Novel Factors Contributing to the Expression of Latent Inhibition

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Behavioral and neural correlates of latent inhibition (LI) during eyeblink conditioning were studied in 2 experiments. In Experiment 1, rabbits (Oryctolagus cuniculus) were conditioned after 8 days of tone conditioned stimulus (CS) presentations or 8 days of context-alone experience. LI was seen in the CS-preexposed rabbits when a relatively intense (5 psi) airpuff unconditioned stimulus was paired with the CS. In Experiment 2, rabbits were given 0, 4, or 8 days of CS preexposures or context-alone experience. Hippocampal activity was monitored from the 8-day CS- or context-exposure rabbits. The LI effect was seen only in rabbits given 4 days of CS preexposure, thus suggesting that LI depended largely on the rate of acquisition in the context-preexposed control group. The neural recordings showed that the hippocampus was sensitive to the relative novelty of the stimuli and the overall context, regardless of whether exposure to stimuli and context promoted LI.

Latent inhibition (LI) is a well-studied phenomenon whereby nonreinforced preexposure to a conditioned stimulus (CS) retards later learning of a conditioned response (CR) to that CS. This retardation of learning has been reported in a variety of research paradigms, including learned taste aversion (Aguado, Symonds, & Hall, 1994; Purves, Bonardi, & Hall, 1995; Reilly, Harley, & Revusky, 1994), appetitive tone–food pairing (Honey & Good, 1993), conditioned emotional response, conditioned suppression (Clark, Feldon, & Rawlins, 1992; Grahame, Barnet, Gunther, & Miller, 1994; Hall & Minor, 1984; Yee, Feldon, & Rawlins, 1995), and aversive eyeblink conditioning (Salafia & Allan, 1980, 1982; Solomon & Moore, 1975). In eyeblink conditioning, preexposure to the tone CS makes an animal require more trials to reach learning criteria and asymptotic responding than are required by control animals.

Models of LI have, for the most part, attributed the retardation to a failure or delay in the formation of a CS–unconditioned stimulus (US) association, possibly due to a CS–context association formed during preexposure sessions (Mackintosh, 1975; Pearce & Hall, 1980; Wagner, 1981). Alternatively, it has been suggested that LI results from a contextually driven failure of the CS to properly retrieve a normal CS–US association (see, e.g., Grahame et al., 1994; Maren & Holt, 2000). In either case, the hippocampus (or the entorhinal cortex, which serves as an interface between the hippocampus and sensory systems) has been postulated to play a role in the emergence of LI, either by controlling the associability of the CS or by controlling how context interferes with retrieval of learned associations (Weiner, 1990). Several recent formal models have attempted to capture the wide array of findings from a variety of LI paradigms, suggesting that neural activity in the hippocampus represents the “novelty” of the context at the time of stimulus (Bunsey & Eichenbaum, 1993; Gluck & Myers, 1997; Myers & Gluck, 1996). The degree to which such components are novel, compressed, or fused then determines in part the speed with which the CS and US can associate to produce the CR.

Some lesion studies have supported these theories, showing that the hippocampus plays an important role in the production of LI during eyeblink conditioning. For example, Solomon and Moore (1975) demonstrated that ablations of the dorsal hippocampus eliminated the retardation effect in CS-preexposed rabbits compared with rabbits that simply sat in the conditioning context for an amount of time equal to that experienced by the CS-preexposed group. Several years ago, we decided to explore the involvement of the hippocampus in LI by recording from hippocampal pyramidal cells during LI and subsequent paired training. To maximize the chances of producing the LI effect in the CS-exposed group, we gave the rabbits eight sessions of CS preexposures before instituting paired CS–US training (i.e., about twice as much LI training as that normally given, see Table 1). To our surprise, however, we could not consistently produce LI in our rabbits (Katz, Rogers, & Steinmetz, 2000; Katz, Shock, & Steinmetz, 1995; Miller, 1994; Rogers, Katz, Gorin, & Steinmetz, 2001). We introduced a variety of parametric manipulations used in conjunc-
<table>
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<tr>
<th>Effect and study</th>
<th>CS/US</th>
<th>ISI (ms)</th>
<th>Trials</th>
<th>Measure</th>
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<td>Tone (800 Hz, 72 dB) Air (1.45 psi)*</td>
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<td>560 (70/day)</td>
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<td>Summation</td>
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<td>300 (100/day)</td>
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<td>324 (108/day)</td>
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<td>500</td>
<td>300 (100/day)</td>
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Note. CS = conditioned stimulus; US = unconditioned stimulus; ISI = interstimulus interval; CR = conditioned response; avg. = averaged.

*75 mm mercury airpuff intensity.
tion with the 8 days of CS preexposure, including lengthening of the interstimulus interval (ISI) to 500 ms to increase CS saliency, increasing the intertrial interval (ITI) used during training, and delivering the last 50 CS-alone presentations in the same session as the initial paired training. None of these parametric manipulations produced the LI effect.

A summary of earlier studies examining LI in eyeblink conditioning reveals that the vast majority of studies of LI during eyeblink conditioning reported positive results (see Table 1). Besides our attempts, we found only one published study (Suboski, Di Lollo, & Gormezano, 1964) that reported negative results, in spite of a number of procedural differences in all of the studies, including different USs (airpuffs and periorbital shocks), different trial delivery schemes (massed trials vs. multiple whole sessions), and different control groups (preexposed to context vs. no context preexposure).

