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In a previous study, a latent inhibition (LI) effect was found to be dictated by a facilitation of the acquisition of a conditioned eyeblink response in context pre-exposed rabbits as opposed to slower learning in tone preexposed rabbits. In the present experiments, we examined the effects of preexposure to the tone conditional stimulus (CS) using a similar paradigm with rats. In Experiment 1, rats were given four or eight days of context (SIT) or CS preexposure (TONE) followed by eight days of paired training. Unlike rabbits, control and eight day SIT groups learned faster than TONE exposed rats and the four day SIT group. In Experiment 2, we controlled for the context preexposure by giving the control rats the same adaptation as Experiment 1 and testing rats given two days of CS preexposure or no preexposure. Again, SIT rats learned faster than TONE rats as well as rats that did not receive any preexposure. In Experiment 3, we tested a frequently-used method for examining LI, whereby paired training began immediately after the last of four sessions of preexposure, but observed no effect. Similar to our previous results, any LI effect produced in the present set of experiments arose from facilitated performance by SIT rats as opposed to deficits in learning in TONE rats. The present results highlight the need for a unifying theory of preexposure effects immune to differences experimental paradigms and parameters in order explain the variety of results obtained in the field.

It has long been appreciated that non-reinforced exposure to the conditional stimulus (CS) impacts the subsequent acquisition of conditional responses (CRs) during associative learning. The effect of CS preexposure is well studied, in part because preexposure may either facilitate or attenuate rates of learning depending on the experimental paradigm used. The contrasting effects of CS preexposure depend on a number of factors such as: amount of preexposure (Ayres, Philbin, Cassidy, & Belling, 1992), type of preexposure (e.g., sensory preconditioning; Nicholson & Freeman, 2000), conditioning context (Channell & Hall, 1981; Lubow, Rifkin, & Alex, 1976), and delay between preexposure and testing (Killcross, Kiernan, Dwyer, & Westbrook, 1998).

The latent inhibition (LI) effect is a well-studied phenomenon of one the many effects that stimulus preexposure has on learning and has produced numerous well-used paradigms. LI is an impairment of acquisition as a result of non-reinforced exposure to the CS prior to conditioning (Lubow & Moore, 1959). Within the first 10 years of Lubow and Moore’s (1959) report of a deficit in the acquisition of a conditioned limb flexion response in ungulates preexposed to the light CS prior to conditioning, LI was produced in an array of learning tasks across a range of species (see Lubow, 1973). More recently the LI paradigm has been used by investigators to examine attentional and cognitive deficits in learning and memory systems with psychological disorders such as schizophrenia (Davis & Gould, 2005; Lubow & Gerwitz, 1995).

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LI has been produced in rats using a variety of associative paradigms including conditioned tasted aversion (De La Casa & Lubow, 2002; Symonds & Hall, 1995) and conditioned suppression (Domjan & Siegel, 1971; Wright, Skala, & Peuser, 1986) as well as classical eyeblink conditioning (Nicholson & Freeman, 2002). Just as LI may be observed with numerous experimental paradigms it may be enhanced or diminished by manipulating different aspects of the experimental design. For example, LI is known to be influenced by the number of non-reinforced CS preexposures, intensity of the unconditional stimulus (US), and the interval between preexposure and testing (Katz, Rogers, & Steinmetz, 2002; Killcross, et al. 1998; Rodriguez & Alonso, 2002; Solomon, Brennan, & Moore, 1974; Solomon, Lohr, & Moore, 1974).

Due to the relative ease in producing LI across a variety of conditioning methods and parametric manipulations, numerous theories have been proposed to account for the phenomenon (see Schmajuk, Lam, & Gray, 1996). The major theories of the LI effect primarily fall into two broad classes of how CS preexposure impairs subsequent learning by affecting (1) formation of the CS-US association or (2) retrieval of an established CS-US association. Within the class of theories that postulate LI arises from impairments in the formation of CS-US associations, CS preexposure influences the subsequent associability of the CS. A decrease in CS associability may be influenced by a change in stimulus salience and efficacy (e.g. McLaren & Mackintosh, 2000) or the overall novelty of the stimulus (e.g. Schmajuk, et al. 1996). Retrieval based theories of LI predict an impairment in the retrieval of normal CS-US associations that have formed during conditioning due to the prior formation of a CS-context association that is formed during preexposure (Escobar, Arcedano, & Miller, 2002).

The choice of control groups in LI experiments is yet another important factor with respect to the observation of differences in learning between groups (Boughner, Thomas, & Papini, 2004; Lubow, et al. 1976). Previous work in this lab looking at LI using eyeblink conditioning in rabbits had found that the extent to which a difference was observed between CS and context preexposed animals depended primarily on learning rates in the latter group (Katz, et al. 2002). If it is the case that context preexposure exerts a facilitative effect on subsequent acquisition it may be problematic for context preexposure to be used as a sole control group. The true test of LI, therefore, would be a difference, or impairment, in conditioning rates in CS preexposed animals not only relative to context preexposure but to no preexposure as well (see Boughner, et al. 2004).

