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Research report

Variations in CS associability and multiple unit hippocampal activity in the rabbit

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Abstract

Hippocampal multiple unit activity was recorded in rabbits during each of four preacquisition treatments and during subsequent classical conditioning of the nictitating membrane response. Three preexposure conditions were employed: CS alone presentations, presentations of the CS paired with a second, neutral stimulus, or unpaired presentations of the CS and second stimulus. It was predicted that (a) CS alone preexposures would produce a decrease in hippocampal activity and a retarded rate of subsequent conditioned response (CR) acquisition and (b) the magnitude of both effects would be attenuated by preexposures of the CS paired with a second stimulus. The results partially supported both predictions. Hippocampal activity was inhibited during CS alone preexposures and that inhibition was attenuated by pairing the CS with a second, neutral stimulus. Behaviorally, all of the preexposure groups showed equivalent, retarded rates of acquisition compared to a nonpreexposed control group. Hippocampal activity throughout acquisition was significantly greater in the nonpreexposed group compared to the group preexposed to the CS alone. Hippocampal activity of the other two groups was intermediate between the nonpreexposed and the CS alone groups. It is suggested that alterations in the magnitude of hippocampal activity may provide a reliable, neuronal correlate of CS associability changes.

Keywords: Latent inhibition; CS associability; Nictitating membrane; Attention; Classical conditioning; Hippocampus

1. Introduction

Latent inhibition is a term coined by Lubow and Moore [20] in reference to the retarded rate of acquisition following multiple, nonreinforced preexposures of a conditioned stimulus (CS). Most theoretical accounts of Pavlovian conditioning attribute the phenomenon of latent inhibition to a decrease in the associability of the CS during the preexposure phase [14,22,23,26,32,33].

Several investigators have noted that the latent inhibition effect can be attenuated if, during the preexposure phase, the CS is paired with a second, relatively neutral stimulus [19,21,39]. Lubow and colleagues [18,22] proposed a conditioned attention theory to account for these results. They hypothesized that the first presentation of a stimulus elicits an attentional response and that subsequent stimulus presentations lead to a decrease in the magnitude of that response. The magnitude of the attentional response is directly related to stimulus associability such that increases in attention to the stimulus enhance associability and decreases in attention reduce stimulus associability. Lubow and colleagues further specified that attention to a stimulus, and therefore, stimulus associability, may be temporarily increased by pairing it with a second attention-eliciting stimulus. As a result of these pairings, conditioned attention would temporarily accrue to the first stimulus of the pair, S_1, until attention to the second stimulus, S_2, begins to wane. Eventually, attention to both stimuli would decline to a minimum. According to the theory, latent inhibition was attenuated in the Lubow et al. [19,21] and Szakmary [39] studies because pairings of S_1

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with an unconditioned stimulus (US) were instituted before the decline in attention to the S_1-S_2 pair reached asymptote during the preexposure phase.

Several limbic system structures have been implicated in the latent inhibition effect. Hippocampal lesions have been shown to abolish the latent inhibition effect in both rats [1,13,16,31] and rabbits [36]. Latent inhibition is also abolished in rats following lesions of nucleus accumbens [40] and by chemical stimulation of the nucleus accumbens with the indirect dopamine agonist, amphetamine [37]. Because nucleus accumbens is known to receive hippocampal afferents via the subiculum in both rats [17] and rabbits [3,9], Weiner [42] suggested a critical neuroanatomical circuit for latent inhibition involving the hippocampus, subiculum and nucleus accumbens. Based on the attentional-associative models of hippocampal function proposed by Schmajuk and Moore [32,33], Weiner suggested that during the conditioning stage of the latent inhibition procedure, the hippocampus assigns the CS a low associability value by averaging the past associability value of the CS with the present CS-US associability value. Because the hippocampus determined CS associability to be low during the preexposure stage, the average value during conditioning will continue to be low and the hippocampus will generate an inhibitory signal which is projected to the nucleus accumbens via the subiculum-accumbens pathway.

If the hippocampus does indeed integrate CS associability over time [32,33] and relay inhibitory signals to nucleus accumbens when CS associability is low [42], then the electrophysiological activity of the hippocampus would be expected to decline as a consequence of CS preexposures. There is some indirect evidence in support of this hypothesis. Vinogradova [41] found that stimulus-evoked, single-unit activity of hippocampal pyramidal cells in the rabbit tended to habituate after 8-20 stimulus presentations. Best and Best [6] reported that rats preexposed to a CS showed less hippocampal responsiveness to the CS during subsequent CS-US pairings than rats who were not preexposed. Although Best and Best did not report any behavioral measure of conditioning, their results and those of Vinogradova suggest that the decline in CS associability assumed to underlie the latent inhibition effect may be indexed by a decrease in hippocampal responsiveness to the CS. The present experiment assesses this possibility in greater detail. Hippocampal activity was monitored during various preacquisition treatments and during subsequent conditioning of the rabbit's nictitating membrane (NM) response. A number of studies have shown that multiple-unit hippocampal activity in the rabbit is highly correlated with acquisition of the classically conditioned NM response [2,4,15,34] but how hippocampal activity is affected by CS preexposures in this preparation is an open question. The preacquisition treatments used in the present study were similar to those used by Lubow et al. [21]. Briefly, three CS preexposure conditions and one nonpreexposure condition were employed. The CS preexposure conditions consisted of either CS alone presentations, presentations of the CS paired with a second, neutral stimulus, or unpaired presentations of the CS and second stimulus. On the basis of conditioned attention theory [18,22] and the model of CS associability changes proposed by Pearce and Hall [27] it was predicted that (a) CS alone preexposures would result in less conditioned responding during acquisition than nonpreexposures and (b) CS alone preexposures would produce less conditioned responding than preexposures of the CS paired with a second stimulus. Because the hippocampus appears to be involved in processing declines in CS associability [1,6,13,16,31–33,36,42], similar predictions were made with regard to hippocampal activity. Specifically, it was predicted that (a) CS alone preexposures would produce less hippocampal responsiveness to the CS than nonpreexposures and (b) CS alone preexposures would produce less hippocampal responsiveness than preexposures of the CS paired with a second stimulus. Finally, unpaired presentations of the CS and second stimulus were used to determine if some factor other than temporal contiguity between the two stimuli might contribute to the latter, predicted difference.

