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Spontaneous Recovery But Not Reinstatement of the Extinguished Conditioned Eyeblink Response in the Rat

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Abstract

Reinstatement, the return of an extinguished conditioned response (CR) after reexposure to the unconditioned stimulus (US), and spontaneous recovery, the return of an extinguished CR with the passage of time, are two of four well-established phenomena which demonstrate that extinction does not erase the conditioned stimulus (CS)-US association. However, reinstatement of extinguished eyeblink CRs has never been demonstrated and spontaneous recovery of extinguished eyeblink CRs has not been systematically demonstrated in rodent eyeblink conditioning. In Experiment 1, US reexposure was administered 24 hours prior to a reinstatement test. In Experiment 2, US reexposure was administered 5 min prior to a reinstatement test. In Experiment 3, a long, discrete cue (a houselight), present in all phases of training and testing, served as a context within which each trial occurred to maximize context processing, which in other preparations has been shown to be required for reinstatement. In Experiment 4, an additional group was included that received footshock exposure, rather than US reexposure, between extinction and test, and contextual freezing was measured prior to test. Spontaneous recovery was robust in Experiments 3 and 4. In Experiment 4, context freezing was strong in a group given footshock exposure but not in a group given eyeshock US reexposure. There was no reinstatement observed in any experiment. With stimulus conditions that produce eyeblink conditioning and research designs that produce reinstatement in other forms of classical conditioning, we observed spontaneous recovery but not reinstatement of extinguished eyeblink CRs. This suggests that reinstatement, but not spontaneous recovery, is a preparation- or substrate-dependent phenomenon.

Keywords

eyeblink conditioning; extinction; reinstatement; spontaneous recovery; contextual

A large body of evidence supports the notion that extinction is not equivalent to unlearning (for a review, see Bouton, 2004). Experiments have shown that nonreinforcement following conditioning does not result in the erasure of the CS-US association. Four experimental phenomena have been widely regarded as demonstrating this: spontaneous recovery, rapid reacquisition, renewal, and reinstatement (e.g., Bouton, 2004; Larrauri & Schmajuk, 2008). These four phenomena show that, under specific experimental conditions, the CR can return to pre-extinction levels.

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Reinstatement of classically conditioned performance (the return of an extinguished CR when animals are re-exposed to the US following extinction training) was first reported by Pavlov (1927) in salivary conditioning of dogs. More recently, reinstatement of extinguished classically conditioned fear CRs in rodents has been shown with multiple indices of conditioned fear, including conditioned suppression (Bouton & Bolles, 1979; Bouton & King, 1983; Bouton, 1984; Bouton & King, 1986; Frohardt, Guarraci, & Bouton, 2000; Rauhut, Thomas, & Ayres, 2001; Rescorla, 2006; Rescorla & Cunningham, 1977; Rescorla & Heth, 1975; Waddell, Morris, & Bouton, 2006; Wilson, Brooks, & Bouton, 1995), conditioned freezing (Gewirtz, Falls, & Davis, 1997; Morris, Furlong, & Killcross, 2005; Morris, Westbrook, & Killcross, 2005; Richardson, Duffield, Bailey, & Westbrook, 1999; Westbrook, Iordanova, McNally, Richardson, & Harris, 2002), and fear potentiated startle (Gewirtz et al., 1997; Waddell, Bouton, & Falls, 2008). In addition, reinstatement of extinguished appetitive CRs in rodents has been demonstrated (Bouton & Peck, 1989; Bouton, Rosengard, Achenbach, Peck, & Brooks, 1993; Delamater, 1997; Fox & Holland, 1998; Moody, Sunsay, & Bouton, 2006). Reinstatement of extinguished conditioned taste aversions has been less studied but has been demonstrated in at least one study (Schachtman, Brown, & Miller, 1985; but see Bouton, 1982) in rodents. Finally, a number of recent studies in humans have demonstrated reinstatement of extinguished fear CRs (Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2004, 2007; Dirikx, Vansteenwegen, Eelen, & Hermans, 2009; Hermans, Dirikx, Vansteenwegen, Baeyens, Van den Bergh, & Eelen, 2005; LaBar & Phelps, 2005; Norrholm, Jovanovic, Vervliet, Myers, Davis, Rothbaum, & Duncan, 2006; Schiller, Cain, Curley, Schwartz, Stern, LeDoux, & Phelps, 2008; Van Damme, Crombez, Hermans, Koster, & Eccleston, 2006). Research has shown that reinstatement critically depends on conditioning of the context in which subsequent reinstatement testing takes place (e.g., Bouton & Bolles, 1979; Bouton & King, 1983, Bouton, 1984; Bouton & King, 1986; Bouton & Peck, 1989; LaBar & Phelps, 2005). The presence of contextual excitation at test has been demonstrated through context preference tests following footshock reexposure after extinction of fear conditioning (Bouton & King, 1983, 1986; Bouton, 1984).

Eyeblink conditioning is a notable exception to the classical conditioning preparations in which reinstatement has been demonstrated. Only two, limited, attempts to produce and measure reinstatement of the extinguished conditioned eyeblink response have been published, both using restrained rabbits as subjects (Napier, Macrae, & Kehoe, 1992; Weidemann & Kehoe, 2004; see also Frey & Sears, 1978, p. 328, for mention of an unpublished earlier attempt). Neither of these two attempts to produce reinstatement of the extinguished eyeblink CR was systematic; rather, each involved a control condition in an experiment conducted for another purpose. Napier et al. (1992) examined reinstatement in an experiment designed to study rapid reacquisition of the extinguished eyeblink CR in rabbits. Previous studies of rapid reacquisition of extinguished eyeblink CRs had failed to rule out the possibility of spontaneous recovery or reinstatement, rather than rapid reacquisition. In Experiment 1, Group 1 received 3 days of CS-US conditioning and 5 days of CS-alone extinction. The day after the final session of extinction, Group 1 received 4 CS-alone trials (to test for spontaneous recovery), 4 US-alone trials (to produce reinstatement), and 4 CS-alone trials (to test for reinstatement) prior to further CS-US trials. Group 2 received the same treatment except for the extinction phase, where they received unpaired CS and US trials. Group 3 was equated on context exposure and received only the final set of CS-US trials. Neither spontaneous recovery nor reinstatement was detected prior to rapid reacquisition in Groups 1 and 2.

More recently, Weidemann and Kehoe (2004) examined reinstatement in an experiment designed to study concurrent recovery in rabbit eyeblink conditioning, the return of responding to an extinguished CS (e.g., CSA) after conditioning of a second CS of a

different modality (e.g., CSB; Macrae & Kehoe, 1999; Weidemann & Kehoe, 2003, 2004, 2005). Like rapid reacquisition, an alternative explanation of concurrent recovery is that it is a reinstatement effect. In Experiment 1, rabbits received CSA-US conditioning followed by extinction. In the final stage, groups received 3 sessions of either CSB-US conditioning or US-alone trials, the latter designed to produce reinstatement. Test trials of CSA-alone were intermixed with these final stage trials. Concurrent recovery of responding to CSA was evident in groups that underwent CSB-US conditioning in the final stage but not in groups that received only US-alone trials. Thus, despite the presentation of many more US-alone trials than in Napier et al. (1992), this experiment also failed to produce reinstatement of the extinguished eyeblink CR.

