

Conditioning-specific reflex modification of the rabbit (*Oryctolagus cuniculus*) nictitating membrane response is sensitive to context

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Conditioning-specific reflex modification occurs when an unconditioned response is modified in the *absence* of the conditioned stimulus as a result of pairings of the conditioned stimulus and an unconditioned stimulus. In two experiments, we assessed conditioning-specific reflex modification in either a novel context (Experiment 1) or a context different from, but equally familiar in relation to, the training context (Experiment 2). Conditioning-specific reflex modification did not demonstrate sensitivity to a novel context but did demonstrate sensitivity to a change in familiar context. The data cannot be explained by unconditioned stimulus preexposure, overtraining, or context insensitivity. The results suggest that conditioning-specific reflex modification models normal stress and may be used to evaluate theories of and treatments for posttraumatic stress disorder.

Conditioning-specific reflex modification occurs when an unconditioned response (UR) is modified in the *absence* of the conditioned stimulus (CS) as a result of pairings of the CS and an unconditioned stimulus (US). After at least 3 days of tone–periorbital stimulation pairings, the rabbit nictitating membrane response (NMR; Gormezano, Schneiderman, Deaux, & Fuentes, 1962) to the US increases in amplitude, peak latency, and area, especially at US intensities weaker than the training intensity (Gruart & Yeo, 1995; Schreurs, Oh, Hirashima, & Alkon, 1995; Wikgren, Ruusuvirta, & Korhonen, 2002). We know that conditioning-specific reflex modification (CRM) is a function of both the level of conditioning (Schreurs et al., 1995) and the intensity of the US (Seager, Smith-Bell, & Schreurs, 2003). In addition, CRM can survive extinction of the conditioned response (CR), can be extinguished with US-alone presentations (Schreurs, Shi, Pineda, & Buck, 2000), and can be generalized from electrodermal stimulation to air puff (Buck, Seager, & Schreurs, 2001). More recent evidence from heart rate measurements suggests that classical conditioning of the NMR increases the aversiveness of the US (Schreurs & Smith-Bell, 2005) and that CRM of heart rate can occur as a function of heart rate conditioning (Schreurs, Crum, Wang, & Smith-Bell, 2005). Our original observation of NMR CRM (Schreurs et al., 1995) was confirmed by Gruart and Yeo (1995)

using electrodermal stimulation and has since been replicated by Wikgren and colleagues using air puff (Wikgren & Korhonen, 2001; Wikgren et al., 2002). Clark and colleagues also reported an increase in UR amplitude in a rabbit air puff study (Clark, Zhang, & Lavond, 1992) but did not attribute the increase to CS–US pairings because Steinmetz and colleagues had observed that UR amplitude increased equally following both paired and unpaired stimulus presentations (Steinmetz, Lavond, Ivkovich, Logan, & Thompson, 1992).

CRM may follow behavioral laws similar to, but not necessarily the same as, those of classical conditioning (Buck et al., 2001; Schreurs, 2003; Schreurs et al., 2000). For example, like classical conditioning, CRM is a function of the number of CS–US pairings (Schreurs et al., 1995) and the intensity of the US (Seager et al., 2003). However, unlike classical conditioning, CRM is not extinguished by CS-alone presentations (Schreurs et al., 2000). CRM does generalize from electrodermal stimulation to air puff but does not generalize from air puff to electrodermal stimulation even though conditioning occurs to comparable levels with both electrodermal stimulation and air puff (Buck et al., 2001). Finally, significant levels of CRM can be obtained only at high air puff intensities even though conditioning is supported by lower air puff intensities (Buck et al., 2001). The same appears to be true for electrodermal stimulation, although CRM can be obtained at lower electrodermal stimulation intensities (Seager et al., 2003).

There is considerable evidence that context is an important factor in learning and memory (Bouton, 1993; Spear, 1978) and that changes in context can significantly disrupt performance in a number of different learning tasks, including fear conditioning (Millin & Riccio, 2004; Zhou & Riccio, 1996), taste aversion learning (Boakes, Westbrook,

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Elliott, & Swinbourne, 1997), conditioned suppression (Gunther, Miller, & Matute, 1997), and eyelid conditioning (Penick & Solomon, 1991). Context is also an important factor in a number of learning phenomena, including blocking (Baker & Mackintosh, 1979; Miller, Jagielo, & Spear, 1993; Randich & Ross, 1984), extinction (Bouton, 2004; Gunther et al., 1997; Westbrook et al., 1995), latent inhibition (Baker & Mackintosh, 1979; Katz, Rogers, & Steinmetz, 2002; Lubow, Rifkin, & Alec, 1976), and CS and US preexposure effects (Hinson, 1982; Lubow et al., 1976; Saladin, ten Have, Saper, Labinsky, & Tait, 1989).

We have suggested previously that our CRM data may have some implications for theories of and treatments for posttraumatic stress disorder (PTSD), a psychological disorder resulting from exposure to a traumatic event (Schreurs, 2003; Seager et al., 2003). The symptoms associated with PTSD include persistent reexperiencing of the traumatic event, persistent avoidance of stimuli associated with the trauma, numbing of general responsiveness, and persistent symptoms of increased arousal (Pitman, 1988; see the *DSM-IV*). One of the hallmarks of PTSD is intense psychological distress and/or physiological reactivity to cues that symbolize or resemble an aspect of the traumatic event. This learning-related component of PTSD may have some bearing on our findings of CRM (Buck et al., 2001; Schreurs et al., 2000). Like a Persian Gulf veteran who may "hit the deck" when he hears a car backfire, rabbits, following CS-US pairings, blink to a mild electrical stimulus as if it were a stronger stimulus. There is evidence that context may play an important role in PTSD (Grillon, Morgan, Davis, & Southwick, 1998; Kaysen, Resick, & Wise, 2003; Pawlyk, Jha, Brennan, Morrison, & Ross, 2005).