2. Materials and methods

2.1. Subjects

Thirty-six New Zealand White rabbits of both sexes were obtained from a licensed, local supplier. Rabbits were individually housed and maintained on ad lib food and water. The colony room was illuminated according to a 12/12-h light/dark cycle. All experimentation took place during the light portion of the cycle. The rabbits' weights at the start of the experiment ranged between 2.0 and 2.8 kg.

2.2. Surgery

The rabbits were anesthetized with halothane (Fluothane) gas. After anesthesia was induced, the animal was placed in a Kopf rabbit stereotaxic headholder such that the bony landmark lambda was 1.5 mm inferior to bregma. Anesthesia was maintained throughout the surgical procedure with the aid of a specially constructed mask [25]. A chronic electrode fashioned from an insulated stainless-steel 00 insect pin and having a tip diameter of 10 μm, a tip exposure of 50 μm, and a tip resistance of 10–30k [26] was targeted for the CA1 layer of the right hippocampus. Each electrode was positioned 4.5 mm posterior and 5.5 mm

lateral to bregma. Electrophysiological activity was monitored as the electrode was lowered to the pyramidal cell layer of the hippocampus. On average, electrodes were positioned 3.7 mm (range: 2.7–4.5) ventral to the overlying dural surface. The electrode was fixed in place with dental cement anchored by skull screws. One skull screw served as a reference electrode. Wires from both the reference and active electrodes were attached to a plastic plug. The plug and a small bolt were fixed in place with denture material and allowed to protrude through the wound. Nitrofurazone powder (Furacin) was applied before closing the wound with suture.

2.3. Apparatus

The conditioning chamber consisted of a deactivated, ventilated refrigerator shell fitted with a copper-screened inner chamber which provided electrical isolation for neural recordings. The copper chamber was dimly illuminated by an externally mounted 15 W lightbulb. Two speakers were mounted above the chamber, directly over the animal's head. One speaker delivered a constant 65 dB (C) white masking noise while the other speaker was used to deliver a 1 kHz, 85 dB (C) tone CS. Tone duration was always 600 ms. The US consisted of a 100 ms puff of compressed air directed at the cornea of the right eye from a distance of about 5 mm. The corneal airpuff was pressurized to 210 g/cm^2 and produced a noise of 82 dB (C). The same airpuff, when directed away from the eye, was used as a neutral, auditory stimulus for part of the experiment.

2.4. Procedure

Three-to-five days after surgery, rabbits were prepared for NM conditioning. The NM conditioning preparation is described in detail elsewhere [12,26]. Briefly, a small nylon loop was sutured to the right NM and tailor hooks were used to hold the eye open. A minitorque potentiometer was mounted on the bolt affixed to the animal's skull. A small hook was slipped through the NM suture and connected by a thread to a counterweighted arm attached to the shaft of the potentiometer. NM movements were transduced to changes in a dc voltage applied across the potentiometer. After each animal was prepared for NM conditioning, it was placed in the conditioning chamber for a period of 100 min. During this initial adaptation session, all stimulating and recording devices were attached to the animal but no stimuli were administered nor data recorded.

2.4.1. Preacquisition

The first stage of training began on the day following the adaptation session and lasted for three sessions. Each of four groups experienced different preacquisition conditions. One group (SIT, $n = 9$) sat in the chamber with all devices attached while 100 dummy trials were presented at an intertrial interval (ITI) of 60 s. Data were recorded on these dummy trials even though no stimuli were presented. A second group (LI, $n = 10$) was presented with 100 nonreinforced tone CSs at an ITI of 60 s. The third group (CS + N, $n = 8$) received 100 paired presentations of the CS and the airpuff noise at a 60 s ITI. The noise overlapped the last 100 ms of the CS yielding an interstimulus interval (ISI) of 500 ms. Finally, the fourth group (CS/N, $n = 9$) received 100 presentations of the CS and 100 presentations of the noise at an ITI of 30 s. CS and noise trials were presented in a quasi-random order with the restriction that no more than two consecutive trials of the same type could occur in a block of 10 trials. The same order was repeated for each trial block.

