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Haloperidol Impairs Classically Conditioned Nictitating Membrane Responses and Conditioning-Related Cerebellar Interpositus Nucleus Activity in Rabbits

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SEARS, L. L. AND J. E. STEINMETZ. *Haloperidol impairs classically conditioned nictitating membrane responses and conditioning-related cerebellar interpositus nucleus activity in rabbits.* PHARMACOL BIOCHEM BEHAV 36(4) 821–830, 1990.—Rabbits, chronically implanted with recording electrodes in the cerebellar interpositus nucleus and following acquisition of a classically conditioned eyelid response, were injected with haloperidol (HAL, 250 μg/kg). HAL significantly reduced the number of conditioned responses when a 75 and 85 dB tone conditioned stimulus (CS) was presented but not when a 95 dB tone CS was used. There was a corresponding decrease in interpositus activity at the 75 and 85 dB CS intensities but not at the 95 dB intensity. HAL appeared to disrupt CRs and interpositus activity by increasing the intensity threshold of the tone CS for eliciting conditioned responses. Possible mechanisms for the effect of HAL on neural circuitry involved in classical eyelid conditioning are discussed.

Haloperidol NM conditioning Cerebellar interpositus nucleus CS pathway

NUMEROUS studies have used the rabbit nictitating membrane (NM)/eyelid conditioning paradigm (7) to look at various behavioral and neural correlates of learning and memory [see (22) for review]. In this form of classical conditioning, a conditioned stimulus (CS) such as a tone or a light is forward paired with an unconditioned stimulus (US) such as an airpuff or paraorbital shock so that the CS eventually elicits an NM conditioned response (CR) that is similar in form to the reflexive unconditioned response (UR) elicited by the airpuff or paraorbital shock. As the number of CRs increases with paired CS-US training, the onset latency of the anticipatory CR decreases gradually as does the latency to the peak of the learned NM movement. Eventually, the rabbit learns to execute CRs that are timed optimally (i.e., the maximum amplitude of the CR occurs around the time the airpuff is delivered to the cornea of the eye).

Using this motor learning paradigm, Harvey and Gormezano (10) demonstrated that the neuroleptic, haloperidol (HAL), delayed CR acquisition and, in trained animals, produced a significant decrease in the number of learned responses when relatively moderate CS tone intensities were presented. Conditioned responding was not impaired when relatively high CS intensities were used and HAL did not impair the execution of the UR. It was concluded from these results that HAL increased the CS intensity threshold for the production of CRs by blocking excitatory properties of the tone CS. These results also indicated clearly that the rabbit classical NM conditioning preparation was sensitive to

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the disruptive behavioral effects of HAL administration. Given the wealth of behavioral and neural information available concerning NM conditioning, this paradigm seems ideal for studying the neuronal systems and processes underlying the behavioral effects of the neuroleptics.

The brain systems involved in classical eyelid conditioning are beginning to be delineated and understood. The involvement of the hippocampus in NM conditioning has been well-documented [e.g., (2)]. However, a number of studies have shown that brain regions above the level of the thalamus are not necessary for acquisition or retention of simple delay NM conditioned responses [e.g., (16,24)], although the hippocampus is apparently important for optimal execution of more complex learned responses such as CRs established with classical trace NM conditioning procedures (20,26). Regions of the brain stem and cerebellum, however, have been demonstrated to be involved critically in NM conditioning [see (30) for review]. For example, lateral and anterior regions of the cerebellar interpositus nucleus appear to be essential for this type of motor learning because complete lesions of these areas of the interpositus nucleus have disrupted the learned eyelid CRs (17,33) and blocked CR acquisition (15). Furthermore, multiple- and single-unit recordings from the interpositus nucleus as well as regions of the cerebellar cortex revealed CS- and US-evoked cells as well as cells whose firing patterns were related to execution of the learned behavioral response (4–6, 18). These studies have generally indicated that the essential site of plasticity underlying the acquisition and retention of the CR is in regions of the interpositus nucleus and/or cerebellar cortex, or in brain stem regions that use the cerebellum as a mandatory efferent for the execution of the CR.

Given the essential involvement of the interpositus nucleus in eyelid conditioning, we designed the present study to examine the effects of HAL on the characteristic CR-related activity seen in the interpositus nucleus during training. In this manner, we hoped to determine if the CR-disrupting effects of HAL also affected activity in the cerebellum, thus providing initial data concerning the neuronal systems and processes underlying the behavioral effects of HAL administration in the NM conditioning paradigm.

METHOD

Subjects

Thirteen male, New Zealand, albino rabbits that weighed 2.0–3.0 kg were used in the present study. Ten of these rabbits had accurate placements of cerebellar recording electrodes and provided data for the analyses reported below. All rabbits were individually housed, given ad lib access to food and water, and maintained on 12/12-hr light/dark cycles.

Surgery

Surgery was performed under aseptic conditions. Surgical anesthesia was initiated and maintained with IM injections of xylazine (6 mg/kg) and ketamine (60 mg/kg). Rabbits were positioned in a stereotaxic headholder with the bregma skull landmark located 1.5 mm above the lambda skull landmark. Insulated, stainless steel, recording electrodes (00 insect pins, 25 μm exposed tips) were then lowered stereotaxically into either the left or right interpositus nucleus using standard coordinates (i.e., 0.7 mm anterior, 5.5 mm lateral, and 15.0 mm ventral to lambda). Final position of the electrode was determined by observing neural activity that was characteristic of the interpositus nucleus. After positioning, the electrode was cemented into place with dental acrylic. A plug assembly designed to hold a headstage device for subsequent behavioral training was also cemented to the skull. During surgery a small loop of suture was placed in the nictitating membrane to allow monitoring of NM movement during subsequent training sessions. Following surgery the rabbits were given a 1-week recovery period prior to behavioral training.