In this article, we report on two experiments that were designed to help us determine why we failed to observe LI given the apparent robustness of the phenomenon, and examine the activity of hippocampal pyramidal cells during LI (i.e., our original objective in undertaking these studies). In examining Table 1, we noted that the majority of studies that obtained positive LI effects used a shock US instead of the airpuff US used in our lab. Although a 3-psi airpuff is commonly used in rabbit eyeblink conditioning experiments, a 5-psi airpuff (a relatively intense US) may be more similar to shock. Therefore, in the first experiment we decided to look at LI effects in rabbits given eight sessions of CS preexposure that trained with either a 3-psi or a 5-psi airpuff US. In a second experiment, we tested the possibility that the amount of context exposure might affect the amount of LI observed in the CS-preexposed group compared with the standard sit group that is given an equal exposure time to the conditioning context. In this experiment, rabbits were given either 0, 4, or 8 days of preexposure (or 0, 4, or 8 days of context-only exposure).

We also recorded hippocampal activity from rabbits given 8 days of CS preexposure or 8 days of context-only experience. To date, there have been no published reports of hippocampal recordings taken from rabbits given this relatively large number of CS preexposures before paired training. Many previous studies have examined hippocampal activity in rabbits given zero days of CS preexposure (e.g., Berger & Thompson, 1978; Sears & Steinmetz, 1990), and Romano (1999) recorded from rabbits given 4 days of CS preexposures. When considered together with these previous recording studies, the present recordings provide a complete assessment of hippocampal unit activity and behavioral responding in rabbits given 0, 4, and 8 days of CS preexposure or equivalent context-alone experience.

Our results showed that systematic parametric manipulation greatly affected whether or not an LI effect was observed. In the first experiment, training with a 5-psi airpuff led the groups preexposed to the CS to produce fewer CRs than those preexposed only to context; no such differences were seen when 3-psi rabbits were compared. The smaller number of CRs noted in the 5-psi LI rabbits was not due to differences noted on the first few sessions of paired training, however, but rather to lower asymptotic responding in the LI group. In the second experiment, we observed an LI effect only when the rabbits given 4 days of CS preexposure were compared with the rabbits given 4 days of context exposure; this difference seemed to be caused by a relative facilitation in learning noted in the context-preexposed group. All other rabbits appeared to learn at similar rates regardless of whether or not they received LI training or context exposure. Finally, in rabbits given 8 days of preexposure, hippocampal activity appeared to reflect an interaction between the novelty of the stimuli themselves and the novelty of less salient facets of the context and did not seem tightly tied to the magnitude of LI. Together, these results suggest new complexities in our understanding of the interaction between contextual and punctate variables during learning.

Experiment 1

As noted above, our initial attempts to investigate the role of the hippocampus in LI (Katz et al., 1995) met with the same failure as the first such study (Suboski et al., 1964). A variety of parameters, including ISI, ITI, and whether or not a half-session ended the preexposure portion of the experiment, were varied to no effect (Katz, Rogers, and Steinmetz, unpublished observations). We reasoned that a more intense US might increase the speed with which control rabbits learn, thus increasing the magnitude of a preexposed versus control difference early in training. In Experiment 1, we examined this possibility by directly comparing the relative rate at which LI could be produced in rabbits trained with a 3- or 5-psi airpuff US.

Method

Subjects

Twenty-five male New Zealand White rabbits (Oryctolagus cuniculus, −1.75–2.00 kg) obtained from Myrtle’s Rabbitry (Thompson Station, TN) were used throughout behavioral training and testing. Rabbits were individually housed and given free access to food (−150 g/day) and water. All rabbits were maintained on a 12-hr light–dark cycle with experimental procedures occurring during the early phase of the light cycle.

Surgery

All surgeries were performed 7 days prior to behavioral training, under aseptic conditions. Rabbits were anesthetized with a combination of ketamine (60 mg/kg im) and xylazine (6 mg/kg sc) and maintained throughout surgery with 1-cc im injections of a ketamine–xylazine cocktail (2:1 ratio) administered every 45 min. Once anesthetized, the rabbits were placed into a stereotactic head-holder with bregma 1.5 mm above lambda, and two ground screws were placed in the skull over the posterior regions of the cerebral cortex. Finally, a head bolt, used to attach the airpuff–blink detector assembly, was embedded within a dental acrylic cap, the scalp was sutured about the head-stage, and Providine ointment was liberally applied to the wound as a prophylactic treatment for infection. The rabbits were treated with antibiotics (Sulmet) during the weeks prior and subsequent to surgery to minimize risk of infection.

Behavioral Training

The rabbits were placed into standard Plexiglas restraint boxes housed within sound- and light-attenuating chambers and given 2 obligatory days of adaptation (60 min/day) to the restraint. After adaptation, all of the rabbits received eight additional sessions (one session/day) of preexposure. For 13 rabbits, each session consisted of 120 presentations of a 350-ms tone CS (1 kHz, 85 dB SPL) within the experimental context (LI groups). ISIs were randomly chosen from the range of 20–30 s. In total, these rabbits experienced 960 repetitions of the tone CS over preexposure.
training. The remaining 12 rabbits were preexposed to the experimental context only (sit groups). The context preexposure for this group was equivalent in duration to the groups described above (i.e., 60 min/day for 8 days).