The purpose of the present experiments was to determine whether the similarity between the behavioral laws of classical conditioning and those of CRM extends to the effects of context change. Another purpose of the experiments was to continue to evaluate the hypothesis that CRM may serve as an animal model that can help one understand PTSD (Schreurs, 2003). In two experiments, context was manipulated as rabbits were presented with a series of US intensities and durations on pretest and posttest and given CS-US pairings to determine the extent to which CRM was context specific. If CRM were not context specific, one might expect that once an association was formed, a weak US could elicit a large UR regardless of where the US was presented. If CRM were context specific, a weak US would only elicit a large UR in the training context. Either outcome might have implications for the role of context in PTSD (Kaysen et al., 2003). For example, if CRM were not context specific, removing a subject from the stress-inducing situation would have no effect on the symptoms of PTSD. If, on the other hand, CRM were context specific, removing a subject from a stress-inducing situation might alleviate the symptoms of PTSD. If CRM and classical conditioning were similar in their sensitivity to a shift in context, we would predict that testing the US in a different context would result in weaker CRM than the CRM that would occur as a result

of testing in the same context as the one in which CS-US pairings took place.

EXPERIMENT 1

The purpose of the present experiment was to determine whether CRM of the rabbit NMR would be altered as a result of testing in a context different from the training context and novel. Permutations of US intensity and duration were presented on pretest in Context A, and 6 days of CS-US pairings took place in Context B. Permutations of US intensity and duration were presented on posttest, which was conducted in either the training context (Context B) or a novel context (Context C). Contexts B and C consisted of altered lighting (dark, flashing light), distinctive smell (sandalwood oil, peppermint oil), and sound (white noise, low-frequency tone) in the training chamber (Penick & Solomon, 1991), as well as altered texture of the training restrainer (sandpaper, foam rubber floor).

In order to determine whether the contexts were discriminable, CS-alone extinction trials were conducted following posttest in either the training context (Context B) or a different context (Context C). There is considerable evidence for an effect of a shift in context between conditioning and extinction in a number of paradigms, including taste aversion learning (Westbrook et al., 1995), fear conditioning (Bouton, 2004), and classical conditioning of the rabbit NMR (Kehoe, Weidemann, & Dartnall, 2004). If the present contexts were discriminable, we might expect to see more CR extinction in rabbits that experienced the context shift between CS-US pairings and extinction than in rabbits that experienced the same context during both CS-US pairings and extinction.

Method

Subjects. Eighteen male New Zealand White rabbits (*Oryctolagus cuniculus*) supplied by Harlan (Indianapolis) weighed approximately 2.0–2.2 kg at the beginning of the experiment. The animals were housed in individual cages, given free access to food and water, and maintained on a 12:12-h light:dark cycle. The rabbits were maintained in accordance with guidelines issued by the National Institutes of Health, and the experimental protocols were approved by the West Virginia University Animal Care and Use Committee.

Apparatus. The apparatus and recording procedures for the NMR have been detailed by Schreurs and Alkon (1990), who modeled their apparatus after those designed by Gormezano (Coleman & Gormezano, 1971; Gormezano, 1966). During pretest, each rabbit was restrained in a Plexiglas box and tested in a sound-attenuating, ventilated chamber (Coulbourn Instruments, Allentown, PA). A stimulus panel containing a speaker and houselight (10-W, 120-V incandescent lamp) was mounted at a 45° angle, 15 cm anterior to and 15 cm above the subject's head. A noise level of 65 dB (SPL, scale C) in each chamber was provided by an exhaust fan. This was the standard context and was designated Context A. Contexts B and C involved the manipulation of sound, light, smell, and texture to make them distinct from each and from Context A (Penick & Solomon, 1991). Specifically, when rabbits subjected to Context B were brought into the training room, the houselights and ventilating fans had been switched off, and prerecorded white noise (PureWhiteNoise.com, Tallahassee, FL) was playing through portable speakers (RCA, Malaysia). The white noise, left on in the room throughout the training session, produced a sound level of 58 dB in each chamber. In Context B, the rabbits were restrained