Behavioral Training

Rabbits were first placed in standard Plexiglas restraint boxes inside a sound-attenuating conditioning chamber and given two 45-min adaptation sessions. The chamber was equipped with a speaker for delivery of a tone CS, an airpuff delivery system for presenting the US, brain recording amplifiers, and a white noise fan for air circulation that generated 54 dB of background white noise. Paired presentations of the CS and US began following the adaptation sessions. Classical eyelid conditioning was accomplished by pairing a 348 msec tone CS with a coterminating 99 msec airpuff (2.1 N/cm^2) thus creating a 249 msec interstimulus interval (ISI). Movement of the NM during training was monitored by a minitorque potentiometer connected by a thread to the suture placed in the rabbit's NM during aseptic surgery. The potentiometer transduced NM movements to voltage signals and thereby allowed for measurement of several NM response parameters such as percent CRs (i.e., 0.5 mm of NM movement during the CS-US interval), response amplitude, and response latencies. The presentation of the CS and the US and the recording of behavioral responses and neural activity (see below) were controlled by a computer programmed in Forth and machine language (14).

Behavioral training consisted of three separate phases. The first phase was initial acquisition training, which lasted until a response criterion was reached (see below). Once the criterion was reached, the rabbits received three consecutive training sessions to test the effects of a saline injection (Sessions 1–3) and these sessions were followed by three consecutive HAL administration sessions (Sessions 4–6). For all training sessions, the airpuff US was presented to the eye ipsilateral to the chronically implanted interpositus recording electrode. An individual training session for each of the three phases consisted of 120 trials that were divided into 12 blocks of 10 trials. The first trial of each block was a tone-alone test trial while the remaining nine trials were paired CS-US presentations. Four intensities of a 1 kHz tone CS were used for each session (i.e., 65, 75, 85, and 95 dB SPL) and each session consisted of three consecutive blocks of training at each CS tone intensity.

Acquisition training. During acquisition training, each CS intensity was presented over three consecutive blocks (e.g., Blocks 1–3 at 75 dB, Blocks 4–6 at 95 dB, Blocks 7–9 at 65 dB, and Blocks 10–12 at 85 dB). The order of CS intensity presentation was varied pseudorandomly for each session to prevent possible CS intensity order effects. The acquisition phase continued until a criterion of 50% CRs at the 75 dB CS level was met. This criterion was chosen to ensure that rabbits had a sufficient amount of training to detect pre- and postinjection differences in behavioral performance or neural activity.

Saline testing. Sessions 1, 2, and 3 following acquisition training assessed behavioral responding and interpositus activity before and after an IV injection of 0.9% saline (0.125 ml/kg). For each 12-block session, either four or eight blocks of trials were presented prior to saline administration. Each CS intensity was presented pseudorandomly for an equal number of consecutive blocks (e.g., Blocks 1–2 at 75 dB, Blocks 3–4 at 65 dB, Blocks 5–6 at 95 dB, and Blocks 7–8 at 85 dB). Upon completion of the four or eight blocks of presaline trials, a saline injection was given and, following a 15-minute wait, the remaining blocks of training were presented using the same order of CS intensities that was presented prior to the injection. Because the number of training

blocks that was presented before saline was not the same as the number presented after the saline injection during an individual session, the number of pre- versus postsaline-injection blocks were counterbalanced across animals. In this manner, the number of blocks of training before and after the saline administration were equalized across animals and across training sessions.

Haldoperidol testing. Sessions 4, 5, and 6 followed the same procedures as described above for the saline control sessions except that a 250-μg/kg IV injection of HAL (Haldol, McNeil Laboratories, Inc., Spring House, PA) was administered upon completion of the initial four or eight blocks of training with the four CS intensities. This dosage was selected because it effectively disrupted CRs in a previous NM conditioning study without producing observable sedative effects or other side effects (10).

Neural Recording and Data Analysis

Interpositus nucleus activity was recorded during all phases of training. The neural activity was amplified, band-pass filtered (500 to 5000 Hz) and routed to a window discriminator that selected spikes that exceeded a preset level. The output of the window discriminator was then routed to a computer that counted discriminated spikes and recorded NM movement during each trial. To facilitate the analysis of neural activity each trial was divided into three 249-msec periods: (a) a pre-CS period defined as the period of time from trial onset to CS onset, (b) a CS period defined as the period of time between CS onset and US onset, and (c) a US period defined as the period of time from onset of the US to the end of the trial. Data acquisition was accomplished by polling the behavioral and discriminated neural data inputs once every 3 msec during the total 747-msec trial period and then storing the behavioral and discriminated neural data on floppy disk after each trial for subsequent off-line analysis. Analysis of behavioral data consisted of calculating behavioral measures such as percent CRs, response amplitude, and response latencies. The summed, discriminated neural activity was converted to standard scores for further analysis. This procedure involved subdividing each pre-CS, CS, and US period into three 83-msec subperiods: a first, second, and third 83-msec subperiod before CS presentation (pre-CS-1, pre-CS-2, and pre-CS-3), a first, second, and third 83-msec subperiod following the presentation of the tone CS (CS-1, CS-2, and CS-3), and a first, second, and third 83-msec subperiod following the airpuff US (US-1, US-2, and US-3). Discriminated units were summed for each subperiod across each block of training producing a Blocks × Subperiods matrix of raw interpositus unit activity. Standard scores were then calculated for each CS and US subperiod of every 10-trial block by subtracting the mean unit activity recorded during the pre-CS-3 subperiod from the respective CS and US subperiod unit activity and then dividing the result by the standard deviation of the entire 12 blocks of pre-CS-3 activity. This procedure produced 72 standard scores for each session (i.e., a CS-1, CS-2, CS-3, US-1, US-2 and US-3 standard score for each block of training).