2.4.2. Acquisition

The second stage of training began on the day following completion of the first stage. All four groups received standard acquisition training. On each of 3 days, 100 pairings of the tone CS and corneal airpuff US were presented at an ITI of 60 s. The airpuff overlapped the last 100 ms of the tone yielding an ISI of 500 ms.

2.5. Data acquisition and analysis

An Apple II/FIRST system [30] interfaced with solid-state devices and relays was used to control stimulus timing and delivery as well as on-line acquisition of behavioral data and off-line analysis of both behavioral and neural data [29]. Hippocampal multiple unit activity was amplified with a battery-powered, solid-state FET amplifier [7] and recorded on magnetic tape. Neural activity was band-pass filtered (Krohn-Hite 3103A) between 5 Hz and 5 kHz before being fed to a spike-height discriminator (Mentor N-750). The output of the discriminator was recorded on a second channel of the tape recorder for subsequent spike counting. Computer-generated timing signals marking the onset of each trial were recorded on a third channel.

NM movements were digitized every 5 ms during the trial and neural spike counts were cumulated in 5 ms time bins. Each trial was segmented into three 500 ms time periods designated as a pre-CS period, a CS period, and a US period. Behavioral and neural data were blocked over 10 trials (20 trials for CS/N preacquisition data). Conditioning was indexed as the percentage of conditioned responding. A conditioned response (CR) was defined as an extension of the NM in excess of 0.5 mm occurring during the CS-US interval.

Standard scores of hippocampal activity during the CS and US periods were computed using the proce-

dures outlined by Berger and Thompson [4]. The window discriminator was set to produce 4–20 pre-CS counts averaged over an entire session. For each block of trials, the mean number of counts in the pre-CS period was subtracted from the mean number of counts in each of the other trial periods. Each difference was then divided by the standard error of the entire session's pre-CS period counts. During preacquisition for CS/N animals, CS period standard scores were computed only during tone presentations and US period standard scores were computed only during noise presentations.

2.6. Histological procedure

Shortly after each animal's last training session, an electrolytic lesion was made by passing 100 μA from a constant-current source through the recording electrode for 1 s. Each animal was then overdosed with sodium pentobarbital (Nembutal) and perfused through the carotid arteries with normal saline followed by a 10% formalin solution. Frozen sections were taken through the electrode track and lesion site at 50 μm intervals. The sections were stained with cresyl violet for optimal viewing of the hippocampal pyramidal cell layer.

Inspection of the electrode track and/or gliosis left by the lesion indicated that 25 of the 36 placements were in or contiguous with the CA1 pyramidal cell layer. The remainder were located in stratum radiatum, just proximal to the CA1 pyramidal layer.

3. Results

3.1. Preacquisition-behavioral data

All four groups exhibited some degree of nonassociative responding during the preacquisition stage (data not shown). The percentage of nonassociative responses was computed separately for the CS and US periods. In general, nonassociative responding during either period was minimal. Thus, during the CS period, no group ever responded on more than 4% of the trials in any trial block. Nonassociative responding was somewhat higher during the US period although no group ever responded on more than 12% of the trials in a trial block. A $4 \times 3 \times 10$ (Group × Session × Trial Block) mixed analysis of variance determined that there were no significant group main or interaction effects during either the CS or US periods. Thus, any group differences in hippocampal activity can be attributed to the differential treatments per se rather than to differences in levels of nonassociative responding.

3.2. Preacquisition-neural data

Standard scores of CS period activity for each preacquisition session are plotted in Fig. 1. Mean hippocampal activity averaged over all three sessions showed little deviation from baseline in the SIT group ($M = 0.10$), decreased slightly in group CS + N ($M = -$

