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ORIGINAL PAPER

Recovery-from-extinction effects in an anuran amphibian: renewal effect, but no reinstatement

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Abstract

Recovery-from-extinction effects in which a conditioned response returns after extinction have been shown in mammals, birds and fish. Thus, these effects appear to be conserved among vertebrates; however, they have yet to be investigated in amphibians. Using prey catching conditioning in the fire-bellied toad (*Bombina orientalis*), we tested if renewal and reinstatement occurred after extinction when subjects were respectively re-exposed to the context or reinforcer used during conditioning. For renewal, a different context was used during extinction and thus renewal tests assessed if external contextual cues associated during conditioning stimulated prey catching performance. For reinstatement, the reinforcer withheld during extinction was simply delivered again prior to a test assessing if internal cues associated with recent prey consumption stimulated prey catching performance. Conditioning followed a fixed ratio 5 schedule of reinforcement, where five attempts to capture a cricket stimulus displayed on a computer screen were reinforced by delivery of a single live cricket. Performance was measured as the time to reach five prey catching attempts. A significant improvement in prey catching performance during conditioning followed by deterioration with extinction was seen in the experiments. Upon return to the context used for conditioning after extinction, toads showed a renewal effect whereby they displayed faster performance during testing compared to the end of extinction. Conversely, toads showed no reinstatement effect because pre-feeding of a cricket did not influence performance during the test that followed extinction. Reinstatement could have been lost in amphibian phylogeny due to secondary simplification of the nervous system.

Keywords Amphibians · Prey catching conditioning · Recovery from extinction effects · Renewal · Reinstatement

Introduction

After an animal learns something useful, conditions can change so that the learned response is no longer relevant and should not be expressed anymore. However, should conditions promoting the usefulness of the learned response return, it would be adaptive to quickly recover the earlier response instead of learning a new one from scratch because learning requires costly investment in time and growth of neuronal circuits. The characteristics of extinction learning seem to meet the above requirements for adaptive value. To wit, there is good evidence that extinction of learned performance after conditioning is usually not due to unlearning. This interpretation is supported by the observation of recovery-from-extinction effects (aka relapse after extinction), where an extinguished conditioned response returns after a time (spontaneous recovery), after a change in context (renewal), or after re-exposure to the reinforcer (reinstatement) used during conditioning (Bouton 2002; McConnell and Miller 2014). Instead, extinction learning appears caused by inhibition of previously established learning (Rescorla 1993; Harris and Westbrook 1998; Delamater 2004; Lingawi et al. 2018). Physiological data showing that neural correlates of original memories established by conditioning are stored for a long time after their behavioural effects have been extinguished also support this interpretation (Hobin et al. 2003; Gale et al. 2004). Nevertheless, unlearning is the favoured candidate mechanism of extinction under certain circumstances, such as during early development (Kim and Richardson 2008) or when extinction follows shortly after conditioning (Lin et al. 2003; Myers et al. 2006). Therefore, mechanisms of extinction are variable.

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At first glance, recovery-from-extinction effects appear conserved in phylogeny because evidence of these phenomena is found in different vertebrate and invertebrate taxa. Abundant work on this topic has been conducted in mammals. For example, reinstatement of salivary conditioning in dogs was reported by Pavlov (1927). Rodent models have often been used for such purposes using different measures of fear conditioning, such as conditioned freezing and potentiated startle responses (Gewirtz et al. 1997), or appetitive conditioning, both Pavlovian and operant (Delamater, 1997; Bouton et al. 2011). Humans also show a broad variety of recovery-from-extinction effects (Havermans et al. 2005; Haaker et al. 2014; Steins-Loeber et al. 2019). Even though recovery from extinction has been observed using a variety of learning tasks in mammals, the phenomenon is not universal. No reinstatement or renewal of conditioned eyeblink responses was seen in rats and human subjects, respectively (Thanellou and Green 2011; Claassen et al. 2016), a mouse strain showed no renewal of extinguished conditioned fear (Waddell et al. 2004), and recovery from extinction in rats is sensitive to manipulations of context and inter-trial intervals during extinction (Bouton and Bolles 1979; Urcelay et al. 2009; Bernal-Gamboa et al. 2017). Work in taxa other than mammals is much more limited. Pigeons (*Columba livia*) show recovery from extinction of food pecking behaviour (Podlesnik and Shahan 2009; Starosta et al. 2016), zebrafish (*Danio rerio*) show renewal and reinstatement of responding to an automated feeder (Kuroda et al. 2017), honeybees (*Apis mellifera*) show spontaneous recovery and reinstatement of the odor-conditioned proboscis extension response (Sandoz and Pham-Delegue 2004; Plath et al. 2012), crayfish (*Orconectes rusticus*) show relapse of drug-seeking behaviour (Nathaniel et al. 2009), and garden snails (*Helix aspersa)* show spontaneous recovery and reinstatement of chemosensory conditioning for food (Alvarez et al. 2014). Despite these findings, the phylogenetic coverage of studies of recovery-from-extinction effects is very limited. In the present study, we investigated if mechanisms of extinction could vary in vertebrate phylogeny by assessing renewal and reinstatement for the first time in an amphibian species.