in a modified rabbit restrainer in which the floor was covered with coarse aluminum oxide sandpaper (60 grade, 3M, St. Paul, MN). Each rabbit was then placed in a training chamber with an open glass vial positioned in front of the restrainer containing a 2×2 gauze pad impregnated with a drop of sandalwood essential oil (Wyndmere Naturals Inc., Minneapolis, MN). When the rabbits subjected to Context C were brought into the training room, the houselights and ventilating fan were off, and a 90-dB, 400-Hz pure tone was playing through portable speakers (Harman/Kardon, Northridge, CA). The low-frequency tone, left on in the room throughout the training session, produced a sound level of 58 dB in each chamber. Peppermint oil (Wyndmere Naturals Inc.) was placed in a petri dish in front of a bench-top fan (Pace, Annapolis Junction, MD) in order to scent the room before the rabbits were brought in. The rabbits were restrained in a modified rabbit restrainer, in which the floor was covered with rubberized foam padding. Each rabbit was placed in a training chamber from which the door had been removed. The training chamber contained an open glass vial positioned in front of the restrainer containing a 2×2 gauze pad impregnated with a drop of the same peppermint essential oil that was used to scent the room. After the rabbits in Context C were placed in the training chambers, the room lights were turned off and a strobe light (Federal Signal, Novi, MI; 175,000 peak candlepower) flashing at a frequency of 3.6 Hz was switched on in the center of the room. In sum, the rabbits in Context A were sitting in an illuminated, closed chamber on a smooth floor with ventilating fans running and no added odor. The rabbits in Context B were sitting in a darkened, closed chamber on a sandpaper floor with a background of white noise in the presence of sandalwood oil. The rabbits in Context C were sitting in an open chamber on a foam rubber floor with a background low-frequency tone in the presence of peppermint oil and a flashing light.

Periorbital electrical stimulation (ES) was delivered to all rabbits by a programmable two-pole shocker (Model E13-35, Coulbourn Instruments) via stainless steel Autoclip wound clips (Stoelting, Wood Dale, IL) positioned 10 mm below and 10 mm posterior to the dorsal canthus of the right eye.

Details of transducing nictitating membrane movements have been reported previously (Gormezano & Gibbs, 1988; Schreurs & Alkon, 1990). In short, a hook connected to an L-shaped lever containing a freely moving ball and socket joint was attached to a 6-0 nylon loop sutured into, but not through, the nictitating membrane. The other end of the lever was attached to a rotary encoder (Vernitron Corp., St. Petersburg, FL) that, in turn, was connected to a 12-bit analog-to-digital converter (5-msec sampling rate; 0.05-mm resolution). Individual analog-to-digital outputs were stored on a trial-by-trial basis for subsequent analysis. Data collection, analysis, and stimulus delivery were carried out using a LabVIEW system (Version 5.1, National Instruments, Austin, TX).

Procedure. Eighteen rabbits received 1 day of adaptation and one 80-trial pretest session in Context A followed by six daily 80-trial sessions of paired CS-US presentations in Context B. Half the rabbits then received an 80-trial posttest session in Context B (Group ABB, $n = 9$), and the other half received an 80-trial posttest session in Context C—the novel context (Group ABC, $n = 9$). Exposure to the contexts was not counterbalanced. Following the posttest session, rabbits in Group ABB were given four daily 80-trial sessions of CS-alone extinction trials in Context C—a context different from the one in which they were given CS-US pairings. The rabbits in Group ABC were given CS-alone extinction trials in Context B—the same context as the one in which they were given CS-US pairings.

On adaptation day, the rabbits were prepared for ES and recording of nictitating membrane movement and then adapted to the training chambers for the length of time of subsequent training sessions (80 min). On both pretest and posttest days, the subjects received a total of 80 trials of ES presented at an average intertrial interval (ITI) of 60 sec (range, 50–70 sec). Each trial involved the presentation of 1 of 20 possible combinations of stimulus intensity (0.1, 0.25,

0.5, 1.0, or 2.0 mA) and duration (10, 25, 50, or 100 msec). Four separately randomized sequences of the 20 stimulus combinations were presented on each testing day, with the restriction that the same intensity or duration could not occur on more than three consecutive trials. Each of the six training sessions consisted of 80 presentations of a 400-msec, 1-kHz, 82-dB tone CS that coterminated with a 100-msec, 60-Hz, 2.0-mA ES US (i.e., 300-msec interstimulus interval). Paired stimulus presentations were delivered, on average, every 60 sec (range, 50–70 sec).

A CR was defined as any extension of the nictitating membrane exceeding 0.5 mm that was initiated after CS onset but prior to US onset. A UR was defined as any extension of the nictitating membrane exceeding 0.5 mm that was initiated within 300 msec of US onset (i.e., the CS-US interstimulus interval used to score CRs during pairings). The UR criterion was based on the observation that, following CS-US pairings, posttest URs at lower US intensities had onset latencies that fell into the range of latencies for CRs (Schreurs et al., 2000). Amplitude of a response was scored in millimeters as the maximum extension of the nictitating membrane. Onset latency of a response was identified as the latency in milliseconds from stimulus onset at which a response rose 0.1 mm above the baseline. Peak latency of a response was determined as the latency in milliseconds from stimulus onset for maximum extension of the nictitating membrane. Area of a response was calculated as the total area under the response curve from US onset to the end of the trial.

Response topographies for each of the US intensities were averaged across subjects and examined for differences by comparing the shape of the averaged response (Seager et al., 2003). To provide a statistical measure of the shape of an averaged response, we analyzed for symmetry (skew) and tail size (kurtosis). A significant positive skew value indicated that the response had a peak toward the beginning of the trial, and a significant negative value indicated that the response had a peak more toward the end of the trial. A significant positive kurtosis value indicated that the response had a long tail, and a significant negative value indicated that the response had a short tail. A skew coefficient was considered significant if the absolute value of skew divided by the standard error of skew was greater than 2, and a kurtosis coefficient was considered significant if the absolute value of kurtosis divided by the standard error of kurtosis was greater than 2 (Systat 8.0, SSI, Point Richmond, CA). Our previous research indicated that URs on pretest reached their peak just after US onset with a long tail to the right. Such response topographies would yield high, significant, positive values for both skew and kurtosis. However, following CS-US pairings, URs tended to have peaks larger and shifted to the right, yielding lower and even significant negative values for both skew and kurtosis (Seager et al., 2003).

