Contents lists available at ScienceDirect

Behavioural Brain Research

Research report

Renewal of extinguished behavior in pigeons (*Columba livia*) does not require memory consolidation of acquisition or extinction in a free-operant appetitive conditioning paradigm

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ABSTRACT

Extinction learning is a fundamental capacity for adaptive and flexible behavior. As extinguished conditioned responding is prone to relapse under certain conditions, the necessity of memory consolidation for recovery phenomena to occur has been highlighted recently. Several studies have demonstrated that both acquisition and extinction training need to be properly consolidated for a relapse of the original acquired memory trace to occur. Does this imply that extinguished responses cannot relapse before memory consolidation? To answer this question, we investigated the renewal effect subsequent to an immediate or a delayed (24 h) extinction in a discriminative operant conditioning paradigm. In three different experiments, we could show (1) that acquisition learning does not need to be long-term consolidated for the occurrence of renewal, (2) that the offset of extinction training is a reliable marker for extinction recall in a free-operant extinction learning paradigm where organisms undergo consecutive acquisition training, extinction training as well as testing of conditioned responding and (3), that immediate and long-term consolidated renewal do not demonstrate any qualitative difference in terms of the behavioral output. Our results indicate on the behavioral level that the inhibitory nature of extinction is already present in free-operant learning paradigms and that it does not seem to be affected by the absence of long-term memory consolidation.

1. Introduction

The capacity to flexibly adapt behavior to changing environmental conditions is a fundamental evolutionary component for survival. Reflecting this fundamental importance, the ability to extinguish already learned behaviors is widespread across animal species. This ability described for rodents or primates [1–4] has also been demonstrated in birds [5,6], fish [7] and invertebrate species as honeybees [8,9]. Ever since its first scientific description by Pavlov [10], this phenomenon became a prime target for research in the field of learning theory. In many models of learning and memory, extinction is described as an erasure process that involves the subsequent loss of the original acquisition memory during the extinction phase [11,12]. However, extinction research has uncovered several phenomena demonstrating that the original memory is inhibited by a secondary memory trace suppressing the initially acquired CS-US association [13]. Since the analyses of these phenomena sometimes went along with a confusing usage of terminology, we will adopt in the following the nomenclature

established by Lonsdorf et al. [14] to clearly differentiate between procedure, theoretical account and the effect in extinction learning.

A prominent example demonstrating that extinction learning is not solely a process of erasure of the acquisition memory is the renewal effect. Renewal occurs when the acquisition training is conducted in Context A and the extinction training takes place in Context B [15–17]. In a test for the return of conditioned responding, subjects are re-tested in the acquisition training context (Context A) following extinction training to measure the relapse of the originally learned response behavior. This procedure is known as ABA renewal [18]. However, renewal has also been found in ABC [19,20] and even AAB experimental designs [21].

An important question in extinction research has been the necessity of memory consolidation for later recall. As memory consolidation relies on protein synthesis which requires at least several hours to occur [22–24], two critical intervals exist where consolidation might occur: following the acquisition training and following the extinction training phase.

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Consolidation after acquisition: Myers et al. [25] demonstrated the critical role of the interval between acquisition training and extinction training. In their study they contrasted a 10 min, 1 h, 24 h and 72 h interval between acquisition training and extinction training. They found that short intervals of 10 min did not elicit a relapse of the original CS-US association, but a 72 h interval elicited robust spontaneous recovery, reinstatement, and renewal. This result indicated that the acquisition memory needs to be properly consolidated for relapse phenomena to manifest. Straus et al. [26] investigated the effects of sleep deprivation following both acquisition and extinction learning on extinction recall. They found that extinction recall was impaired only if the participants were sleep deprived following the initial conditioning. As sleep enhances memory consolidation [27,28], these results also indicate that memory consolidation plays an important role after the initial acquisition for proper extinction retrieval.

An important phenomenon relating to potential effects of memory consolidation following the acquisition training is the immediate extinction deficit [29]. The immediate extinction deficit refers to a decrement in long-term suppression of conditioned responding if extinction training is conducted immediately after acquisition training, a result completely opposed to that of Myers et al. [25]. Many studies found evidence that the relapse of conditioned responding following extinction training is more severe if extinction training is applied immediately after acquisition training [30–36]. However, the immediate extinction deficit has so far only been demonstrated in Pavlovian fear conditioning and is therefore mostly associated with elevated stress levels suggesting that it is not due to improper memory consolidation of the acquisition memory, but rather due to a deficit in extinction learning itself [29,37].

Consolidation after extinction: In contrast to research on potential effects of the interval between acquisition training and extinction training, studies investigating the effects of different test intervals after extinction training with respect to the return of conditioned responding are sparse. While several studies have shown that extinction recall is impaired if memory consolidation is disrupted by administering e.g. anisomycin [38], β-blockers [39], PKA-inhibitors [39], NMDA receptor antagonists [40], TTX [41], scopolamine [42] or PACAP [43] into central structures of the extinction network prior to extinction training, the systematic effects of delayed vs. non-delayed extinction recall are still widely unknown. Kim and Richardson [44] were the first to systematically modulate the extinction-test interval by varying the consolidation period following extinction training. They hypothesized that because the extinction memory consists of two separate memories (a CS-no US memory and a context memory, [45]), these two memory traces might consolidate at a different rate and therefore could not allow for renewal to occur. The extent of renewal was tested both following a 10 min and a 24 h interval between extinction training and the return of conditioned responding test. In the 10 min condition they found no evidence of a return of conditioned responding in an ABA compared to an ABB design indicating that the consolidation of contextual information during extinction training is necessary for renewal to occur. However, while some studies found renewal in continuous paradigms ([46,47,48];), no other study systematically varied the interval between extinction training and the subsequent test of a returned conditioned response.

In summary, for both critical intervals there are indications that memory consolidation is a prerequisite for proper acquisition and/or extinction recall. However, these results are either controversial in case of the acquisition-extinction interval as several studies found considerable post-extinction learning response relapse even in the absence of an acquisition training consolidation phase (e.g. [33,49–53]) or stem from a single study in case of the extinction-test interval. To extend the lacking body of research, we used a free-operant appetitive learning paradigm to investigate the effects of memory consolidation on extinction learning and renewal. Since the majority of research on extinction consolidation has been conducted in Pavlovian fear

conditioning, we aimed to find out if corresponding results could be found in appetitive free-operant conditioning. Especially for renewal, it has been proposed that Pavlovian fear conditioning and free-operant conditioning are subjected to different mechanisms of inhibition (cue inhibition vs response inhibition, [54]). It is therefore important to investigate extinction learning and relapse phenomena in free-operant appetitive conditioning paradigms. In experiment 1, we investigated if relapse of conditioned responding occurs under conditions of immediate extinction training and recall. We hypothesize that response relapse will occur in the absence of an acquisition consolidation phase similar to previous experiments [33,49–53]. In the second experiment, we addressed the question if the observed response recovery was true renewal or simply due to poor extinction recall. We hypothesize that the response recovery is not related to poor extinction recall similar to other studies using an immediate test phase following extinction training (e.g. [48]). In experiment 3, we systematically investigated the effects of a consolidation interval between extinction training and the return of conditioned response test phase on the extent of the renewal effect. Here, we hypothesize that the extent of renewal should be reduced if no memory consolidation occurs in accordance with the findings of Kim and Richardson [44].