For the present report, three experiments were conducted to examine LI effects during eyeblink classical conditioning in rats. We had two basic purposes for conducting these studies. First, we wanted to systematically examine the effects of context exposure and CS exposure on subsequent conditioning, as we had previously done in the rabbit (see Katz, et al. 2002). Second, we wanted to compare the LI effect in rats and rabbits given procedural differences that exist in training, especially noting the fact that rabbits are conditioned while restrained whereas rats are conditioned while free to move and explore the conditioning chamber. In replicating the Katz, et al. (2002) study, our control rats in Experiment 1 were exposed to the experimental context during a 2-day adaptation period. In Experiment 2, therefore, we controlled for that context preexposure by testing rats given 2 days of CS preexposure as well as testing rats that received no
preexposure prior to conditioning. Finally, in Experiment 3, we examined the
effect of the delay between preexposure and the start of paired training by using
the oft-used method for examining the LI effect, whereby paired training begins
immediately after the last session of preexposure.

Similar to previous results, LI effects produced in the present set of
experiments appear to be caused by facilitated performance of rats preexposed to
the conditioning context, as opposed to deficits in learning in rats preexposed to
the CS. Unlike rabbits, 8 days of context preexposure facilitated learning more
than 4 days, although in neither study was there a linear relationship for the rate of
learning and the amount of context or CS preexposure (see Katz, et al. 2002).
Furthermore, in no instance did any preexposed group exhibit poorer learning than
the group that received no preexposure. These results suggest that a better
understanding of LI will arise with an incorporation of negative results (i.e., no LI
effect) into current theoretical explanations of the phenomenon. Specifically, why
does preexposure to an intermittent distinct stimulus, like a CS, inconsistently
produce LI? Oftentimes LI depends more on variables such as exposure to
continuous compound stimuli (e.g., context) and US intensity than on the CS
preexposure itself. If preexposure to stimuli can either facilitate or attenuate
subsequent learning, then theories of preexposure effects must accommodate the
variety of outcomes obtained in the field.

Experiment 1: The effect of 4 or 8 days of preexposure to the conditioning
context or the conditional stimulus (CS) on the expression
of the conditioned eyeblink response

The amount of CS preexposure has been shown to play an important role in
the magnitude of the LI effect (Ayres, et al. 1992; Solomon, Lohr, et al., 1974). In
a previous study, however, neither 4 nor 8 sessions of CS preexposure resulted in
an increase in the magnitude of an LI effect in rabbits (Katz, et al. 2002). In
Experiment 1, we tested the traditional conceptualization of LI (i.e., amount of
preexposure affects magnitude of differences between groups) by assessing the
effects of 0, 4, or 8 days preexposure to the CS and context followed by 8 days of
paired training in rats. This experiment was conducted as a replication of a
previous LI study in rabbits, conducted in this laboratory (Katz, et al. 2002), to
compare differences between the two species.

Method

Subjects. A total of 45 male and female Long Evans Blue Spruce rats purchased from Harlan
Indianapolis United States were used (m = 24; f = 21). Rats were housed by sex in groups of up to 4
rats in standard laboratory cages (48 cm x 20 cm x 26 cm) in the animal colony in the Department of
Brain and Psychological Sciences at Indiana University. Food and water were available ad libitum.
The animal colony was maintained on a 12:12 hr light/dark cycle (lights on at 0700 hrs).

Surgical procedures. When rats were approximately 3 months old (96.4 ± 2 days) they were
anesthetized with an i.m. injection of anesthetic cocktail. The cocktail consisted of 9 mg/kg sterile
physiological saline, 74.0 mg/kg ketamine, 3.7 mg/kg xylazine, and 0.74 mg/kg acepromazine.
Injection volume was 2.0 mL/kg body weight. When fully anesthetized, rats were fitted with 2
Teflon-coated stainless steel electromyographic (EMG) electrodes, implanted in the anterior portion
of the orbicularis oculi of the left eyelid. A ground wire was secured to one of three skull screws and
the ground wire and electrodes were attached via gold pins to a connector that was secured to the
skull with dental acrylic. A bipolar stimulating electrode (Plastics One, Roanoke, VA) was implanted subdermally, dorsocaudal to the left eye. The stimulating electrode was contained in a plastic connector that was also secured with dental acrylic. The surgical site was sutured around the headstage and antibiotic ointment (Povidine) was applied liberally to the scalp. Rats were housed individually after surgery and handled once daily during the 5-day recovery period.

Behavioral training. Preexposure and conditioning took place in operant boxes (Coulburn Instruments, Allentown, PA) placed in sound attenuating chambers. The operant boxes had two Plexiglas and two stainless steel walls and a stainless steel floor grid. A speaker for the delivery of the CS sat directly above the box. After the recovery period rats were placed in one of the conditioning chambers and EMG and bipolar connectors were plugged into a tether composed of light weight wire, allowing the rats to move freely.

After the recovery period all rats received two adaptation sessions (1 session/day) where they were placed in one of the conditioning chambers and EMG and bipolar connectors were plugged in but no stimuli were presented. Each adaptation session was 60 min in duration. After adaptation, rats were assigned to one of 4 preexposure groups or the control group (n = 9). For preexposure groups, rats were assigned to one of two durations of preexposure (i.e., 4 or 8 days) and one of two preexposure conditions (i.e., TONE or SIT).

