main effect for recording interval was also found, $F(1, 40) = 36.79$, $p < 0.001$, indicating that more responding was seen during the stimuli compared to the post stimulus periods. No other significant main effects or interactions were obtained.

3.3.3. Delayed non-matching-to-place—Data from the non-matching to place task are presented in Fig. 4(lower panel) above. The figure shows mean percent correct during the test. The left side depicts the data from subjects trained on the ABA task, while the right side displays similar data from subjects trained with the ABC renewal task. The figure suggests that DH lesioned subjects from all renewal training conditions performed with less accuracy than sham operated subjects. The data were analyzed with a Surgical Condition $\times$ Renewal type (ABA, ABC) factorial ANOVA. The only significant effect obtained was a main effect for surgery, $F(1, 38) = 6.96$, $p < 0.05$, confirming that hippocampally lesioned subjects

performed worse than sham subjects. Supplementary analyses showed no significant interactions between the factor of Surgery and Gender as well as Replication.

3.3.4. Histology—Histological results are presented in Fig. 5. This figure depicts the extent of damage induced in this study throughout the DH. The shading corresponds to the damage observed during histological evaluation. Subjects for whom portions of CA fields or the dentate gyrus remained intact despite undergoing experimental surgery were excluded from all analyses. The minimum (black) and maximum (grey) acceptable damage for inclusion in the study are shown on the left half of each atlas slide while the right side shows the target structure (DH) itself. While the extent of the lesion continued caudally, the ventral hippocampus was spared, with the dorsal damage conforming to the minimum maximum specifications presented in Fig. 5.

4. Discussion

The results of Experiment 1 demonstrated ABA renewal of magazine approach responding and showed that DH inactivation did not impair this effect. Experiment 2 demonstrated ABC renewal of magazine approach, and that inactivation of the DH did not impair this effect as well. The results of Experiment 3 replicated the ABA and ABC renewal effects seen in Experiments 1 and 2, respectively, and also revealed no effects of post-training neurotoxic DH lesions. However, in all of these studies, the neural manipulations did result in impaired performance on the delayed non-matching to place task conducted after renewal testing concluded, suggesting that these manipulations did have a functional impact. These results differ in some respects to those found in fear conditioning studies and so some discussion will follow concerning any similarities and discrepancies.

In fear conditioning, Corcoran and Maren [26] similarly showed that ABA renewal was unimpaired by DH inactivation. However, because Corcoran and Maren [26] used test contexts that differed in their associative history with the shock US, the nature of the psychological process (summation or occasion setting) underlying the behavioral results remains unclear. On the other hand, the design for Experiment 1 of the current paper did equate the conditioning history of the test contexts. Thus, the present studies can be seen as extending those of Corcoran and Maren [26] to an appetitive task and to an occasion setting renewal design. Because each context was the acquisition context for one stimulus and the extinction context for the other, simple summation-based accounts do not adequately account for renewal under these conditions. Bouton's [2,3] modulatory account of extinction offers a more plausible explanation of renewal using such a design. Because Experiment 1 did not find an effect of DH inactivation on renewal, this suggests that the DH is not involved in the conditional control of extinguished appetitive learning in an ABA renewal design.

Ji and Maren [18], however, reported that electrolytic DH lesions did impair ABA renewal of fear conditioning. Therefore, it was unexpected to find that ABA renewal of magazine approach was not disrupted by DH lesions in Experiment 3. One potential reason for the discrepancy between Ji and Maren [18] and the ABA renewal findings in Experiment 3 of the current paper may be that aversive and appetitive extinction learning processes are fundamentally different from one another and involve different brain structures. However, other research suggests that this idea may not be entirely correct. For example, lesions of the infralimbic region of the vmPFC have been reported to increase spontaneous recovery in aversive [16] and appetitive conditioning preparations [27]. Additionally, the amygdala has been found to be involved in fear conditioning and extinction (e.g., [15,28]) as well as in appetitive processes (e.g., [29]). This suggests that at least some of the underlying circuitry in appetitive and aversive extinction learning overlaps.

Another difference between the findings of Ji and Maren [18] and those of Experiment 3 worth noting is the technique used to lesion the DH. Electrolytic lesions are known to destroy axonal fibers in the vicinity of the electrode tip leaving open the possibility that communication between structures in other parts of the brain may have additionally been compromised. The NMDA lesions used in Experiment 3 are known to selectively destroy cell bodies in the vicinity of the diffusion zone without affecting fibers of passage. It may be the case that collateral damage in the Ji and Maren [18] study could have resulted in impaired ABA renewal of fear conditioning. Finally, another difference between the two studies is that the experimental design used in Experiment 3 likely assessed the contribution of conditional control processes in renewal whereas simple context-US summation processes could have controlled performance in the Ji and Maren [18] study. Any one of these differences could account for the discrepant findings and future work will be required in order to resolve the discrepancy.