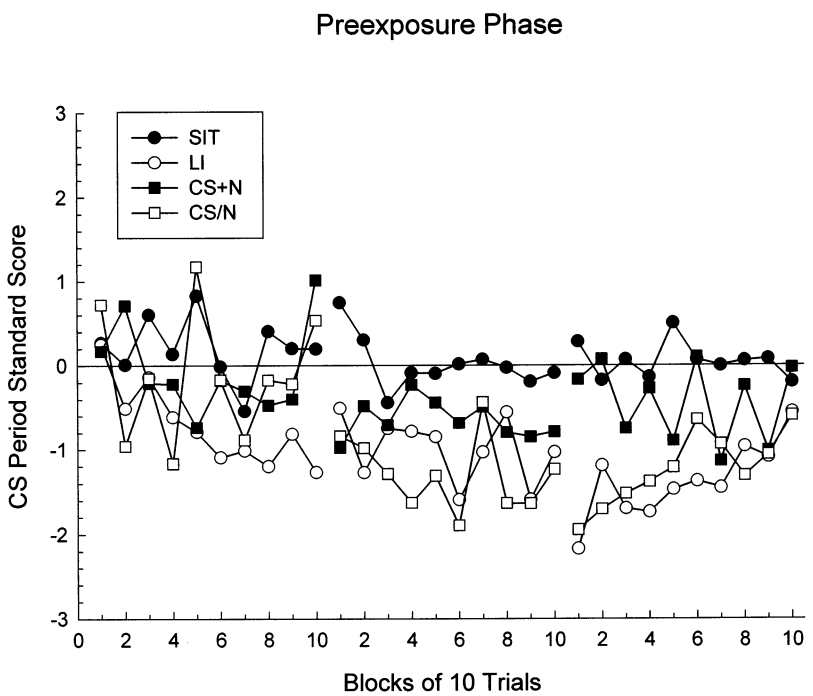

Fig. 1. CS period hippocampal activity for each group expressed as standard scores and averaged over trial blocks for each of three preexposure sessions. Group abbreviations refer to preexposure treatments. SIT animals ($n = 9$) were the nonpreexposed control group. LI animals ($n = 10$) received 100 daily CS-alone preexposures. CS + N animals ($n = 8$) were presented with 100 daily preexposures of the CS paired with a noise. CS/N animals ($n = 9$) were presented with 100 explicitly unpaired presentations of the CS and noise stimuli during each preexposure session.

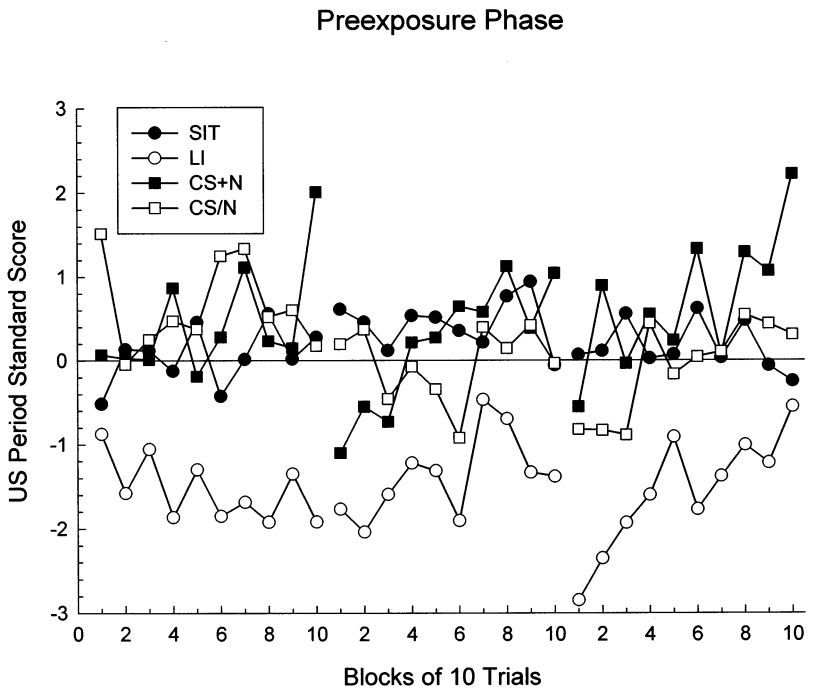

Fig. 2. US period hippocampal activity for each group expressed as standard scores and averaged over trial blocks for each of three preexposure sessions. As in Fig. 1, group abbreviations refer to the preexposure treatments. For CS/N animals, US period activity is displayed only for the 100 daily, noise alone trials.

0.38), and dropped well below baseline in the CS/N ($M = -0.88$) and LI ($M = -1.05$) groups. Although activity in the latter two groups appeared to decrease over the three sessions, both groups showed a within-session increase in activity during the last session (see Fig. 1). The analysis of variance determined that there were significant main effects due to both the group variable, $F_{(3,32)} = 5.75$, $P < 0.005$, and the session variable, $F_{(2,64)} = 10.32$, $P < 0.0001$. Collapsed over the four groups, hippocampal activity during the CS period tended to decrease over the three preacquisition sessions, decreasing from a mean of -0.19 during Session 1 to a mean of -0.79 during Session 3. There was also a significant Session X Trial Block interaction, $F_{(18,576)} = 1.75$, $P < 0.05$, as a consequence of the within-session decrease in activity during Sessions 1 and 2 compared to the within-session increase in activity during Session 3. No other sources of variation were significant.

Planned comparisons (one-tailed) determined that standard scores in the LI group were significantly lower than standard scores in the SIT group, $t_{(32)} = 3.83$, $P < 0.0005$, and standard scores in the CS + N group, $t_{(32)} = 2.17$, $P < 0.025$. Post-hoc Newman–Keuls comparisons also found that standard scores in the CS/N group were significantly lower than scores in the SIT group, $q_{(3,36)} = 0.98$, $P < 0.01$.

Standard scores of hippocampal activity during the US period are plotted in Fig. 2. Averaged over the three sessions, the standard score of LI animals remained below baseline during the US period ($M = -1.49$) even though the CS occupied only the first 100 ms of that period. By contrast, SIT animals ($M = 0.22$), CS + N animals ($M = 0.45$), and CS/N animals ($M = 0.18$) showed only small, positive deviations from baseline during the US period. The differences among the four group means were significant, $F_{(3,32)} = 5.75$, $P < 0.005$. Post-hoc Newman-Keuls comparisons determined that LI animals exhibited lower standard scores than SIT animals, $q_{(3,32)} = 1.71$, $P < 0.05$, CS + N animals, $q_{(4,32)} = 1.94$, $P < 0.05$, and CS/N animals, $q_{(2,32)} = 1.67$, $P < 0.01$. The only other source of significant variation was that due to the trial block variable, $F_{(9,288)} = 2.36$, $P < 0.025$, with standard scores of US period activity tending to increase within each session, increasing from an average of -0.54 on the first trial block to an average of 0.07 on the last trial block.