Twenty-four hours after the last preexposure session, rabbits were returned to the experimental context to begin a subsequent 8 days (one session/day) of standard delay conditioning. Rabbits from both LI and sit groups were randomly assigned to one of two possible training conditions that differed only with regard to the intensity of the US used (i.e., 3 vs. 5 psi). Training sessions consisted of 108 paired trials in which the 350-ms tone CS coterminated with a 100-ms airpuff US directed at the cornea of the left eye. This resulted in an ISI of 250 ms. ITIs were randomly chosen from the range of 20–30 s. The remaining 12 trials were CS-alone probe trials that occurred on the last trial within each training block of 10 trials. These probe trials are a common method for evaluating the CR by removing the contribution of unconditioned responding.

Eye movements were monitored with an infrared detection system (Thompson, Moyer, Akase, & Disterhoft, 1994) routed to a computer that controlled trial delivery and behavioral data acquisition (Chen & Steinmetz, 1998). On each trial, a 250-ms pre-CS interval was acquired. If eyelid movements of 0.5 mm or greater were detected during this interval, the trial was discarded and was not used in the analysis. The probe trials differed from CS-alone trials only with regard to the period of time that was monitored for conditioned responding. Specifically, on paired and CS-alone trials, a CR was defined as a 0.5 mm or more closure of the eyelid that occurred during the ISI (250 ms post-CS onset). In contrast, CRs produced during probe trials could occur anytime during the 350-ms post-CS-onset period.

**Statistical Analysis**

Initially a $2 \times 2 \times 8$ analysis of variance (ANOVA) was conducted (Group $\times$ US Intensity $\times$ Session). This was followed by two-factor $(2 \times 8)$ ANOVAs with one repeated measure for each US intensity, in which Factor 1 was the type of experience during the preexposure phase (tone + context [LI groups] or context only [sit controls]) and Factor 2 was the number of conditioning sessions. Comparisons of asymptotic responding (in the 5-psi groups) involved an identical ANOVA calculated on the data from the latter half of the training sessions.

**Results**

Figure 1 presents the percentage of CRs for four groups based on the type of preexposure training (i.e., LI or sit) and the US intensity during acquisition training (i.e., 3 or 5 psi). As expected, no differences in responding were seen during the 8 days of preexposure to the tone (LI group) or the context (sit group). As expected (and confirmed with a three-way ANOVA), the rate of learning was faster for rabbits trained with a 5-psi airpuff than for those trained with a 3-psi airpuff, $F(1, 21) = 24.48$, $p < .001$. There was also a Group $\times$ Session interaction, $F(7, 147) = 5.46$, $p < .001$, that was analyzed with post hoc tests and ANOVAs on earlier and later (last four sessions) portions of the data set. Differences in percentage of CRs were only noticeable during the last five paired training sessions where the LI rabbits appeared to reach lower asymptotic responding levels, $F(1, 11) = 7.42$, $p > 0.05$.
.02; neither session nor the interaction achieved significance. Few differences in conditioning were seen when the LI and sit groups that received training with a 3-psi airpuff were compared; in the analysis of these groups, only the effect of session approached significance, F(3, 30) = 2.89, p = .055. If anything, the LI group showed slightly higher rates of learning on the 3rd and 4th days of paired training when compared with the corresponding sit group.

Discussion

In Experiment 1, we replicated our earlier unsuccessful attempts (Katz et al., 1995; Miller, 1994) to produce LI by using 8 days of CS preexposure and a 3-psi airpuff US: There was no detectable difference between the 3-psi LI and sit groups. As measured by overall percentage of CRs, we did observe what could be described as an LI effect when we compared the LI and sit groups that received a 3-psi airpuff US. A lower percentage of CRs was seen in the LI group versus the sit group. However, the effect we observed was, at best, atypical of LI, which normally is manifested as a delay in initial learning. In our 3-psi data set, by contrast, early learning was identical for the LI and sit groups, but rabbits preexposed to the CS eventually reached asymptote at lower CR levels. The Experiment 1 findings are very similar to those reported previously using similar stimulus parameters (Robinson, Port, & Stillwell, 1993), and they may provide an explanation of why many previous eyelid conditioning experiments successfully reported LI effects: The successful earlier LI studies and the present study used either a shock US or a relative intense airpuff US. Also, many studies used percentage of CRs or total numbers of CRs (i.e., measures of overall CR performance across trials) as the measure of LI (e.g., Salafia & Allan, 1980, 1982; Solomon, Brennan, & Moore, 1974; Solomon, Lohr, & Moore, 1974; Solomon & Moore, 1975) instead of (or in addition to) reporting trials to a conditioning criterion (i.e., a measure of rate of initial learning). Differences in asymptotic responding in these previous studies could result in overall differences in the percentage or number of CRs observed and thus could be reported as an LI effect.

At this point, we do not have a clear explanation of why using a more intense US after CS preexposure would cause a lowering of asymptotic responding, except to speculate that at the strong US intensity, preexposure to the context and the CS lowers the salience of the overall testing environment. In essence, the gains in overall strength of conditioning seen when the more intense US is used may be offset by the exposure the subject had to the CS during the eight sessions that preceded paired training.

Experiment 2

Another major difference between earlier LI studies and our early attempts to demonstrate the retardation phenomenon is the number and distribution of CS preexposures that were given before paired training. Frequently, fewer than 500 CS preexposures have been given over 3–5 days. Sometimes, 24 hr was allowed to elapse between the last CS-alone trial and the first paired trial, but often paired training was begun within a training session immediately after a block of CS-alone presentations. Rabbits in our Experiment 1 received 960 CSs over 8 days of preexposure training, ostensibly to maximize the LI effect. We allowed 24 hr between preexposure training and paired training. It is important to keep in mind that this manipulation affected both the LI and sit groups—the more preexposure trials that were given, the longer the sit rabbits remained in the context exposure condition.