During a preexposure session, rats were placed in the conditioning chambers and given either 120 tone CS presentations (group TONE) or 120 blank trials (group SIT) with an average intertrial interval of 30 s (range 20-40 s). Triggering trials and delivery of the tone CS (2.8 kHz, 450ms, 85dB) was accomplished by a custom computer program (Chen & Steinmetz, 1998). Rats received 1 session of preexposure per day. The 4D and 8D TONE groups (n = 10 and n = 8, respectively) received either 4 or 8 days of preexposure resulting in 480 and 960 non-reinforced tone presentations, respectively. The 4D and 8D SIT groups (n = 10 and n = 8, respectively) also received 4 or 8 days of preexposure resulting in approximately 240 or 480 min of context exposure, respectively.

Twenty-four hours after the last preexposure session, or the 2nd adaptation session for control rats, paired training began. Training consisted of 8 sessions of 120 trials (1 session/day). The trials were delivered in 12 blocks of 10 trials and were 90% reinforced (9 paired and 1 CS-alone trial/block), resulting in 108 paired trials and 12 CS-alone trials per session. An average intertrial interval of 30 s (range 20-40 s) was used. For all trials there was a 350 ms pre-CS period followed by a 450 ms tone CS (2.8 kHz, 85dB). During paired trials, the US, a 1.5 mA, 100ms periocular stimulation, co-terminated with the CS producing a 350 ms interstimulus interval (ISI). Data for two control rats and one 4D SIT rat were excluded due to poor quality EMG records.

Data acquisition. Trials were triggered for data acquisition and stimulus delivery was relayed to the chambers by a customized computer program (Chen & Steinmetz, 1998). Raw EMG data were amplified before being acquired by a computer data acquisition system (Spike2, CED, London, UK) whereupon the raw EMG signal was rectified and smoothed for subsequent analysis.

Data analysis. EMG data from each session were converted into comma-separated value files and then analyzed by a custom data analysis program (DataMunch; King & Tracy 1999) that computed the total number of trials in which a conditioned response (CR) was detected. Thresholds for detecting CRs were set for values exceeding eight standard deviations above mean EMG activity during the pre-CS period. Elevated EMG activity during the 100 ms period immediately after CS onset was considered an alpha response and not included as a CR. Trials where EMG activity was elevated during a 100 ms period immediately preceding the CS were deemed bad trials and were excluded from analysis.

Data were analyzed using repeated-measures analysis of variance (ANOVA) with a nested design to capture differences in CR production early versus late in training (i.e., acquisition versus asymptotic CR performance). Simple repeated-measures ANOVAs, single factor ANOVAs, paired t-tests and Fisher’s PLSD were used as post-hoc tests when significant main effects and interactions were obtained. Alpha level was set at .05 for all tests. For missing data, values were either interpolated from appropriate group means or the averages of individual values immediately prior to and after the missing value for a given subject. These instances of interpolation were rare (less than 4% of all cells) and were used only for repeated-measures analyses.
Results and Discussion

Figure 1 presents the percentage of conditional responses (%CR) for all context preexposed (SIT) and CS preexposed (TONE) groups from the last day of preexposure, and for the control group from the last adaptation session, throughout paired training. No differences were observed between groups during the session immediately prior to paired training. As rats exhibited increases in the number of CRs over acquisition repeated-measures ANOVA revealed a significant effect of phase (early versus late training) and session, as well as an interaction between phase and session, $F(1, 37) = 40.5, p < .0001$ and $F(3, 111) > 25.4, ps < .0001$, respectively. Specifically, for all groups, the CS-US association strengthened rapidly during early paired training, the first four sessions, $F(3, 111) = 58.2, p < .0001$, whereupon it plateaued during late paired training, the final four sessions, $F(3, 111) = .43$, NS. Repeated-measures ANOVA for the early phase of paired training also revealed an effect of group, $F(4, 37) = 4.8, p < .005$. During sessions 1 and 3, single factor ANOVAs revealed a significant effect of group, $F(4, 37) = 5.9, p < .005$ and $F(4, 36) = 4.8, p < .005$, respectively. Specifically, rats in the 8D SIT group and control rats exhibited significantly greater percent CRs on paired trials than all other groups during session 1 ($ps < .05$) and both SIT groups and control rats exhibited greater percent CRs than either TONE group during session 3 ($ps < .05$).

![Figure 1](image-url)

**Figure 1.** Mean (± SEM) percentage of conditioned responses (%CR) for rats receiving 4 or 8 days (4D or 8D) of tone or context preexposure (TONE or SIT) and control rats. Data are presented from the last session of preexposure (last adaptation session for control rats) through the 8 sessions of paired CS-US training. Both 8D SIT and control rats showed higher percentage CRs over the early part of training.