3.3. Acquisition-behavioral data

The rate of acquisition for each group is shown in Fig. 3. Conditioned responding increased in all four groups over the three sessions with the SIT group exhibiting the fastest rate of learning and highest level of conditioned responding, averaging 47% over the three sessions. Levels of conditioned responding were considerably lower for LI animals ($M = 22\%$), CS + N animals ($M = 19.61\%$), and CS/N animals ($M = 28.65\%$). The planned, one-tailed comparison between SIT and LI animals was significant, $t_{(32)} = 2.60$, $P <$

0.01, whereas the comparison between CS + N and LI animals was neither significant nor was it in the predicted direction. Reference to Fig. 3 suggests that there was a trend in the predicted direction during Session 2; however, that trend was reversed during Session 3. Although the overall analysis determined that all three variables exhibited significant main effects, these were of little interest due to the presence of significant interaction effects. All groups increased their levels of conditioned responding during Session 2, $F_{(9,640)} = 18.12$, $P < 0.001$, and Session 3, $F_{(9,640)} = 2.12$, $P < 0.05$. As reference to Fig. 3 suggests, however, there was a significant interaction between the group and session variables, $F_{(6,64)} = 2.92$, $P < 0.05$. Newman–Keuls comparisons among the group means established that SIT animals responded at a higher level than LI animals during Session 2, $q_{(4,96)} = 40.02$, $P < 0.05$, and Session 3, $q_{(2,96)} = 32.62$, $P < 0.05$. During the latter session, SIT animals were also superior to CS + N animals $q_{(4,96)} = 51.54$, $P < 0.01$, and CS/N animals, $q_{(3,96)} = 40.59$, $P < 0.01$. Thus, all three CS preexposure conditions significantly retarded the rate of acquisition and the magnitude of this effect was most pronounced in the LI group.

3.4. Acquisition-neural data

Standard scores of hippocampal activity during the CS period are shown in Fig. 4. Although all four groups showed higher standard scores during acquisition relative to preacquisition (compare with Fig. 1), the three CS preexposure groups still tended to exhibit lower standard scores than SIT animals. Mean standard scores over the three acquisition sessions for SIT, CS + N, CS/N, and LI animals were 1.97, 0.58, 0.33, and −0.40, respectively. This ordering of means tended to be preserved during each session. The planned comparison between SIT and LI animals was significant, $t_{(32)} = 3.03$, $P < 0.005$. The difference between CS + N and LI animals, although in the predicted direction, failed to achieve the appropriate level of significance, $t_{(32)} = 1.21$, $P < 0.15$. Although the overall group main effect was significant, $F_{(3,32)} = 3.17$, $P < 0.05$, subsequent Newman–Keuls comparisons failed to detect a group difference other than that between SIT and LI animals.

The significant main effects of session and trial block were not examined in detail because of the presence of a significant interaction between these two variables, $F(18,576) = 2.16$, $P < 0.005$. The increase in CS period standard scores appeared to parallel the increase in conditioned responding. Thus, standard scores were fairly stable during Session 1, $F_{(9,640)} = 1.05$, and increased significantly during the course of Session 2, $F_{(9,640)} = 4.15$, $P < 0.001$. However, unlike the percentage of CRs, which continued to increase during Session 3, standard scores were fairly stable during this last session, $F_{(9,640)} = 1.46$.

Mean standard scores of US period activity ranged from a low of 8.26 for LI animals to a high of 20.77 for SIT animals. Despite these apparently large group differences, the only reliable effect to emerge from the

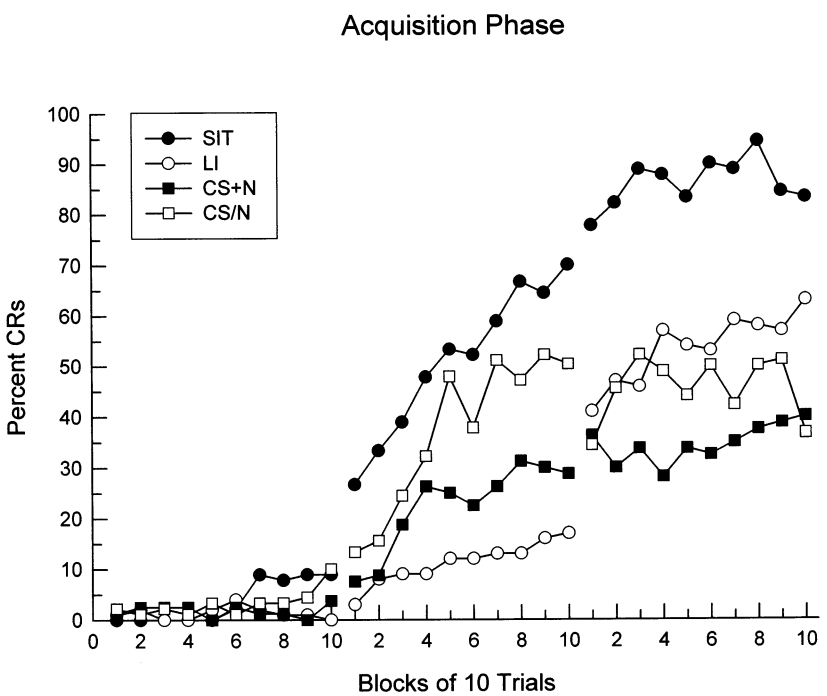

Fig. 3. Mean percentages of conditioned responses exhibited by each group during each of three acquisition sessions. As in Fig. 1, group abbreviations refer to the preexposure treatments.