The findings of Experiments 2 and 3 are contrary to those reported by Maren and colleagues [20,26], in which ABC renewal of fear conditioning was impaired by DH inactivation. As noted above, the design used by Corcoran and Maren [26] was quite different from the design used in Experiments 2 and 3 and is, therefore, amenable to an analysis in terms of context-US summation processes. Therefore, the present results are not, strictly speaking, in direct conflict with these particular findings. However, Maren and Hobin [20] used a nearly identical design to the ABC design used in the current paper. Therefore, the failure to observe impaired ABC renewal in DH-inactivated subjects in the present situation was unexpected. The results of the delayed non-matching to place task suggests that the neural manipulation was effective, so the failure to find an effect on renewal was not due to a poor neural manipulation. However, two procedural differences between our experiments may have played an especially important role. Maren and Hobin [20] used a fixed ITI throughout training and extinction, whereas variable ITIs were used in the present tasks. In addition, during the conditioning phase trials strictly alternated in Maren and Hobin's [20] study whereas they were pseudo randomly interspersed in the present studies. As a result of these differences, subjects in Maren and Hobin's [20] experiment may have used the time between stimuli as effective "context" cues that helped differentiate the acquisition and extinction contexts.

More specifically, during training, Maren and Hobin [20] used a fixed ITI of 62 s and two different 10 s stimuli were trained in an alternating manner. This resulted in each individual CS being presented every 134 s. During extinction, each CS was extinguished in a distinct extinction context and was presented every 60 s. Thus, each CS was separated by a longer ITI during training than during extinction. The longer ITI could have set the occasion for the target stimulus being reinforced, while the shorter ITI could have set the occasion for nonreinforcement of the target stimulus. In the current ABC renewal studies, stimuli did not strictly alternate during acquisition, and variable ITIs were used. Therefore, subjects could not have as easily used the ITI to differentially cue acquisition and extinction trials. It seems possible that the DH may especially be important in the conditional control of extinction when temporal cues act as occasion setting stimuli.

One final point should be mentioned regarding the failure to observe effects of DH manipulations on ABC renewal. Overall session responding was very quickly reduced in the first session of extinction in our ABC renewal task and fell no lower as a result of the following sessions in these studies, although a more fine-grained trial $\times$ trial analysis revealed some evidence of extinction. Nevertheless, while the length of the extinction phases for the ABA and ABC tasks were the same, the subjects trained with the ABC task may have acquired extinction more rapidly than ABA-trained subjects because the extinction contexts used in the ABC studies were not previously used as acquisition

contexts. Additionally, magazine training was not conducted in these contexts. In other words, residual excitation activated by the context after initial training may have slowed extinction for subjects trained on the ABA task, but not the ABC task. The trial by trial extinction data from Experiment 1 presented in Fig. 2 support this claim. These subjects appeared to show more responding to the CS early during the extinction phase.

Caution should be used, however, in interpreting why responding was so strongly attenuated during the first session of extinction. A perhaps simpler explanation (than a faster extinction rate) is that the low levels of CRs seen during extinction may be attributable to the external inhibition produced by the novel situation presented during extinction. Subjects were placed in novel contexts and then experienced CS presentations for the first time in these new environments. These conditions could have masked the full expression of excitatory conditioning to the CS resulting from the initial training phase. The trial $\times$ trial extinction data supports this view since CS responding was only very modestly higher than Pre CS responding from the beginning of extinction. Had the rate of extinction been accelerated in the ABC condition, then we would have expected to see much higher levels of responding during the early extinction trials.

An additional explanation for low responding during extinction in the ABC studies is based on the notion of generalization decrement that has sometimes been reported to occur when a stimulus is tested outside of its acquisition context. Although, Bouton has rarely seen evidence of such decrement in aversive learning situations (e.g., [39]), evidence of such decrement is sometimes seen in aversive (e.g., [30]) and appetitive settings (e.g., [31]). If magazine approach responding were specific to its training context in the present situation, however, then there would be little evidence of responding when stimuli were tested outside of their training context. In the ABC tasks reported here we observed relatively low levels of responding, but, nevertheless, we did observe statistically reliable increases in responding when the CSs were tested outside of their extinction contexts both in the ABA and ABC experiments. Furthermore, we observed that the CSs commanded higher levels of responding during extinction in the ABA than the ABC studies, but in both cases the CSs were extinguished outside of their acquisition context. We would not expect generalization decrement to differ in these two cases, but whereas in the ABA studies the extinction context was not truly novel it was in the ABC studies. This more strongly supports the external inhibition account described above.