Given these considerations, Experiment 2 was designed to systematically look at the LI effect in groups of rabbits given different numbers of CS-exposure or context-exposure sessions. In this experiment, we also examined hippocampal unit activity in rabbits given CS or context exposure. A recent article (Romano, 1999) noted the appearance of robust LI in a paradigm very similar to that used in our laboratory, comparing an LI group that received 4 days of CS preexposure to a control group that received an identical amount of contextual preexposure. Romano (1999) examined multi-unit hippocampal activity in these four-session preexposure groups and reported that CS- and learning-related hippocampal activity was lower in rabbits exposed to the CS than in rabbits exposed to the context. In Experiment 2, we compared hippocampal recordings from groups given eight sessions of CS exposure or eight sessions of context exposure. The Romano (1999) data provided an examination of unit activity in rabbits given 4 days of preexposure; many studies have already examined hippocampal unit activity with zero days of preexposure (see, e.g., Berger & Thompson, 1978; Romano, 1999; Sears & Steinmetz, 1990). Zero days of preexposure constituted a “normal” conditioning group that received no preexposure (save the traditional two sessions of adaptation). Together with previous unit data collected from rabbits given 0 or 4 days of preexposure, the unit data collected from the 8-day preexposure rabbits in the present experiment provide a complete characterization of hippocampal unit responsiveness in rabbits given a wide range of CS preexposures.

Method

Subjects

Thirty-four male New Zealand White rabbits, housed and maintained as described in Experiment 1, served as the subjects of Experiment 2.

Surgery

All rabbits were surgically prepared for behavioral conditioning as described for Experiment 1. Specifically, each rabbit had a head bolt implanted for the purpose of attaching the airpuff–blink assembly. In addition, 18 of the rabbits were prepared for the acute recording of hippocampal single units. During surgery on these rabbits, a 5-mm-diameter hole was drilled in the skull over the left anterior hippocampus and subsequently filled with bone wax. A single-unit recording well was cemented over the hole with dental acrylic and filled with petroleum jelly.

Behavioral Training

The rabbits were placed into standard Plexiglas restraint boxes housed within sound- and light-attenuating chambers and given 2 days of adaptation (60 min/day). After adaptation, all of the rabbits were randomly assigned to one of five possible groups on the basis of the duration and type of preexposure training. Specifically, rabbits received 8, 4, or 0 days of preexposure training (8d, 4d, or 0d). The 8d and 4d rabbits were further assigned to either an LI or a sit condition. Preexposure sessions were identical to those described in Experiment 1.

Twenty-four hours after the last preexposure session, the rabbits were returned to the experimental context to begin a subsequent 8 days (one session/day) of standard delay conditioning. All groups received identical
training sessions, parameters of which were identical to those described in Experiment 1. For all rabbits, the US intensity was 3 psi.

**Neural Recording**

From the eighteen 8d rabbits, single units were recorded from area CA1 of the dorsal hippocampus during the preexposure sessions, as well as on both the 1st day of conditioning and the sessions surrounding the achievement of the learning criterion of eight CRs out of nine trials. Prior to the start of a session, a microdrive was firmly attached to the single-unit well, and a single stainless steel recording electrode (1.5 to 3 MΩ impedance) was lowered into the brain. Proper electrode placement was determined by measurement of the ventral position of the electrode tip and confirmed through observation of typical hippocampal bursting activity. During trial delivery, hippocampal activity was amplified ×10,000 and bandpass filtered between 300 and 3000 Hz before being routed to a computer. Multiple-unit activity was digitally sampled at a rate of 20 kHz for 750 ms during each trial and stored for offline analysis by a microcomputer equipped with commercially available waveform acquisition software and hardware (Spike2, CED, Cambridge, England). Units were typically held through six blocks of training trials, after which the electrode was moved by approximately 100–150 μm, and additional units were collected.

**Statistical Analysis**

Data from Experiment 2 were subjected to a three-factor ANOVA with one repeated measure, in which Factor 1 was the duration of preexposure training (i.e., 0, 4, or 8 days), Factor 2 was the type of experience during the preexposure phase (tone + context [LI groups] or context only [sit controls]), and Factor 3 was the number of conditioning sessions. Hippocampal unit activity, recorded from the 8d rabbits, was analyzed by extracting individual neurons’ waveforms from digitized sessions by means of a template-matching algorithm (Spike2). The responsiveness of each isolated unit was determined by examining its pattern of firing during the 250-ms period immediately after CS onset. To accomplish this, peristimulus histograms of spiking activities during the 350 ms before (the pre-CS period) and the 250 ms after (the CS period) CS onset were summed across the entire session. Next, the histograms were subdivided into eight 50-ms bins: five bins of pre-CS period activity and three bins of CS-period activity. The spike count in each CS-period bin was standardized by t-scores transformation, and significant (p < .05) increases and decreases in spiking in the CS and US periods, compared with the pre-CS baseline period, were determined for each unit (Katz & Steinmetz, 1997).