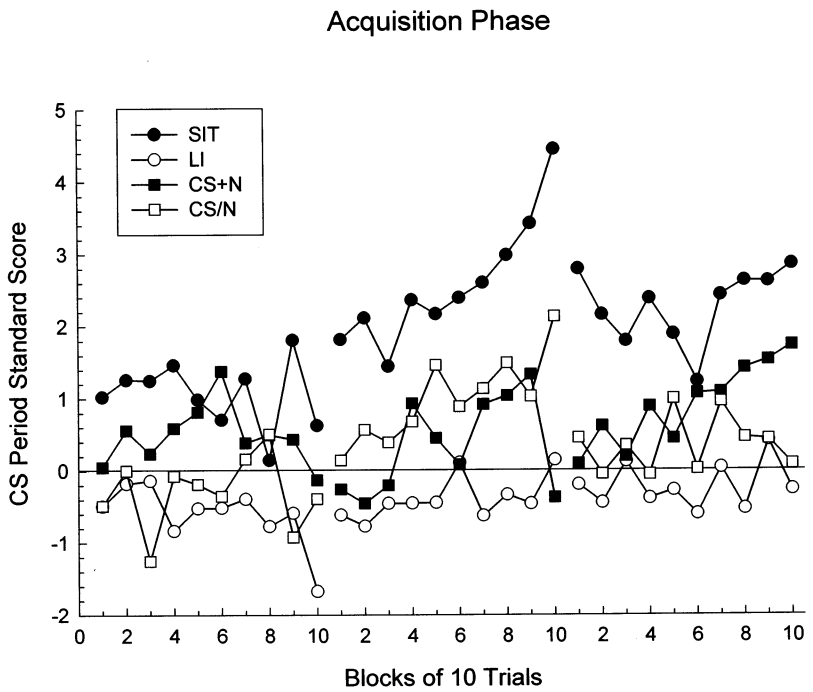

Fig. 4. CS period hippocampal activity for each group expressed as standard scores and averaged over trial blocks for each of three acquisition sessions. As in Fig. 1, group abbreviations refer to the preexposure treatments.

analysis was that due to the trial block variable, $F_{(9,288)} = 6.34$, $P < 0.0001$. In general, US period activity tended to increase over successive trial blocks (data not shown).

4. Discussion

The working hypothesis for this study was that hippocampal multiple unit activity could be used as an index of CS associability and would be differentially affected by treatments which purportedly influence decrements in CS associability. The preacquisition results generally supported this hypothesis. Behaviorally, all groups preexposed to the CS showed a latent inhibition effect during acquisition. However, contrary to prediction, paired preexposures of the CS and a second, neutral stimulus failed to significantly attenuate the latent inhibition effect. Similarly, the CS-alone preexposure group showed reduced hippocampal activity compared to the nonpreexposed group and pairings of the CS with a second stimulus failed to significantly attenuate the reduction in hippocampal activity.

The electrophysiological results of the preacquisition phase partially replicate earlier studies which showed that stimulus-evoked hippocampal activity habituates after repeated stimulus presentations. Vinogradova [41] reported that single units in both the CA1 and CA3 pyramidal cell fields showed an initial responsiveness to a variety of innocuous sensory stimuli. The initial response, whether it was excitatory or inhibitory, declined over 8–20 stimulus presentations. By contrast, in the present study, ongoing multiple unit activity in field CA1 was inhibited as a consequence of repeated stimulus presentations. Multiple unit activity showed a steady decrease below a prestimulus baseline period and that decrease occurred over 100–200 stimulus presentations distributed over a two-day period. Given the limitations of multiple unit recordings, it is not possible to determine if the reduction in hippocampal activity was due to an decrease in excitatory single-unit responsiveness, an increase in inhibitory unit responsiveness, or a combination of both. In addition to the finding that hippocampal activity was inhibited as a consequence of repeated stimulus presentations, a unique finding in the present study was that neutral stimulus pairings attenuated the suppressive effect of repeated stimulus presentations. More importantly, this attenuation was dependent upon temporal contiguity between the two neutral stimuli; that is, the attenuation was dependent upon an associative mechanism. The alterations in hippocampal activity during preacquisition are consistent with the alterations in CS associability postulated to occur by a number of current theories. For example, both the conditioned attention theory of latent inhibition proposed by Lubow and colleagues [18,22] and the more general theory of conditioning proposed by Pearce and Hall [27] would predict an attenuated decrease in CS associability in the CS + N group in the present study.