Conveniently, prey catching can be conditioned in amphibians. Prey catching conditioning exploits the innate tendency of anuran amphibians to display predation toward visual stimuli of moving prey (Roster et al. 1995). We previously showed that learning is involved in accelerating prey catching responses directed at a visual prey stimulus in fire-bellied toads (*Bombina orientalis*) reinforced with food, and that performance declined in extinction after moderate but not extended conditioning (Ramsay et al. 2013). This procedure is well suited to implement the experimental manipulations needed to assess recovery-from-extinction effects. First, we used distinct environmental contexts during prey catching conditioning and extinction before renewal tests in the conditioning context in two experiments that used different amounts of extinction. Experiment 1 tested for renewal after a short period of extinction (four sessions), while Experiment 2 tested for renewal after a longer period of extinction (six sessions). Second, in an experiment that used a single environmental context, we administered a food reinforcer after conditioned prey catching was extinguished to enable a reinstatement test (Experiment 3). Any deviation from the expected pattern of broad conservation of recovery-from-extinction effects across phylogeny could inform our understanding of the evolution of cognition. Conversely, the presence of these learning phenomena in an amphibian could broaden the pool of model organisms available for their study. Both outcomes would increase our understanding of the origin and necessity of recovery-from-extinction effects in a larger phylogenetic context.

Methods

Subjects

Fire-bellied toads of mixed sexes were held in groups of up to 6 individuals in terrariums laid with a gravel substrate and provided with various stones and fragments of clay pots for cover. A total of 68 toads naïve to the prey catching conditioning task were used in this study. They were purchased from a commercial supplier (National Reptile Supply, Mississauga, ON). Individual toads were identified based on dorsal marking patterns. A collection of toad pictures was consulted for accurate identification during the experiments. Photoperiod was 12–12 h light–dark. Toads had continuous access to water in a dish and were fed crickets (*Acheta domesticus*) ad libitum weekly until 2 weeks prior to the commencement of experiments. Toads were not fed for 2 weeks before the experiments to increase prey catching motivation. During conditioning, they were fed up to 6 crickets per day, 3 days a week. During extinction, toads were fed ad libitum once a week outside of the training context on a day that was not used for trials (Sunday).

Testing apparatus

Two different contexts were used in renewal experiments (Fig. 1A, B). Context A was a modified Bussey-Saksida rat touchscreen chamber (Lafayette Instrument, IN, USA) equipped with three black walls and a computer screen ($l \times w \times h$: 32 × 27 × 25 cm). A matte screen protector was applied to reduce reflections, as toads can be attracted to their own images reflected on glossy surfaces. The floor of context A was smooth, clear plexiglass with a view of the underlying patterned metal subfloor. Context B was a custom-made 39 × 39 cm platform fitted at the edge of a regular