Results

Conditioning. Figure 1 shows the mean ($\pm SEM$) percent CRs for subjects in Group ABB and Group ABC across the 6 days of CS-US pairings. As can be seen from the figure, the acquisition functions are virtually identical and both groups acquired CRs rapidly, reaching an asymptotic level of over 90% CRs by Day 2 and maintaining that level across the remaining 4 days of pairings. An ANOVA revealed a significant main effect of days [$F(5,80) = 128.63, p < .001$], but there was no main effect of group [$F(1,16) < 1$] or interaction of group \times days [$F(5,80) < 1$]. Analysis of the four 20-trial blocks of responding on Day 1 revealed a significant main effect of blocks [$F(3,48) = 28.25, p < .001$] but no main effect of group [$F(1,16) < 1$] or interaction of group \times blocks [$F(3,48) < 1$].

US testing. Figure 2 shows average response topographies during the first 20 US posttest trials for Groups ABB

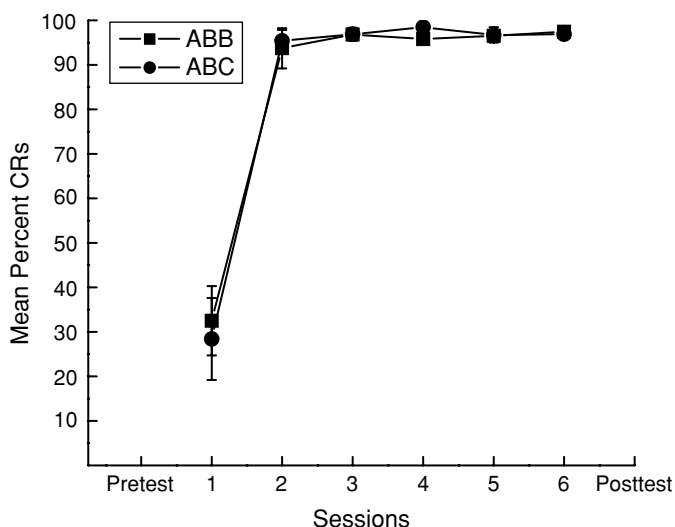


Figure 1. Mean (\pm SEM) percent CRs for rabbits in Groups ABB and ABC across the 6 days of CS-US pairings. Each of the six paired-conditioning sessions consisted of 80 presentations of a 400-msec, 1-kHz, 82-dB tone CS that coterminated with a 100-msec, 60-Hz, 2.0-mA US. Note that all SEM values are included but are often smaller than the data points.

(solid lines) and ABC (dotted lines). The plots are arranged in descending order of US intensities at which responses occurred (2.0–0.25 mA), collapsed across US duration. The inset shows average response topographies to a 0.5-mA, 25-msec US on pretest (dashed lines) and posttest (solid lines) for Groups ABB and ABC. Although

the difference in UR topography between pretest and posttest for both groups in the inset shows the increase in size and shift in peak latency characteristic of CRM, Figure 2 shows no evidence of a difference in the average posttest topographies between the two groups. Analysis of skew and kurtosis revealed no significant differences in aver-

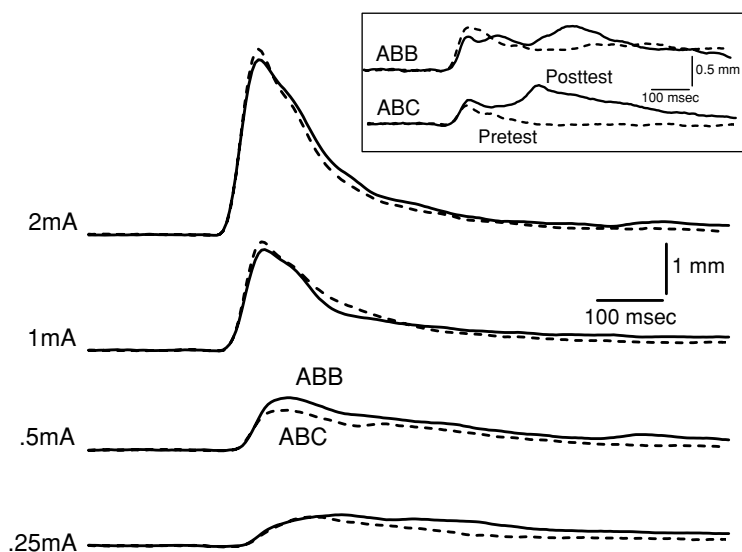


Figure 2. Averaged response topographies for Group ABB (solid lines) and Group ABC (dashed lines) during the first 20 posttest trials arranged in descending order of US intensity at which responses occurred (2.0 mA to 0.25 mA) and collapsed across US duration. The inset shows average response topographies to a 0.5-mA, 25-msec US on pretest (dashed lines) and posttest (solid lines) for Groups ABB and ABC illustrating the shift in peak latency and increase in size of the UR that is characteristic of CRM.

aged response topography between the groups except at 0.25 mA, where Group ABB had significantly less skew (-1.27 vs. 3.64) and significantly more negative kurtosis (-3.49 vs. -1.58) than did Group ABC. Analysis of the mean percent URs for all five US intensities collapsed across US duration yielded a significant main effect of intensity [$F(4,64) = 98.02, p < .001$], but no main effect or interactions of the group factor (largest $F = 1.85, p = .19$). Analysis of the mean UR peak latency and amplitude at US intensities where responses occurred (2.0 – 0.25 mA) revealed significant main effects of intensity [$F(4,64) = 263.75, p < .001$] but no main effect or interactions of group ($F_s < 1.15$). Analysis of the mean UR area yielded no significant main effects or interactions ($F_s < 1.0$).