2. Experiment 1

2.1. Methods

2.1.1. Subjects

Eight pigeons (*Columba livia*) obtained from local breeders served as subjects in the first experiment. Birds were housed in individual cages or local aviaries made of wire-mesh within a colony room controlled for temperature, humidity and light cycles (12 h light/dark cycles starting at 8am). All animals had free access to water and were maintained between 80% and 90% of the free-feeding body weight to keep the animals engaged in the experimental procedures. Subjects were treated in accordance with the German guidelines for the care and use of animals in science and all experimental procedures were approved by a national ethics committee of the State of North Rhine-Westphalia, Germany and were in agreement with the European Communities Council Directive 86/609/EEC concerning the care and use of animals for experimental purposes.

2.1.2. Apparatus

The experiments were performed in custom-built operant-chambers (35*35*35 cm [55] situated in sound-attenuated cubicles (80*80*80 cm). Each operant-chamber contained three potential pecking sites that were horizontally arranged on the rear wall. In the experiments, either touch screens or translucent response keys combined with a mounted LCD flat screen monitor were used to record pecking responses depending on the type of the operant chamber used. A food hopper was mounted below the pecking sites to provide food during the experiments. The boxes were illuminated by white LED strips attached to the ceiling of the box. Furthermore, red LED strips were mounted to the ceiling to induce contextual changes during the paradigm. Successfully registered key pecks were followed by an audio feedback. The hardware was controlled by a custom written MATLAB code using the Biopsychology toolbox (The Mathworks, Natick, MA, USA [56]).

2.1.3. Procedure

We employed a modified version of a consecutive extinction learning paradigm in which animals undergo acquisition training, extinction training and a subsequent return of conditioned responding test phase within one session [55,57]. The experiment employed a forced-choice paradigm in which the animals had to associate stimuli with corresponding choices. In the experiment, one single stimulus was presented per individual trial that signalled the animal to make either a
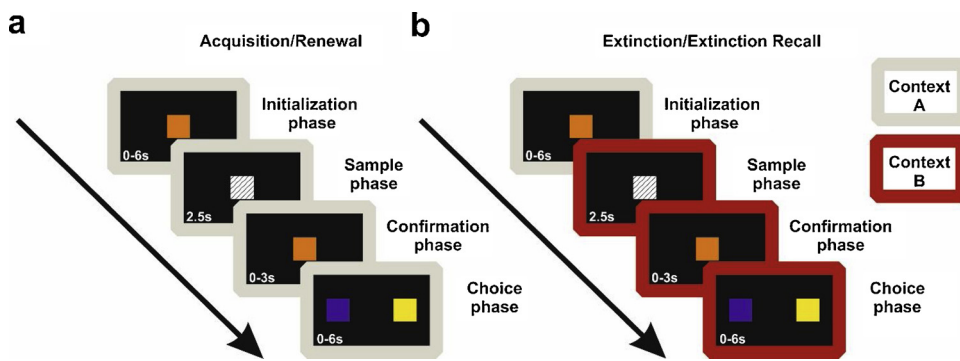
Fig. 1. Procedure of individual trials within the experiment. (a) Procedure during acquisition and test phase. During acquisition training and in the return of conditioned responding test condition, trials took place under house-light conditions (Context A; indicated by the white surrounding of the screen). Here, an initialization key appeared for up to 6 s. A peck onto this response key started the sample phase during which one of the four experimental stimuli was shown for a fixed period of 2.5 s. Following the sample phase, the animals had to confirm that they attended the sample by pressing a confirmation key that was visible for up to 3 s. After the confirmation, the choice keys were illuminated for a maximum of 6 s to allow the animal to make a choice. After the choice phase, an outcome phase of 2 s followed. Trials were separated by an ITI of 4 s. (b) Procedure during extinction training and extinction recall phase. The trial procedure was identical in the extinction training phase, but after a successful initialization, the house-light (Context A) was changed to a red LED (Context B) illumination to induce a context change. The red context remained until the end of the outcome phase or was shut off in the case of punishment. (For interpretation of the references to colour in the figure legend and text, the reader is referred to the web version of this article.)

left or a right choice at the end of the trial depending on the stimulus identity.

In brief, each trial started with the presentation of an initialization key for up to 6 s. A successfully registered key peck to the center response key triggered the 2.5 s sample presentation. As a sample, one of four stimuli was presented on the center key. The animals were then required to confirm that they attended the target stimulus by pecking on the center key once more following the stimulus presentation. After pecking on the confirmation key, the center key stimulus disappeared and the two choice keys were illuminated. The animal had to decide on a left or a right choice depending on the identity of the stimulus during the choice phase. If the animals made a correct choice, a 2 s long reward period commenced during which the food hopper was illuminated and food was presented. In the case of an incorrect choice, the lights in the operant-chamber were turned off for 2 s as mild punishment. Consecutive trials were separated by an inter-trial-interval (ITI) of 4 s duration. The structure of the trials for the different experimental phases is given in Fig. 1.

During the experiment, the animals were confronted with four different stimuli that were presented in a pseudorandomized order. Two of the stimuli were associated with a left choice and the other two stimuli were associated with a right choice (Fig. 2). During a single trial, only one of the four stimuli was presented on the center key. Therefore, the animal had to decide on a left or a right choice depending on the identity of this stimulus during the choice phase. Animals were pre-trained on two of the stimuli prior to the experimental session. Thus, two of these stimuli were familiar to the animals and they both served as control stimuli as well as fix points during the entire procedure. The other two stimuli were novel and the stimulus-response associations

had to be learned in an acquisition training phase through trial and error. The acquisition training phase followed the above described procedure and comprised a minimum of 200 trials. To finish the acquisition training phase the animals had to initialize 85% of the trials, perform above 85% correct responses towards the novel stimuli and above 80% correct responses towards the familiar stimuli. All these values were calculated as a running average over the past 100 trials.

The extinction training phase was marked by two key differences to the acquisition training procedure: (1) one of the novel stimuli was randomly chosen as extinction stimulus i.e. it was neither followed by reward nor by punishment after the choice of the animal (Fig. 2). Instead, a 2 s long period void of feedback regarding the pigeon's decision replaced the outcome phase. (2) After the initialization of the animal, a red LED light (light indicator of context B) replaced the white house light shown in the acquisition training phase (light indicator of context A, see Fig. 1b). The red LED light remained turned on until the end of the trial or a punishment condition was met. To counteract effects of the physical identity of context B, we also used a green LED light as indicator for contextual changes. Red and green extinction contexts were counterbalanced across animals and did not influence the animals' performance during the task. The extinction training phase comprised a minimum of 150 trials. To finish the extinction training phase, the animals had to initialize 85% of the trials, perform above 80% correct responses towards the non-extinction stimulus and more than 75% correct responses towards the control stimuli. For the extinction stimulus they had to perform below 20% correct choices. All these values were calculated as a running average over the past 100 trials.