**Histology**

Electrode placements into area CA1 were confirmed at the completion of training for all rabbits in which neural recordings were made. Small electrolytic lesions were made after the final recording session by passing 100 μA constant current through the recording electrodes for 1 s. Each rabbit was then overdosed with an intravenous injection of pentobarbital (4 cc) and transcardially perfused with 0.9% (wt/vol) saline followed by 10% (wt/vol) Formalin. The brains were removed, placed in a Formalin–sucrose solution for at least 10 days, and subsequently blocked in albumin–gelatin. Frozen coronal sections (80 μm) were taken through the electrode track and lesion sites. The sections were then stained for ferrous deposits (Prussian blue) and cell bodies (cresyl violet), and coverslipped.

**Results**

**Behavioral Results**

Figure 2A presents the percent conditioned response for both the preexposure and acquisition phases of conditioning. The data are further organized into the five groups based on the type (sit vs. LI) and duration (0, 4, or 8 days) of preexposure training. As is evident from the figure, there was an overall learning effect of session,

$$F(7, 203) = 65.56, p < .001$$

The overall effect of preexposure type (LI vs. sit) was also significant,

$$F(1, 29) = 13.75, p < .01$$

but the overall effect of preexposure duration failed to reach significance. The highest-level significant effect was the three-way Session × Preexposure Type × Preexposure Duration interaction,

$$F(7, 203) = 2.16, p < .05$$

This effect suggests that the LI effect (the difference in learning rate between sit and LI groups) was not equivalent across preexposure duration groups. The most obvious source of this effect is the swift learning of the sit 4d group and the impaired late performance of the LI 4d group: These facts translate into a strong LI effect after 4 days of preexposure, compared with virtually none after 8 days of preexposure.

Individual means were compared for each group at each session by means of the Bonferroni correction. The results indicate that sit 4d rabbits exhibited significantly higher rates of conditioned responding than LI 4d rabbits on all sessions except for Sessions 1 and 3 (p < .001–.002), and exhibited higher responding compared with all other groups, including those rabbits that did not receive preexposure (i.e., sit 0d) on Session 2 (p < .001). The main portion of Figure 2B extracts the learning curves for the sit 0d, sit 4d, and sit 8d groups; the overall rate of responding is shown in the inset. Four days of preexposure to context increased the learning (and overall response) rate compared with both days 0 and 8 days ("normal" conditioning) of preexposure.

In summary, the retardation in learning that is associated with the LI effect was only identified in rabbits that received 4 days of preexposure; this retardation appears to be largely a consequence of the rapid rate of learning exhibited by the context-only preexposed control group (i.e., sit 4d).

**Hippocampal Activity in 8d Rabbits**

**Electrode placements.** In our analyses of hippocampal unit activity, we included only those recording sites that were verified by histological reconstruction as being located in the CA1 pyramidal cell layer of the hippocampus. Great care was taken to verify electrode placement because, as described below, we failed to see the characteristic learning-related activity in the CA1 population during paired training that has been reported often in past studies (e.g., Berger & Thompson, 1978). Figure 3 shows a CA1 recording site together with a sample of raw unit activity recorded from the population of pyramidal cells at that site.

In total, 73 neurons were isolated during paired training. Units were recorded from a subset of LI rabbits on both the 1st day of training (n = 20 units) and the day on which the learning criterion was met for each rabbit (n = 14 units). Additional units were recorded from a subset of sit rabbits on both the 1st day of training (n = 22 units) and criterion day (n = 17 units). Analysis of observed firing patterns is presented by day of training to facilitate direct comparisons between the groups. As the number of significance tests was high, lengthy discussion centers only on firing rate modulations that persisted for at least two of three consecutive time bins or appeared in a large percentage of cells.

**Recording during Li and sit training.** In our preliminary studies (e.g., Katz et al., 1995) we never observed systematic changes in unit activity during the CS- or context-preexposure sessions.
presented to the LI and sit groups. In these preliminary studies, we first examined whether or not the spiking rates of neurons changed over 4–8 days of context exposure in sit rabbits. Not surprisingly, changes in the baseline spiking rates of neurons were never seen when the sit data were analyzed. In these preliminary studies, we also examined whether changes in hippocampal unit activity could be seen during the preexposure phase of training in rabbits in the LI group. Analysis of multiple-unit activity during this training phase in the LI group revealed no significant changes in baseline firing rates of CS-period unit responsiveness over the 8 days of CS preexposure.

Given the negative results of these preliminary studies, we decided to record during the CS preexposure training phase of only a subset of rabbits from the present 8d LI group. A total of 103

Figure 2. A: Mean (± SEM) percentage of conditioned responses (CRs) recorded from rabbits given 0, 4, or 8 days (d) of conditioned stimulus + context preexposure (latent inhibition [LI] groups) or context-alone preexposure (sit groups). B: Mean (± SEM) percentage of CRs recorded from the three sit groups that were included in Panel A. The insert shows the mean number of CRs recorded for the three sit groups.
single units were recorded from these 5 rabbits across the 8 days of preexposure training. No changes in baseline firing rates in the LI rabbits were found. With the exception of a few isolated bins in which random increases in spiking were observed, no systematic changes in CS-period unit firings were seen over the course of preexposure training. For the sit group, these data are in agreement with the results reported by Romano (1999). However, Romano reported a slight decrement in hippocampal responsiveness across the 4 days of CS preexposure given to LI rabbits, something we did not observe. These data indicate a lack of systematic changes in hippocampal responsiveness during the preexposure phase of LI training.