Because a different number of blocks were given before and after the saline or HAL injections for an individual session, a selection procedure was developed so that pre- versus postinjection differences could be compared. As noted earlier, the number of blocks of training given before and after the injection was equalized across all sessions for all rabbits through the counterbalancing procedure. To ensure that statistical comparisons of pre- versus postinjection blocks were based on an equal number of trials in both groups, one block out of the two presented at each CS intensity during each session was randomly selected for inclusion in the data analysis.

Upon completion of training sessions, rabbits were overdosed with an IV injection of pentobarbital (4 cc) and perfused via the ascending aorta with saline followed by 10% formalin. A 100-μA DC was passed through the recording electrode for 10 sec to mark recording sites. The brains were then removed and placed in a 10% formalin/30% sucrose solution for at least 1 week at which time they were blocked in albumin/gelatin. Frozen coronal sections were taken through the interpositus nucleus. The sections were mounted on gelatinized slides and stained with cresyl violet and potassium ferrocyanide. The location of the recording electrode was determined by viewing the stained section under a microscope.

RESULTS

Due to technical difficulties with the recording apparatus, data were not available for all 10 rabbits across all training days. Data from ten rabbits was available for the first HAL injection session (Session 4), data from nine rabbits were available for Session 5, and data from eight rabbits were available for the third day of HAL injection (Session 6). In addition, analyses of saline control sessions are based on data from seven rabbits (i.e., three rabbits were not given saline injections but were given equivalent amounts of behavioral training).

Percent CRs and interpositus unit activity were analyzed for both saline and HAL administration sessions using a repeated measures analysis of variance. When appropriate, significant main or interaction effects were further analyzed with a Tukey HSD test (all $ps<0.05$). Statistical analyses of CR amplitudes were also conducted. The results of these analyses were virtually identical to the percent CRs analyses and are therefore not reported here. Because no significant main or interaction effects were found when saline or HAL sessions were compared (i.e., Session 1 vs. Session 2 vs. Session 3 and Session 4 vs. Session 5 vs. Session 6), data for subsequent analyses were collapsed across training days. Also, because the number of CRs at the 65 dB CS intensity was near the spontaneous blinking rate (about 2–4% per session) and because no training-related activity was seen in the interpositus during any training session for this CS intensity, data for the 65 dB CS intensity was not included in the statistical analyses.

Saline Testing

Behavioral responses. Percent CRs recorded during saline-injection sessions were analyzed for differences in the number of pre- and postsaline CRs for the three CS intensities. This analysis revealed only a main effect for CS intensity, $F(2,12)=8.00$, with the percentage of CRs at 95 dB significantly greater than percent CRs at 75 dB. The mean percent CRs for saline testing are shown in Fig. 1.

Interpositus unit activity. Standard scores of interpositus activity were analyzed with two repeated measures analyses of variance; one for the CS and one for the US period. Significant differences across CS intensities, $F(2,12)=5.47$, were observed with standard scores of interpositus activity at 95 dB greater than the 75 dB CS intensity. No pre- versus postsaline injection differences were observed. Analysis of US period activity revealed only a main effect for US subperiod, $F(2,12)=5.30$, with the US-1 subperiod being significantly larger than the US-3 subperiod. Again, no pre versus postsaline injection differences were observed. Mean standard scores of interpositus activity scores are shown in Fig. 1.