The differences seen in %CR, in the present experiment, are consistent with the LI effect. Specifically, rats receiving non-reinforced exposure to the tone CS...
prior to paired training exhibited impaired learning. In contrast to the majority of LI studies, however, the differences seen here between SIT and TONE rats were not consistent across preexposure durations (i.e., 4 and 8 days of preexposure) and the greatest differences were seen on the first day of paired training. To better assess differences between the present results and previous LI studies using eyelink conditioning in rats (e.g., Nicholson and Freeman, 2002) we analyzed percent CRs by blocks for session 1. Figure 2 presents percent CRs for session 1 in 20 trial blocks. Repeated-measures ANOVA revealed a significant effect of group and block, $F(4, 37) = 10.0$ and $F(5, 185) = 33.5$, respectively, $ps < .0001$. All five groups, showed an increase in percent CRs during the first session $Fs > 5.2$, $ps < .001$, indicating a significant amount of learning on the first day of paired training. The 8D SIT rats produced more CRs than both TONE groups across all blocks of session 1 ($ps < .05$) and even produced more CRs than 4D SIT rats over trials 21-60 ($ps < .005$). The 4D SIT rats only differed from 4D TONE rats for one block of trials (81-100; $p < .05$) and rates of learning were equivalent between 4D and 8D TONE rats for the entire session. There were no differences between 8D SIT rats and the control group, although the control rats produced a significantly greater number of CRs during session 1 relative to both TONE groups ($ps < .05$; not different from 8D TONE trials 81-100) and differed significantly from 4D SIT rats for the first 80 trials ($ps < .05$).

![Figure 2](image-url)

**Figure 2.** Mean (± SEM) %CR for rats receiving 4 or 8 days (4D or 8D) of tone or context preexposure (TONE or SIT) and control rats. Data are presented for the first session of paired CS-US training, in blocks of 20 trials followed by the average %CR for session 1. Both 8D SIT and control rats showed rapid learning during the first session of paired training.

There were no significant differences between groups in either the average latency to the CR peak or in CR amplitude. As animals learned across training days, however, the peak of the CR shifted to immediately prior to US onset.
vs. Late phase: $F(1, 37) = 52.2, \ p < .0001$. Specifically, during session 1 the average latency to the peak of the CR on tone alone trials was $366.5 \pm 13$ ms but by session 8 the latency was much shorter, $282.9 \pm 10$ ms, $t(41) = 5.97, \ p < .0001$. Concordant with changes in the timing of the learned response, CR amplitude increased as animals learned [Early vs. Late phase: $F(1, 37) = 21.2, \ p < .0001$] increasing on average 70% from the first to last day of conditioning, $t(40) = 3.1, \ p < .005$.

Experiment 2: The effect of 2 days of preexposure to the conditioning context or the CS on the expression of the conditioned eyeblink response

When replicating the previous study conducted in this laboratory (see Katz, et al. 2002) our control group received 2 days of preexposure to the conditioning context during the adaptation phase prior to conditioning. Control rats in Experiment 1, therefore, essentially comprised a 2 day SIT condition (2D SIT) necessitating a comparison to conditioning rates in rats preexposed to the tone CS for 2 days (2D TONE) prior to conditioning. In addition, since LI studies most often use context exposure in the absence of CS exposure as a control condition (see Boughner et al. 2004), we added a control group that received neither adaptation nor preexposure prior to conditioning to assess whether or not differences in rates of learning were due to facilitation in context preexposed or to deficits in learning in CS preexposed animals.

Method

Subjects. A total of 24 male and female Long Evans Blue Spruce rats purchased from Harlan Indianapolis United States were used (m = 11; f = 13). Rats were housed, as described in Experiment 1, in the animal colony in the Department of Brain and Psychological Sciences at Indiana University. Food and water were available ad libitum. The animal colony was maintained on a 12:12 hr light/dark cycle (lights on at 0700 hrs).

Surgical procedures. Rats underwent surgery at approximately 3 months of age ($95.6 \pm 6$ days). Surgical procedures were performed as described in Experiment 1.

Behavioral training. Preexposure and conditioning took place in operant boxes (Coulburn Instruments, Allentown, PA) placed in sound attenuating chambers, as described in Experiment 1. After the recovery period rats were placed in one of the conditioning chambers and EMG and bipolar connecters were plugged into a tether composed of lightweight wire, allowing the rats to move freely.

After the recovery period rats were assigned to one of two preexposure groups or the control group. For preexposure groups, rats received two sessions of preexposure to either the CS (2D TONE; n =8) or the conditioning context (2D SIT; see Experiment 1). The 2D TONE group received two days of preexposure resulting in 240 non-reinforced tone presentations and the 2D SIT group received two days of preexposure resulting in approximately 120 min of context exposure. Twenty four hours after the last preexposure session, paired training began. Control rats (n = 8) began paired training immediately after the recovery period. Paired training was conducted as described in Experiment 1. Data for two control rats and two 2D rats were excluded due to poor quality EMG records.

Data acquisition. Trials were triggered for data acquisition and stimulus delivery was relayed to the chambers by a customized computer program (Chen & Steinmetz, 1998). Raw EMG data were amplified before being acquired by a computer data acquisition system (Spike2, CED, London, UK) whereupon the raw EMG signal was rectified and smoothed for subsequent analysis.
**Data analysis.** EMG data from each session were converted into comma-separated value files and then analyzed by a custom data analysis program (DataMunch; King & Tracy 1999) that computed the total number of trials during which a conditioned response (CR) was detected. Thresholds for detecting CRs were set for values exceeding eight standard deviations above mean EMG activity during the pre-CS period. Elevated EMG activity during the 100 ms period immediately after CS onset was considered an alpha response and not included as a CR. Trials where EMG activity was elevated during a 100 ms period immediately preceding the CS were deemed bad trials and were excluded from analysis.