Conditioning Session 1. Neurons recorded from LI 8d rabbits during the first session of training (a session that generally preceded substantial production of CRs) showed little modulations of firing rate in the CS period (that is, the 250 ms between tone and airpuff onset). Only 25% of the neurons generated significant activity in more than one bin; all but one of the modulations were excitatory. In summary, very little conditioning-related activity was found during this session. In the US period, however, LI 8d hippocampal neurons were more active, with 45% demonstrating substantial US response (at least two of the first three US period bins); all but one of these neurons showed firing rate increases. The hippocampus of a rabbit preexposed to a tone CS appears to primarily code US presentation in the first session of training.

Figure 4A shows some examples of patterns of unit activity recorded from LI and sit rabbits in Session 1. Session 1 hippocampal activity in the sit 8d rabbits was far greater than that observed in LI 8d rabbits. The vast majority of recorded neurons (83%) proved to be modulated in some way. The firing rate of 35% of the neurons increased early in the CS period. Another 23% showed significantly reduced firing rates during the CS period. As was true in LI 8d rabbits, inhibitory modulations seen in this sample typically occurred later in the CS period than excitatory modulations. Overall, sit 8d neurons appeared to code both stimuli in Session 1.

Figure 5A presents a summary of these data, showing mean (± SEM) absolute standard scores of neural activity in each time bin. The difference between groups in the CS period, and the elevated

Figure 3. A: Brain section showing a Prussian-blue stained indication of the location of a recording electrode from a sit rabbit used in Experiment 2. B: Brain section showing two recording sites from a latent inhibition rabbit used in Experiment 2. C: Representative behavioral response (top) and trace of multiple-unit activity (bottom) recorded from the left electrode site shown in Panel B for a paired conditioned stimulus–unconditioned stimulus (CS-US) trial. The vertical lines mark CS and US onsets.
Learning criterion session. Hippocampal single-unit firing patterns recorded from both LI 8d and sit 8d rabbits showed little significant activity during the session in which learning curves were at their steepest (see Figure 4B for some examples). The lack of recorded activity modulations was particularly striking in light of the fact that this is generally the period of maximal hippocampal coding of eyeblink conditioning in rabbits not given preexposure training (i.e., the 0d groups). In the LI 8d sample, only 21% cells were more than phasically modulated in the CS period, and the number of significant bins was spread fairly evenly across the ISI. The neurons that did show substantial modulation consisted entirely of firing rate decreases for brief periods in the latter half of the CS period. The percentage of neurons showing substantial modulation in the US period was highly similar, with the only difference being that the majority of these were US-responsive.
consecutive trials.

session, defined as the first time eight CRs were observed on any nine activity. A: Neural activity recorded on the first session after CS and/or – bins (150 ms) of the US period (US-1 unconditioned stimulus (CS – in inhibition (solid lines) rabbits during the conditioned stimulus

Figure 5. Mean (± SEM) standard scores of neural activity recorded from the CA1 layer of the hippocampus of sit (dashed lines) and latent inhibition (solid lines) rabbits during the conditioned stimulus–unconditioned stimulus (CS–US) period (CS–1–CS–5) and the first three bins (150 ms) of the US period (US–1–US–3). All bins represent 50 ms of activity. A: Neural activity recorded on the first session after CS and/or context preexposure. B: Neural activity recorded on the training criterion session, defined as the first time eight CRs were observed on any nine consecutive trials.

 increases in firing rate, similar to (but significantly less strong than) those seen in Session 1. In rabbits preexposed to the tone CS, the hippocampus appears to have done nothing during the criterion session.

Only 12% of the neurons recorded from sit 8d rabbits during criterion sessions showed substantial changes in firing rate. Figure 5B summarizes these results, again showing the mean absolute standard scores for each group. The scores are uniformly low and similar for the two groups. No significant effects were found in either CS- or US-period ANOVAs.

Discussion

Our behavioral data are quite clear: Four sessions of preexposure induced LI, whereas eight sessions of preexposure did not. In fact, the sit 4d group learned more quickly than the group that received no preexposure (the 0d group). This fact, surprising upon initial examination of the data, makes a certain sense when it is remembered that even the 0d group received 2 days of adaptation that was identical to subsequent contextual preexposure sessions. Although it has not been systematically studied, most researchers who conduct eyeblink conditioning experiments have observed that a certain amount of preexposure to the testing context leads to more rapid learning. More surprising is the simple fact that further contextual preexposure sessions (in this case, 8 days) counteract the felicitous effect of the 4-day preexposure. How this inverted U-shaped function of contextual preexposure might fit into a theory of LI will be taken up in the General Discussion section. But whatever the exact reason, examining 0d, 4d, and 8d preexposures in the same experiment allowed us to demonstrate a very interesting effect of contextual preexposures, and of LI in general: The latent inhibition effect produced here was actually a facilitation of learning as a result of experience with the context.

Under normal circumstances, (i.e., in 0d rabbits that receive no preexposure training) the hippocampus codes both stimuli early in training and comes to model the learned response during the criterion session (Moyer, Thompson, & Disterhoft, 1996; Sears & Steinmetz, 1990). Romano (1999) has recently shown that hippocampal multiple-unit clusters respond during the CS-period during early paired training of sit rabbits, and less strongly during early training of LI rabbits; he concluded that the hippocampus was signaling the associability of the CS to the eyeblink conditioning system, thus playing a major role in the induction of LI. Here, however, we have shown that hippocampal single-neuron CS-period responses are stronger in sit rabbits than in LI rabbits, even under conditions in which no LI is induced (i.e., in the 8d groups). The hippocampal neurons appeared to respond to whatever stimuli were novel in the lengthily preexposed context. Furthermore, in this situation there was no evidence of the traditionally observed hippocampal activity at the time of fastest learning.