Haloperidol Testing

Behavioral response. A significant HAL Injection × CS-

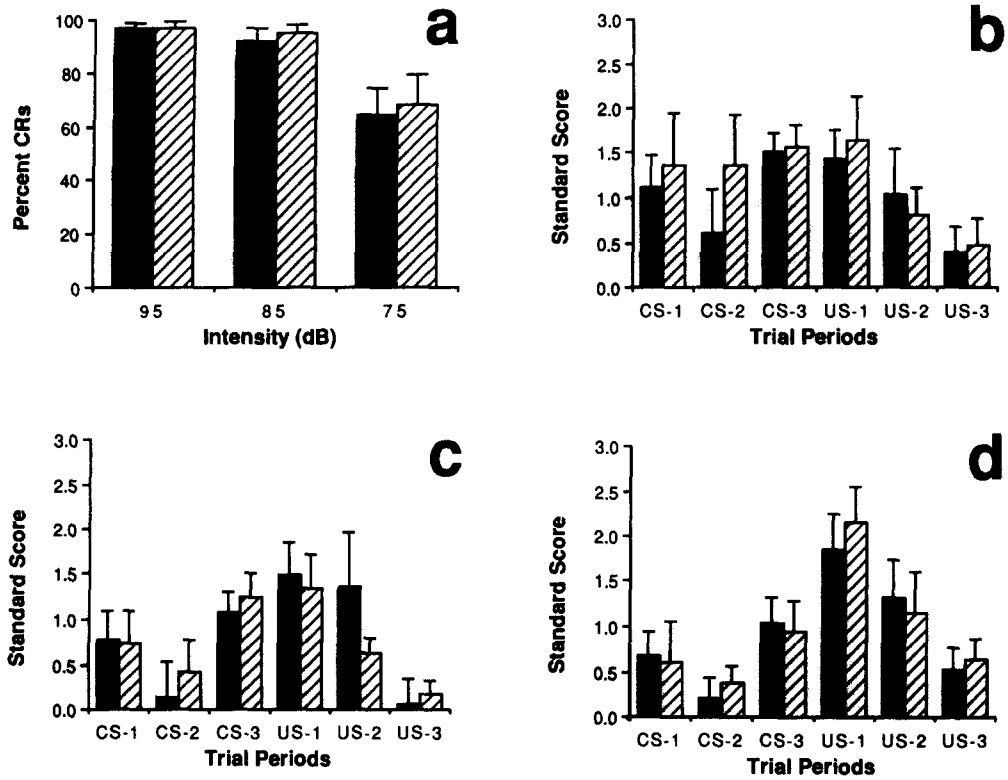

FIG. 1. (a) Percent CRs for 75, 85, and 95 dB CSs collapsed across three days of classical NM conditioning before (dark bars) and after (cross-hatched bars) saline injection. Standard scores of interpositus nucleus activity are also shown before (dark bars) and after (cross-hatched bars) saline injection at CS intensities of 95 dB (b), 85 dB (c), and 75 dB (d).

Intensity interaction was found in the analysis of the percent CRs during HAL injection sessions, $F(2,52) = 15.73$. There was a significant decrease in CRs at 75 and 85 dB but not at the 95 dB level. Mean percent CRs recorded during HAL testing are shown in Fig. 2.

In order to assess whether the behavioral effect of HAL was due to a disruption in the timing of the learned response rather than to a failure to perform the CR altogether, a comparison was made of the onset latencies of the NM movement before and after administration of HAL on 95, 85, and 75 dB trials. Only CS-alone test trials were used for the latency analysis because the NM movement produced by presentation of the US (i.e., the UR) could mask late CRs that were performed during the US period. Also, only trials that contained a response somewhere within the 498-msec period following the tone presentation were used (i.e., 81% of the pre-HAL trials and 43% of the post-HAL trials). The mean response latency recorded before HAL was 204 msec while the mean response latency recorded after HAL was slightly shorter at 190 msec. These values were not significantly different thus indicating that when a NM movement was executed, the onset latency was not affected by HAL. In short, HAL blocked execution of the learned NM response and did not simply disrupt timing of the learned response.

To assess further the possible effects of HAL on the motor response a comparison of the UR amplitude at the 65 dB CS level was made. As described above, no CRs were recorded on 65 dB trials thus permitting an analysis of UR amplitude in the absence of a learned response. First, our analysis revealed no changes in UR amplitude over the course of conditioning. Moreover, no pre- and postinjection differences were observed in the amplitude of the UR, a finding that is in basic agreement with previous observations by Harvey and Gormezano (10). In short, HAL did not affect execution of the reflexive NM response elicited by the airpuff US.

Interpositus unit activity. An initial analysis was conducted to assess changes in the spontaneous firing rate of the interpositus before and after HAL injection. A repeated measures analysis of variance of raw unit activity for all CS intensity levels across the entire pre-CS period indicated that HAL did not affect the spontaneous firing rate of the interpositus. Analysis of CS period data revealed a significant three-way interaction, $F(4,104) = 2.60$, that was due to a decrease in interpositus activity that occurred after the HAL injection in period CS-3 at the 75 and 85 dB CS intensity. The Tukey analysis also revealed a significant decrease at 95 dB in the CS-1 and CS-2 subperiod activity after HAL administration, but not during the CS-3 subperiod. These results were likely due to a suppression of interpositus activity evoked by the tone CS. This finding is consistent with previous reports of decreasing activity in early portions of the CS period as training progresses [e.g., (18)]. Because decreases in activity during early portions of the CS period were not observed when 75 and 85 dB CSs were presented, it seems likely that the presence of the tone-evoked activity in the interpositus nucleus is dependent on the intensity of the tone. Analysis of US-period neural activity revealed a significant HAL Injection × US subperiod interaction, $F(2,52) = 3.95$. The US-1 and US-2 subperiods decreased significantly following HAL injection. Finally, an analysis of the relationship between interpositus activity and percent CRs was done for 75 and 85 dB CS intensities after the HAL injection. The correlation between CS-3 interpositus activity and the percent CRs was calculated to be 0.82 ($p<0.05$). Mean standard scores of