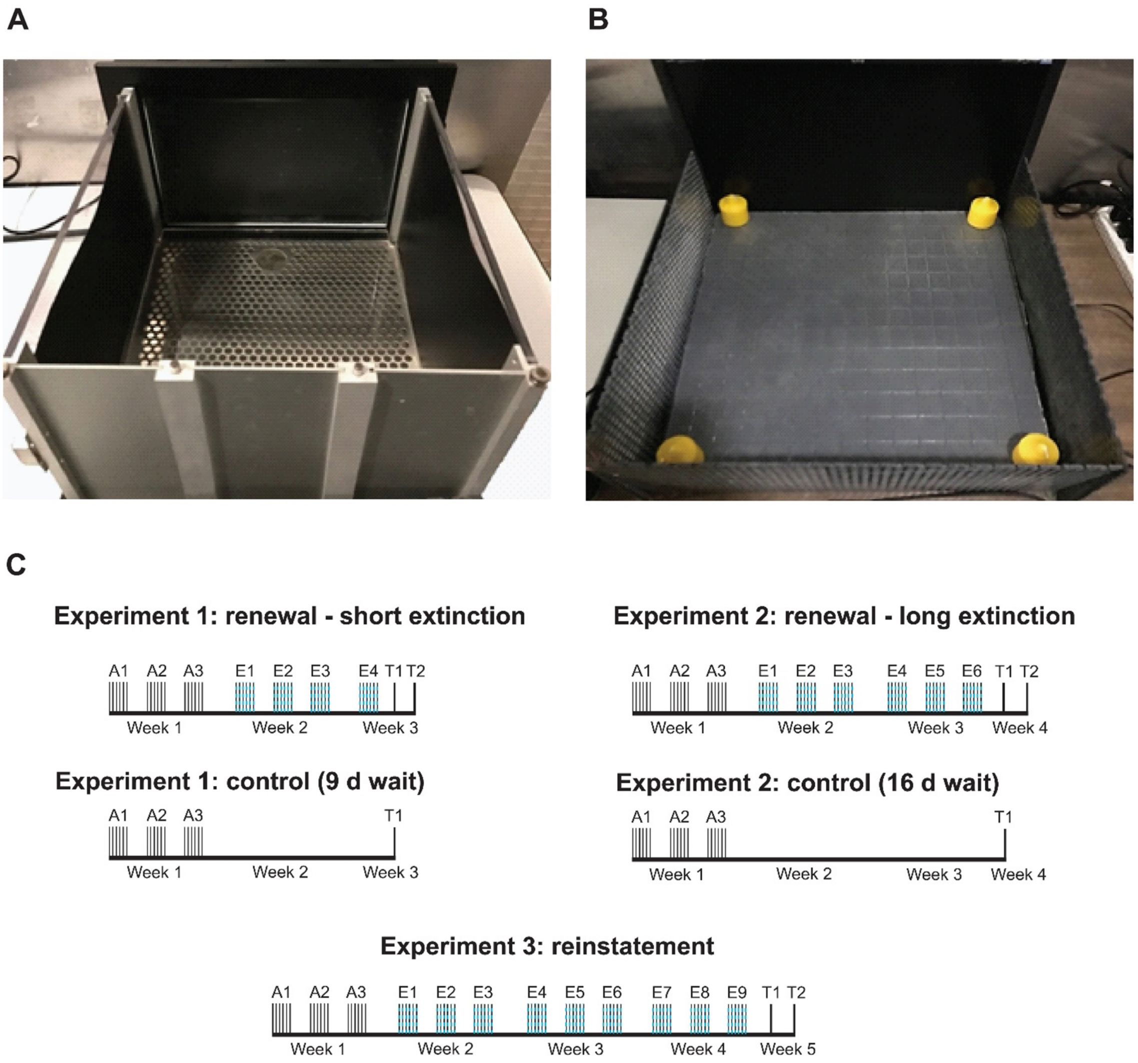

Fig. 1 Experimental procedures. Panels A and B show a top-front view of the two distinct experimental contexts used in renewal experiments (Experiments 1 and 2). **A** Context A is a modified rat touch screen chamber with a smooth floor and three opaque black walls. **B** The platform of context B has a textured plastic floor and three mid-height perforated gray plastic walls in addition to scented candles in each corner. Context B without the textured floor and candles was used in the reinstatement experiment (Experiment 3). **C** Diagrams illustrate the sequence of events in each experiment (*A* acquisition sessions, *E* extinction sessions, *T* test trials)

computer screen (LP 1965 LCD, Hewlett-Packard, Palo Alto, California; 18 cm high). The floor of the platform was made of semi-transparent textured plastic to provide tactile cues and the three mid-height walls were made of perforated gray plastic. In the renewal experiments, additional visual and olfactory cues distinguishing context B from context A were provided by yellow citrus-smelling (Experiment 1) or beige vanilla-scented (Experiment 2) candles in each corner of the platform. Only context B was used for the reinstatement experiment (Experiment 3), albeit without candles and textured floor. Instead, a smooth gray plastic platform was used. There was no ceiling on top of the chambers to allow manual administration of live cricket reinforcers by the experimenters.

Procedure

Toads were distributed evenly between experimental groups based on body weights before training. Prior to the experiments, one shaping trial was conducted with each toad to reduce behavioural inhibition in the training contexts. The shaping trial in renewal experiments (Experiments 1 and 2) consisted of each toad being placed in a context for 2 min without presentation of the prey stimulus on screen or attribution of food. Prior to the reinstatement experiment (Experiment 3), the shaping trial involved the feeding of one cricket after 30 s in the context, again without presentation of the prey stimulus on screen, because we noticed in advance that the different batch of toads used for this experiment was less responsive to presentation of the prey stimulus. We hoped that the context-cricket pairing would stimulate prey catching and facilitate conditioning in these toads. Shaping trials occurred 2 days prior to the beginning of conditioning. Conditioning and extinction trials occurred on Mondays, Wednesdays and Fridays throughout the course of the experiments. Each toad was used at approximately the same time of day to avoid potential circadian influences on prey catching performance.

Conditioning trials for acquisition of a faster prey catching response followed the same procedure in all experiments and in both contexts used for the renewal experiments. Briefly, a cricket stimulus video was played on the screen at the same level as the platform floor. The video was a recording of five crickets moving around in an enclosed clear plastic tube against a white background. As described in Ramsay et al. (2013), a trial began by placing the toad 10 cm away from the screen and ended once the toad had snapped at the cricket stimulus on the screen 5 times (FR5 schedule of reinforcement), after a maximum time of 180 s had elapsed, or after two attempts to escape the platform. A snap had to hit the area of the screen displaying the stimulus in order to count. Once 5 snaps were reached, the video stimulus was paused, and the toad was immediately fed a cricket manually by the experimenter. No cricket reinforcer was attributed if a toad did not perform 5 snapping responses or attempted to escape twice during a trial. In such cases, the maximal time of 180 s was attributed. Extinction trials followed the same procedure, except that no reinforcer was delivered after 5 snaps. The time to perform 5 snaps at the cricket stimulus was measured. Acquisition and extinction trials were grouped into sessions of 6 trials per day with an inter-trial interval of 1 min. Each toad was conditioned for 3 sessions (total 18 trials) in 1 week before the onset of extinction the following week (Fig. 1C). Extinction lasted for 4 (Experiment 1) and 6 (Experiment 2) sessions before the renewal tests and 9 sessions before the reinstatement test (Experiment 3). In context A, the cricket video was displayed using ABET VideoTouch version 2.18.10.2 and the ABET II software was used to automatically record and retrieve snap data (Lafayette Instrument). In context B, all data was manually recorded with a digital timer.