CR Extinction. Figure 3 depicts the mean ($\pm SEM$) percent CRs across 20-trial blocks for subjects in Group ABB and Group ABC on each of the four daily sessions of CS-alone extinction trials. The inset shows percent CRs for the 4 days of CS-alone extinction, collapsed across the 20-trial blocks. Examination of Figure 3 shows that both groups exhibited a decrease in CRs within each session and across the 4 days of extinction. There is a suggestion of faster extinction in Group ABB, the group trained in Context B and extinguished in a different context (Context C), than in Group ABC, the group trained and extinguished in the same context (Context B). However, as noted in a number of other rabbit NMR extinction studies (Kehoe & White, 2002; Macrae & Kehoe, 1999; Schreurs, 1993), the variance is considerably larger than that shown during CR acquisition (Figure 1) and is often due to 1 or 2 rabbits that do not extinguish responding. This is certainly true in the present experiment, in which

1 rabbit in each group was consistently responding at a level of over 80% CRs up to and through the final 20-trial block of Day 4 of extinction. In contrast, 5 other rabbits in each group extinguished to a level of 6% CRs (approaching baseline) during the final 20-trial block of Day 4 of extinction.

Analysis of responding across the 20-trial blocks for the 4 days of CS-alone extinction revealed significant main effects of days [$F(3,48) = 10.71, p < .001$] and blocks [$F(3,48) = 28.19, p < .001$], as well as an interaction of days \times blocks [$F(8,144) = 4.13, p < .001$], but no main effect or interaction of group (largest $F = 1.0$). However, a Wilcoxon signed-rank test confirmed that there was a significant overall difference between Group ABB and Group ABC on Day 1 of extinction ($p < .008$) and that this difference was in fact significant for each of the 20-trial blocks (all $p_s < .039$) of Day 1. Thus, although the variance was large, if ranked, rabbits in Group ABB had significantly lower CR levels than did the correspondingly ranked rabbits in Group ABC. A similar overall difference between Group ABB and Group ABC was found on Day 3 ($p < .008$) but not on Day 2 ($p < .08$) or Day 4 ($p < .11$).

Discussion

The principal finding of the present experiment was that CRM of the rabbit NMR appeared to be at least as strong after testing in a novel context as it was after testing in the training context. Thus, there does not appear to be an effect of context shift on CRM of the rabbit NMR in the same way that such effects have been observed in classical conditioning of the rabbit NMR (Penick & Solomon, 1991)

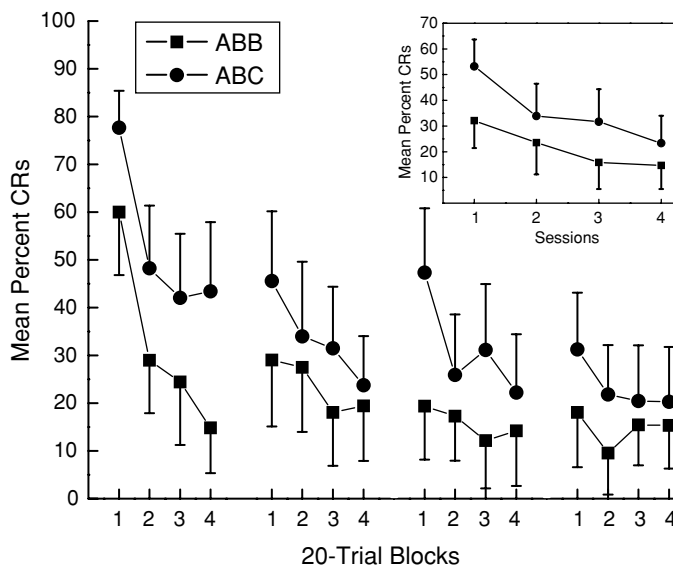


Figure 3. Mean ($\pm SEM$) percent CRs for rabbits in Groups ABB and ABC across 20-trial blocks for each of the 4 days of CS-alone extinction sessions. The inset shows responding collapsed across the 20-trial blocks for each of the four sessions of extinction. Each of the four CS-alone extinction sessions consisted of 80 presentations of a 400-msec, 1-kHz, 82-dB tone CS.

and in other forms of learning, including fear conditioning (Millin & Riccio, 2004; Zhou & Riccio, 1996), taste aversion learning (Boakes et al., 1997), and conditioned suppression (Gunther et al., 1997). A second finding of the present experiment was that rabbits were sensitive to the manipulation of the context. This sensitivity to context was indexed by a more rapid rate and a stronger level of extinction in a context different from the training context—the context shift extinction effect (Bouton, 2004).