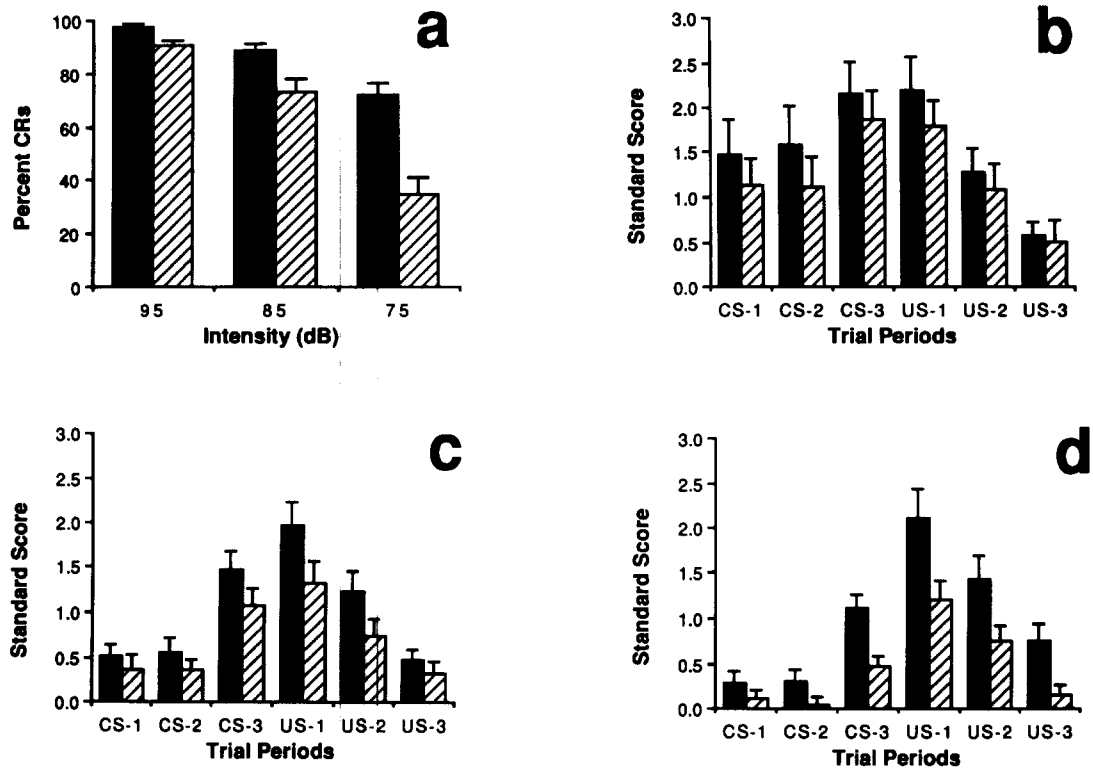

FIG. 2. (a) Percent CRs for 75, 85, and 95 dB CS collapsed across three days of classical NM conditioning before (dark bars) and after (cross-hatched bars) haloperidol injection. Standard scores of interpositus nucleus activity are also shown before (dark bars) and after (cross-hatched bars) haloperidol injection at CS intensities of 95 dB (b), 85 dB (c), and 75 dB (d).

interpositus activity during HAL-administration sessions are shown in Fig. 2. Examples of behavioral responses and interpositus unit activity taken before and after HAL administration can be seen in Fig. 3, while Fig. shows examples of raw multiple-unit neural activity recorded on trials with and without HAL.

Histological Analysis

Placements of recording electrodes in the cerebellum are shown in Fig. 5. Ten rabbits had electrodes located within anterior regions of the interpositus nucleus (four in the left interpositus and six in the right interpositus). The location of electrodes for the three rabbits that failed to show learning-related activity are also included. These electrodes were lateral, dorsal, and posterior to the interpositus nucleus.

DISCUSSION

The present data indicated that injections of haloperidol (HAL) disrupted classically conditioned NM responding when 75 or 85 dB tone CSs were used but not when a 95 dB tone CS was used. Furthermore, the HAL injections disrupted conditioning-related activity in the cerebellar interpositus nucleus, a structure suspected to be involved critically in learning and performance of the classically conditioned response.

The observed reduction in percent CRs at moderate tone CS intensities but not at high tone CS intensities was similar to previous findings reported by Harvey and Gormezano (10). In the earlier study, classical conditioning of the NM response was accomplished by pairing a tone or light CS with a paraorbital shock US. Both HAL and pimozide retarded the rate of conditioning in naive rabbits and the retardant effect of HAL was found not to be due to a variety of nonassociative effects such as sensitization, pseudoconditioning, increases in baseline responding, or changes in UR amplitude. Similar to the present study, Harvey and Gormezano (10) also evaluated the effects of HAL on trained rabbits. They found a significant increase in the intensity threshold of a tone CS for elicitation of CRs as conditioning was disrupted when tones between about 50 and 75 dB tones were used but not when greater CS intensities were presented. From these data it was argued that HAL blocked the excitatory properties of the tone CS, thus accounting for the ability of HAL to retard the rate of CR acquisition and disrupt conditioning at medium to low tone CS intensities.

The behavioral results from the present study are in agreement with Harvey and Gormezano's (10) results. We observed no decreases in the number of CRs executed when 95 dB tones were presented but observed a significant decrease in CRs when 85 and 75 dB tones were presented. The 65 dB tone in the present study failed to produce CRs, possibly because the 65 dB tone was only 11 dB above the background white noise level of the conditioning chamber or because more training trials at this tone intensity were needed to establish conditioning. The post-HAL reduction in percent CRs at 75 and 85 dB, however, was similar to the reduction in CRs observed by Harvey and Gormezano (10) at medium to low CS intensities and, similarly, suggests that HAL significantly elevates the intensity threshold of a tone CS for eliciting CRs. Furthermore, our results argue that HAL did not produce the CR-disrupting effects by affecting motor pathways essential for expression of the behavioral response. First, HAL failed to cause a reduction in percent CRs when a 95 dB tone was