Renewal

In Experiment 1 (renewal—short extinction), half of the toads were conditioned in context A ($n = 10$) and the other half were conditioned in context B ($n = 10$). Two toads were rejected early in the experiment (1 control and 1 renewal); one because it did not perform prey catching toward the cricket stimulus and another because it had trouble aiming at the stimulus, likely due to poor vision. For 9 conditioned toads, the context was changed in extinction. The remaining 9 conditioned toads were used as controls that did not undergo extinction but rather waited in their housing containers for the time that extinction lasted in the other toads involved in this experiment. After this waiting period, control toads were tested for one trial in the context in which they were originally conditioned to assess memory decay over time (memory decay test trial). The toads that underwent extinction were submitted to a renewal test trial in the original conditioning context 1 day after the end of extinction. On the following day, these toads were again tested in the extinction context to verify contextual control of the conditioned prey catching response. No reinforcer was administered in the memory decay, renewal and contextual control test trials.

In Experiment 2 (renewal—long extinction), 10 toads were conditioned in context A and 9 toads in context B. Five toads were rejected (3 control and 2 renewal) because they failed to reach 5 snaps for 9 or more trials during the conditioning sessions. For ten conditioned toads (5 in each context), the context was changed in extinction. Four conditioned toads (2 in each context) were used as controls that did not undergo extinction but waited in their housing containers for the time that extinction lasted in the other toads involved in this experiment. As in Experiment 1, control toads were tested for one trial in the context in which they were originally conditioned after the waiting period. The toads that underwent extinction were submitted to a renewal test trial in the original conditioning context 2 days after the end of extinction. Two days later, these toads were again tested for a trial in the extinction context. On three sessions (one acquisition and two extinction in different toads) conducted in context A in this experiment, computerized retrieval of data on the first trial failed. In these cases, the time to perform 5 snaps on the second trial of the session was used.

Reinstatement

Conditioning was attempted with a total of 29 toads in Experiment 3. The different batch of toads used for this experiment had many individuals that were less responsive to presentation of the prey stimulus. As a result, 13 toads had to be rejected because of failure to reach 5 snaps in 9 or more trials during the conditioning sessions. A total of 7 control and 9 reinstatement toads remained after rejections. Following extinction, a reinstatement test was conducted in which toads of the reinstatement group were fed one cricket in the context just before the cricket stimulus was played on screen and their prey catching response measured. On the same day, toads of the control group were placed into the context without pre-feeding a cricket but waited 15 s (the approximate time reinstatement toads spent eating the cricket) before the stimulus on the screen was turned on and their prey catching response measured. Since we expected the control toads to maintain extinction behaviour during that trial, 2 days following the reinstatement test control toads were fed one cricket outside the testing context in a box before conducting a prey catching trial. This provided an additional test of 'outside-context' reinstatement.

Statistics

Because prey catching performance tended to decrease in the later trials of sessions, conditioning and extinction were analyzed using the time to perform 5 snaps during the first trial of each session. Behavioural data is often skewed, as was the case here, and do not meet the assumptions of parametric statistics (Siegel 1956). Therefore, we used separate generalized estimating equations (GEE) models to assess if prey catching performance varied over the acquisition and extinction sessions in each experiment. Least significant difference (LSD) pairwise contrasts were used to determine differences between sessions. For renewal, GEE and LSD pairwise contrasts were also used to compare prey catching performance between the first trial of the last extinction session, the renewal test trial and the return trial in the extinction context. For reinstatement, the Mann–Whitney test on ranked data was used to compare prey catching performance between control and in-context reinstatement groups. In this test, the time to perform 5 snaps in the reinstatement test was divided by the time to 5 snaps on the first trial of the last extinction session (ratio of test/E9) to account for individual variation in prey catching activity by the end of extinction. A ratio below one in the reinstatement group would be expected if recovery from extinction was occurring. Finally, the performance of toads that consumed a cricket out of context in the second reinstatement test was compared with their own prior control performance using a Wilcoxon matched-pairs test. GEE statistics were computed using SPSS 26 (IBM, Armonk, NY) while reinstatement tests on ranked data were computed using Prism version 8 (GraphPad Software, La Jolla, CA, USA). The statistical threshold was set at 0.05.

Results

Experiment 1: renewal—short extinction

Toads in this experiment significantly reduced their time to reach 5 snaps during conditioning as shown by a significant effect of session in the GEE analysis of the acquisition period ($\chi^2(2)=31.8$, $P<0.001$; Fig. 2A). LSD pairwise contrasts showed that the time to 5 snaps in the first trial decreased with training (sessions 1–2, $P=0.03$; sessions 1–3, $P=0.002$; sessions 2–3, $P=0.01$). Thus, the analysis showed that conditioning quickly improved prey catching performance after the first session. Conversely, toads increased their times to reach 5 snaps over the 4 extinction sessions in this experiment (Fig. 2A). The time to 5 snaps in the first trial of sessions significantly differed over the extinction period ($\chi^2(3)=14.1$, $P=0.003$) and LSD contrasts indicated a higher time to 5 snaps compared to the first extinction session at session 4 ($P=0.05$), but not at sessions 2 and 3 ($P>0.2$). This analysis suggests that the toads had undergone significant extinction just before the renewal test.