Spontaneous recovery, unlike reinstatement, is a well-established phenomenon in eyeblink conditioning in humans (e.g., Beeman & Grant, 1961; Beeman, Hartman, & Grant, 1960; Franks, 1963; Froseth & Grant, 1961; Grant, Hunter, & Patel, 1958; Hartman & Grant, 1960, 1962; Howat & Grant, 1958; Prokasy, 1958) and in nictitating membrane conditioning in rabbits (e.g., Berger & Thompson, 1982; Haberlandt, Hamsher, & Kennedy, 1978; Hardiman, Ramnani, & Yeo, 1996; Macrae & Kehoe, 1999; Napier et al., 1992; Schneiderman, 1966; Schneiderman & Gormezano, 1964; Schreurs, 1993; Weidemann & Kehoe, 2003, 2004, 2005; see also Schneiderman, Fuentes, & Gormezano, 1962 for a possible, but unanalyzed example in rabbits using conditioning of the outer eyelids). We know of no documented reports of spontaneous recovery of eyeblink CRs in rodents. Nicholson, Sweet, and Freeman (2003), in a demonstration of rapid reacquisition of the eyeblink CR in rats, make brief mention of a lack of spontaneous recovery in their study (see also Figure 3A of Kishimoto & Kano, 2006 for a likely, but unanalyzed, example of spontaneous recovery of eyeblink CRs in mice).

The present experiments were designed to more systemically examine whether reinstatement of the extinguished conditioned eyeblink response is possible and, if not, why not. A secondary aim was to document spontaneous recovery in rodent eyeblink conditioning and to determine whether observation of spontaneous recovery was necessary for observing reinstatement. Demonstration of reinstatement in eyeblink conditioning would extend the generality of reinstatement as a behavioral phenomenon in Pavlovian conditioning beyond specific preparations. The ability for extinguished CRs to be reinstated by reexposure to the US alone is generally regarded as a cardinal feature of extinction and, as such, is a litmus test for models designed to explain extinction (e.g., Larrauri & Schmajuk, 2008). If reinstatement cannot be produced in eyeblink conditioning, it would challenge the view that reinstatement (and perhaps other aspects of extinction) is a phenomenon independent of specific procedures that engage particular brain and/or response systems. Furthermore, whether or not reinstatement of extinguished eyeblink CRs can be produced could further inform the debate on the neural substrates of reinstatement (Fox & Holland, 1998; Frohardt, Guarraci, & Bouton, 2000; Waddell, Morris, & Bouton, 2006; Wilson, Brooks, & Bouton, 1995), given the well-mapped neural circuit in eyeblink conditioning.

Experiment 1

In Experiment 1, we examined whether a significant number of US-alone trials (100 trials) after extinction training is sufficient to reinstate the extinguished conditioned eyeblink response 24-hr later.

Method

Subjects—Twenty-four male Long-Evans (80–90 days old on arrival) were obtained from a commercial supplier (Harlan, Indianapolis, IN). After arrival, the rats were housed individually for approximately one week prior to surgery with ad libitum chow and water.

The colony was maintained on a 12 hour light-dark cycle (lights on at 7 am). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont.

Surgery—Rats were anesthetized using 3% isoflurane in oxygen and, using aseptic surgical procedures, each rat was surgically prepared with differential electromyographic (EMG) recording wires for recording eyeblinks and a bipolar periocular stimulation electrode for delivering the stimulation US. In addition, a ground wire was connected to three stainless steel skull screws.

The EMG wires for recording activity of the external muscles of the eyelid, the orbicularis oculi, were constructed of two strands of ultra-thin (75 μ m) Teflon-coated stainless steel wire soldered at one end to a mini-strip connector. The other end of each wire was passed subdermally to penetrate the skin of the upper eyelid of the left eye and a small amount of the insulation was removed. The bipolar stimulation electrode (Plastics One, Roanoke, VA) was positioned subdermally immediately dorsocaudal to the left eye. The mini-strip connector and the bipolar stimulation electrode were cemented to the skull with dental cement. The wound was salved with antibiotic ointment (Povidone), and an analgesic (buprenorphine) was administered (s.c.) immediately after surgery and twice the following day. Rats were given 7 days to recover prior to eyeblink conditioning.

Apparatus—Eyeblink conditioning took place in one of four identical testing chambers (30.5 $\times$ 24.1 $\times$ 29.2 cm; Med-Associates, St. Albans, VT), each with a grid floor. The top of each chamber was modified so that a 25-channel tether/commutator could be mounted to it. Each testing chamber was housed within an electrically-shielded, sound-attenuating chamber (45.7 $\times$ 91.4 $\times$ 50.8 cm; BRS-LVE, Laurel, MD). A fan in each sound-attenuating chamber provided background noise of approximately 60 dB sound pressure level (SPL). A speaker was mounted in each corner of the rear wall and a houselight (off during testing) was mounted in the center of the rear wall of each sound-attenuating chamber. The sound-attenuating chambers were housed within a walk-in sound-proof chamber.

Stimulus delivery was controlled by an IBM PC-compatible computer running custom software (Chen & Steinmetz, 1998). A 290 ms, 2800 Hz, 80 dB tone, delivered through the left speaker of the sound-attenuating chamber, served as the conditioned stimulus (CS). A 10 ms, 4.0 mA unipolar periorbital stimulation, delivered from a constant current stimulator (model A365D; World Precision Instruments, Sarasota, FL), served as the unconditioned stimulus (US). Recording of eyelid EMG activity was controlled by a computer interfaced with a Power 1401 high-speed data acquisition unit and running Spike2 software (Version 5). The eyelid EMG signals were amplified (10k) and bandpass filtered (100–1000 Hz) prior to being passed to the Power 1401 and from there to a computer running Spike2. Spike2 was used to full-wave rectify, smooth (10 ms time constant), and time shift (10 ms, to compensate for smoothing) the amplified EMG signal.

Procedure—At the beginning of each session, each rat was plugged in, via the connectors cemented to its head, to the 25-channel tether/commutator, which carried leads to and from peripheral equipment and allowed the rat to move freely within the testing box. On Day 1 (adaptation), rats were plugged in but no stimuli were delivered. They remained in the chamber for 60 min (the approximate length of a training session). On Days 2–7 (conditioning), 100 trials per day were delivered, with an average inter-trial interval (ITI) of 30 sec (range = 20–40 sec). Trials consisted of a 290 ms tone (CS) which coterminated with a 10 ms eyelid stimulation (US) except for every tenth trial, in which the CS was presented alone (for inspection of long latency responses). On Days 8–13 (extinction), 100 trials per day were delivered at an average ITI of 30 sec (range = 20–40 sec), each consisting of a 290

ms tone CS. On Day 14, half of the animals received a reinstatement treatment (US-alone trials) during which 100 trials were delivered at an average ITI of 30 sec (range = 20–40 sec), each consisting of the 10 ms US stimulation. The other half of the animals stayed in the conditioning chamber without receiving any US presentations. On Day 15 (testing), all animals received 100 trials, at an average ITI of 30 sec (range = 20–40 sec), each consisting of a 290 ms tone CS.