Data were analyzed using repeated-measures analysis of variance (ANOVA) with a nested design to capture differences in CR production early versus late in training (i.e., acquisition versus asymptotic CR performance). Simple repeated-measures ANOVAs, single factor ANOVAs, paired t-tests and Fisher’s PLSD were used as post-hoc tests when significant main effects and interactions were obtained. Alpha level was set at .05 for all tests. For missing data, values were either interpolated from appropriate group means or the averages of individual values immediately prior to and after the missing value for a given subject. These instances of interpolation were rare (less than 4% of all cells) and were used only for repeated-measures analyses.

**Results and Discussion**

Figure 3 presents the percent CRs for all SIT and TONE groups from the last day of preexposure throughout paired training. No differences were observed between SIT and TONE groups during the session immediately prior to paired training. As in Experiment 1, rats exhibited increases in the number of CRs over acquisition rapidly forming a CS-US association. Repeated-measures ANOVA revealed a significant effect of the repeated measurements of phase (early versus

![Figure 3. Mean (+ SEM) %CR for rats receiving 2 days of tone or context preexposure (2D SIT and 2D TONE, respectively) and control rats. Data are presented from the last session of preexposure for SIT and TONE animals through the 8 sessions of paired CS-US training. Two days of context exposure resulted in a very high mean percentage CR relative to tone preexposure or no preexposure, especially during session 1.](image-url)
late training) and session, as well as an interaction between phase and session, $F(2, 16) = 29.9, p < .0001$ and $F(3, 48) > 12.5, p < .0001$, respectively. For all groups, acquisition occurred early during paired training, the first four sessions, $F(3, 48) = 26.0, p < .0001$, reaching asymptotic levels of CR production shortly thereafter, $F(3, 48) = .15$, NS. Group differences were again confined to the early phase of training, specifically in the present experiment, session 1. This group by phase interaction, however, only approached significance, $F(2, 16) = 3.0, p < .08$, for the first four sessions of paired training. A single factor ANOVA revealed a significant effect of group during only the first session of paired training, $F(2, 16) = 5.3, p < .05$. Specifically, rats in the 2D SIT group exhibited significantly greater percent CRs on paired trials than all other groups ($p < .05$).

Figure 4, presents percent CRs for all groups during session 1 in 20 trial blocks. Repeated-measures ANOVA revealed a significant effect of group and block, $F(2, 16) = 17.2, p = .0001$ and $F(5, 80) = 4.7, p < .001$, respectively. The 2D SIT rats exhibited higher rates of CR production relative to control rats for every block of 20 trials ($p < .05$). Furthermore, 2D SIT rats exhibited higher rates of CR production relative 2D TONE rats for the first 80 trials ($p < .05$).

![Figure 4](image_url)

*Figure 4. Mean (± SEM) %CR for rats receiving 2 days of tone or context preexposure (2D SIT and 2D TONE, respectively) and control rats. Data are presented for the first session of paired CS-US training, in blocks of 20 trials followed by the average %CR for session 1. 2D SIT rats showed rapid learning during the first session of training. 2D TONE rats also showed an increase in the percent CR during the first session and although they learned to a lesser extent than context pre-exposed rats 2D TONE rats consistently showed higher percent responding than control rats.*

Interestingly, 2D TONE rats exhibited higher rates of CR production relative to control rats as well. Specifically, during trials 21-60 and 81-100 %CR was higher for 2D TONE rats ($p < .05$). In keeping with the poor learning seen in control rats in the present experiment, only 2D SIT and TONE rats showed significant increases in %CR during the first session of paired training: 2D SIT,
$F(5, 30) = 7.6, p = .0001$; 2D TONE, $F(5, 25) = 2.6, p < .05$. Control rats in contrast, showed no evidence of learning the CS-US association during that first day, $F(5, 25) = .34, \text{NS}$.

Similar to the results of Experiment 1, the peak of the CR changed and shifted to immediately prior to US onset over training [Early vs. Late phase: $F(1, 16) = 9.8, p < .01$]. In contrast to the findings of Experiment 1, however, latency to the peak of the CR differed between groups $F(2, 16) = 5.4, p < .05$. Specifically, control rats showed significantly longer latencies for the peak of the CR than 2D SIT rats during sessions 2 and 4 ($ps < .05$; Figure 5). Despite groups differences, there was a significant decrease in the average latency of the peak of the CR over training, $t(16) = 3.0, p < .01$. During session 1 the average latency of the peak of the learned response, $382.1 \pm 27 \text{ ms}$, occurred just after US onset. In contrast, by session 8 the average latency of the peak shifted forward in time to $307.1 \pm 20 \text{ ms}$ or just prior to US onset. There were no significant differences between groups for CR amplitude. CR amplitude, however, increased as animals learned [Early vs. Late phase, $F(1, 16) = 7.1, p < .05$], increasing on average 48% from the first to the last day of conditioning, $t(17) = 3.1, p < .01$.

**Figure 5.** Mean (± SEM) of the average latency to the peak of the CR during tone alone trials for preexposure (PE) and sessions 1-4. The latency was shorter for 2D SIT rats, preceding US onset times by session 1. In contrast, Control rats exhibited consistently longer latencies to the peak of the CR. The difference in these latencies is a further reflection of the facilitated learning in 2D SIT.