There was a clear trend for reduced time to 5 snaps during the renewal test and a return to poor performance similar the to end of extinction a day later back in the extinction context (T1 and T2 in Fig. 2B, respectively). This trend was supported by a significant effect of trial in the GEE analysis ($\chi^2(2)=25.5$, $P<0.001$) and significant LSD contrasts between the first trial at the fourth extinction session and

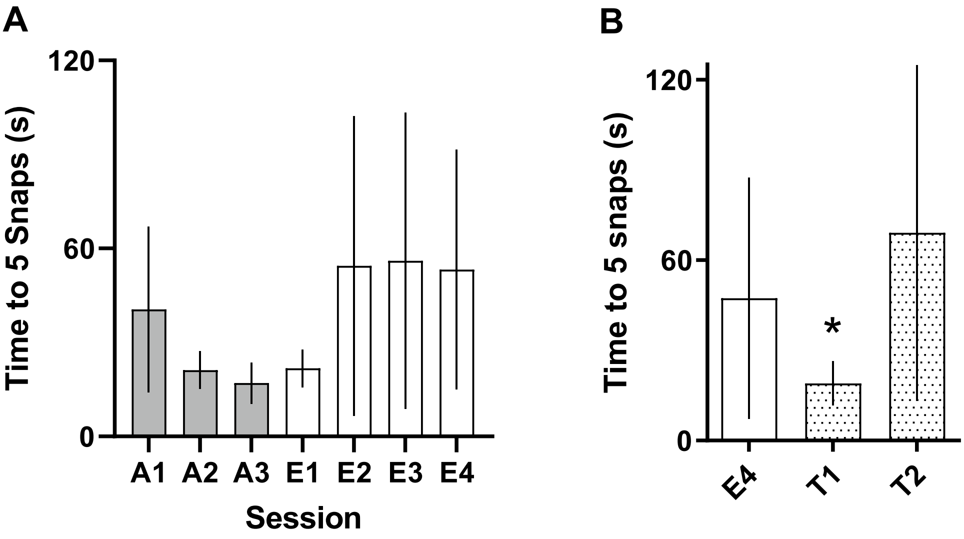

Fig. 2 Prey catching performance of fire-bellied toads during the first renewal experiment (Experiment 1). **A** Time to 5 snaps on the first trial of sessions during acquisition (A1–A3; gray bars) and extinction training (E1–E4; white bars). **B** Time to 5 snaps on the first trial of the last extinction session (E4) and the renewal and return to extinction context tests (T1 and T2, respectively; bars with dotted pattern). Bars show means ± 95% confidence intervals. The asterisk in panel B indicates that time to 5 snaps was lower at T1 compared to both E4 and T2

the renewal test ($P = 0.04$) as well as between the renewal test and the subsequent return to the extinction context ($P = 0.02$). There was no difference in prey catching performance between the last extinction session and the test in the extinction context that followed the renewal test ($P = 0.15$).

Experiment 2: renewal—long extinction

Since extinction was limited to the last session just before the renewal test in Experiment 1, we conducted a second experiment with more extinction sessions before testing for renewal. As in the previous experiment, toads showed decreased time to 5 snaps during the 3 conditioning sessions (GEE: $\chi^2(2) = 8.5$, $P = 0.01$; Fig. 3A), but this time the significant decrease was limited to the comparison between sessions 1–3 (LSD contrasts: sessions 1–2, $P = 0.2$; sessions 1–3, $P = 0.02$; sessions 2–3, $P = 0.34$). Again, toads increased their times to reach 5 snaps over the extinction period (GEE: $\chi^2(5) = 93.9$, $P < 0.001$; Fig. 3A). LSD contrasts showed that the time to 5 snaps in the first trial of sessions significantly differed between the first extinction session and sessions 4–6 ($P < 0.05$), but not sessions 2 and 3 ($P > 0.14$). Therefore, Experiment 2 effectively produced more extinction before the tests of renewal and return to the extinction context.

Again, there was a trend for reduced time to 5 snaps during the renewal test and a return to poor performance similar the to end of extinction 2 days later back in the extinction context (Fig. 3B). This trend was supported by a significant effect of trial in the GEE analysis ($\chi^2(2) = 9.2$, $P = 0.01$) and significant LSD contrasts between the first trial at the sixth extinction session and the renewal test ($P = 0.04$) as well as between the renewal test and the subsequent return to the extinction context ($P < 0.001$). There was again no difference in prey catching performance between the last extinction session and the test in the extinction context after the renewal test ($P = 0.56$).

Control toads in Experiment 1 waited 9 days before a test trial to assess if memory of the prey catching conditioned response had decayed, while toads in Experiment 2 waited 16 days. Figure 4 shows that the time to 5 snaps after these waiting periods did not change much compared to the first trial of the last conditioning session (A3). GEE analysis showed that there was no significant difference in prey catching performance after the 9- and 16-days waiting periods ($\chi^2(2) = 0.6$, $P = 0.73$). Therefore, we conclude that the conditioned prey catching response is remembered for at least 16 days.