Data Analysis—For conditioning sessions, paired trials were subdivided into four time periods: (1) a “baseline” period, 280 ms prior to CS onset; (2) a “startle” period, 0–80 ms after CS onset; (3) a “CR” period, 81–280 ms after CS onset; and (4) a “US period”, 65–165 ms after US onset (the first 65-ms was obscured by the US electrical artifact). Eye blinks that exceeded mean baseline activity by 0.5 arbitrary units (range = 0.0 to 5.0) during the CR period were scored as CRs. Eye blinks that met this threshold during the startle period were scored as startle responses (SRs). The dependent measure of CR acquisition was percentage of CRs across all CS-US trials of each session. For extinction and testing sessions, CS alone trials were subdivided into three time periods: (1) a “baseline” period, 280 ms prior to CS onset; (2) a “startle” period, 0–80 ms after CS onset; and (3) a “CR” period, 81–440 ms after CS onset. Thus, the 440-ms end of the “CR” period was 150-ms after the offset of the 290-ms CS. Similar to conditioning sessions, eyeblinks that exceeded mean baseline activity by 0.5 arbitrary units during the CR period were scored as CRs whereas eyeblinks that met this threshold during the startle period were scored as startle responses.

Data were analyzed using SPSS 17.0.2. An alpha level of 0.05 was used as the rejection criterion for all statistical tests.

Results

Of the 24 rats who began Experiment 1, 15 ($n=8$ in Group Reinstatement; $n=7$ in Group Control) were included in analyses. Of the 9 rats that were not included, 7 rats (3 from Group Reinstatement and 4 from Group Control) had poor eyelid EMGs, 1 rat (from Group Reinstatement) lost its headcap prior to the test session, and 2 rats (1 from each group) were classified as “poor learners” (fewer than 30% CRs in Session 5 or 6 of conditioning).

Conditioning and Extinction—At the session level, groups demonstrated equivalent conditioning and extinction (Figure 1A). This was confirmed by a 2 (Group) X 6 (Acquisition Session) repeated-measures ANOVA that revealed a significant acquisition session effect, $F(5,60) = 16.13$, $p < 0.05$ and a 2 (Group) X 6 (Extinction Session) repeated-measures ANOVA that revealed a significant extinction session effect, $F(5,60) = 46.91$, $p < 0.05$. There were no significant group or interaction effects.

Spontaneous Recovery and Reinstatement—Neither spontaneous recovery (a greater percentage of CRs in the first 10 trials of Extinction Sessions 2–6 compared to the last 10 trials of Extinction Sessions 1–5, respectively) nor reinstatement (a greater percentage CRs in Group Eyeshock Reinstatement compared to Group Control in the first 10 trials of the Test Session) of extinguished eyeblink CRs was evident (Figure 1B). For analysis of spontaneous recovery, extinction sessions and the test session were analyzed in blocks of 10 trials. A series of six 2 (Group) X 2 (Last Block of 10 Trials of Previous Session vs. First Block of 10 Trials of Next Session) repeated-measures ANOVAs was conducted comparing percentage of CRs. There were no significant differences in these analyses, indicating no spontaneous recovery in extinction sessions 2–6 or in the test session.

For analysis of reinstatement, Group Eyeshock Reinstatement was compared to Group Control in the first block of 10 trials of the test session. A one-way ANOVA failed to reveal group differences in percentage of CRs, $F < 1$.

Discussion

Experiment 1 failed to reveal either spontaneous recovery or reinstatement of the extinguished eyeblink CR in the rat. The failure to find reinstatement replicates earlier failures in rabbit eyeblink conditioning (Napier et al., 1992; Weidemann & Kehoe, 2004). While spontaneous recovery has been demonstrated numerous times in rabbit eyeblink conditioning (e.g., Macrae & Kehoe, 1999; Napier et al., 1992; Weidemann & Kehoe, 2003, 2004, 2005), we know of no published reports of spontaneous recovery of eyeblink CRs in the rat.

Experiment 2

US reexposure has been hypothesized to produce reinstatement of extinguished CRs by returning animals to the conditioned or perhaps unconditioned emotional context of conditioning, which has been hypothesized to underlie reinstatement in other preparations as an ABA renewal type of effect (Bouton, 2004). This hypothesis suggests that reinstatement might be strongest immediately after US reexposure, given the likelihood that emotional effects of the US reexposure would weaken over time. Others have argued that US reexposure restores a US representation degraded by conditioning (Rescorla & Heth, 1975). If this US representation degrades over time, reinstatement should be strongest right after US reexposure (cf. Delamater, 1997). This also suggests that reinstatement should be strongest immediately after US reexposure. Finally, reinstatement of drug-seeking instrumental responses after exposure to footshock is typically measured within a few minutes after footshock (e.g., Shaham & Stewart, 1995; Shalev, Highfield, Yap, & Shaham, 2000; Shalev, Morales, Hope, Yap, & Shaham, 2001). With the above in mind, we conducted US reexposure and testing within a single session in Experiment 2.

Method

Subjects, Surgery and Apparatus—Twenty-four male Long-Evans (80–90 days old on arrival) were obtained from a commercial supplier (Harlan, Indianapolis, IN). All housing and care was identical to those of Experiment 1. The surgical procedures, apparatus and data analyses for Experiment 2 were also identical to those described for Experiment 1.

Procedure—The conditioning and extinction phases were identical to Experiment 1. Following the last day of extinction training, on Day 14, half of the animals received a reinstatement treatment (US-alone trials) during which 100 trials were delivered at an average ITI of 30 sec (range = 20–40 sec), each consisting of the 10 ms US stimulation (as in Experiment 1). The other half of the animals stayed in the conditioning chamber without receiving any US presentations. After the end of the US-alone trials session, we waited for 5 min in order for any immediate sensitization effects of the US to dissipate and then tested for reinstatement of the extinguished conditioned response by continuing the session with 100 CS-alone trials (290 ms tone) delivered at an average ITI of 30 sec (range = 20–40 sec).

Results

Of the 24 rats who began Experiment 2, 14 ($n=7$ in Group Reinstatement; $n=7$ in Group Control) were included in analyses. Of the 10 rats that were not included, 7 rats (3 from Group Reinstatement and 4 from Group Control) had poor eyelid EMGs, 1 rat (from Group Control) lost its headcap prior to the test session, and 2 rats (from Group Reinstatement) were classified as “poor learners” (fewer than 30% CRs in Session 5 or 6 of conditioning).

Conditioning and Extinction—At the session level, groups demonstrated equivalent conditioning and extinction (Figure 2A). This was confirmed by a 2 (Group) X 6 (Acquisition Session) repeated-measures ANOVA that revealed a significant acquisition session effect, $F(5,60) = 28.11$, $p < 0.05$ and a 2 (Group) X 6 (Extinction Session) repeated-measures ANOVA that revealed a significant extinction session effect, $F(5,60) = 16.67$, $p < 0.05$. There were no significant group or interaction effects.