It should be noted, however, that learning-related activity in the hippocampus is somewhat transient. For example, Sears and Steinmetz (1990) reported that learning-related activity in the hippocampus increased over the first 3–4 days of paired training, then steadily declined to baseline levels over an additional 5–8 days of training. The learning-related activity could be reinstated when training was shifted from one eye to the other, but the activity eventually decreased to baseline levels with additional paired training. Even though stimulus or learning-related activity was not seen during the preexposure phase of the present experiment, it is possible that the transient nature of hippocampal excitability explains the lack of learning-related activity seen in the CS-exposed rabbits during paired training. In other words, after 3–4 days of CS-alone presentations, the capacity or potential for plasticity in the hippocampus declines to baseline levels, and further increases in learning-related activity are not seen during paired training. The transient nature of learning-related activity in the hippocampus may also explain the recording data of the sit group: After 8 days of contextual experience, a very rapid increase in CS-period activity was seen on the 1st day of paired training, after which time hippocampal activity diminished over the next several sessions. Thus, when behavioral responding was at asymptotic levels, because of the transient nature of hippocampal excitability changes,
learning-related activity in the hippocampus was already back to pretraining, baseline levels.

General Discussion

The findings of these experiments can be summarized as follows:

1. Latent inhibition fails to emerge when rabbits are given 8 days of preexposure to a tone CS and a 3-psi airpuff is used as a US.
2. Differences between rabbits preexposed to stimuli and context do appear when the US intensity is raised to 5 psi, but the differences have to do with asymptotic responding, and not with initial learning rate.
3. We replicated the behavioral findings of Romano (1999) and others (e.g., Solomon & Moore, 1975), in that LI did appear after 4 days of preexposure, but our data suggest that this effect has to do with facilitation of learning in the group preexposed to the context, rather than with slowing of the LI group’s learning.
4. The hippocampus responded to novel stimuli in early training sessions after 8 days of preexposure, despite the fact that this preparation did not support LI.
5. After 8 days of preexposure, the hippocampus was not responsive during the peak of learning, a clear departure from previous studies involving rabbits that were not preexposed to the CS and/or training context.

Complexities in the Production of LI

These data suggest, first and foremost, that LI is not a simple matter of experience with the experimental stimuli; that is, it is not simply the case that manipulating the salience or frequency of the stimuli causes linearly related changes in LI magnitude. It appears, rather, that experience with contextual variables plays a large role in determining LI magnitude, and that this experience may interact somehow with processing of the CS and US. As such, our data are consistent with work suggesting that LI is a phenomenon of contextual retrieval (Grahame et al., 1994; Maren & Holt, 2000)—that the associations of the context with itself, with the stimuli, and with irrelevance enable or impede the retrieval of appropriately made CS–US associations. Put another way, it is possible that the early-in-training development of CS–US associations proceeded normally in all of our subject groups, but that the behavioral index of this development was masked by the effect of experience with other variables.

Further, the data from Experiment 2 suggest that the LI effect may be more a product of a failure of the LI group to show the facilitation in learning that is normally seen in the sit group. In Experiment 2, only the 4d sit group differed significantly from the other groups, and this was because this group’s rate of acquisition was extremely rapid. Indeed, it appears that the retardation effect associated with LI was actually a failure-to-facilitate effect.

Our data are, at least in part, consistent with several recent models, including those of Schmajuk, Gray, and colleagues (Schmajuk, Gray, & Lam, 1996) and of Gluck and Myers (1997). Schmajuk et al. suggested that a vital link in the LI-generating process is the computation of overall novelty, which is proportional to the sum of mismatches between predicted and experienced occurrences of the CS, the US, and the training context (see Figure 2 in Buhusi et al., 1998). According to this model, LI occurs because CS preexposure reduces novelty, thereby reducing attention to the CS during training and retarding conditioning. The Myers and Gluck model takes a different approach, suggesting that the internal representations of stimuli that co-occur to similar impact—in the case of the LI groups, the representations of CS and context—become compressed into unified compounds. LI then occurs because, for LI rabbits, the CS representation must be reindividuated from the compressed compound before it can be associated with the US representation.

Both models can explain our 4d data, although both have a certain degree of difficulty explaining why the effect appears to be a latent enhancement of the sit group’s performance (both predict that preexposure to the CS should slow learning compared with that of any non-preexposed group). Both have more trouble explaining the overall pattern, wherein LI appears as the preexposure period is lengthened from 0 to 4 days and then disappears as it lengthens to 8. Perhaps the data reveal a floor effect in associability (easily conceptualized as minimum novelty because contextual novelty is specifically proposed to have an impact on CS processing, but perhaps alternatively conceptualized as maximum compression) within the overall paradigm, reached after 8 days of preexposure to the context or after 4 days of preexposure to both ambient context and CS.