Experiment 3: reinstatement

In this experiment, toads that underwent successful conditioning reduced their time to reach 5 snaps with training (Fig. 5A), a trend supported by a significant effect of session in the GEE analysis ($\chi^2(2) = 25.7$, $P < 0.001$). LSD contrasts showed that the time to 5 snaps in the first trial significantly differed between acquisition sessions 1–2 ($P = 0.03$) and 1–3 ($P = 0.03$), but not between sessions 2–3 ($P = 0.09$). Thus, the analysis showed that conditioning quickly improved prey catching performance after the first session. Conversely, toads increased their times to reach 5 snaps over the 9 extinction sessions (Fig. 5A). The time to 5 snaps in the first trial of sessions significantly differed over the extinction period ($\chi^2(8) = 323$, $P < 0.001$) and LSD contrasts

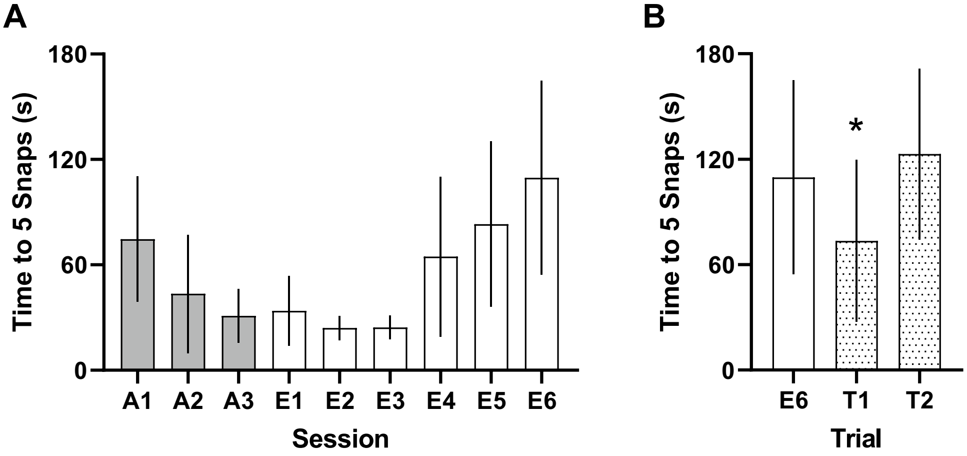

Fig. 3 Prey catching performance of fire-bellied toads during the second renewal experiment (Experiment 2). **A** Time to 5 snaps on the first trial of sessions during acquisition (A1–A3; gray bars) and extinction training (E1–E6; white bars). **B** Time to 5 snaps on the first trial of the last extinction session (E6) and the renewal and return to extinction context tests (T1 and T2, respectively; bars with dotted pattern). Bars show means ± 95% confidence intervals. The asterisk in panel B indicates that time to 5 snaps was lower at T1 compared to both E6 and T2

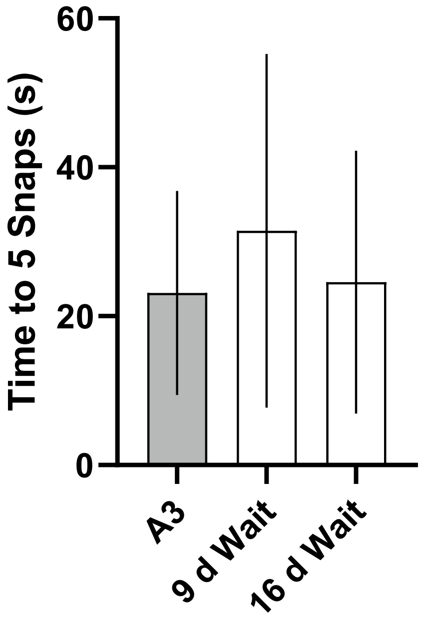

Fig. 4 Prey catching performance of control fire-bellied toads tested during the renewal experiments (Experiments 1 and 2). Time to 5 snaps on the first trial of the last session of acquisition training (A3; gray bar) was compared to performance on a test trial after waiting periods of 9 or 16 days (white bars), which corresponded to the duration of the extinction training periods in toads that underwent renewal tests. There was no effect of waiting on performance. Bars show means ± 95% confidence intervals

indicated sustained higher times to 5 snaps compared to the first extinction session beginning at session 3 ($P \leq 0.006$). This analysis showed that the toads had undergone significant extinction before the reinstatement tests.

During the in-context reinstatement test, control and treatment groups showed no difference in time to 5 snaps corrected for individual performance at the end of extinction (Fig. 5B; $U = 25.5$, $n_1 = 9$, $n_2 = 7$, $P = 0.53$). The reinstatement toads subsequently fed outside the context also showed no significant difference in time to 5 snaps compared to their own performance of 2 days prior (Fig. 5C; $\mathrm{W}(7) = -11$, $P = 0.19$).