Spontaneous Recovery and Reinstatement—Spontaneous recovery of extinguished eyeblink CRs was evident only in Extinction Session 6 (Figure 2B). As in Experiment 1, reinstatement was not produced (Figure 2B). A series of six 2 (Group) X 2 (Last Block of 10 Trials of Previous Session vs. First Block of 10 Trials of Next Session) repeated-measures ANOVAs was conducted comparing percentage of CRs. These analyses revealed a significantly greater percentage of CRs at the beginning of Extinction Session 6 compared to Extinction Session 5 (i.e., spontaneous recovery), $F(1,12) = 7.64$, $p < 0.02$. There was no spontaneous recovery in any other extinction session or in the test session.

For analysis of reinstatement, Group Eyeshock Reinstatement was compared to Group Control in the first block of 10 trials of the test session. A one-way ANOVA failed to reveal group differences in percentage of CRs, $F < 1$.

Discussion

As in Experiment 1, in Experiment 2 we failed to observe reinstatement. The group that received eyeshock reexposure failed to differ in percentage of eyeblink CRs in the test session from the group that received context exposure. However, unlike Experiment 1, we did obtain some evidence of spontaneous recovery of the extinguished eyeblink CR. Specifically, we observed spontaneous recovery in the final session (Session 6) of extinction. Lack of spontaneous recovery in some other sessions of extinction (e.g., Session 5) and in the test session may have been due to a failure to fully extinguish CRs by the end of the previous session. For example, in several sessions of extinction in Experiment 2, CRs showed inconsistent decreases and increases across blocks. This slowing of extinction may have been due to the partial reinforcement procedure used in acquisition. Specifically, in acquisition, every 10th trial was a test trial so that rats were on a 90% reinforcement schedule. This is a standard design of eyeblink conditioning experiments. However, rats may have learned that trials without a US were a signal that further trials would include a US. This may have precluded stable extinction performance by the end of each extinction session (particularly in early extinction sessions), a necessary condition for assessing spontaneous recovery in the subsequent extinction session. These test trials were removed in Experiments 3 and 4. In addition, Experiments 3 and 4 were designed to assess whether a failure of context conditioning during US reexposure might explain our failure to observe reinstatement. Experiment 3 focused on the salience of the context.

Experiment 3

Experiment 3 added several procedural details that were expected to increase the chances of observing reinstatement. The most important of these modifications were designed to make the context as salient as possible to facilitate context conditioning during US reexposure, since reinstatement critically depends on conditioning of the context in which subsequent reinstatement testing takes place (e.g., Bouton & Bolles, 1979; Bouton & King, 1983, Bouton, 1984; Bouton & King, 1986; Bouton & Peck, 1989; LaBar & Phelps, 2005).

In experiments designed to examine whether contexts can be pretrained to have a modulating influence on the performance of conditioned eyeblink responses at the time of testing, Wagner and colleagues employed a long but discrete stimulus to serve as a “context”

in rabbit eyeblink conditioning (Bombace, Brandon, & Wagner, 1991; Brandon, Betts, & Wagner, 1994; Brandon, Bombace, Falls, & Wagner, 1991; Brandon & Wagner, 1991; Gewirtz, Brandon, & Wagner, 1998). For example, Gewirtz et al. (1998) paired a 30 sec auditory stimulus with eyelid stimulation and produced conditioned fear to the long auditory stimulus, as measured by potentiated startle (head jerk to an airpuff to the auditory canal), but no conditioned eyeblinks. They also demonstrated that this long auditory stimulus could modulate subsequent eyeblink conditioning to a 1 sec light paired with an eyelid stimulation US to the opposite eyelid from that used to produce conditioned fear. This was hypothesized to occur because the long auditory stimulus functioned like a contextual stimulus. Following this line of thought, in Experiment 3 we attempted to create a “context” using a long and discrete visual cue during all experimental phases, hypothesizing that such a discrete cue might allow contextual conditioning by the brief, localized US used in eyeblink conditioning (see also Kehoe, 2006). We used this “context” in all phases to avoid confounding reinstatement and renewal types of effects (which might occur with a context change between extinction training and testing) and because reexposure to the US and subsequent testing must occur in the same physical context for reinstatement to occur (see Bouton, 2004). In addition, we included a salient odor cue (a coconut scent that was present during all training phases) to further facilitate contextual conditioning during US reexposure (cf., McKinzie & Spear, 1995).

The two previous attempts to demonstrate reinstatement of the extinguished eyeblink CR (Napier et al., 1992; Weidemann & Kehoe, 2004) may have failed to do so because of the extended extinction given prior to US reexposure and test. Both studies were designed to continue extinction beyond the point of spontaneous recovery and, although the presence of rapid reacquisition (Napier et al., 1992) and concurrent recovery (Weidemann & Kehoe, 2004) suggested the presence of residual CS excitatory strength, it is conceivable that reinstatement does not survive extended extinction of the eyeblink CR (see also Schachtman et al., 1985). Thus, we decreased the number of extinction training days to shorten the amount of extinction training.

Finally, in order to increase our chances of observing at least spontaneous recovery during extinction and test, we excluded the 10 CS-alone trials included during acquisition sessions in Experiments 1 and 2. This was intended to make acquisition and extinction phases as distinct as possible in order to facilitate robust within-session extinction. This within-session extinction is a necessary prerequisite for detecting spontaneous recovery at the beginning of a subsequent extinction or test session.

Method

Subjects—Twenty-five male Long-Evans (80–90 days old on arrival) were obtained from a commercial supplier (Harlan, Indianapolis, IN). All housing and care was identical to Experiments 1 and 2. The surgical procedures and apparatus for Experiment 3 were also identical to those described for Experiment 1 and 2. The data analysis for Experiment 3 was identical to Experiments 1 and 2, except that we also scored eyeblinks during the first 500-ms of the long light context (described below), using the same scoring criteria as we used for scoring eyeblinks to the CS.

Procedure—For all days, an odor cue was added to the conditioning chambers by addition of a coconut scent (McCormick Imitation Coconut Extract) in a 3.5” x 3.5” weigh boat outside the chamber. Adaptation day was identical to Experiments 1 and 2. On Days 2–7 (conditioning), 100 trials were delivered each day. Trials consisted of a 290 ms tone CS which coterminated with a 10 ms US. A discrete, long (19.5 sec; 25 lux at the level of the rat) houselight served as a “context” within which each trial occurred. Specifically, the CS-

US trial occurred 18.5-sec after the houselight onset which, in turn, terminated 1 sec after the CS-US trial. The inter-trial interval between houselight stimuli was 20 sec. On Days 8–10 (extinction), 100 trials were delivered each day; trials were identical to those used during conditioning, except that the US was omitted. On Day 11, half of the animals received a reinstatement treatment (US-alone trials) during which 100 trials were delivered, each consisting of a 10 ms US stimulation in the presence of the 19.5 sec houselight at the same time point it occurred during paired trials. The other half of the animals stayed in the conditioning chamber without receiving any US presentations but only the 19.5 sec houselight. After 5-min, all animals received 100 trials in the presence of the houselight, each consisting of a 290 ms tone CS at an ITI of 20 sec between houselight stimuli.