The return of conditioned responding test phase mirrored the overall procedure of the acquisition training phase as the context was
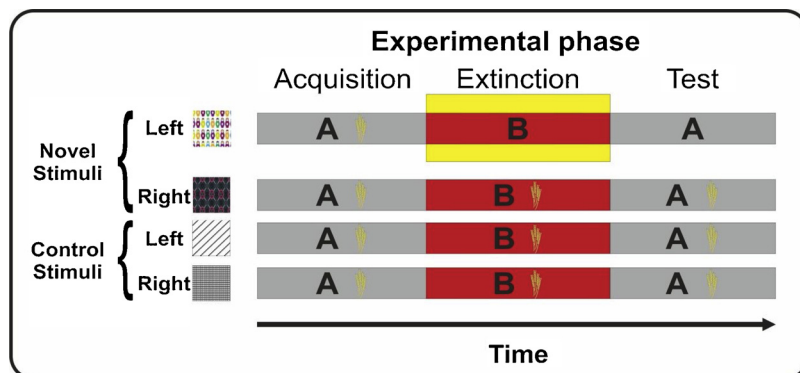

Fig. 2. Experimental procedure from experiment 1. The experiment was divided into the three distinct phases of learning. During acquisition training, animals learned the stimulus-response association for two previously unknown stimuli under white house-light conditions (context A). They were also confronted with two control stimuli that they had already acquired in a pre-training phase. After reaching the learning criterion of the acquisition training phase, animals went directly into the extinction training phase. Here, the houselight was replaced by a red LED light in each trial to induce a contextual change (context B). Of the two novel stimuli, one stimulus was randomly chosen for the extinction training. The extinction training stimulus was no longer followed by reward or punishment in case of a correct or incorrect choice. After the behavioral criterion for the extinction phase was reached, the testing phase began immediately. The reward contingencies were identical to those of the extinction phase, but

trials were again conducted in the acquisition context A to test for renewal, i.e. under white houselight conditions. (For interpretation of the references to colour in the figure legend and text, the reader is referred to the web version of this article.)

switched back to house light conditions thus signaling the animals a return to the acquisition context A (Fig. 1a). Importantly however, the extinction stimulus remained without feedback to measure the renewal effect (Fig. 2). The return of conditioned responding test phase lasted for a fixed number of 250 trials and required no behavioral criterion to be reached in order to finish the experiment.

2.1.4. Data analysis

In order to compare the acquisition training, extinction training and return of conditioned responding test phase across animals, we divided the total number of trials conducted for each individual stimulus into six even blocks. This was a necessary procedure as both the acquisition training and extinction training phase were of varying duration across animals as some animals reached the criterion sooner than others. While the number of trials in the return of conditioned responding test phase was fixed, not all animals continued to perform until all trials were concluded. For each experimental period, we then calculated the number of correct responses per stimulus as well as the number of pecks elicited by each stimulus during the stimulus presentation. Furthermore, we calculated active avoidance for the extinction stimulus during the extinction phase. Since the experiment was a forced-choice paradigm, the animals had to respond to advance to the outcome phase. Any non-response led to an abortion of the current trial. Active avoidance was calculated as the number of non-responses either during the confirmation or during the choice phase in extinction stimulus trials.

To estimate the amount of renewal, we quantified the return of correct responses across the whole return of conditioned responding test phase for the extinction stimulus and compared these responses to the last block of the extinction training phase using a paired t-test. This analysis was then repeated for each individual block of the return of conditioned responding test phase. Since the block-wise analysis required a total number of six paired t-tests, we used a significance threshold of $p < .008$ in accordance with a Bonferroni correction. Furthermore, we conducted the same analysis for pecking rates elicited by the extinction stimulus. Pecking rates were quantified as the number of pecking responses per stimulus presentation.
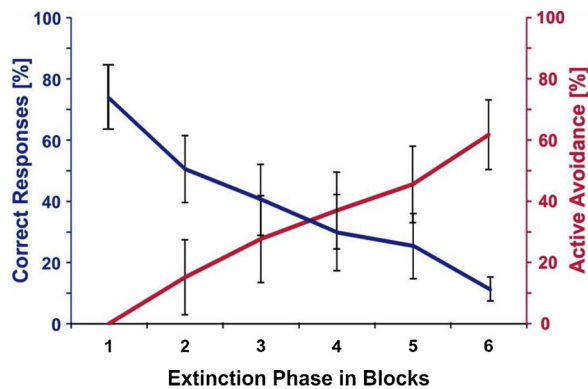

Fig. 4. Correct responses and avoidance behavior towards the extinction stimulus during the extinction training phase. To analyze whether the animals truly refrained from responding towards the extinction stimulus, we also computed the active avoidance after the presentation of the extinction stimulus. Performance therefore consisted of both random responses and active disengagement from responding towards the extinction stimulus at the offset of the extinction training phase. Error bars represent SEM.

2.2. Results and discussion

Learning curves for acquisition and extinction training are shown in Fig. 3. Active avoidance is plotted separately to demonstrate its interaction with the behavioral performance towards the extinction stimulus (Fig. 4). Correct responses were significantly increased compared to the last block of extinction training across the entire test phase ($t_{(7)} = 3.87$, $p = .006$, $d = 1.45$, Fig. 5a). Comparing the individual blocks of the return of conditioned responding test phase with the last block of extinction training revealed a significant difference for the first ($t_{(7)} = 8.04$, $p < .001$, $d = 3.21$) and the second block ($t_{(7)} = 4.75$, $p = .002$, $d = 1.80$). For pecking frequencies elicited by the extinction stimulus, we found an overall increase during the return of conditioned responding test phase compared to the last block of extinction training ($t_{(7)} = 3.35$, $p = .012$, $d = 1.91$, Fig. 5b). A block-wise analysis revealed a significant increase during the first block of the return of conditioned responding test phase ($t_{(7)} = 6.20$, $p < .001$, $d = 3.70$).

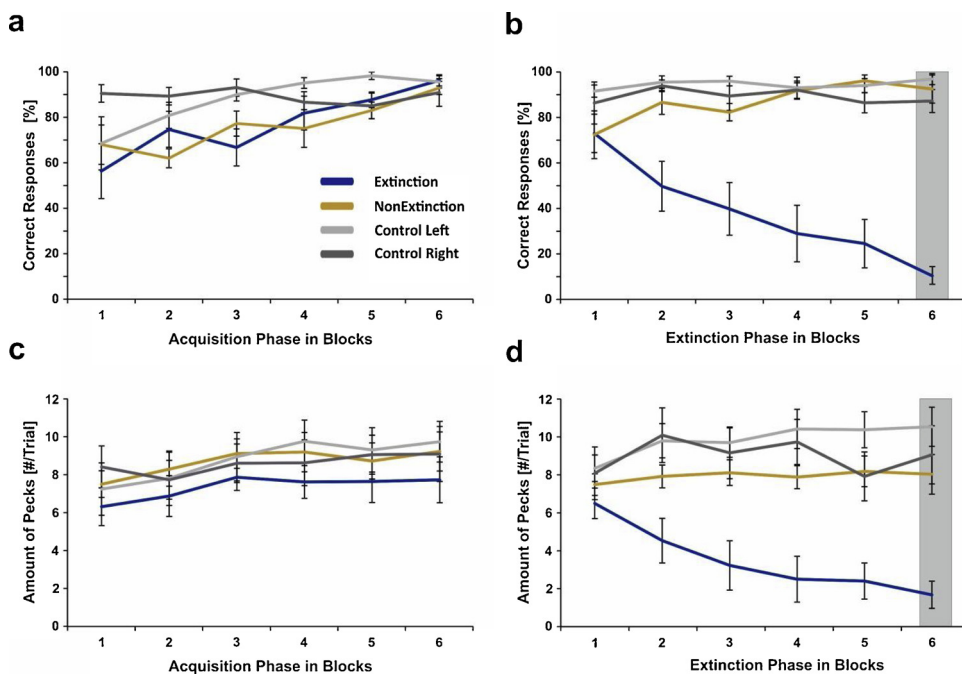In contrast to the findings of Myers et al. [25], we were able to