Discussion

Fire-bellied toads that had a conditioned prey catching response reduced by extinction in a distinct experimental context than the one used during acquisition showed renewal of rapid prey catching responding when brought back to the acquisition context. This renewal effect showed that contextual cues unrelated to the visual prey stimulus targeted by the toads can modulate prey catching performance. The reduced prey catching activity that followed a return to the extinction context after renewal further supported a role for contextual cues in modulation of the conditioned response. To our knowledge, this is the first report of a role for contextual cues in the control of amphibian conditioned behaviour. Conversely, toads showed no difference in performance following consumption of a cricket in the reinstatement tests administered after extinction. The absence of reinstatement using a conditioning paradigm that produced renewal suggests that chemosensory and visceral cues modifying the

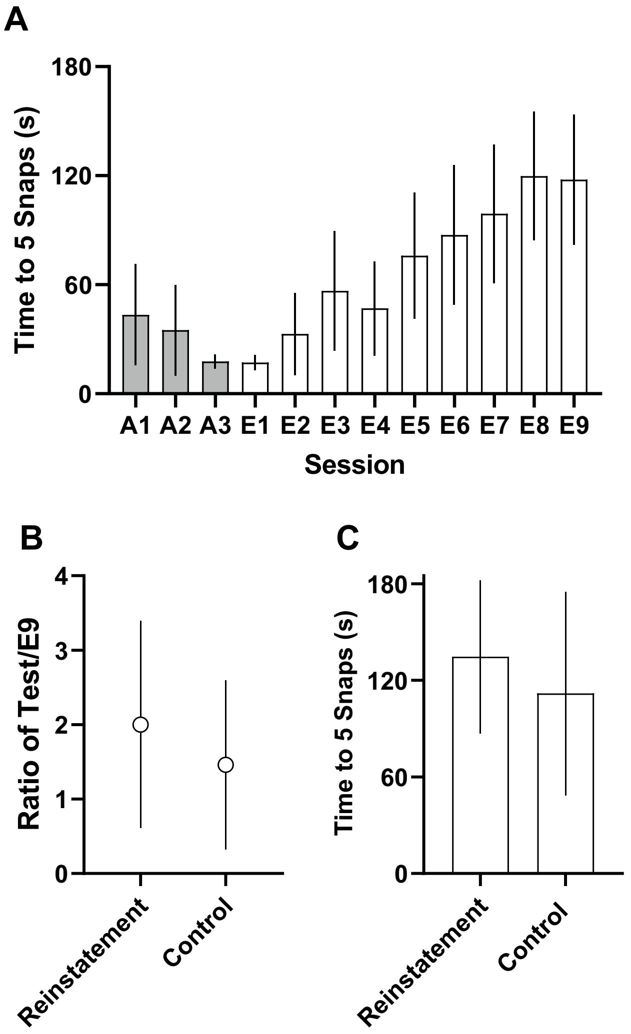

Fig. 5 Prey catching performance of fire-bellied toads during the reinstatement experiment (Experiment 3). **A** Time to 5 snaps on the first trial of sessions during acquisition (A1–A3; gray bars) and extinction training (E1–E9; white bars). **B** Time to 5 snaps of reinstatement and control toads during the in-context test. Because two different groups of toads were compared in this test, performance was divided by time to 5 snaps at the first trial of the ninth extinction session (ratio of test time/E9 time) to account for individual variation in performance by the end of extinction. **C** Time to 5 snaps of reinstatement and control trials during the out-of-context test. In this case, performance of the same toads was compared on subsequent trials. There was no effect of the reinstatement manipulations on performance. Bars and symbols show means ± 95% confidence intervals

‘interoceptive context’ (sensu Bouton 2002) associated with prey consumption do not modulate the conditioned prey catching response and that reinstatement might be altogether absent in this species.

Extinction of conditioned prey catching is not caused by forgetting

The control toads used in renewal experiments showed no evidence of memory decay when tested 9 or 16 days after the end of conditioning, ruling out forgetting as a contributing factor to the decreasing prey catching performance seen during extinction. This contrasts with the findings of Puddington et al. (2013) who showed that dehydrated terrestrial toads (*Rhinella arenarum*) trained on a runway task lost their tendency to speed along the runway after 8 days of waiting following conditioning with 30 s of water access for reinforcement or after 16 days of waiting with 300 s of water access during conditioning. This evidence was used to propose that memory decay contributes to the reduction of instrumental behaviour of toads in extinction and that stronger reinforcement produces better memory retention. What could explain the differences between studies? Besides the obvious possibility of species differences, it is possible that food reward produces stronger reinforcement than water reward, resulting in enhanced memory retention. We have previously showed that an extended period of daily prey catching conditioning can produce strong resistance to extinction in *B. orientalis* (Ramsay et al. 2013), a phenomenon that does not seem to apply to runway conditioning for water in *R. arenarum* (Muzio et al. 2006). Alternatively, the massed training schedule we used differed from the single daily runway trials used by Puddington et al. (2013) and thus could also have produced stronger reinforcement and better memory retention. However, the present study used the shortest amount of training producing significant acquisition of the prey catching response to allow for extinction to happen and even under this short training condition, memory was retained for at least 16 days. The ability of food reinforcement to produce resistance to extinction and long memory retention after minimal training suggests that it produces stronger learning than water reinforcement in amphibians.