There are a number of possible reasons for the apparent lack of an effect of context on CRM. First, although Penick and Solomon (1991) were able to show that a change in context similar to the change employed in the present experiment was effective in showing a context effect during classical conditioning of the rabbit NMR, the context changes in the present experiment may not have been discriminable; the rabbits simply generalized between the contexts (Rau, DeCola, & Fanselow, 2005). However, as noted above, a more rapid rate and a stronger level of extinction in a context different from the training context indicates that the rabbits were sensitive to the manipulation of the context during extinction (Bouton, 2004). Second, CRM may not obey the same behavioral laws as those of classical conditioning. For example, we have shown that CRM, unlike classical conditioning, does not extinguish if the CR is extinguished with CS-alone presentations (Schreurs et al., 2000). Although Penick and Solomon (1991) were able to show that a change in context similar to the change employed in the present experiment was effective in producing a drop in the level of responding during conditioning, if CRM and classical conditioning do not follow the same behavioral laws, we might not see sensitivity of CRM to a shift in context. Third, there may well be a context shift effect to which CRM is sensitive, but the effect may have been masked by the significant amounts of overtraining required to induce CRM. Using avoidance conditioning in rats, Millin and Riccio (2004) have shown that the effects of a shift in context can be overcome by overtraining. In the present experiment, rabbits reached an asymptotic level of CR acquisition in excess of 90% by Day 2 (160 trials), and overtraining in the form of continued CS-US pairings occurred for 4 additional days (320 additional trials). Fourth, there could have been US-preexposure effects as a result of US presentations on pretest that affected CRM. There is considerable evidence that US preexposure can retard CR acquisition (Matzel, Brown, & Miller, 1987; Mis & Moore, 1973; Rau et al., 2005; Saladin & Tait, 1986) and that this effect can be overcome by a change in context (Cole, VanTilburg, Burch-Vernon, & Riccio, 1996; Matzel et al., 1987; Tomie, Murphy, Fath, & Jackson, 1980). However, in the present experiment, any amelioration of potential US preexposure effects by a change in context would have been the same for both groups, because all rabbits were shifted from pretest in Context A to CS-US pairings in Context B. Fifth, the present results might be explained by an unequal amount of exposure to the different contexts, because Group ABB received 6 more days of exposure to the context in which the posttest occurred than

did Group ABC, for which the posttest context was novel (Millin & Riccio, 2004). As a consequence, Group ABC may have experienced contextual fear to Context C during the unsignaled US presentations of posttest, and this contextual fear may have summated with the responses to the US (Rau et al., 2005). In contrast, Group ABB received signaled and unsignaled USs in the same context and so may not have had the same level of contextual fear summate with the responses to the US on posttest. Finally, the novelty of Context C may also have played a role, given the animal and human literature on the behavioral and neural effects of novelty (Davis, Jones, & Derrick, 2004; Macken, 2002; Nyberg, 2005; Stein, 1966). For example, hippocampal long-term potentiation is enhanced when induced in a novel environment (Davis et al., 2004), and human EEG and cerebral blood flow increase with novelty (Nyberg, 2005). Using identical experimental designs, Lubow et al. (1976) showed that children and rats had enhanced levels of learning when a new stimulus was presented in an old environment or an old stimulus was presented in a new environment (Lubow et al., 1976). Finally, Izquierdo and colleagues showed that exposure to a novel context before acquisition enhanced retrieval of an avoidance task in rats (Izquierdo, Barros, Medina, & Izquierdo, 2003). It is conceivable that the novel context in the present experiment may have increased responsiveness to the US and overcome any detrimental effects of a change in context on CRM.

EXPERIMENT 2

Although the previous experiment established that rabbits can discern manipulations of context, important issues that remain unresolved about the effects of context on CRM are the potential effects of different amounts of exposure to the various contexts (Millin & Riccio, 2004; Rau et al., 2005) and differences in the familiarity/novelty of those contexts (Honey, Pye, Lightbrown, Rey, & Hall, 1992; Izquierdo et al., 2003; Lubow et al., 1976). The purpose of the second experiment was to determine whether equating exposure to each of the contexts and eliminating the novelty of the posttest context would reveal an effect of context change on CRM. To control for differences in exposure to the training and testing contexts and eliminate the novelty of the testing context, the rabbits in the present experiment were exposed to Context B or Context C for 6 days before classical conditioning in Context C or Context B, respectively, and the posttest for all rabbits was conducted in Context C.

Method

Unless otherwise noted, the methods and procedures were identical to those in Experiment 2.

Subjects. Eighteen male New Zealand White rabbits (*Oryctolagus cuniculus*) supplied by Covance (Denver, PA) weighed approximately 2.0–2.2 kg at the beginning of the experiment. The rabbits were housed and treated identically to those in Experiment 1.

Apparatus. Contexts A, B, and C were the same as those used in Experiment 1, except that the strobe light in Context C flashed at a frequency of 1.6 Hz.

Procedure. The rabbits were randomly assigned to one of two groups. All rabbits received 1 day of adaptation and one 80-trial pretest session in Context A, followed by six daily sessions of exposure to either Context B or Context C with no further stimulus presentations. The rabbits exposed to Context B were then given six daily 80-trial sessions of paired CS–US presentations in Context C (Group ABCC, $n = 9$) and those exposed to Context C were then given six daily 80-trial sessions of paired CS–US presentations in Context B (Group ACBC, $n = 9$). Finally, all 18 rabbits received an 80-trial session of posttest in Context C. The group designations of ABCC and ACBC reflect each group's history of sequential exposure to Contexts A, B, and C.