Long Light Conditioning Data Analysis—Responding in the tone CS was analyzed as in the preceding experiments. The first 500-ms of the long light “context” was subdivided into three time periods: (1) a “baseline” period, 280 ms prior to light onset; (2) a “startle” period, 0–80 ms after light onset; and (3) a “CR” period, 81–500 ms after CS onset. Eye blinks that exceeded mean baseline activity by 0.5 arbitrary units (range = 0.0 to 5.0) during the CR period were scored as CRs. Eye blinks that met this threshold during the startle period were scored as SRs.

Results

Of the 25 rats who began Experiment 3, 16 ($n=9$ in Group Eyeshock Reinstatement; $n=7$ in Group Control) were included in analyses. Of the 9 rats that were not included, 1 rat (from Group Control) had a poor eyelid EMG, 1 rat (from Group Eyeshock Reinstatement) lost its headcap prior to the test session, 2 rats’ data (from Group Eyeshock Reinstatement) were discarded because of equipment failure, and 7 rats (2 from Group Reinstatement and 5 from Group Control) were classified as “poor learners” (fewer than 30% CRs in Session 5 or 6 of conditioning).

Conditioning and Extinction—At the session level, groups demonstrated equivalent conditioning and extinction (Figure 3A). This was confirmed by a 2 (Group) X 6 (Acquisition Session) repeated-measures ANOVA that revealed a significant acquisition session effect, $F(5,70) = 38.04$, $p < 0.05$ and a 2 (Group) X 3 (Extinction Session) repeated-measures ANOVA that revealed a significant extinction session effect, $F(2,28) = 20.45$, $p < 0.05$. There were no significant group or interaction effects.

Spontaneous Recovery and Reinstatement—Spontaneous recovery of extinguished eyeblink CRs was evident in Extinction Sessions 2 and 3 (Figure 3B). As in Experiments 1 and 2, reinstatement was not produced (Figure 3B). A series of three 2 (Group) X 2 (Last Block of 10 Trials of Previous Session vs. First Block of 10 Trials of Next Session) repeated-measures ANOVAs was conducted comparing percentage of CRs. These analyses revealed a significantly greater percentage of CRs at the beginning of Extinction Session 2 compared to Extinction Session 1 (i.e., spontaneous recovery), $F(1,14) = 13.37$, $p < 0.01$ and at the beginning of Extinction Session 3 compared to Extinction Session 2, $F(1,14) = 8.46$, $p < 0.02$. There was no spontaneous recovery in the test session.

For analysis of reinstatement, Group Eyeshock Reinstatement was compared to Group Control in the first block of 10 trials of the test session. A one-way ANOVA failed to reveal group differences in percentage of CRs, $F < 1$.

Long Light Conditioning—Eye blinks during the first 500-ms of the long light were low in the first session of acquisition ($M=9.02\% \pm 2.43\%$ SEM for Group Eyeshock Reinstatement; $M=11.21\% \pm 4.43\%$ for Group Control) and declined significantly across

acquisition sessions in both groups. This was confirmed with a 2 (Group) X 6 (Acquisition Session) repeated-measures ANOVA that revealed a significant session effect, $F(5,70) = 3.02$, $p < 0.02$ but no group or interaction effects. In extinction sessions, eyeblinks during the first 500-ms of the long light were still low and did not change across extinction sessions but were significantly higher overall in Group Control compared to Group Eyeshock Reinstatement ($M=7.24\% \pm 0.86\%$ SEM for Group Eyeshock Reinstatement; $M=12.41\% \pm 1.76\%$ SEM for Group Control). This was confirmed with a 2 (Group) X 3 (Extinction Session) repeated-measures ANOVA that revealed a significant group effect, $F(1,14) = 5.44$, $p < 0.04$. Finally, in the test session, eyeblinks during the first 500-ms of the long light were still low ($M=4.77\% \pm 1.44\%$ SEM for Group Eyeshock Reinstatement; $M=3.26\% \pm 0.69\%$ SEM for Group Control) and did not differ between groups.

Discussion

As in Experiments 1 and 2, we failed to observe reinstatement in Experiment 3. Specifically, there were no group differences in percentage of CRs in the first block of trials of the test session. We did, however, observe robust spontaneous recovery in Experiment 3. Specifically, there was a significantly greater percentage of CRs at the beginning of Extinction Sessions 2 and 3 compared to the end of Extinction Sessions 1 and 2, respectively. It is possible that the inclusion of CS-alone test trials in conditioning in Experiments 1 and 2 contributed to the lack of spontaneous recovery in those experiments. For example, it is possible that occasional CS-alone trials during acquisition (every 10th trial, beginning with trial 1) came to serve as a signal or a context for the presence of CS-US trials and this carried over into extinction (cf., Bouton, Woods, & Pineno, 2004). This would tend to prevent the within-session extinction necessary to detect spontaneous recovery at the beginning of the subsequent session, which is usually done by showing that responding at the beginning of an extinction session is significantly greater than responding at the end of the previous extinction session. We eliminated these CS-alone test trials in the acquisition phase in Experiment 3 and were able to observe robust spontaneous recovery, suggesting that this account might have some validity (but see Gormezano & Coleman, 1975). The results of Experiment 4 (see below) also support this interpretation.

Surprisingly, the reexposure phase between extinction and testing, where the long light was presented either by itself or with a US intended to produce reinstatement, appeared to abolish spontaneous recovery during the test. Since we didn't include a group that underwent the reexposure phase without the long light, we can't be certain that exposure to the long light in the absence of the CS abolished spontaneous recovery. Nevertheless, the change from robust spontaneous recovery in both groups at the beginning of Extinction Session 3 to the complete absence of spontaneous recovery in either group in the test session was striking. According to the model of Larrauri and Schmajuk (2008), the context develops inhibition during extinction that protects the CS from losing all of its excitatory strength. When excitation to the CS and inhibition to the context balance, no more responding is observed. The more salient the context, the stronger the inhibition that develops during extinction. Spontaneous recovery is observed because attention is drawn to the excitatory CS first, which promotes responding. After a few trials, attention is drawn to the inhibitory context, which suppresses responding. If attention was drawn to the inhibitory context quickly, there would be an absence of spontaneous recovery. It may be the case that, during the reexposure phase, the presentation of the salient long light "context" for the first time without the CS drew attention to the inhibitory "context". When CS presentations within the long light "context" resumed, attention was still high to the inhibitory "context". Although a caveat to this account is there have been several notable failures to demonstrate context inhibition after extinction (e.g., Bouton, 1984; Bouton & Swartzentruber, 1986), more recent, unpublished data (Laborda, Polack, Miguez, & Miller, 2010) suggest that contexts

can indeed acquire inhibitory properties during extinction. Furthermore, Larrauri and Schmajuk (2008) have explained the failures to demonstrate context inhibition in terms of a decreased attention to the context, which makes the context appear neutral in both summation and retardation tests for inhibition.

An alternative explanation is that we failed to observe spontaneous recovery in the test session because rats had already been in the context for some time (recall that the groups underwent either US reexposure or context exposure for approximately one hour and then were given CS test trials) and early session cues, such as handling, had extinguished (cf. Skinner, 1950). While spontaneous recovery can be observed in the middle of a test session (e.g., Robbins, 1990), suggesting that this explanation of spontaneous recovery is not complete, we cannot rule it out as an explanation of the lack of spontaneous recovery in Experiment 3.