Again, the differences seen in percent CR, in the present experiment, are consistent with the LI effect. Specifically, rats receiving non-reinforced exposure to the tone CS prior to paired training exhibited impaired learning relative to rats preexposed to only the conditioning context. Preexposure to the tone, however, did not impair conditioning relative to a control group of rats receiving no preexposure prior to conditioning. In fact, only the two preexposed groups showed
evidence of early learning during the first session of paired training whereas rats that received no preexposure did not.

**Experiment 3: Using a massed trial LI eyeblink conditioning procedure to assess the role of a delay between preexposure and conditioning following four sessions of preexposure**

Context preexposure led to facilitated learning in Experiments 1 and 2. Tone preexposure resulted in diminished CR production relative to context preexposure although deficits were inconsistent over all durations of preexposure. Furthermore, rats that had no preexposure produced the fewest CRs during the early phase of training. One difference between the previous two experiments and a number of other LI paradigms that have been used in the past was a 24 hr delay that elapsed between the last preexposure session and the first session of paired training. Although some LI studies using classical eyeblink conditioning and produced LI, the majority of LI studies have no delay between the end of the preexposure phase and the beginning of conditioning. Retention interval is one of the many parameters of the experimental design that may influence the occurrence of LI. Previous studies have found that LI is abolished by inserting a delay between CS preexposure and testing (Aguado, Symonds, & Hall, 1994; Killcross, et al. 1998), therefore, in the present experiment we tested two groups of rats receiving four sessions of either context (LI SIT) or CS (LI TONE) preexposure. Rats began with three daily sessions of preexposure. On the fourth day rats had an abbreviated preexposure session (60 versus 120 trials) followed immediately by half a session of paired training.

**Method**

**Subjects.** A total of 13 male and female Long Evans Blue Spruce rats purchased from Harlan Indianapolis United States were used (m = 7; f = 6). Rats were housed, as described in Experiment 1, in the animal colony in the Department of Brain and Psychological Sciences at Indiana University. Food and water were available ad libitum. The animal colony was maintained on a 12:12 hr light/dark cycle (lights on at 0700 hrs).

**Surgical procedures.** Rats underwent surgery at approximately 3 months of age (101.2 ± 5 days). Surgical procedures were performed as described in Experiment 1.

**Behavioral training.** Preexposure and conditioning took place in operant boxes (Coulburn Instruments, Allentown, PA) placed in sound attenuating chambers, as described in Experiment 1. After the recovery period rats were placed in one of the conditioning chambers and EMG and bipolar connectors were plugged into a tether composed of light weight wire, allowing the rats to move freely.

After the recovery period rats were assigned to one of two preexposure groups. Rats received four sessions of preexposure to either the CS (LI TONE; n = 6) or the conditioning context (LI SIT; n = 7). The LI TONE group received four days of preexposure, a total of 420 non-reinforced tone presentations, and the LI SIT group received four days of preexposure, a total of approximately 210 min of context exposure. The first three preexposure sessions consisted of 120 non-reinforced tone CS presentations or 120 blank trials. The fourth preexposure session consisted of 60 tone presentations or 60 blank trials followed immediately by the first session of paired training, consisting of six blocks of 10 trials (i.e., 54 paired and 6 CS alone trials). The following seven sessions of paired training were conducted once daily and consisted of 120 trials (i.e., 108 paired and 12 CS alone trials).
**Data acquisition.** Trials were triggered for data acquisition and stimulus delivery was relayed to the chambers by a customized computer program (Chen & Steinmetz, 1998). Raw EMG data were amplified before being acquired by a computer data acquisition system (Spike2, CED, London, UK) whereupon the raw EMG signal was rectified and smoothed for subsequent analysis.

**Data analysis.** EMG data from each session were converted into comma-separated value files and then analyzed by a custom data analysis program (DataMunch; King & Tracy 1999) that computed the total number of trials in which a conditioned response (CR) was detected. Thresholds for detecting CRs were set for values exceeding eight standard deviations above mean EMG activity during the pre-CS period. Elevated EMG activity during the 100 ms period immediately after CS onset was considered an alpha response and not included as a CR. Trials where EMG activity was elevated during a 100 ms period immediately preceding the CS were deemed bad trials and were excluded from analysis.

Data were analyzed using repeated-measures analysis of variance (ANOVA) with a nested design to capture differences in CR production early versus late in training (i.e., acquisition versus asymptotic CR performance). Simple repeated-measures ANOVAs, single factor ANOVAs, paired t-tests and Fisher’s PLSD were used as post-hoc tests when significant main effects and interactions were obtained. Alpha level was set at .05 for all tests. For missing data, values were either interpolated from appropriate group means or the averages of individual values immediately prior to and after the missing value for a given subject. These instances of interpolation were rare (less than 4% of all cells) and were used only for repeated-measures analyses.

**Results and Discussion**

Figure 6 presents the percentage of CRs for both SIT and TONE groups from the last session of preexposure throughout paired training. No significant differences were observed between SIT and TONE groups during the session immediately prior to paired training. As in both previous experiments, rats exhibited increases in the number of CRs over acquisition rapidly forming a CS-US association. Repeated-measures ANOVA revealed a significant effect of the repeated measurements of phase (early versus late training) and session, as well as an interaction between phase and session, $F(1, 10) = 14.6, p < .005$ and $F_s(3, 30) > 3.1, p < .05$, respectively. For both groups, acquisition occurred during the early phase of paired training, the first four sessions, $F(3, 30) = 6.6, p < .005$, reaching asymptotic levels of CR production during the late phase, $F(3, 30) = .42, NS$. Group differences were not evident in the present experiment and the analysis of percent CRs over blocks during session 1 revealed an effect of block that approached significance, $F(2, 22) = 3.3, p = .057$ (Figure 7), suggesting that both groups began forming CS-US associations early in training.