Fig. 3. Learning curves across acquisition training and extinction training during experiment 1. (a) Correct responses for the control stimuli (grey) and the test stimuli (extinction = blue, non-extinction = yellow) during the acquisition training phase. The phases were divided into six even blocks for each individual stimulus due to varying phase durations. (b) Same as in (a), but for the extinction training phase. The highlighted last block serves as a reference point to quantify the return of conditioned responding in all experiments. (c) Same as in (a), but for the number of elicited pecks during each trial per stimulus. (d) Same as in (c), but for the extinction training phase. Learning curves for experiment 2 and 3 can be found in the supplementary materials. Error bars represent SEM. (For interpretation of the references to colour in the figure legend, the reader is referred to the web version of this article.)
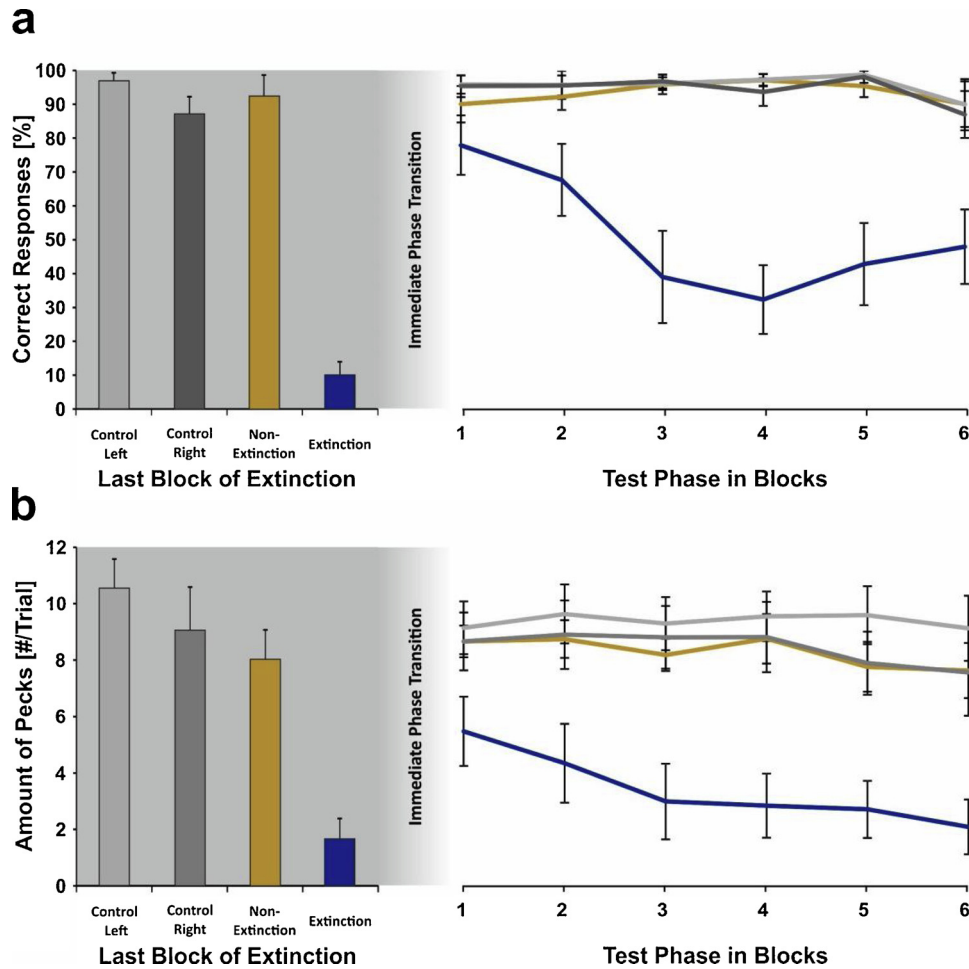
Fig. 5. Correct responses and pecking frequencies across stimuli during the last block of extinction training and the return of conditioned responding test for experiment 1. (a) The performance across stimuli during the last block of extinction training is shown on the left. Performance during the return of conditioned responding test divided into six even blocks is shown on the right. (b) Same as in (a), but for the number of pecks elicited by each stimulus. Error bars represent SEM.
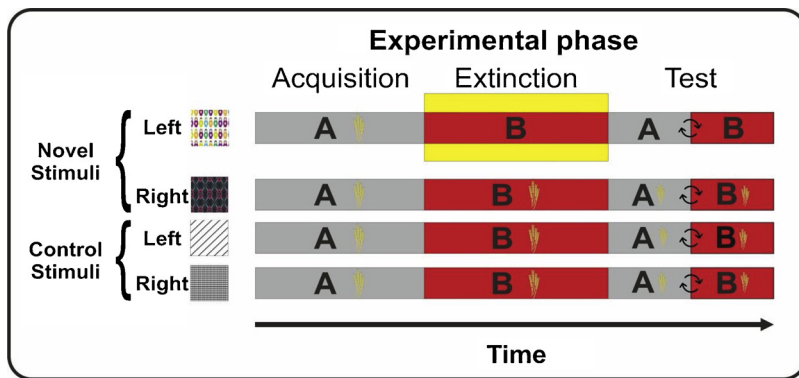

Fig. 6. Experimental procedure from experiment 2. Acquisition training and extinction training were conducted identically to experiment 1. However, the return of conditioned responding test phase took place in both the acquisition training and extinction training context to test renewal and extinction recall in parallel. At the onset of the return of conditioned responding test phase, a single context A trial using a control stimulus signified the transition from extinction training to the return of conditioned responding test phase. Afterwards, context A and B trials were presented in a pseudorandomized order.

observe robust renewal without an acquisition training — extinction training interval to allow for consolidation. However, the results of this experiment might stem from the possibility that the return of correct conditioned responses to the CS was a result of poor extinction recall. We therefore conducted a second experiment that tested the conditioned response in the acquisition training and extinction training context in parallel during the return of conditioned responding test phase. The experiment was designed to identify whether extinction learning, i.e. the decrement in conditioned responding that results from extinction training, is a reasonable marker for extinction recall during the return of conditioned responding test phase in a continuous

extinction paradigm.

4. Experiment 2

4.1. Methods

4.1.1. Subjects and apparatus

Eight pigeons served as subject in the second experiment. Housing conditions, ethics approval and the behavioral apparatus were identical to experiment 1.

4.1.2. Procedure

Blocking of the three experimental phases was conducted identically to experiment 1. After the animals had reached the behavioral criterion during extinction training, a single fixed trial of a control stimulus took place in context A (white house-light) to indicate the change from the extinction training to the return of conditioned responding test. Following this trial, all four experimental stimuli were presented in both context A and B. In both contexts, the extinction stimulus was followed without feedback (Fig. 6). As in experiment 1, context colors during extinction training were counterbalanced across animals and did not have an effect on the behavioral responses. The total number of trials in the return of conditioned responding test were subdivided into context A (renewal trials) and context B (extinction recall trials). Stimulus sequences were pseudorandomized as before.