Fire-bellied toads encode information about environmental context

The presence of a renewal effect in an anuran amphibian showed that they are capable of encoding information about the environmental contexts that they experience despite showing no obvious exploratory behaviour. Learning of the features of experimental contexts happened even under the distracting influence of a prey stimulus that engaged predatory behaviour. Since the intensity of prey catching responses was subject to specific context modulation, it suggests that the brain systems encoding environmental information additionally encode reinforcement history. Learning of contextual information is a widespread phenomenon in animals that has been demonstrated using renewal and context occasion setting effects in many vertebrates (Bouton 2002; McConnell and Miller 2014; Kenney et al. 2017; Trask et al. 2017) and invertebrates (Colwill et al. 1988a,b; Matsumoto and Mizunami 2004; Brembs and Wiener 2006). Its novel demonstration in an amphibian supports the view that the ability to encode contextual information is a conserved feature of animals with brains. Integrating complex information from multiple sensory modalities to remember the environmental contexts in which animals live might be the primary pressure that led to the independent elaboration of brains in different groups of animals (Northcutt 2012). Pairing this ability with memories of reinforcement history would allow adaptive adjustment of behaviour according to the features of each environment experienced by an individual.

Absence of a reinstatement effect

Fire-bellied toads showed no reinstatement of extinguished prey catching when fed the cricket reinforcer that was used during conditioning. This result differs from what is seen in a variety of animals that display reinstatement after consumption of food reinforcers, including zebrafish (Kuroda et al. 2017), rats (Ghitza et al. 2006; Nair et al. 2009) and pigeons (Podlesnik and Shahan 2009; Miranda-Dukoski et al. 2016). Thus, it appears that *B. orientalis* does not undergo reinstatement of conditioned prey catching behaviour despite renewal of the same behaviour based on context changes. Nevertheless, it is difficult to prove the absence of a learning phenomenon and possible that *B. orientalis* could show reinstatement under different circumstances. For example, reinstatement could be specific to some forms of learning, as suggested by its absence following extinguished eyeblink conditioning in rats (Thanellou and Green 2011). Prey catching conditioning is based on the innate predation tendency of anurans and as such might differ fundamentally from the other tasks that have been used to show reinstatement after consumption of food reinforcers. Another possibility is that reinstatement might rely on a reinforcer or cues uniquely experienced during conditioning. Here, we used normal cricket food for prey catching conditioning, a food that the toads also experienced in their normal housing. Conditioning and reinstatement testing using a novel prey that is not used for normal feeding might be able to support reinstatement if it elicits an interoceptive state distinguishable from the one experienced during normal feeding. Further work will be needed to evaluate this possibility.

If reinstatement is indeed not present in *B. orientalis*, and possibly other amphibians, why is this the case? Contextual cues about the environment might be more important than priming cues of food availability for amphibians making decisions about foraging, and as such they would have no need for reinstatement. Since amphibians generally rely on an irregular food supply and can tolerate long periods without feeding (Wells 2007), the interoceptive state associated with food consumption that supports reinstatement in other vertebrates could be absent in these animals. Alternatively, it is now well established that lungfishes and modern amphibians have undergone substantial secondary simplification of their nervous systems during phylogeny; a phenomenon accompanied by increased genome and cell sizes (Roth and Walkowiak 2015). The end result of this simplification in amphibians is a reduced ability to pack high numbers of neurons in brains that are already comparatively small for vertebrates (Striedter and Northcutt 2020). Secondary simplification of the brain in amphibians could have come at cognitive costs, with loss of the ability to display reinstatement one such cost.

Conclusion

The absence of forgetting of the prey catching conditioned response in control toads and recovery from extinction by renewal suggest that inhibitory learning established during extinction reduced prey catching performance. Thus, the neural substrate for inhibitory extinction learning is present in the relatively simple brains of amphibians. A good candidate brain region to enact such inhibition is the medial pallium because its lesion prevents extinction of runway training in *R. arenarum* (Muzio et al. 1993). The inhibitory learning mediating the renewal effect involves environmental cues unrelated to the prey catching response and the stimulus that releases it. Remembering environmental cues associated with the presence of food could be important to the expression of behaviours in amphibians that encounter food resources only intermittently. On the other hand, the absence of reinstatement of extinguished prey catching conditioning suggest that amphibians may have lost the ability to represent interoceptive contexts as other vertebrates do; a loss possibly due to secondary simplification of the nervous system. Recovery from extinction effects may not be as widespread as previously thought. Further research is needed to better understand brain structure–function relationships in vertebrates and amphibians could provide promising examples of cognitive differences associated with brain simplification.

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Author contributions JM and AR contributed equally to this manuscript. JM conducted the reinstatement experiment. ML conducted the first renewal experiment. AR conducted the second renewal experiment. FL analysed the data and wrote the manuscript with draft contributions from all authors.

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Declarations

Conflict of interest We declare no conflict or competing interests.

Ethical approval All procedures were approved by the University of Guelph animal care committee (AUP #3590) under the guidelines of the Canadian Council on Animal Care.

Data availability Data are available as supplementary material with the article.

Code availability Not applicable.

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