There were no significant differences between groups for CR amplitude. Conditioned response amplitude, however, increased during the early phase of training, $F(3, 30) = 4.3, p < .05$. A comparison between the average amplitude of the CR peak on session 1 and session 8 indicated that there was an increase in response amplitude by 34%. Similarly, there was an average decrease in the latency to the peak of the learned response. Specifically, during session 1 the average latency of the peak of the learned response, $366.9 \pm 30$ ms, occurred just after US onset. By session 8 the average latency of the peak shifted forward in time to $348.5.1 \pm 20$ ms, or immediately prior to US onset.
Figure 6. Mean (± SEM) %CR for rats receiving 4 sessions of tone or context preexposure (LI TONE and LI SIT, respectively). The 4th session of preexposure was followed immediately by paired training. Data are presented for the last 60 preexposure trials (PE) through the 8 sessions of paired CS-US training.

Figure 7. Mean (± SEM) percentage of conditioned responses (%CR) for rats receiving 4 sessions of tone or context preexposure (LI TONE and LI SIT, respectively). The 4th session of preexposure was followed immediately by paired training. Data are presented for the first session of paired CS-US training in blocks of 20 trials followed by the average %CR for session 1.
In the present experiment LI was not produced even though there was no delay between the last session of preexposure and the first session of conditioning. Figure 8 presents the percent CRs for the first 60 trials of conditioning for preexposed animals that had either no delay (0 hrs) or a 24 hr delay prior to the start of training (4D SIT and TONE groups from Experiment 1). In neither instance did tone preexposure result in LI. A 2-factor ANOVA indicated a significant effect of delay, $F(1,27) = 6.9, p < .05$, but no effect of preexposure condition, $F(1,27) = .52, \text{NS}$. Specifically, rats that had no delay between the last session of preexposure and the first session of conditioning exhibited greater %CRs than rats that had a 24 hr delay before conditioning began, $p < .05$.

![Figure 8](image_url)

**Figure 8.** Mean (± SEM) %CR for the first 60 trials of session 1 for rats receiving 4 sessions of tone or context preexposure (TONE or SIT, respectively) with no delay prior to first session of paired training or with a 24 hr delay prior to the first session of paired training. * 0 hr delay significantly greater %CR than 24 hr delay, $p < .05$.

**General Discussion**

The results of the present study are consistent with previous findings (e.g., Katz, et al. 2002), showing that the difference between rates of acquisition in context and CS preexposed animals arises from a facilitation of acquisition in context preexposed (SIT) animals as opposed to impaired learning in CS preexposed (TONE) animals. With rabbits, four daily sessions of context preexposure but not eight daily sessions resulted in facilitated learning relative to CS preexposed rabbits. In contrast, rats preexposed to the conditioning environment over 8 daily sessions but not 4 daily sessions exhibited higher rates of acquisition than CS preexposed rats. The results of Experiment 1 taken together with the results of Katz et al. (2002) suggest that (1) the amount of preexposure, to either the conditioning context or the CS, impacts acquisition in a non-linear
fashion and (2) rats and rabbits differ with respect to how the amount of context preexposure impacts learning. Specifically, stimulus and preexposure parameters greatly affect the occurrence of LI and, similarly, species differences in stress responses, habituation or conditioning environment (i.e. free moving exploration in rats versus restraint used for rabbits) can also affect subsequent learning.

In Experiment 2, two daily sessions of context preexposure also facilitated acquisition of the CS-US association relative to CS preexposure. In that experiment, however, two days of tone preexposure did not impair learning relative to a control group that received no preexposure to either the context or the CS prior to training. The issue of control groups is pertinent not only for LI but for perceptual learning as well (Lubow, et al. 1976), where preexposed animals learn more rapidly than animals that receive no preexposure. In fact, Lubow et al. (1976), proposed an early theory of these positive and negative effects of preexposure on learning by stating that learning is impaired in the group that experiences the least amount of contrast between stimuli and context during conditioning. Specifically, animals preexposed to the CS in the conditioning context and animals that do not undergo preexposure learn more slowly because for the former the US is introduced with an old stimulus in an old environment whereas for the latter the US is introduced with a new stimulus in a new environment, two situations that are not optimal for learning. The typical comparison group in many LI studies is a context preexposed group. Given that context preexposure often facilitates learning (Boughner, et al. 2004) the results and conclusions of such studies in which deficits are found between tone preexposed animals and context preexposed controls may not be indicative of impaired acquisition.