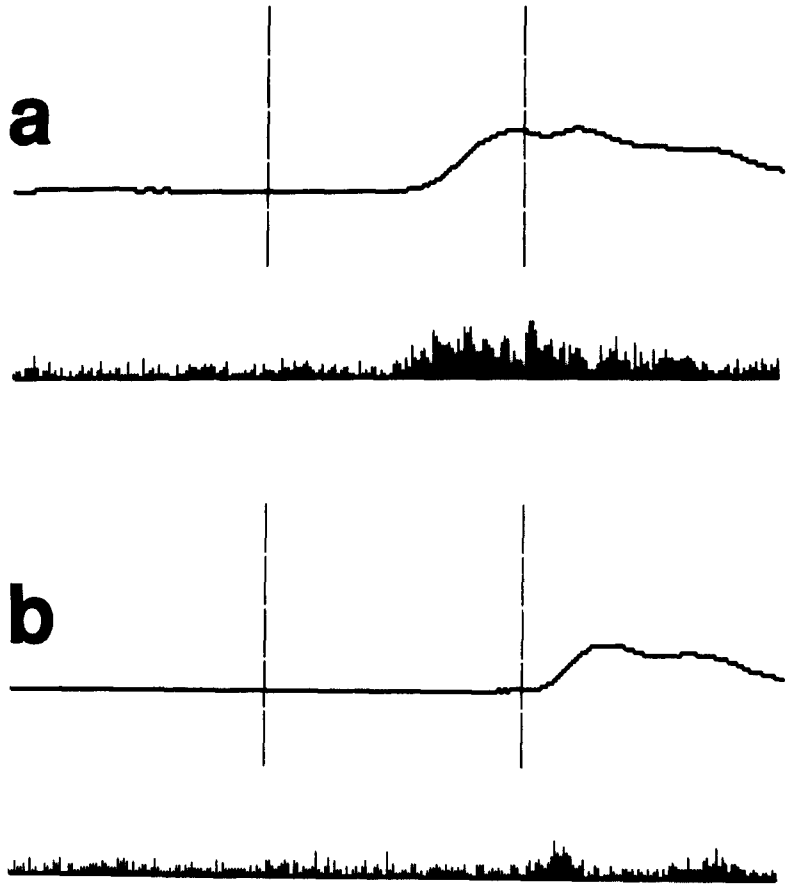

FIG. 3. Examples of behavioral responses and interpositus nucleus activity during two blocks of training before (a) and two blocks of training after (b) haloperidol administration. A 75 dB tone was used as a CS during these training blocks. The upward deflection of the upper traces in (a) and (b) show average extension of the NM while the lower traces in (a) and (b) are peristimulus histograms of discriminated interpositus nucleus activity. The first vertical line indicates when onset of the tone CS occurred while the second vertical line indicates the onset of the air puff US.

presented even though the number of CRs declined when 85 and 75 dB tones were used. This finding indicated that motor pathways needed to execute the CR were not impaired after HAL administration. Second, CR and UR amplitudes on 95 dB trials and UR amplitudes on 65 dB trials (i.e., when on CRs were observed) were not altered significantly by the injection of HAL. Third, analysis of tone-alone trials revealed that the decrease in percent CRs was not due to a delay in the execution of the learned NM response. When pre- and post-HAL trials were compared (i.e., CS-alone trials during which NM responses were observed), no significant differences in NM response onset latencies were discerned. This analysis indicated that the rabbits were capable of executing properly timed CRs thus suggesting that the decrease in percent CRs was likely not due to an increase in NM response latency caused by disruptions of the motor output system. The behavioral evidence presented here further supports the conclusion that HAL disrupts the excitatory properties of a tone CS, thus causing an increase in the intensity threshold for detecting an effective tone CS.

Previous studies have demonstrated a high correlation between execution of the CR and multiple unit activity in the cerebellar interpositus nucleus [e.g., (18)]. These studies have shown that the onset of neuronal firing in the interpositus nucleus precedes execution of the learned NM response and that the amount of cellular discharge is correlated highly with the amplitude of the CR. In short, the pattern of neuronal discharges within the interpositus nucleus forms an amplitude/time-course "model" of the CR which is characteristic of a brain region that may be responsible for performance of the learned response. The present study replicated these findings as high levels of interpositus nucleus activity were present on CR trials during training sessions when saline was given and during blocks of trials presented before HAL was injected. As expected, the interpositus nucleus activity was especially prominent during the CS-2 and CS-3 subperiods (i.e., about 150 msec prior to US onset) as this is the trial period during which onset of the anticipatory CR occurs. Moreover, the present study provides physiological evidence that HAL affects at least one neural system known to be involved in eyelid conditioning, the cerebellar interpositus nucleus. Our results showed a reduction in CS period interpositus activity after administration of HAL on trials when 75 and 85 dB tones were presented but not on trials when 95 dB tones were presented, a result that parallels the behavioral findings. In fact, a rather high positive correlation existed ($r = +.82$) between CR execution and interpositus activity