Most likely, the nonlinear effect of changing from 0 to 4 to 8 days of preexposure reflects the action of (at least) two separate processes, both related to the rabbits’ ability to process the stimuli. It seems reasonable to propose that as rabbits in the sit group become adapted to the context, decreases in the rabbits’ restraint-related stress (reductions in the novelty of the context, or the progressive compression of contextual elements) may increase their ability to focus on the more salient stimuli presented by the experimenters. Only after this process of increasing attention has progressed to some “asymptote” would the process of novelty reduction of the stimuli (or of compression of the stimulus representations) be expected to have a noticeable impact.

Hippocampal Involvement in LI

Neurobiological theories of learning and memory have implicated the hippocampus in the process (or processes) thought to produce LI (Weiner, 1990). The hippocampus, it has been suggested, gates the associability of the CS in the conditioning situation; information regarding a US in which an animal has been preexposed is transmitted by the limbic portion of the memory systems only poorly, and thus is less potent for the purposes of participating in plastic processes. In support of this theory, numerous studies have reported the elimination of LI after aspiration lesions of the hippocampus. More than one study, in fact, has demonstrated that aspiration lesions of the hippocampus eliminate LI in the eyetrack conditioning paradigm (Schmajuk et al., 1996; Solomon & Moore, 1975). Such results make a strong case for hippocampal involvement in LI.

Attempts at replication using excitotoxins have challenged the above conclusion, however. Far from eliminating LI, excitotoxic hippocampal lesions have often spared, enhanced, or even decontextualized LI. Honey and Good (1993) have suggested that the hippocampus’ role in the conditioning setting has more to do with the retrieval of context than with stimulus associability (see also
Maren & Holt, 2000), leaving open the possibility that LI depends on cortical regions that are typically damaged in the process of creating hippocampal aspiration lesions (but see Han, Gallagher, & Holland, 1995).

Buhusi et al. (1998) have attempted to use the Schmajuk et al. (1996) model to explain the above bewildering array of results. Specifically, they suggested that the total amount of preexposure influences exactly how the hippocampus affects LI, by changing the direction of hippocampectomy’s effect on novelty. This model, and its success at explaining seemingly contradictory effects of hippocampal lesions, suggests that the hippocampus contributes to the calculation of a testing situation’s novelty, but that it does not control or determine that calculation. Such a conception is consistent with our findings. The hippocampal neurons in our sample coded the status of the CS and US, but these codes were only a part of the novelty measurement that controlled the occurrence (or, in this case, the lack of occurrence) of LI. It is possible, as suggested by Buhusi et al. (1998), that the output of the hippocampus is routed to a downstream site at which an overall novelty measurement is calculated, and that this value is used to determine LI. Certainly, our results suggest that the hippocampus’ failure to transmit information concerning stimuli to which the animal has been preexposed does not, in and of itself, result in LI.

Recent data (Shohamy, Allen, & Gluck, 2000; Yee et al., 1995) have suggested that the role previously attributed to the hippocampus during LI is actually performed by the entorhinal cortex, which serves as a portal through which input to and output from the hippocampus must flow. Our data certainly support the view that some area other than the hippocampus is responsible for representing the important differences between LI and sit groups; between-group differences in hippocampal activity do not appear to depend on whether LI appears. Perhaps the entorhinal cortex effectively determines whether hippocampal processing will be used to change the rate of conditioning.

The fact that 8 days of preexposure (and, to some extent, 4 days of preexposure, see Romano, 1999) eliminated the normal tendency of the hippocampal neurons to become modulated during peak learning sessions (Katz, Shock, & Steinmetz, 1995; Sears & Steinmetz, 1990) provides further evidence that the hippocampus is neither coding delay eyeblink conditioning itself nor reflecting learning-related activity in the cerebellum or elsewhere in the brain (see Berthier & Moore, 1990; Gould & Steinmetz, 1996; Sears & Steinmetz, 1990). This basic idea is supported by recordings made during eyeblink classical discrimination–reversal conditioning, a training procedure that also appears to involve the hippocampus (Miller & Steinmetz, 1997). In that study, CR-related hippocampal unit activity was not observed on the 1st day of reversal training, even though the number of behavioral CRs were very high on both CS+ and CS− trials. This result may be unsurprising given the fact that hippocampectomy does not impair delay conditioning (Schmaltz & Theios, 1972) and suggests that perhaps, when the novelty of the testing situation is brought to its floor level, the hippocampus no longer processes the association between CS and US. This idea is compatible with previous observations of the transient nature of hippocampal excitability during eyeblink conditioning (e.g., Sears & Steinmetz, 1990). Alternatively, perhaps the hippocampus, during delay eyeblink conditioning, is specifically sensitive to combinations of contextual factors (consistent with influential theories of hippocampal memory, see Eichenbaum, Otto, & Cohen, 1994), such that it codes shifts in these combinations, with more “surprising” shifts leading to larger signals. These shifts could then be used for more complicated calculations that enter into determination of associability and retrievability. Our data, however, argue that the factors that determine the “surprisedness” of a shift in context are somewhat complicated: For example, 4 days of contextual processing leads to more notable shifts than 8 days of contextual processing, perhaps because the novelty function for context is not linear in nature.

In summary, the present data demonstrate that the LI inhibition phenomenon is far from the simple effect that is often portrayed in the literature. There appears to be a fascinating interaction between CS, context, and US that determines the ultimate rate and level of conditioning reached after preexposure trials. Further, our behavioral and recording results are in agreement with Romano (1999) and suggest that the hippocampus is involved in encoding these interactions, rather than only simple CS–US associations. Further studies are needed to delineate more precisely the role of this structure in producing the LI effect.

References