In Experiment 3, we failed to replicate the classic LI effect using a procedure that has often been employed to study LI. Specifically, there were no differences in learning rates between rats preexposed to either the conditioning context or the tone CS over four sessions when paired training followed preexposure with no delay. Typically, the insertion of a delay between preexposure and testing attenuates the LI effect (Killcross, et al. 1998). The comparison of learning rates in rats preexposed to either the CS or the conditioning context for four preexposure sessions prior to subsequent paired training suggested that the largest effect of delay was on learning during the first 60 conditioning trials. Specifically, rats that had no delay between preexposure and conditioning produced more CRs during the first 60 conditioning trials than rats given a 24 hr delay. Finally, although there was not a linear effect of preexposure on rates of learning across experiments context preexposed rats, on average, produced more CRs than CS preexposed rats early in paired training regardless of the duration of preexposure although the difference was not always significant. In no instance, however, did tone preexposed animals perform more poorly than the actual control condition that began conditioning with no prior preexposure to the conditioning context or stimuli (Figure 9).

Given that LI is a widely used experimental paradigm and a typically robust phenomenon it is important to consider variables in the present study that may have prevented the observation of LI. It was noted above that US intensity can modulate the magnitude of the LI effect (Katz, et al. 2002). In previous studies of rat eyeblink conditioning examining the LI effect different conditioning
parameters were used, as well as different US intensities. In one experiment, the US was a 5 psi air puff to the cornea in restrained rats (Schmajuk et al. 1994) while another used a titrated 25 ms eyeshock in freely moving rats that ranged from 2-3 mA (Nicholson & Freeman, 2002). Although the latter study used a more intense shock US than used here (i.e., we used a 1.5 mA eyeshock) the duration of the US was much shorter, 25 ms versus 100 ms, precluding a direct comparison of the intensities.

Far fewer studies of the LI effect have used eyeblink conditioning in rats and numerous studies in human adults also failed to find an LI effect using eyeblink conditioning (see Lubow, 1973). Perhaps for unrestrained rats, classical eyeblink conditioning is not the optimal experimental paradigm for producing LI. Positive LI effects are robustly produced in rats using paradigms with highly salient aversive or appetitive stimuli (i.e., fear conditioning or appetitive tone-food pairing; Davis & Gould 2005; Honey & Good 1993). Similarly, paradigms with multiple phases of shaping, pre-training and training, such as conditioned suppression (Kremer, 1972; Wheeler, Chang, & Miller, 2003), also produce a more robust LI effect, due perhaps to the fewer number of conditioning trials and the varying difficulty of the learning paradigm for the subjects.

Numerous brain regions have been implicated in the LI effect, all of which play some role in stimulus processing, context encoding, attention or motivation. The hippocampus has received the most attention as a neural structure mediating...
Hippocampal aspiration and hippocampal ablation have been shown to abolish the LI effect in CS preexposed rats and rabbits using eyeblink and nictitating membrane conditioning paradigms, respectively (Schmajuk, Lam, & Christiansen, 1994; Solomon & Moore, 1975). More discrete lesions of the hippocampus, however, have produced conflicting results (Buhusi, Gray, & Schmajuk, 1998; Honey & Good, 1993; Shohamy, Allen, & Gluck, 2000) and electrical stimulation of the hippocampus has been shown to either enhance or attenuate the LI effect (Salafia & Allan, 1980, 1982). The hippocampus is important for processing multiple aspects of the conditioning environment during learning (i.e. CS, context and/or US; Katz, et al. 2002) but may not be necessary to produce LI.

More recent data have implicated the entorhinal and cingulate cortices in LI (Nicholson & Freeman, 2002; Shohamy, et al. 2000; Talk, Stoll, & Gabriel, 2005). Lesions and neural recording in thalamus, amygdala, substantia nigra and nucleus accumbens have indicated the involvement of these brain regions in LI as well (Nicholson & Freeman, 2002; Schiller, Zuckerman, & Weiner, 2006; Talk, et al. 2005). LI, therefore, appears to be a broadly distributed neural phenomenon. Specifically, at the neural level there is a reduction in short latency neural activity to the CS across numerous brain regions in preexposed animals as opposed to animals that are naïve to the CS (Talk, et al. 2005). This neural response to CS preexposure supports the notion that the CS can have unconditioned excitatory effects that attenuate during preexposure and result in decreased salience of the stimulus (Kremer, 1972; Rescorla, 1971).

Due to the distributed neural representation of LI it is not surprising that the effect is not reliably reproduced across all experimental paradigms. The most comprehensive models of LI accommodate a variety of LI theories, such as changes in novelty, motivation and attention (Buhusi, et al. 1998; Schmajuk, et al. 1996). These models also accommodate the numerous brain regions implicated in LI, encompassing the hippocampal formation, nucleus accumbens and ventral tegmental area, as well as other cortical and subcortical regions (Buhusi, et al. 1998; Myers, Gluck, & Granger, 1995; Schmajuk, et al. 1996). These models have been successful at capturing a great deal of the variety of findings in the LI literature across paradigms. The task still remains, however, to determine why context preexposure reliably facilitates learning whereas CS preexposure, in the conditioning context, may impair, facilitate or have no effect on learning.

In summary, the present results add to a small yet growing number of LI results suggesting that there is still more to discover about this phenomenon. Specifically, the role of CS and context processing as well as the association formed between the two during preexposure has not been fully elucidated. Furthermore, the interaction of preexposure with the formation of the CS-US association during subsequent conditioning requires more investigation to assess the impact of factors like US intensity or salience on LI. The present results indicate that a better understanding and incorporation of the facilitative effects of context preexposure may shed light on the LI phenomenon and that context processing should be considered when computational and neural models of LI are discussed.
References