Experiment 4

Experiment 4 had three purposes. First, we hypothesized that the failure to observe reinstatement in Experiments 1–3 might be due to a failure of context conditioning during US reexposure, which left the context neutral during the test session. As a direct measure of the presence or absence of context excitation, in Experiment 4 we measured freezing immediately prior to test presentations of the CS. If the eye shock US conditioned the context, we should observe freezing during this period. Second, we included a group in Experiment 4 that received footshocks, rather than eyeshocks, in between extinction and testing. We hypothesized that this group would show significant context excitation (as assessed by freezing) during the test session and reinstatement of the extinguished eyeblink CR, and they provided a look at whether fear conditioned to a contextual cue was sufficient to enhance responding to an extinguished eyeblink CS (cf., Bombace et al., 1991; Brandon et al., 1991, 1994; Brandon & Wagner, 1991; Gerwitz et al., 1998). Third, we wanted to determine whether the absence of partial reinforcement during conditioning or the presence of the extra-salient context was behind the observation of robust spontaneous recovery in Experiment 3. Therefore, in Experiment 4, all phases took place under standard conditions (i.e., without the long light context or odor cues).

Method

Subjects—Twenty-four male Long-Evans (80–90 days old on arrival) were obtained from a commercial supplier (Harlan, Indianapolis, IN). All housing and care was identical to Experiments 1–3. The surgical procedures, apparatus and data analyses for Experiment 4 were also identical to those described for Experiments 1–3, except that stimulus delivery, in addition to data recording, was controlled by a computer interfaced with a Power 1401 high-speed data acquisition unit and running Spike2 software (Version 5).

Procedure—Adaptation (1 session), conditioning (6 sessions), and extinction (3 sessions) were identical to Experiment 3 with the omission of the long light stimulus and the coconut scent. On the day after the final extinction session, Group Eyeshock Reinstatement received 100 eyeshocks and Group Control received no stimuli, comparable to Experiments 1 and 2. Group Footshock Reinstatement 10 footshocks. The first 3 footshocks were delivered at an intertrial interval of 3 minutes. The subsequent 7 footshocks were delivered at an intertrial interval of 7 minutes. This trial spacing ensured that Group Footshock Reinstatement received the same time in the context as the other two groups and ensured that freezing levels would be high at the beginning of the test session the next day. On Day 12 (testing), all rats were videotaped for 6-min in the context alone and then received 100 CS-alone trials to test for reinstatement.

Context Conditioning Data Analysis—Freezing was scored by an observer blind to group identity in 6-sec samples of time across the first 6-min of the test session. Freezing was defined as an absence of body movement, except that needed for respiration. Percentage of 6-sec samples in which a rat was freezing was calculated for three 2-min blocks.

Results

Of the 24 rats who began Experiment 4, 20 ($n=6$ in Group Eyeshock Reinstatement; $n=6$ in Group Control; $n=8$ in Group Footshock Reinstatement) were included in analyses. Of the 4 rats that were not included, 3 rats (1 from Group Eyeshock Reinstatement; 2 from Group Control) had poor eyelid EMGs and 1 rat (from Group Reinstatement) was classified as a “poor learner” (fewer than 30% CRs in Session 5 or 6 of conditioning).

Conditioning and Extinction—At the session level, groups demonstrated equivalent conditioning and extinction (Figure 4A). This was confirmed by a 2 (Group) X 6 (Acquisition Session) repeated-measures ANOVA that revealed a significant acquisition session effect, $F(5,85) = 82.57$, $p < 0.05$ and a 2 (Group) X 3 (Extinction Session) repeated-measures ANOVA that revealed a significant extinction session effect, $F(2,34) = 41.83$, $p < 0.05$. There were no significant group or interaction effects.

Spontaneous Recovery and Reinstatement—Spontaneous recovery of extinguished eyeblink CRs was evident in Extinction Session 3 and the Test Session (Figure 4B). As in Experiments 1–3, reinstatement was not produced (Figure 4B). A series of three 2 (Group) X 2 (Last Block of 10 Trials of Previous Session vs. First Block of 10 Trials of Next Session) repeated-measures ANOVAs was conducted comparing percentage of CRs. These analyses revealed a significantly greater percentage of CRs at the beginning of Extinction Session 3 compared to Extinction Session 2 (i.e., spontaneous recovery), $F(1,17) = 5.03$, $p < 0.04$. Furthermore, there was a significantly greater percentage of CRs at the beginning of the Test Session compared to Extinction Session 3 (i.e., spontaneous recovery), $F(1,17) = 15.44$, $p < 0.01$. The Group effect in this final ANOVA was also significant, $F(2,17) = 4.74$, $p < 0.03$ and appeared to be driven primarily by a lower percentage of CRs in the first block of the test session in Group Footshock Reinstatement. However, the interaction effect between group and block (last block of Extinction Session 3 vs. first block of Test Session) approached, but did not attain statistical significance, $F(2,17) = 2.66$, $p = 0.099$.

For analysis of reinstatement, groups were compared in the first block of 10 trials of the test session. A one-way ANOVA revealed group differences in percentage of CRs, $F(2,20) = 5.11$, $p < 0.02$. Post-hoc test between groups using the Bonferroni method revealed significantly lower responding in Group Footshock Reinstatement than Group Eyeshock Reinstatement, $p < 0.02$. Although Group Footshock Reinstatement also showed fewer CRs than Group Control, this difference failed to achieve statistical significance, $p > 0.18$. There was no reinstatement in Group Eyeshock Reinstatement or Group Footshock Reinstatement. Instead, in Group Footshock Reinstatement, there was a reduction in percentage of CRs.

Context Conditioning—Freezing levels were high during the initial 6-min of context exposure prior to tone CS presentations in Group Footshock Reinstatement and negligible in Groups Eyeshock Reinstatement and Control (Figure 5). This suggests the presence of significant context excitation at test only in Group Footshock Reinstatement. A 3 (Group) X 3 (2-min Block) repeated-measures ANOVA confirmed this, showing a significant Group effect, $F(2,17) = 13.16$, $p < 0.01$. Simple contrasts between groups revealed a significant difference between Group Footshock Reinstatement and Group Control ($p < 0.01$) but not between Group Eyeshock Reinstatement and Group Control. The percentage of eyeblink CRs in the first 10-trial block of the test session was negatively correlated with the

percentage of freezing in the last 2-min prior to the first CS presentation, $r = -.59$, $p < 0.01$. Thus, less freezing to the context, rather than more freezing, was associated with more conditioned eyeblinks to the tone.

Discussion

As in Experiments 1–3, we failed to observe reinstatement of extinguished eyeblink CRs in Experiment 4 in rats given eyeshock US reexposure between extinction and test. Furthermore, we showed that these rats exhibited very little freezing to the context at the beginning of the test session, suggesting that a lack of context conditioning by the eyeshock might explain the lack of reinstatement. However, the high level of freezing in Group Footshock coupled with its low level of eyeblink CRs suggests that context conditioning (and freezing) are not sufficient to cause reinstatement in this preparation. Furthermore, as in Experiment 3, we observed robust spontaneous recovery in all three groups at the beginning of Extinction Session 3 (before they had received separate treatment). This suggests that demonstrable residual excitation to the CS is also not sufficient to enable reinstatement.