Although CS-period hippocampal activity during preacquisition closely paralleled the theoretical alterations in CS associability, some discrepancies were also evident. For example, most models of latent inhibition predict a decline in CS associability to an asymptote near zero in the traditional latent inhibition group. However, as reference to Fig. 1 indicates, the two groups expected to show the greatest declines in CS associability in the present study (the LI and CS/N groups), showed decreases in hippocampal activity which were restricted to the first two preacquisition sessions. During the third session, hippocampal activity in these two groups was maximally inhibited at the beginning of the session and then tended to increase, approaching baseline levels by the end of the session. In the context of Weiner's 'switching' model of latent inhibition [42], these results suggest that the hippocampus may play an active role in mediating the decline in CS associability for only a limited period of time. Once the decrement in CS associability reaches an asymptote, participation by the hippocampus is no longer required and other sites such as subiculum and/or nucleus accumbens may be responsible for maintaining a low level of CS associability.

There is an alternative interpretation for the increase in CS-related hippocampal activity seen in LI and CS/N animals during the last preacquisition session. It is possible that the decrement in CS associability is somewhat labile and may cycle over the course of several preexposure sessions. Thus, if additional preexposure sessions had continued to produce increases in hippocampal activity in CS/N and LI animals, it is possible that no subsequent retardation of learning would have occurred. In this regard, there have been several published failures to obtain a robust latent inhibition effect using the rabbit NM/eyelid preparation despite the use of a large number of CS preexposures and multiple preexposure sessions. Suboski et al. [38] failed to obtain a latent inhibition effect altogether despite 280 preexposures of a tone CS distributed over four sessions and Plotkin and Oakley [28] failed to find any effect of 150 tone CS preexposures distributed over three sessions. By contrast, Siegel [35] reported a latent inhibition effect following either 100 or 1300 tone CS preexposures distributed over 0.5 or 6.5 sessions, respectively. However, there was no significant difference in the initial performance of the two groups of preexposed animals; both groups required an equal number of CS-US pairings before achieving a criterion of five conditioned responses. In light of the Suboski et al. [38] and Plotkin and Oakley [28] data, it is difficult to argue that 100 preexposures produced a near maximal decrement in CS associability such that 1200 additional preexposures were relatively ineffective. Hernández et al. [14] reported mixed findings following 232 tone preexposures distributed over two sessions and part of a third. A small but statistically significant latent inhibition effect was found following preexposures of a 75-db tone but not following preexposures of either a 60- or 90-db tone. Thus, a large number of stimulus preexposures does not guarantee that a latent inhibition effect will occur, at least in the rabbit NM/eyelid preparation. If CS associability does fluctuate from session to session, perhaps covarying with uncontrolled organismic variables, then the total number of preexposures may have less of an impact on the magnitude of the latent inhibition effect. The use of an independent measure of CS associability during the preexposure phase would clearly be the best predictor of subsequent performance.

One unexpected finding in the present study was that CS preexposures produced a relatively large decrease in US-period activity even though the CS occupied only the first 100 ms of the US period. This effect was restricted to the LI group; none of the other preexposure groups showed a such a pronounced decrease in US-period activity. This last result is not surprising given the presence of the noise during the US-period in the CS + N group. The presence of the noise during CS + N presentations apparently disinhibited hippocampal activity such that US-period activity returned to baseline levels. Moreover, noise-evoked hippocampal activity in CS/N animals showed little change throughout the preexposure phase, suggesting that preexposures of brief stimuli may not produce a latent inhibition effect. In reviewing the literature pertaining to the effects of stimulus duration on the magnitude of the latent inhibition effect, Lubow [18, pp.63–64] noted that the magnitude of the effect appears to be a direct function of stimulus duration, at least in the conditioned suppression and conditioned taste aversion paradigms. The electrophysiological results of the CS/N group in the present experiment suggest that the same may be true for the rabbit NM/eyelid preparation. Assuming that the CS-US interval is not allowed to covary with CS duration, brief CSs may not produce appreciable levels of latent inhibition in the rabbit preparation. Finally, US-period activity in LI animals was similar to CS-period activity in showing an increase during the course of the last preexposure session. Thus, whatever processes are initiated by the onset of the CS apparently persist for some period of time following the offset of the CS. This last result suggests that a latent inhibition effect can be obtained even with the use of a trace conditioning procedure as long as the preexposed CS is of sufficiently long duration.

The behavioral results are in general agreement with those of previous studies in demonstrating a retarded rate of NM/eyelid conditioning following CS preexposures [8,35,36]. A robust latent inhibition effect was clearly present in all CS preexposure groups. Thus, in contrast to the predictions made by conditioned atten-

tion theory [18,22] and by Pearce and Hall's [27] model, pairings of the CS with a neutral stimulus during preacquisition failed to attenuate the latent inhibition effect. Both Lubow's [18] conditioned attention theory and the Pearce-Hall [27] model predict that CS associability would decline with repeated stimulus preexposures but that decline could be attenuated by pairing the CS with a second, neutral stimulus during the preexposure phase. Both theoretical positions also indicate that this attenuating effect of the second stimulus is only transient and that CS associability would ultimately decline to the same low level as that of the traditional LI group. Given the large number of preacquisition stimulus pairings used in the present study, it is quite likely that CS associability in CS + N animals had declined sufficiently that an attenuated latent inhibition effect could not be observed. It is also possible that the use of the same noise during preacquisition and acquisition may have contributed to the inability of the noise to attenuate the latent inhibition effect. It should be recalled that the noise produced during preacquisition was the same noise produced by the corneal airpuff during CS-US pairings. It may be necessary to use a unique stimulus as the second, neutral, stimulus in the preacquisition phase in order to obtain an attenuated latent inhibition effect.