4.1.3. Data analysis

Acquisition training and extinction training were analyzed as described in experiment 1. The return of conditioned responding test phase was evaluated using a two factorial context*block ANOVA. The factor context was constituted by the number of correct responses in either context A or context B trials for extinction stimulus presentations. The factor block referred to the six individual blocks in the test session. Furthermore, correct responses during the entire test phase were compared to the number of correct responses to the extinction stimulus in the last block of extinction using a paired t-test to identify differences during the phase transition. As in experiment 1, we compared each individual block of the test phase to the last block of extinction training using paired t-tests. Here, only three blocks were computed as the return of conditioned responding test phase was evenly divided into context A and context B trials. The significance threshold was set to $p < .013$ in accordance with Bonferroni's correction. The identical analysis was repeated for pecking rates.

4.2. Results and discussion

Learning curves for experiment 2 can be found in supplementary Fig. 1. The ANOVA revealed a significant main effect of context for correct responses towards the extinction stimulus ($F_{(1,7)} = 92.59$, $p < .001$, $\eta_p^2 = 0.93$) with more correct responses occurring in context A than in context B during the test phase (Fig. 7a). The interaction context*block also reached significance ($F_{(2,14)} = 5.23$, $p = .020$, $\eta_p^2 = 0.43$). A Bonferroni corrected block-specific analysis demonstrated that this result was true for all three blocks of the test phase (1. block: $p = .001$, 2. block: $p = .022$, 3. block: $p = .031$). Comparing the last block of extinction training with the performance during the return of conditioned responding test revealed a significant increase only for context A ($t_{(7)} = 5.80$, $p = .001$, $d = 2.05$), but not for context B ($t_{(7)} = 0.12$, $p > .250$, $d = 0.04$). For individual blocks, only the comparison of the first block of the return of conditioned responding test in context A reached significance ($t_{(7)} = 4.91$, $p = .002$, $d = 1.85$). For context B, no block of the return of conditioned responding test demonstrated a significant change in behavior compared to the last block of extinction training (all p's > .250). For pecking frequencies, we found a significant main effect of context ($F_{(1,7)} = 11.02$, $p = .013$, $\eta_p^2 = 0.61$) and a significant interaction of context*block ($F_{(2,14)} = 19.08$, $p < .001$, $\eta_p^2 = 0.73$, Fig. 7b). Post hoc tests revealed that only the first block of the return of conditioned responding test demonstrated a significant pecking difference between context A and context B ($p = .001$). A comparison between the last block of extinction training and the pecking rates across the whole return of conditioned responding test showed a significant increase for context A ($t_{(7)} = 2.72$, $p = .030$, $d = 1.51$), but a significant decrease for context B ($t_{(7)} = 2.53$, $p = .040$, $d = 1.01$). A block-specific analysis demonstrated no significant effects for all context B blocks, but a significant increase in pecking for the initial block of context A ($t_{(7)} = 4.59$, $p = .001$, $d = 2.64$).

In experiment 2, we could demonstrate that the offset during the extinction training phase is a reliable marker for the level of extinction recall in a continuous learning paradigm as there was no difference between the performance towards the extinction stimulus at the end of the extinction training phase and during the return of conditioned responding test phase. In contrast to Kim and Richardson [44], we were able to find renewal without an extinction training and return of conditioned responding test interval as the learning paradigm was entirely continuous. However, it still remains unclear whether the renewal effect, albeit existent, is attenuated following an immediate return of conditioned responding test after extinction training. We therefore conducted a third experiment in which pigeons underwent the procedure of experiment 1 (*immediate ABA* without extinction training consolidation), a *delayed ABA* procedure with extinction training consolidation of 24 h and a *delayed ABB* control for extinction recall following a 24 h extinction training and return of conditioned responding test interval.

5. Experiment 3

5.1. Methods

5.1.1. Subjects

Eight pigeons served as subjects in the third experiment. Housing conditions, ethics approval and the behavioral apparatus were identical to experiment 1 and 2.

5.1.2. Procedure

All eight animals were tested in three different experimental conditions. The first procedure was identical to experiment 1 (immediate ABA, Fig. 8a). In the second and third procedure, the animals only underwent acquisition training and extinction training on day 1 (void of an acquisition training and extinction training interval), but went back to their home cages after a successful extinction training phase. On day 2, animals were tested either in the acquisition training context (delayed ABA, Fig. 8b) or in the extinction training context (delayed ABB, Fig. 8c). The three procedures were presented in a random order to each animal to exclude potential learning effects across sessions. As before, contextual cues were also counterbalanced across sessions and animals and did not influence the choice behavior of the animals.

5.1.3. Data analysis

To assess the extent of response recovery towards the extinction stimulus in each condition, we compared the correct number of responses in the last block of extinction training with the number of correct responses for the initial block of the return of conditioned responding test and across the entire test phase using paired t-tests. Furthermore, we conducted a repeated measures ANOVA with the factors condition*block to identify if there was a difference in response recovery across the three conditions. The factor condition was constituted by the experimental procedures (immediate ABA, delayed ABA or delayed ABB) whereas the factor block referred to the six individual blocks within the test phase. The identical analysis was performed for pecking frequencies elicited by the extinction stimulus. Post hoc corrections were performed using Bonferroni's method.