The fact that a group given footshock between extinction and test (Group Footshock Reinstatement) did not show reinstatement, despite high levels of freezing to the context at the beginning of the test session, was especially surprising. It is notable that this group also showed a complete lack of spontaneous recovery in the test session. The most obvious explanation for these observations is that the footshock-promoted freezing to the context interfered with expression of the conditioned eyeblink. There is some evidence that freezing to the context occurs early in rat eyeblink conditioning and, as freezing declines across sessions, eyeblink CRs begin to emerge (Britton & Astheimer, 2004). This inverse relation suggests that a response competition explanation of the test session results in Group Footshock Reinstatement warrants experimental testing. However, experiments by Wagner and colleagues suggest that conditioned fear facilitates the amplitude of the eyeblink CR (Bombace et al., 1991; Brandon et al., 1991, 1994; Brandon & Wagner, 1991; Gerwitz et al., 1998). In these experiments, rabbits were pretrained with 30-sec tone stimuli that terminated in either an eyeshock (Bombace et al., 1991; Brandon et al., 1991, 1994; Brandon & Wagner, 1991; Gerwitz et al., 1998) or in a hindlimb shock (Bombace et al., 1991; Brandon et al., 1991). Both eyeshock and hindlimb shock conditioned fear to the long tone “context” in rabbits as measured by potentiated startle to an airpuff to the ear in the presence of this “context” (Brandon et al., 1991). Rabbits showed facilitated performance of the eyeblink CR to a standard, 1-sec CS in these aversive “contexts” (defined by the tone stimulus) compared to a non-aversive context (no tone stimulus). Thus, in Group Footshock Reinstatement in Experiment 4, conditioned fear suppressed eyeblink CRs while in the experiments of Wagner and colleagues, conditioned fear augmented eyeblink CRs.

One possible reconciliation of these seemingly discrepant findings is that the eyeshock and the hindlimb shock used in the experiments of Wagner and colleagues may have produced a lower level of context fear than the footshock used in our studies, so there would be no response competition between the eyeblink CR and freezing in their experiments. While Wagner and colleagues have reported that the eyeshock and hindlimb shock produced equivalent fear of a context, as measured by potentiated startle to an airpuff to the ear (Brandon et al., 1991), the footshock that we used produced a significantly greater amount of context freezing than the eyeshock we used. If we assume that the eyeshock used by Wagner and colleagues and our eyeshock are roughly equivalent in the amount of contextual fear they can produce, this would suggest that the hindlimb shock that Wagner and colleagues used produced less contextual fear than the footshock we used. Besides that assumption, this explanation relies on the second assumption that the potentiated startle test used by Wagner and colleagues is more sensitive to low levels of fear than is freezing. If this account is correct, there may have been low, undetected levels of fear to the eyeshock during

the test in Group Eyeshock Reinstatement. Importantly, however, this group did not show reinstatement. In contrast, even low levels of fear in a test session (low enough to require sensitive context preference tests to detect), are sufficient for reinstatement of extinguished fear CRs (Bouton & King, 1983, 1986; Bouton, 1984).

General Discussion

In four experiments using rat subjects, we failed to find evidence for reinstatement of extinguished eyeblink CRs, suggesting that reinstatement is not a universal feature of extinction. We demonstrated that a highly salient context, demonstrable contextual conditioning, and the presence of residual CS excitation (indexed by spontaneous recovery) are not sufficient to produce reinstatement.

In Experiments 3 and 4, we proposed that the failure to observe reinstatement in Experiments 1 and 2 might be due to a failure of context conditioning during US reexposure, which has been shown to be necessary for reinstatement of extinguished fear CRs (e.g., Bouton & Bolles, 1979; Bouton & King, 1983, Bouton, 1984; Bouton & King, 1986). However, neither an increased salience of the context (Experiment 3) nor the production of context conditioning by use of a footshock (Experiment 4) produced reinstatement of the extinguished eyeblink CR. This latter result suggests that while context conditioning may be *necessary* for reinstatement of extinguished CRs, it is not *sufficient*. In fear conditioning, the footshock US may be associated more easily with the context during acquisition since it “comes from” the context (floor of the apparatus) and is thus spatially contiguous with it. In contrast, in eyeblink conditioning, the US is delivered from a local source to a discrete body location (the eye). It may be that it is nearly impossible to associate this local US with a diffuse, temporally extended context. Consistent with this idea, Kehoe and colleagues have proposed that the eyeblink CR, unlike other types of CRs, may be sensitive only to very temporally local contextual cues that overlap with the CS and US during conditioning (Kehoe, Weidemann, & Dartnall, 2004; Weidemann & Kehoe, 1997). They arrived at this proposal after showing that, despite the fact that most of an eyeblink conditioning session consists of context exposure (i.e., intertrial intervals), rather than CS or US exposure, simply placing rabbits in the conditioning context after conditioning will reduce subsequent responding to the CS (Kehoe et al., 2004). Kehoe and colleagues hypothesized that, during post-conditioning context exposure, temporally local features of the context that were associated with the CS during conditioning activate a representation of the CS and this representation then undergoes extinction. The fact that only some, local, features of the context can activate the CS representation would explain why the post-conditioning context exposure decrements in the CS representation don’t occur during acquisition, since those local features would be in temporal proximity to the US.

It may be instructive to compare our results with Kehoe and colleagues’ demonstrations of concurrent recovery in rabbit eyeblink conditioning (Macrae & Kehoe, 1999, Weidemann & Kehoe, 2003, 2004, 2005). As described previously, concurrent recovery refers to the recovery of responding to an extinguished CS after pairings of a new CS with the US. Concurrent recovery is not due to cross-modal generalization (Kehoe & Weidemann, 2004; Macrae & Kehoe, 1999), spontaneous recovery (Weidemann & Kehoe, 2003), or reinstatement by the US (Weidemann & Kehoe, 2004). Furthermore, concurrent recovery occurs only to an extinguished CS (Weidemann & Kehoe, 2004) and appears to be due to shared associative linkages of the extinguished CS and the new CS with the CR and/or the US (Weidemann & Kehoe, 2005).

One could think of the production of concurrent recovery as a reinstatement treatment, but with a new CS paired with the US rather than the context paired with the US (recognizing