Despite these possibilities, the renewal effects reported here likely reflect an underlying conditional learning process in which the extinction context sets the occasion for retrieval of the extinction memory and hence reduced CRs. The results suggest that the DH is not needed for conditional control of extinction by physical contexts in an appetitive task. This does not rule out that other parts of the hippocampus may be more important than previously thought for context-dependent learning and memory. For example, Sotres-Bayon et al. [32] recently reported that cells in the ventral hippocampus are required for gating activity in the prelimbic cortex after, but not before extinction. Another possibility is that other structures may be involved – Frankland and colleagues [33] have suggested that the anterior cingulate gyrus plays some role in tasks that are hippocampal-dependent. It seems possible that manipulations of this structure would have an effect on the kinds of conditional control processes that we have identified in our study of appetitive renewal. Future work will be required to address these possibilities.

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HIGHLIGHTS

- Our appetitive renewal designs controlled for test contexts' associative histories.
- Neither lesions nor inactivation of the dorsal hippocampus impaired renewal.
- Both neural manipulations impaired performance on probe tasks.
- Renewal may sometimes depend on different underlying psychological mechanisms.
- The dorsal hippocampus may not be involved in all instances of renewal.

Fig. 1.

Acquisition and extinction data for Experiments 1 (upper left), 2 (upper right) and 3 (lower left for ABA, lower right for ABC trained subjects). Data are presented in the form of difference scores (CS-Pre CS) and are collapsed across the modality of CS.

Fig. 2. Trial by trial extinction data for Experiments 2 (top) and 3 (GP ABC; bottom). Mean magazine responding during the pre and CS periods are presented for each trial during the extinction phase.

Fig. 3.

Test data for Experiments 1 (upper left), 2 (upper right) and 3 (lower left for ABA, lower right for ABC trained subjects). Data are presented in the form of difference scores (CS–Pre CS) and are collapsed across test session as well as the modality of CS and presented in terms of test location (i.e., ABB, ABA or ABC).

Fig. 4.

Test data from the delayed non-matching-to-place task for Experiments 1 (upper left), 2 (upper right) and 3 (lower center). Data are presented in terms of percent correct. Data for Experiment 1 are collapsed over 60 and 120 s delays, while data for Experiment 2 maintain this factor. Data for Experiment 3 are presented for subjects trained on the ABA task on the left portion of the figure and on the right for ABC trained subjects. Experiment 3 tests were conducted with a 3 min delay.

Experiment 1**Experiment 2****Experiment 3**

Fig. 5. Histological figures for Experiments 1 (left), 2 (middle) and 3 (right). For Experiments 1 and 2, subjects infused with vehicle during the renewal task have their injection sites labeled with white boxes while subjects infused with muscimol during the renewal tasks have the sites labeled with black boxes. For Experiment 3, the minimum (black) and maximum (grey) extent of the lesions for subjects included in the study are presented. The anterior-posterior site of each section relative to bregma is displayed on the right in mm.

Table 1

The designs for Experiments 1 (upper), 2 (central), and 3 (lower). CX 1, 2 and 3 are the contexts used throughout the studies. S1 and S2 stand for the Tone and Light stimuli, while + and – stand for reinforced (food pellet) and non-reinforced training respectively. The orders of surgery and infusion events are indicated with arrows.

Experiment 1: The Effects of DH Inactivation on ABA Renewal of Magazine Approach

<u>Acquisition</u>	<u>Extinction</u>	<u>Infusions</u> ↓	<u>Test 1</u>	<u>Rest</u> 5 Days	<u>Infusions</u> ↓	<u>Test 2</u>
CX 1: S1+	CX 1: S2-		CX 1: (VEH) S1-, S2- ?			CX 2: (VEH) S1-, S2- ?
CX 2: S2+	CX 2: S1-		CX 1: (MUS) S1-, S2- ?			CX 2: (MUS) S1-, S2- ?

Experiment 2: The Effects of DH Inactivation on ABC Renewal of Magazine Approach

<u>Acquisition</u>	<u>Extinction</u>	<u>Infusions</u> ↓	<u>Test 1</u>	<u>Rest</u> 5 Days	<u>Infusions</u> ↓	<u>Test 2</u>
CX 1: S1+, S2+	CX 2: S1-		CX 2: (VEH) S1-, S2- ?			CX 3: (VEH) S1-, S2- ?
	CX 3: S2-		CX 2: (MUS) S1-, S2- ?			CX 3: (MUS) S1-, S2- ?

Experiment 3: The Effects of Post Training DH Lesions on ABA and ABC Renewal of Magazine Approach

	<u>Acquisition</u>	<u>Extinction</u>	<u>Surgery</u> ↓	<u>Test 1</u>	<u>Rest</u> 5 Days	<u>Test 2</u>
GP ABA	CX 1: S1+ CX 2: S2+	CX 1: S2- CX 2: S1-		CX 1: S1-, S2- ?		CX 2: S1-, S2- ?
GP ABC	CX 1: S1+, S2+	CX 2: S1- CX 3: S2-		CX 2: S1-, S2- ?		CX 3: S1-, S2- ?