5.2. Results and discussion

Learning curves for experiment 3 can be found in supplementary Fig. 3. For the immediate ABA condition, conditioned responses increased significantly for both the entire return of conditioned responding test ($t_{(7)} = 6.37$, $p < .001$, $d = 2.36$) and the first block of the return of conditioned responding test ($t_{(7)} = 13.51$, $p < .001$, $d = 4.85$, Fig. 9a). The same results were evident in the delayed ABA condition as responses relapsed significantly across the entire session ($t_{(7)} = 5.57$, $p = .001$, $d = 2.13$) as well as the first block of the return
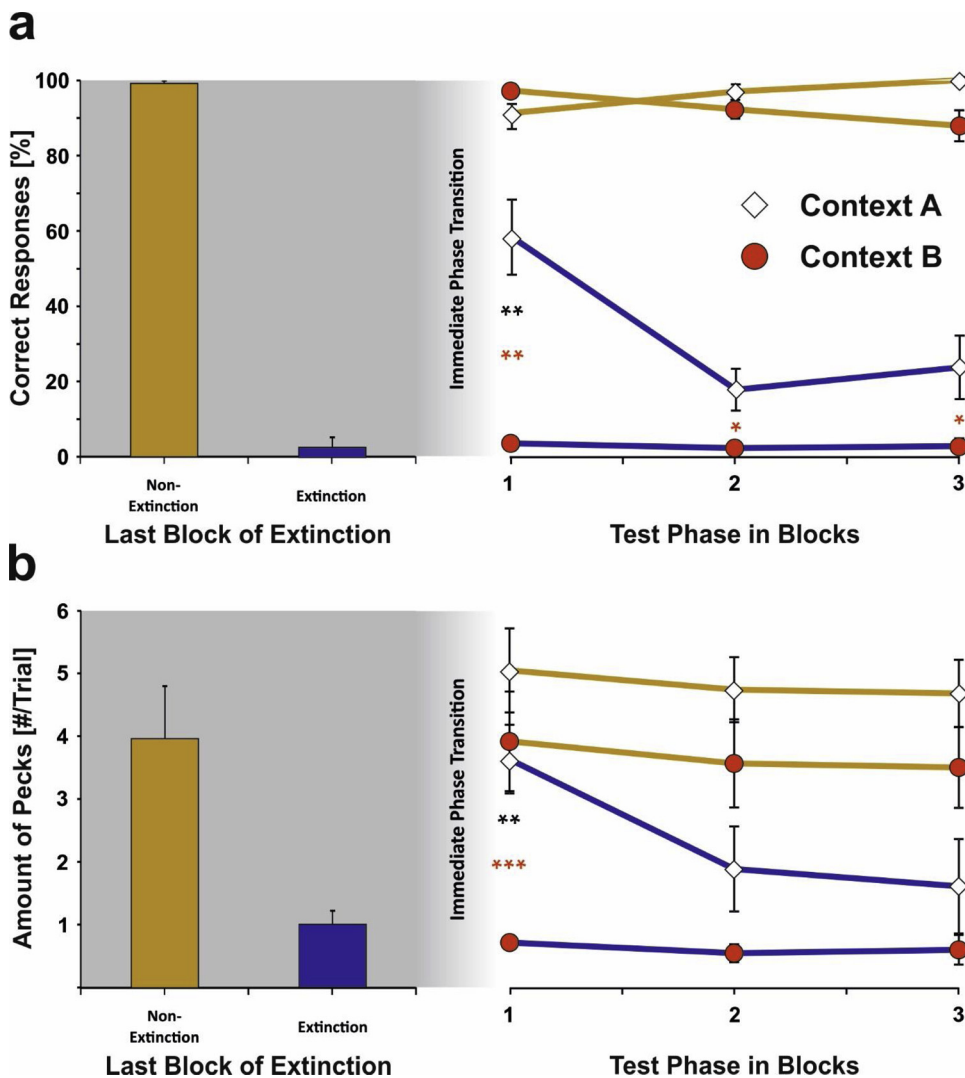
Fig. 7. Correct responses and pecking frequencies for the two novel stimuli during the last block of extinction training and the return of conditioned responding test for experiment 2. As results for both contexts are present during the return of conditioned responding test here, results for the control stimuli have been shifted to the supplementary Fig. 2 for convenience. (a) The performance for the extinction and non-extinction stimulus during the last block of extinction training is shown on the left. The performance during the return of conditioned responding test is divided into three even blocks per context and shown in the right panel. (b) Same as in (a), but for the number of pecks elicited by the two stimuli. Error bars represent SEM.

of conditioned responding test ($t_{(7)} = 11.54$, $p < .001$, $d = 4.91$, Fig. 9b). For the delayed ABB condition, conditioned responses towards the extinction stimulus increased significantly only for the first block of the return of conditioned responding test ($t_{(7)} = 2.51$, $p = .041$, $d = 1.00$), but not across the entire return of conditioned responding test ($t_{(7)} = 0.95$, $p > .250$, $d = 0.37$, Fig. 9c). The repeated measures ANOVA demonstrated a significant main effect of condition ($F_{(2,14)} = 15.78$, $p < .001$, $\eta_p^2 = 0.69$) and of block ($F_{(5,35)} = 24.44$, $p < .001$, $\eta_p^2 = 0.78$, Fig. 9d). After post hoc correction, a comparison between the ABA conditions did not exhibit a significant difference ($p > .250$), but both the immediate and delayed ABA condition differed from the delayed ABB condition ($p = .003$ and $p = .004$, respectively). The interaction also reached significance ($F_{(10,70)} = 2.07$, $p = .039$, $\eta_p^2 = 0.23$). Post hoc analyses revealed significant differences between the delayed ABB condition and both ABA conditions in the first ($p's < .020$), second ($p's < .007$), third ($p's < .012$) and fourth block ($p's < .048$) as the ABA conditions demonstrated more correct responses towards the extinction stimulus.

For pecking rates, we found similar results as for correct responses. Comparing pecking frequencies elicited by the extinction stimulus resulted in a significant increase from the last block of extinction training to the first block of the return of conditioned responding test in the immediate ABA condition ($t_{(7)} = 6.04$, $p = .001$, $d = 2.67$, Fig. 9e), the delayed ABA condition ($t_{(7)} = 4.41$, $p = .003$, $d = 1.66$, Fig. 9f) and the delayed ABB condition ($t_{(7)} = 2.60$, $p = .036$, $d = 1.53$, Fig. 9g). Across the whole return of conditioned responding test, only the immediate

ABA condition demonstrated significant differences to the last block of extinction training ($t_{(7)} = 3.99$, $p = .005$, $d = 1.46$). Pecking rates reached significance for the factor condition ($F_{(2,14)} = 7.83$, $p = .005$, $\eta_p^2 = 0.53$), block ($F_{(5,35)} = 22.74$, $p < .001$, $\eta_p^2 = 0.77$) and their interaction ($F_{(10,70)} = 6.34$, $p < .001$, $\eta_p^2 = 0.48$, Fig. 9h). Post hoc tests demonstrated a significant difference in pecking rates between the immediate ABA condition and the delayed ABB condition ($p = .039$). Immediate ABA vs delayed ABA and delayed ABA vs delayed ABB did not reach significance ($p > .250$ and $p = .076$, respectively). The condition*block interaction demonstrated a significant difference between both the immediate and delayed ABA compared to the delayed ABB condition during the first ($p's < 0.45$) and the second block ($p's < 0.35$).

In this experiment, we found reliable renewal in both ABA conditions. Furthermore, no difference at any time point across the return of conditioned responding test could be detected between the behavioral expression of renewal in an immediate and a delayed test. These results indicate that a 24 h period of post-extinction memory consolidation is not required to find renewal of similar quality. We will discuss our results in the next section.