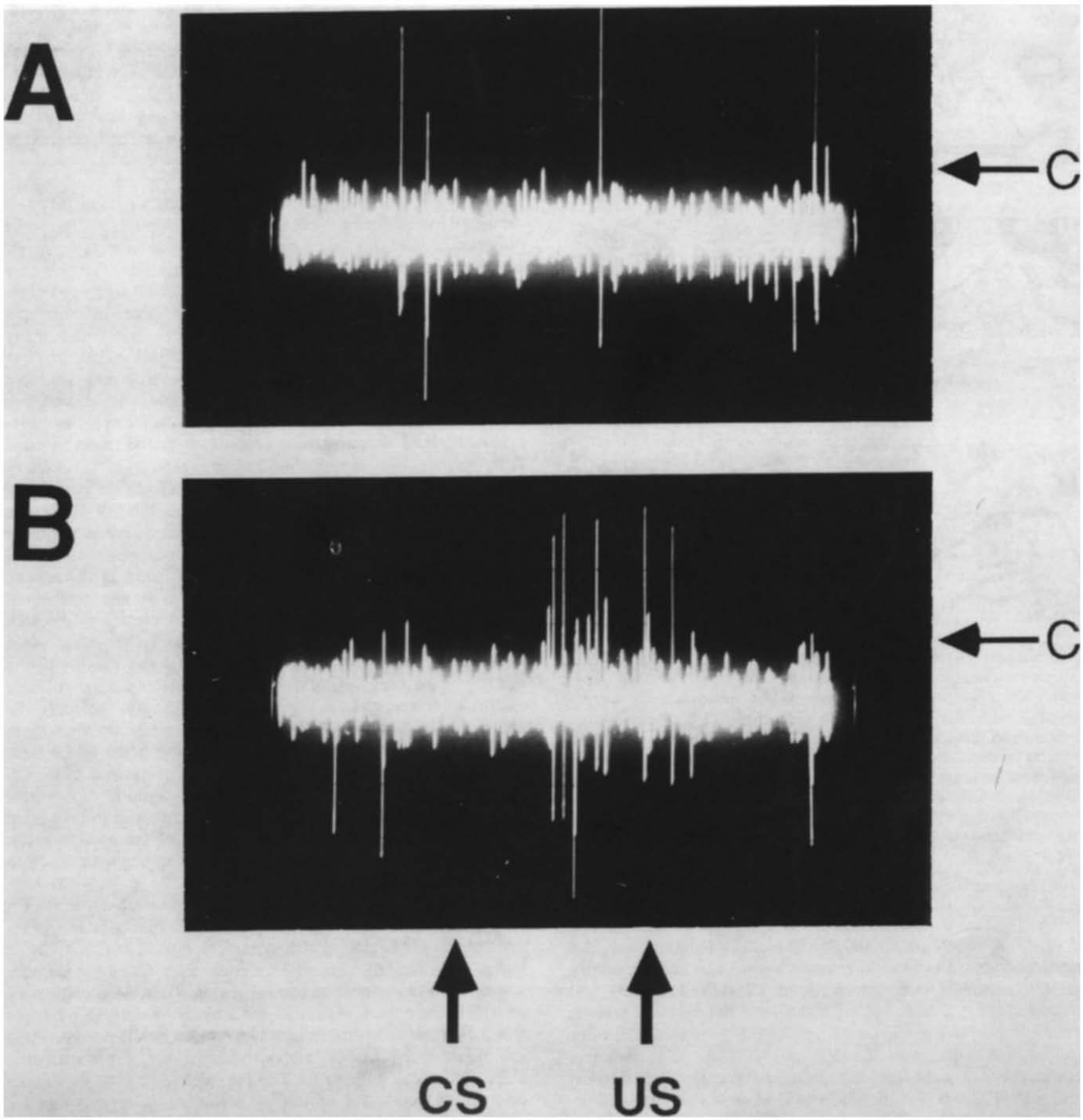

FIG. 4. Representative recordings from the interpositus nucleus of a rabbit during conditioning trials showing the absence of learning-related activity after haloperidol administration (A) and the learning-related discharges present after saline administration (B). The trace of activity is 750 msec in duration and the onsets of the CS and the US are identified at the bottom. No CR was observed on the trial depicted in A while a CR was present on the trial shown in B. The approximate levels at which the comparators were set for discriminating units are shown on the right side of the figure (i.e., the Cs).

recorded both before and after administration of HAL.

Our results also indicate that HAL also affected interpositus activity on 75 and 85 dB trials during the US period as unit activity decreased significantly during the US-1 and US-2 subperiods after HAL injections. Although the behavioral data of Harvey and Gormezano (10) and the results of the present study indicated that processing of the US was not affected by HAL, these recording data suggest that US-related activity in the interpositus nucleus may be affected by the HAL administration. However, data from the 65 and 95 dB training trials argue against this possibility. On these trials, no significant reduction in CS period (95 dB) or US period (65 and 95 dB) activity was seen after HAL injection. Because US intensity was constant on all training trials it is doubtful that HAL would selectively disrupt US activity on 75 and

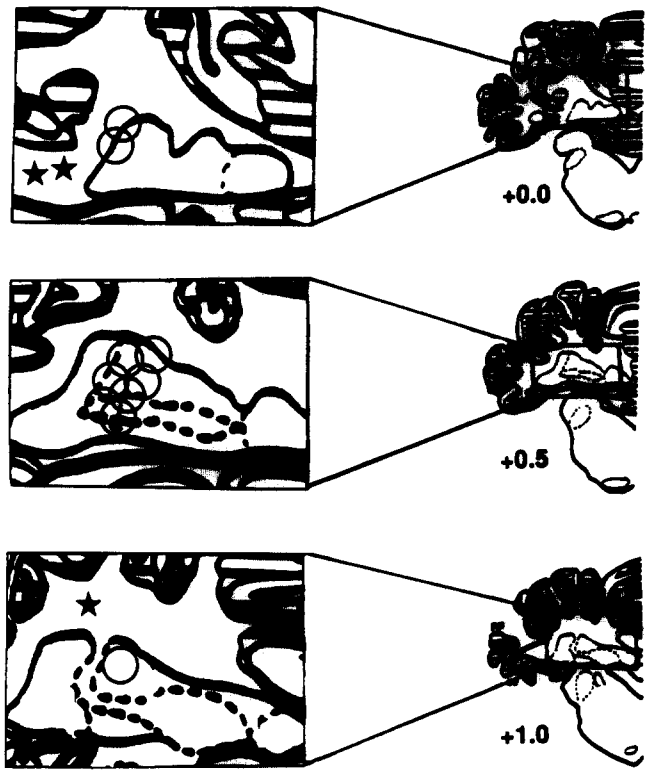

FIG. 5. Locations of recording electrodes in the cerebellum. Numbers indicate the distance of each coronal section from lambda. Open circles denote sites where training-related interpositus nucleus activity was observed (N = 10). For simplicity, both right-side and left-side placements are shown on these left-side drawings. (The actual right-side placements included the dorsal-most site in Section +0.0 and the four dorsal-most and one ventral-most site in Section +0.5.) The stars represent left-side sites where cell activity did not increase above baseline at any time during training (N = 3). The location of the lateral-most ineffective recording site was actually located 0.5 mm posterior to the +0.0 coronal section shown here.