Results

Conditioning. Figure 4 shows the mean (\pm SEM) percent CRs for subjects in Group ABCC and Group ACBC across the 6 days of CS–US pairings. As can be seen from the figure, the acquisition functions are very similar; both groups acquired CRs rapidly, reaching an asymptotic level of over 90% CRs by Day 3, and maintained that level across the 3 remaining days of CS–US pairings. An ANOVA revealed a significant main effect of days [$F(5,80) = 54.62, p < .001$], and, as expected, there was no main effect of group [$F(1,16) < 1$] or interaction of group \times days [$F(5,80) < 1$]. Analysis of the four 20-trial blocks of responding on Day 1 revealed a significant main effect of blocks [$F(3,48) = 21.80, p < .001$], but no main effect of group [$F(1,16) < 1$] or interaction of group \times blocks [$F(3,48) < 1$].

US testing. Figure 5 shows average response topographies during the first 20 US posttest trials for Groups ABCC (solid lines) and ACBC (dashed lines). The plots are arranged in descending order of US intensities at which responses occurred (2.0–0.25 mA), collapsed across US duration. The inset shows average response topographies

to a 0.5-mA, 50-msec US on pretest (dashed lines) and posttest (solid lines) for Groups ABCC and ACBC. Examination of the figure shows clear evidence of larger average responses for Group ABCC than for Group ACBC at US intensities of 0.25 and 0.5 mA. The inset shows the difference in UR topography between pretest and posttest, illustrating the clear increase in size and shift in peak latency characteristic of CRM, a difference that is absent in Group ACBC. Analysis of skew and kurtosis confirmed the shift in topography for Group ABCC by yielding significant differences in averaged response topography between the groups at both 0.25 and 0.5 mA. For example, whereas Group ABCC had significant negative skew and kurtosis values at 0.5 mA (-2.68 and -3.46 , respectively), Group ACBC had significant positive or neutral skew values (6.33 and 1.90 , respectively).

Analysis of the mean percent URs for all five US intensities collapsed across US duration yielded a significant main effect of intensity [$F(4,64) = 98.02, p < .001$], but no main effect or interactions of group (largest $F = 1.85, p = .19$). In contrast, analysis of response peak latency confirmed a significant difference between the groups at 0.25 mA [$F(1,16) = 26.84, p < .001$], and analyses of response amplitude and area yielded significant main effects of group [$F(1,16) = 11.75, p < .005$, and $F(1,16) = 11.62, p < .005$, respectively], which were attributable to significant differences at US intensities of 0.25 mA [$F(1,16) = 11.38, p < .005$, and $F(1,16) = 23.95, p < .001$, respectively] and 0.5 mA [$F(1,16) = 11.51, p < .005$, and $F(1,16) = 9.48, p < .01$, respectively]. These significant differences between groups in peak latency, amplitude, and area corroborate the stronger CRM observed in Group ABCC that was suggested by the topographical analysis.

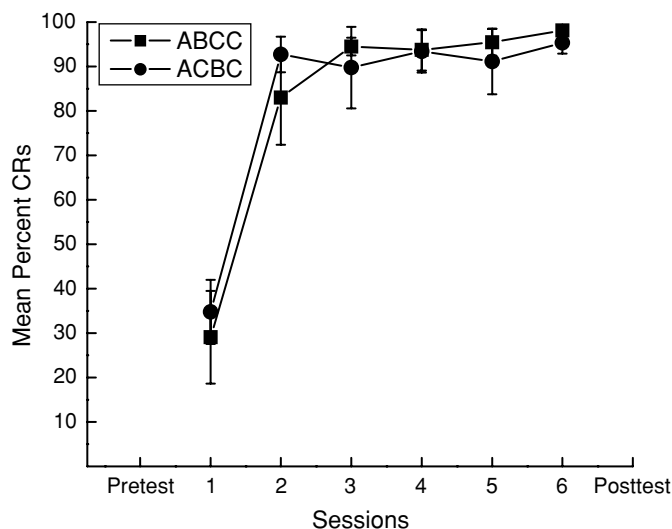


Figure 4. Mean (\pm SEM) percent CRs for rabbits in Groups ABCC and ACBC across the 6 days of stimulus CS–US pairings. Each of the six paired-conditioning sessions consisted of 80 presentations of a 400-msec, 1-kHz, 82-dB tone CS that coterminated with a 100-msec, 60-Hz 2.0-mA US.

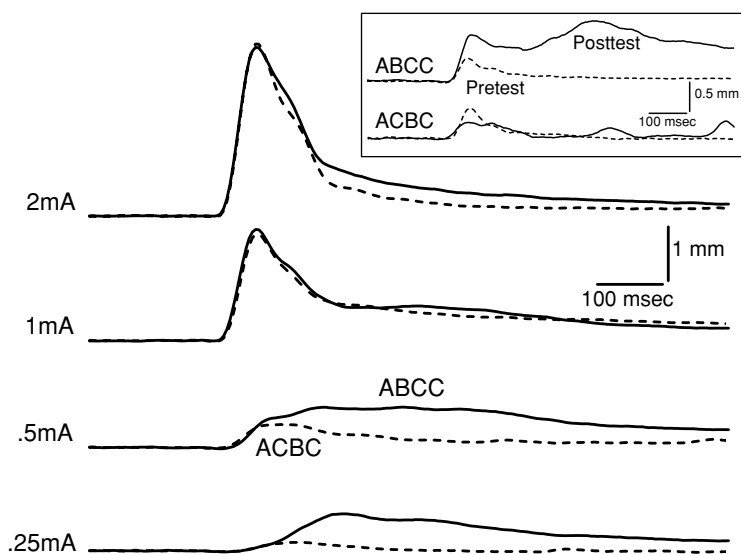


Figure 5. Averaged response topographies for Group ABCC (solid lines) and Group ACBC (dashed lines) during the first 20 posttest trials arranged in descending order of US intensity for which a response occurred (2.0 mA to 0.25 mA) collapsed across US duration. The inset shows average response topographies to a 0.5-mA, 50-msec US on pretest (dotted lines) and posttest (solid lines) for Groups ABCC and ACBC.