6. General discussion

In the present study we conducted three different experiments to assess under which boundary conditions renewal from extinction learning occurs. In experiment 1, we investigated whether renewal
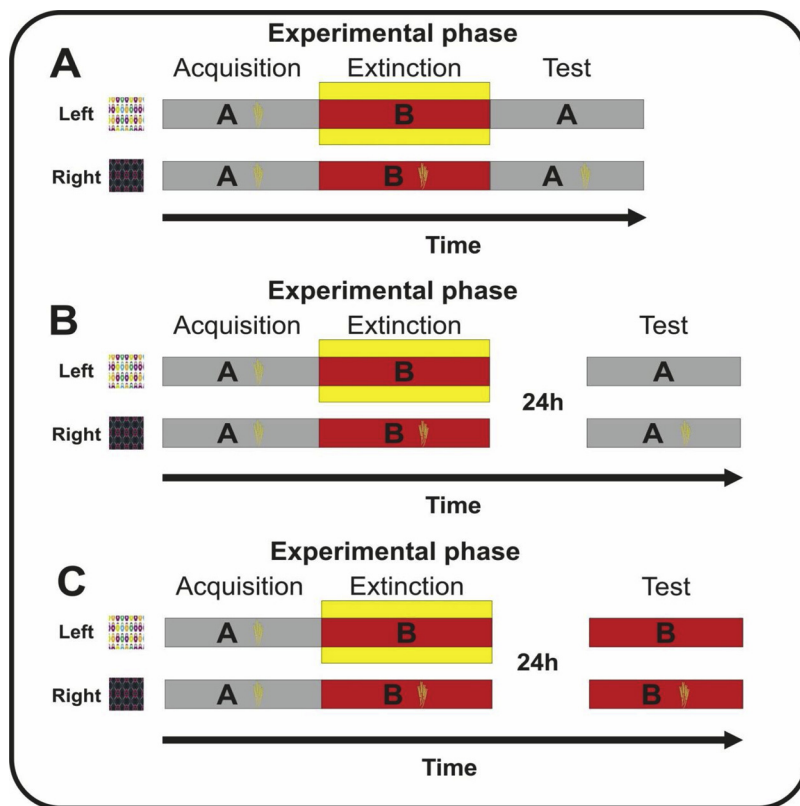
Fig. 8. Experimental procedure from experiment 3. In this experiment, all animals went through three experimental conditions in a randomized order. The first condition (A) mirrored the procedure from experiment 1 (immediate ABA renewal). In the second condition (B), the behavioral session ended after reaching the criterion in the extinction training phase and was restarted after a 24 h delay in the return of conditioned responding test in context A. The last condition (C) also ended on day 1 after the end of the extinction training, but following the 24 h delay, the animals were reintroduced to context B to measure extinction recall. Control stimuli were continued to be shown in all experimental phases.

occurs if extinction training is conducted immediately after acquisition training. We were able to find renewal despite an immediate switch from acquisition training to extinction training. In experiment 2, we validated the paradigm by demonstrating that observed renewal was not due to poor extinction recall. In a third experiment, we tested whether consolidation after extinction training is a necessary condition for renewal to occur. Both with and without a consolidation interval, renewal did occur. Furthermore, there was no difference between immediate and delayed return of conditioned response testing regarding the extent of the renewal effect.

In all experiments, an immediate switch from acquisition to extinction training had no effect on conditioned response recovery. Different from Myers et al. [25] and in line with several other studies [33,49,50,52,53], a secondary inhibitory learning during extinction training does not seem to be based on a long "acquisition training – extinction training" interval. Importantly however, our study did not systematically evaluate the acquisition-extinction interval as opposed to the extinction-test interval in experiment 3. Therefore, interpretations of the findings in our experiments should be viewed cautiously with regard to a role of memory consolidation. While we can determine that renewal occurred in the absence of an acquisition-extinction interval, a result opposing the findings of Myers et al. [25], it does not inform us about a possible difference between a consolidated and non-consolidated acquisition memory trace. It could be that there is a qualitative difference in the extent of the renewal effect if an acquisition-extinction interval is implemented.

Regarding the immediate extinction deficit, we found that extinction recall was unaffected in experiment 2 indicating that immediate extinction did not lead to a failure to retrieve the extinction memory. However, the immediate extinction deficit is usually associated with long-term deficits in extinction recall which our study did not investigate [29]. Furthermore, the lack of manipulation of the acquisition-extinction interval unfortunately does not allow us to draw definite conclusions if it also applies to an appetitive experimental design. However, given that it has been mostly associated with elevated stress

levels due to fear conditioning [29,31–33,37], it seems unlikely to generalize to appetitive conditioning paradigms, a result that can be potentially inferred from the successful extinction recall in experiment 2. Future studies should investigate the nature of the immediate extinction deficit systematically in appetitive paradigms to identify if it truly only applies to Pavlovian fear conditioning.

The necessity for extinction training consolidation has been highlighted in several studies demonstrating that disrupted memory consolidation leads to extinction recall deficits [38,53]. We therefore tested both ABA renewal and ABB extinction recall in parallel in experiment 2 to investigate whether the observed renewal in experiment 1 was true renewal or simply poor extinction recall. The results indicate that the relapse in responding towards the extinction stimulus was not due to improper extinction training consolidation as the extinction recall was unimpaired. Given the pervasive evidence of consolidation being fundamental for extinction recall, this finding could stem from the fact that a continuous paradigm relies mainly on short term memory rather than long term memory processes. In a continuous paradigm, short term network alterations might be sufficient for subsequent recall, but for the formation of long term memory traces, a proper memory consolidation period is required.

While both experiment 1 and 2 already found indications that the appearance of the renewal effect is not dependent on extinction training consolidation as suggested by Kim and Richardson [44], we further investigated a qualitative difference in experiment 3 between immediate and delayed testing of renewal. The results indicate that the magnitude of renewal does not depend on a memory consolidation phase as the return of conditioned responses in presence of the extinction stimulus was comparable across all individual blocks of the test phase. However, a difference in quality could be observed in the third experiment when taken into consideration that extinction recall was impaired after a 24 h delay. As shown by experiment 2, extinction recall can be well estimated using the last block of extinction training in a continuous design. In the delayed paradigm, the extinction recall control demonstrated a significant return of conditioned responses in an
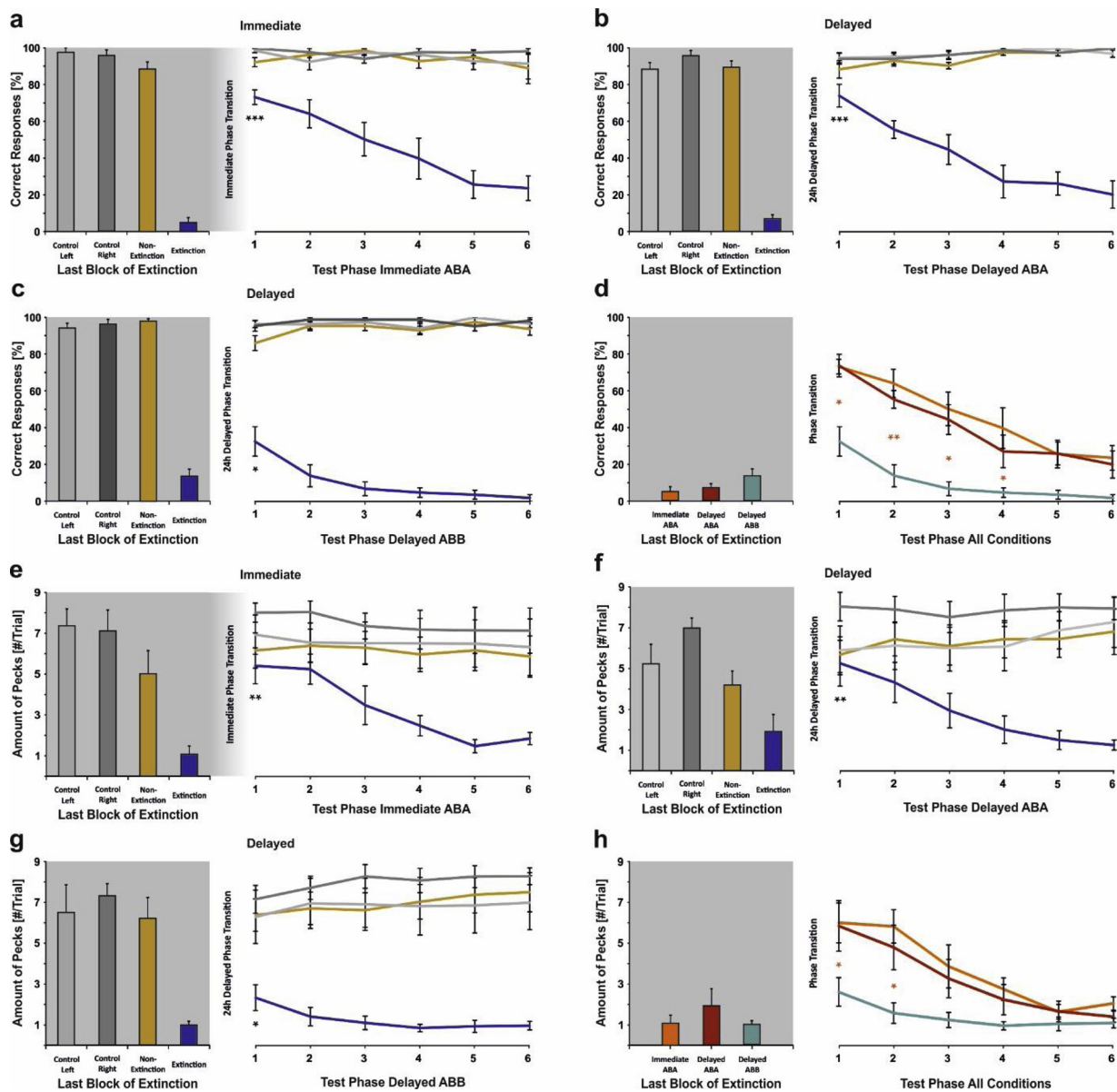
Fig. 9. Correct responses and pecking frequencies across stimuli during the last block of extinction and the test phase for experiment 3 across all three experimental conditions. (a) Correct responses in the immediate ABA condition across stimuli during the last block of extinction are shown on the left. Correct responses during the test phase divided into six even blocks for this condition are shown on the right. (b) Same as in (a), but for the delayed ABA condition. (c) Same as in (a), but for the delayed ABB condition. (d) Performance overlay for the three experimental conditions both during the last block of extinction training and the return of conditioned responding test phase. (e) Same as in (a), but for pecking frequencies. (f) Same as in (e), but for the delayed ABA condition. (g) Same as in (e), but for the delayed ABB condition. (h) Pecking frequency overlay for the three experimental conditions both during the last block of extinction training and the return of conditioned responding test phase. Error bars represent SEM.