that the test phase for reinstatement involves a compound of a conditioned context and the extinguished CS, rather than just the extinguished CS as in concurrent recovery). It is conceivable that recovery of extinguished eyeblink CRs requires a discrete CS (cf., Weidemann & Kehoe, 2004), either old or new, paired with the US, as in rapid reacquisition and concurrent recovery, respectively. Mauk and colleagues have suggested that rapid reacquisition occurs at the level of the brainstem-cerebellar circuit that mediates eyeblink conditioning (Mauk & Donegan, 1997; Medina, Garcia, & Mauk, 2001). In this circuit, the cerebellar cortex tonically inhibits the interpositus nucleus, which is responsible for initiating generation of the eyeblink CR. During conditioning, plasticity is established first in the cerebellar cortex through conjoint activation of parallel fibers (by the CS) and climbing fibers (by the US), which produces long-term depression (LTD) at parallel fiber-to-Purkinje cell synapses and releases the interpositus from cortical inhibition. Once released, long-term potentiation (LTP) is established at mossy fiber-to-interpositus synapses via conjoint activation of mossy fibers (by the CS) and climbing fibers (by the US), and an eyeblink CR is generated when the CS comes on. Extinction reverses LTD at the parallel fiber-to-Purkinje cell synapses first, which returns inhibition of the interpositus nucleus and prevents mossy fiber-to-interpositus synapses from undergoing complete reversal of LTP. A very similar idea is proposed by Kehoe in the conceptual terms of a connectionist mode of classical conditioning, with his “external connections” equivalent to parallel fiber-to-Purkinje cell synapses and his “internal connections” equivalent to mossy fiber-to-interpositus synapses (Kehoe, 1988; Weidemann & Kehoe, 2004, 2005; see also Mauk & Donegan, 1997). When CS-US pairings are resumed, LTD is again restored to parallel fiber-to-Purkinje cell synapses and the interpositus is again released from cortical inhibition. The residual LTP at mossy fiber-to-interpositus synapses leads to a rapid return of the eyeblink CR. It may be the case that reinstatement doesn’t occur in eyeblink conditioning because this mechanism is not engaged when there is no CS present when US reexposure occurs. For example, there is evidence that LTD at parallel fiber-to-Purkinje cell synapses requires a large intracellular Ca^{2+} signal in Purkinje cells, which is provided via both Ca^{2+} entry through voltage-gated Ca^{2+} channels in the Purkinje cell dendrite (from depolarization induced by climbing fiber US input) and metabotropic glutamate receptor 1 (mGluR1)-mediated release of Ca^{2+} from intracellular stores (from mGluR1 binding of glutamate released from parallel fiber terminals that carry the CS) (Wang, Denk, & Hausser, 2000). Whether this mechanism explains the presence of spontaneous recovery is not completely clear.

Our observation of a greater level of eyeblink CRs at the beginning of an extinction session compared to the end of the previous extinction session fulfills the standard criteria for spontaneous recovery. We are not aware of any previous demonstrations of spontaneous recovery of the extinguished rat eyeblink CR. As Rescorla and colleagues have pointed out, an especially strong demonstration of spontaneous recovery could involve comparison of responding to the extinguished CS with responding to a novel stimulus, a comparison which is rarely made (Rescorla, 1997, 2004, 2007; Robbins, 1990). Without a novel comparison stimulus, what looks like spontaneous recovery could be due simply to a general increase in responding at the beginning of an extinction or test session (see Rescorla, 2004 for a discussion). Furthermore, to infer that spontaneous recovery specifically represents the temporary loss of extinction learning, comparison should be made with a non-extinguished CS that has been trained and elicits a level of responding comparable to the extinguished CS (e.g., Rosas & Bouton, 1996; see Rescorla, 2004 for a discussion). Without this comparison stimulus, it may be that what looks like spontaneous recovery has nothing to do with extinction learning. Thus, our observation of what looks like spontaneous recovery is novel for rat eyeblink conditioning but requires further work to ensure that it really is spontaneous recovery and that it is due to a temporary loss of extinction learning.

In conclusion, the present experiments represent the first documented demonstration of spontaneous recovery in rodent eyeblink conditioning and the only systematic attempt to produce reinstatement in eyeblink conditioning. Our inability to see reinstatement of the extinguished eyeblink CR is an intriguing result, especially in light of how we were able to demonstrate spontaneous recovery. The presence of this classic “relapse” effect suggests that extinction of the eyeblink CR did not erase the CS-US association. However, it appears to be the case that reinstatement, another classic “relapse” effect that occurs in many conditioning preparations, may be a phenomenon that occurs only with specific procedures that engage particular brain and/or response systems.

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Figure 1.
 (A) Percentage of eyeblink conditioned responses as a function of conditioning and extinction session in Experiment 1. In this and all subsequent figures the error bars indicate SEM. Groups underwent 100 trials per session (90 CS-US; 10 CS-alone) of 280 ms delay eyeblink conditioning in acquisition and 100 CS-alone trials per session in extinction. (B) Percentage of eyeblink conditioned responses as a function of 10-trial extinction and test block in Experiment 1. The day after the final extinction session, the Reinstatement Eyeshock Group received 100 presentations of the US alone and the Control Group received equivalent exposure to the conditioning context. The next day, both groups received a test session consisting of 100 presentations of the CS alone. No reinstatement was observed in

the Reinstatement Eyeshock Group in the test session. No spontaneous recovery was observed in extinction sessions or in the test session in either group.

Figure 2.

(A) Percentage of eyeblink conditioned responses as a function of conditioning and extinction session in Experiment 2. Groups underwent 100 trials per session (90 CS-US; 10 CS-alone) of 280 ms delay eyeblink conditioning in acquisition and 100 CS-alone trials per session in extinction. (B) Percentage of eyeblink conditioned responses as a function of 10-trial extinction and test block in Experiment 2. The day after the final extinction session, the Reinstatement Eyeshock Group received 100 presentations of the US alone and the Control Group received equivalent exposure to the conditioning context. After a 5 min delay, both groups received a test session consisting of 100 presentations of the CS alone. No

reinstatement was observed in the Reinstatement Eyeshock Group in the test session. Spontaneous recovery was present in both groups in extinction session 6 only.

Figure 3.

(A) Percentage of eyeblink conditioned responses as a function of conditioning and extinction session in Experiment 3. Groups underwent 100 CS-US trials per session of 280 ms delay eyeblink conditioning in acquisition and 100 CS-alone trials per session in extinction. In acquisition and in all subsequent sessions, a houselight came on 18.5 sec prior to the start of each trial and went off 1 sec after the trial was over. (B) Percentage of eyeblink conditioned responses as a function of 10-trial extinction and test block in Experiment 3. The day after the final extinction session, the Reinstatement Eyeshock Group received 100 presentations of the US alone and the Control Group received equivalent exposure to the conditioning context. After a 5 min delay, both groups received a test

session consisting of 100 presentations of the CS alone. No reinstatement was observed in the Reinstatement Eyeshock Group in the test session. Spontaneous recovery was present in extinction sessions 2 and 3 in both groups but not in the test session.

Figure 4. (A) Percentage of eyeblink conditioned responses as a function of conditioning and extinction session in Experiment 4. Groups underwent 100 CS-US trials per session of 280 ms delay eyeblink conditioning in acquisition and 100 CS-alone trials per session in extinction. (B) Percentage of eyeblink conditioned responses as a function of 10-trial extinction and test block in Experiment 4. The day after the final extinction session, the Reinstatement Eyeshock Group received 100 presentations of the US alone, the Reinstatement Footshock Group received 10 presentations of a footshock, and the Control Group received equivalent exposure to the conditioning context. The next day, all groups received a test session consisting of 100 presentations of the CS alone. No reinstatement was

observed in the Reinstatement Eyeshock Group or in the Reinstatement Footshock Group. Spontaneous recovery was present in extinction session 3 and in the test session in all groups, although it was attenuated in the Reinstatement Footshock Group in the test session.

Figure 5. Percentage of time spent freezing as a function of 2-minute block prior to the beginning of the test session in Experiment 4. Context freezing was evident only in the Reinstatement Footshock Group.