Hippocampal unit activity in the nonpreexposed, SIT animals exhibited changes during acquisition similar to those reported in previous studies [2,4,15,34]. Hippocampal responsiveness to the CS increased in conjunction with the increase in conditioned responding during the second session. During the third session, hippocampal activity stabilized at a somewhat lower level than on the previous session whereas conditioned responding continued to increase. The decrease in hippocampal activity on this last acquisition session is consistent with what is predicted for CS associability by both the Pearce-Hall model [27] and conditioned attention theory [18]. Both views suggest that CS associability ultimately declines with continued CS-US pairings even as the associative value of the CS increases.

In contrast to the nonpreexposed group, the CS-alone preexposure group failed to show any increase in CS-evoked hippocampal activity even though conditioned responding increased. CS-period hippocampal activity of LI animals was significantly reduced compared to SIT animals throughout acquisition. These electrophysiological results are consistent with those of Best and Best [6] in demonstrating reduced CS-evoked hippocampal activity as a consequence of CS preexposures. The present results extend the findings of Best and Best in a number of ways. Firstly, a different species and response system were employed. Secondly, the behavioral consequences of CS preexposures were included in the present study. Thirdly, and most importantly, repeated measures of hippocampal activity were recorded concurrently with ongoing changes in learned, behavioral responding.

While CS-alone preexposures affected hippocampal activity throughout acquisition, preexposures of the CS paired with a second stimulus failed to attenuate that effect in acquisition. Although these results are contrary to prediction, they are also somewhat ambiguous in that hippocampal activity of CS + N animals was also not significantly different from that of SIT animals. Thus, there was no apparent preexposure effect on hippocampal activity for CS + N animals even though their behavior was significantly different from that of SIT animals. Furthermore, CS-evoked hippocampal activity of CS/N animals was also not significantly different from either LI or SIT animals even though CS/N animals showed a retarded rate of learning compared to SIT animals. Thus, during acquisition, hippocampal activity was less sensitive to the effects of the preexposure treatments than was behavioral responding.

One of the striking differences between SIT and LI animals during acquisition was the dissociation between hippocampal activity and behavioral responding in LI animals compared to SIT animals. For example, reference to Fig. 3 indicates that the behavior of LI animals during Session 3 was essentially identical to the behavior of SIT animals during Session 2. By contrast, the magnitude of hippocampal activity in LI animals never achieved the same level as that of SIT animals. These results are in contrast to the results of Berger et al. [2] and Berger and Thompson [4] who reported that the magnitude of hippocampal activity was positively correlated with the level of conditioned responding. However, a number of subsequent studies have also found a dissociation between the magnitude of CS-evoked hippocampal activity and the level of conditioned responding. For example, Berger and Thompson [5] reported that on the second day of extinction training, hippocampal activity had declined to spontaneous levels even though the percentage of conditioned responding had declined to only about 50%. Steinmetz and colleagues have also noted that the magnitude of CS-evoked hippocampal activity does not always correlate well with the level of conditioned responding. Miller and Steinmetz [24] found a pronounced dissociation between learned behavioral responding and hippocampal activity in a discrimination-reversal task. On the day when animals achieved a behavioral discrimination criterion, hippocampal activity paralleled the behavioral results: multiple unit activity was greater on CS + trials than on CS- trials. However, during the early days of reversal training, hippocampal activity dropped to baseline levels on both CS + and CS − trials even though conditioned responding on those trials was at or above 70%. In a related finding, using a simpler training paradigm, Sears and Steinmetz [34] reported that while conditioned responding increased over the course

of extended acquisition training, hippocampal activity instead exhibited an inverted U-shaped function. This last result is similar to what was observed for the nonpreexposed, SIT group in the present experiment. The SIT group showed its greatest level of hippocampal activity during the second acquisition session compared to either the first or third sessions. Gabriel and colleagues have reported similar, inverted U-shaped functions for training-related neuronal activity in their discriminative avoidance paradigm. Gabriel et al. [11] reported that the upper layers of posterior cingulate cortex exhibited maximal training-related activity during the early stages of behavioral discriminative performance and less activity during the stage of criterial performance. Similar findings have been reported for basolateral amygdala and entorhinal cortex [10]. Thus, regardless of the learning preparation, a number of brain regions appear to encode different aspects of the learning situation during different stages of training.

In summary, hippocampal activity during CS preexposures decreases in a manner consistent with the decline in CS associability postulated to occur by several current theories of conditioning. The dissociation between hippocampal activity and behavioral responding during acquisition is also consistent with predictions regarding CS associability declines coupled with increases in associative strength. Taken together, these results strongly suggest that hippocampal activity can be used as a reliable index of CS associability.

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