ABB design which indicates that not all the observed renewal in the delayed ABA condition was due to a context change, but also partly due to poor extinction recall. Thus, the magnitude of renewal may be slightly higher in an immediate compared to a delayed test.

A potential explanation for the differences in results compared to the study of Myers et al. [25] and Kim and Richardson [44] is the distinct experimental procedure. We used an appetitive operant conditioning paradigm to measure the extent of renewal which could possibly explain mechanistic differences between operant conditioning using food reward and Pavlovian conditioning using fearful stimuli such as foot shocks. Especially for contextual learning in operant conditioning, it was suggested that the context does not serve as a negative occasion setter as it does in Pavlovian fear conditioning [58]. It was rather demonstrated that the context directly inhibits the conditioned response itself [54,59,60]. This account does not seem to apply to the

present experiments as the control stimuli required the identical conditioned response as the novel stimuli that were then randomly chosen for extinction. If the conditioned response was directly inhibited by the context, it should have affected the response for the corresponding control stimulus as well. Such a finding could not be observed during extinction learning in our experiments indicating against this possibility. However, it still might have been that the transfer of initial conditioning from context A to context B was only partial or incomplete as we could observe significant drops in performance for the non-extinction stimulus in all three experiments even though the reward contingencies remained unchanged for this stimulus. Overall, given that (1) other studies have found reliable renewal and spontaneous recovery without an acquisition training consolidation phase in fear conditioning paradigms, (2) operant conditioning proceeds similarly to Pavlovian conditioning in extinction learning [15], and (3) that appetitive and

aversive extinction learning appear to involve the same underlying neural circuits [6,61], we do not deem the possibility of differences in experimental procedure accounting for the difference in results to Myers et al. [25] and Kim and Richardson [44] likely.

The use of continuous extinction designs is a rather recent development and these studies consistently show that response recovery is observable in such paradigms. Bustamante et al. [48] were able to demonstrate both ABA and ABC renewal in a continuously conducted predictive learning task [62]. In their study, they also tested for extinction recall immediately after the extinction training phase and could demonstrate that participants were able to robustly recall the extinction from context B in line with our results from experiment 2. The same task was used in a series of neuroimaging studies Refs. [46,47,63,64],). They consistently found that humans either exhibit renewal or do not exhibit renewal at all. While the absence of renewal in some participants could be attributed to unlearning of the acquisition memory, roughly half the participants showed a significant recovery of the conditioned response. Thus, the short time lapse between extinction and re-testing had no general effects across individuals. The lack of renewal in these experiments could stem from the neutral nature of the task as the participants were neither punished nor rewarded over the course of the study. A study by Ernst et al. [65] demonstrated rapid reacquisition (another extinction recovery phenomenon in which a response is relearned faster after extinction training than during the original acquisition training [13]) in a continuous aversive eye blink conditioning paradigm. This demonstrates that response recovery without a consolidation phase is not limited to appetitive or neutral learning paradigms.

A significant drawback of the present study is the lack of a systematic investigation of the acquisition-extinction interval in experiment 1 comparable to our design in experiment 3. This missing experimental procedure unfortunately does not allow to draw final conclusions about the necessity of a consolidation interval between acquisition and extinction training. Furthermore, a purely behavioral approach does not inform about the underlying neural mechanisms of our results. Finally, the sample size of this study was low and these results should therefore be viewed with caution. However, the effect sizes in each respective experiment were very high indicating that the observed results are robust and are likely to be found in a larger sample.

The drawbacks of this study directly translate into possible future experiments as these experiments should be replicated using larger sample sizes and experiment 1 should be extended to mirror the experimental design of experiment 3. Another interesting question that should be further investigated is if the results from our experiments apply to procedures that are known to attenuate the renewal effect. If extinction learning for example is conducted in multiple contexts or extinction learning is severely prolonged (massive extinction), the extent of the renewal effect is reduced [66–69]. Our experimental design can easily be accommodated to such task features to test if the present results can be extrapolated beyond a simple ABA renewal design. Furthermore, most of these effects have been found in the context of Pavlovian fear conditioning. If corresponding results could be found for discriminative operant appetitive conditioning, it would indicate that these phenomena can be generalized across extinction procedures.

In conclusion, we found robust renewal in the absence of a 24 h memory consolidation after acquisition training and extinction training. The extent of renewal in an immediate test phase was at least on par with the levels of renewal in a 24 h delayed test condition. These results contrast previous findings that concluded that a consolidation phase is necessary for extinction recall and response recovery. A possible explanation could derive from differences in short term and long-term memory processes and the underlying mechanisms of consolidation. While the behavioral phenotype of renewal might be very similar in the absence or presence of a 24 h consolidation period, the underlying neural mechanism might be distinct. Future research could further investigate the physiological and molecular level of renewal to address this yet unresolved issue.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bbr.2019.111947.

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