85 dB trials but not on 65 and 95 dB trials. It is more likely that the HAL-induced decline in interpositus nucleus activity during the US period reflects alteration of CR-related activity. As evidenced on CS-alone trials, CR-related interpositus activity often is found in the US period just as the behavioral CR often ovelaps and sums with the UR [e.g., (18)]. It is therefore reasonable to assume that the decrease in interpositus activity during the US period is the effect of HAL on CR-related activity that is present in the US period.

A major reason for conducting the present experiment was to begin an analysis of the neural mechanism by which HAL disrupts classical NM conditioning. We have demonstrated that HAL disrupts normal CR-related activity in the interpositus nucleus and therefore can begin speculating about the locations of potential sites of action of HAL. Previous studies have shown that CR-related activity is projected from the interpositus nucleus to the red nucleus and then to brain stem motor nuclei that are responsible for movement of the NM [e.g., (9,23)]. Because our data indicated that HAL did not affect directly the motor systems responsible for executing the CR and UR, it is unlikely that the CR-disrupting effects of HAL occurred in the structures that make up the CR output pathway (e.g., the red nucleus or cranial nerve nuclei responsible for NM movement). Also, because no changes in UR amplitudes were observed during 95 and 65 dB trials, it is unlikely that HAL affected neural conduction in structures that make up the US pathway. Rather, because no training-related interpositus activity was observed after HAL administration when intermediate tone intensities were used, it appears that HAL disrupted conditioned responding by either directly affecting activity in the cerebellum (i.e., in the interpositus nucleus or cerebellar cortex) or by affecting neural activity in regions involved in projecting auditory CS information to the cerebellum.

A major requirement, of course, for a brain site to qualify as a candidate for mediating the HAL-induced disruption of CRs, is the presence of HAL-binding receptors at the brain site. Two major classes of receptors are known to act as high-affinity binding sites for HAL: dopamine receptors (especially D_2) and sigma receptors [e.g., (11,13)]. Because the auditory brain stem structures and pathways and the cerebellum are not known to contain many dopaminergic receptors (11), it seems unlikely that HAL disrupts conditioning by binding at dopaminergic receptors in these brain regions. However, HAL-binding sigma receptors are abundant in the cerebellar cortex (19) and possibly in the deep cerebellar nuclei (8). Also, HAL-sensitive sigma receptors can be found in brain stem regions like the pontine nuclei as well as in auditory structures such as the inferior colliculus (8). Given the availability of these sigma receptors, it is therefore possible that HAL may disrupt classical conditioning by acting at sigma receptors in the cerebellum or at sigma receptors in specific locations along the CS pathway. Some recent progress has been made in delineating pathways that may project CS information to the cerebellum during conditioning [see (28) for review]. One line of research suggests that the tone CS information may be projected via a rather direct pathway from the cochlea of the ear through a number of auditory structures (e.g., inferior colliculus, cochlear nuclei) to converge in a region surrounding and including the lateral pontine nuclei (27,29). We have proposed that these pontine inputs then project auditory CS information to the cerebellum where convergent information about occurrence of the US is also present (27). It is possible that HAL could disrupt normal activity in the auditory structures that are involved in projecting the CS to the cerebellum and/or in regions of the pontine nuclei that have been implicated to be involved in relaying the tone CS to the cerebellum.

It is also possible that HAL directly disrupts activity in the CS projection system by first affecting activity in higher brain regions that contain HAL-binding receptors. The alteration of activity in higher brain regions then, in turn, could alter activity at auditory structures or in the pontine nuclear region. Possible alterations of activity in the pontine region are particularly interesting in light of recent data that show that at least two regions rich in dopaminergic and sigma receptors, the hippocampus (3) and the neostriatum (32), send projections to the pontine nuclei. In fact, the hippocampal and neostriatal projections terminate in regions that are adjacent to or overlap with pontine regions that have been hypothesized to project CS information to the cerebellum. It is possible that HAL may alter activity in the hippocampus and/or neostriatum which, in turn, alters CS-related activity in the pontine nuclear region. This possibility is supported by behavioral data which have implicated the hippocampus and neostriatum in eyelid conditioning. The hippocampus is not essential for NM conditioning in the standard delay paradigm (24) but appears to be involved in relatively more complex conditioning paradigms such as in trace NM conditioning (20,26) and in discrimination reversal learning (1). In addition, altering hippocampal activity with injections of scopolamine or penicillin has been shown to disrupt conditioning (25). Likewise, it appears that the nigro-striatal system also has a modulatory effect on brain stem eyelid conditioning circuit. Lesions of the substantia nigra disrupt CR acqui-

sition (12) as do caudate nucleus lesions (21). In light of these data that suggest a modulatory role for the hippocampus and neostriatum in NM conditioning, it seems possible that the effects of HAL on NM conditioning is through the disruptive actions of the drug at receptors in these higher, modulatory brain regions.

In summary, the present study illustrates the usefulness of the rabbit classical NM conditioning paradigm for studying the effects of HAL on behavioral and neural aspects of a simple form of motor learning. We expect that future studies involving this paradigm, including studies designed to evaluate the effects of direct HAL infusion into specific brain structures, will delineate the neural bases for the HAL-induced disruption of the classically conditioned NM response, a disruption that is apparently mediated through alterations in brain activity that affects the processing of the auditory CS.

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