Discussion

The principal finding of the second experiment was that CRM of the rabbit NMR was sensitive to a change in context if rabbits were exposed equally to each of the different contexts (Millin & Riccio, 2004). Specifically, rabbits in Group ACBC that were given posttest US presentations in Context C after CS-US pairings in Context B showed significantly less CRM than did rabbits in Group ABCC that were given posttest US presentations in Context C after CS-US pairings in Context C. Importantly, both groups were completely equated in terms of total exposure to Contexts A, B, and C, making none of the contexts novel. Differences in the level of CRM were indexed by significant differences in response topography, peak latency, amplitude, and area that are consistent with our previous reports of CRM (Buck et al., 2001; Schreurs et al., 2005; Schreurs et al., 1995; Schreurs et al., 2000; Seager et al., 2003).

GENERAL DISCUSSION

We assessed CRM in a novel context different from the training context (Experiment 1) and in a context different from the training context but equally familiar (Experiment 2). When exposure to the different contexts was equated in Experiment 2, CRM demonstrated sensitivity to a context change. In fact, CRM all but disappeared when the US was tested in a context different from the training context.

A context-dependent reduction in responding during classical conditioning of the rabbit NMR has been demonstrated by Penick and Solomon (1991), who reported that rabbits given CS-US pairings in one context showed a

drop in responding of 50% CRs when given CS-US pairings in a different context where the visual, tactile, and olfactory characteristics had been altered. This reduction in responding as a result of a context shift during classical conditioning of the rabbit NMR has been reported in numerous other learning paradigms including fear conditioning (Millin & Riccio, 2004; Zhou & Riccio, 1996), taste aversion learning (Boakes et al., 1997), and conditioned suppression (Gunther et al., 1997). Consistent with this ubiquitous context shift effect, the present data show that if exposure to the contexts is equated (Millin & Riccio, 2004), CRM can be significantly reduced by a shift in context. Consequently, we see that CRM continues to obey a number of the behavioral laws of classical conditioning (Buck et al., 2001; Schreurs et al., 2005; Schreurs et al., 1995; Schreurs et al., 2000; Seager et al., 2003).

There are a number of possible explanations for the difference in results between the present experiments. These explanations include a difference in the behavioral laws governing classical conditioning and those governing CRM, detrimental effects of overtraining, inability to discriminate between the contexts, differential amounts of exposure to the contexts, and context novelty. Experiment 1 established that the ability to discriminate between the contexts was not a factor in the inability to detect a strong effect of context on CRM. By equating exposure to all the contexts and removing novelty, Experiment 2 established that CRM is sensitive to a change in context. This sensitivity to a change in context suggests that overtraining was not a factor in our inability to detect the effects of context on CRM in Experiment 1 and that CRM obeys yet another behavioral law of classical conditioning.

We have suggested previously that our CRM data may have some implications for theories and treatments of PTSD (Schreurs, 2003; Seager et al., 2003). In the present experiments, it was only when the US was tested in the same context as the training context that we saw the clearest evidence of an “exaggerated” response. When the US was tested in a different context, the increased response to the ES was considerably reduced. Similarly, a veteran may have “hit the deck” in the Persian Gulf but will only flinch when a car backfires in his or her hometown. It is when this “normal” responsiveness to stimuli is compromised by “hitting the deck” on Main Street that the exaggerated responses of PTSD occur. Thus, the observation that CRM is weaker after learning in a different context is consistent with the idea that trauma experienced in a different environment such as a desert or jungle may produce less profound effects in response to a stressful event when that stressful event is experienced at home (Kaysen et al., 2003). Like the drug addictions that failed to materialize when veterans returned from Vietnam (Robins & Slobodyan, 2003)—perhaps because they were removed from the context of drug using (Siegel, 1999)—the traumatic events veterans experience during war normally do not cause “posttraumatic” stress. Importantly, increased exposure to the traumatic context, defined as the environment that creates an atmosphere of fear (e.g., back-to-back tours of duty), has been found to increase PTSD in combat veterans, just as staying in the abusive or violent home affects victims of sexual abuse and domestic violence (Kaysen et al., 2003). Others have shown that veterans with PTSD exhibit exaggerated startle responses only in contexts perceived as stressful (Grillon, 2002; Grillon et al., 1998). It is the exaggerated or abnormal reaction following the trauma of war, sexual abuse, or other violence that characterizes PTSD (Pole, Neylan, Best, Orr, & Marmar, 2003). In fact, some have suggested that it is the inability to put the originally stressful event into context that is a sign of PTSD (Ehlers, Hackmann, & Michael, 2004; Michael, Ehlers, Halligan, & Clark, 2005).

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