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Harmaline-induced Impairment of Pavlovian Conditioning in the Rabbit

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In this study we examined the effects of harmaline on Pavlovian conditioning of the rabbit's nictitating membrane response. The acquisition of conditioned responses was determined during a single session consisting of 120 pairings of a tone-conditioned stimulus with a corneal air puff unconditioned stimulus. Harmaline severely retarded (5 mg/kg) or completely blocked (10 and 20 mg/kg) acquisition of conditioned responses. The blocked or retarded acquisition of conditioned responses could still be detected when the rabbits were tested 2 d after cessation of drug injections, suggesting that harmaline was affecting acquisition and not the motoric expression of conditioned responses. Control experiments established that harmaline (5 mg/kg) did not affect (1) baseline levels of responding, (2) the level of nonassociative responding to the conditioned stimulus, (3) the amplitude or any of the temporal characteristics of the unconditioned response, (4) the development of habituation to the unconditioned stimulus, and (5) the threshold of the unconditioned stimulus for eliciting the unconditioned response. However, harmaline did produce a 12 dB increase in the intensity threshold of the conditioned stimulus for eliciting conditioned responses. We concluded that the primary effect of harmaline was to impair stimulus processing within brainstem circuits such as to reduce the excitatory properties of the conditioned stimulus, thus retarding its entry into associative learning. The results were discussed with respect to the possible role of the inferior olive in associative learning.

[Key words: harmaline, Pavlovian conditioning, rabbit, nictitating membrane, associative learning, olivocerebellar system, stimulus processing]

Evidence has been accumulating for a role of the inferior olivary nucleus in several forms of learning. Inactivation of the inferior olive by means of destructive lesions, reversible lesions, or interruption of olivary projections to the cerebellum has been reported to produce both a loss of previously learned responses and the inability to acquire new responses. Llinás et al. (1975) found that chemical lesions of inferior olive, produced by 3-acetylpyridine, prevented rats from compensating for the postural abnormalities produced by unilateral vestibular lesions. Subsequently, it was reported that changes in gain of the vestibulo-ocular reflex were also prevented by destructive (Haddad et al., 1980) or reversible (Demer and Robinson, 1982) lesions of the inferior olive in the cat. Pavlovian conditioning of nictitating membrane (NM) extension in the rabbit also appears to require the normal activity of the inferior olive. For example, destruction of the inferior olive by means of electrolytic lesions (Yeo et al., 1986) and interruption of olivary projections to the cerebellum by means of knife cuts of the olivary decussation (Türker and Miles, 1986) were reported to abolish the performance of conditioned responses (CRs) and their subsequent reacquisition in the rabbit. A partial exception to these findings was the report by McCormick et al. (1985) of a retention followed by an extinction of CRs after inferior olive lesions in the rabbit.

It is well known that the tremorogenic effects of harmaline are mediated through an action on the inferior olive that results in an enhancement of the rhythmic bursting of neurons in the olivocerebellar pathway (de Montigny and Lamarre, 1973; Llinás and Volkind, 1973). Türker and Miles (1984) reported that harmaline retarded the acquisition of the rabbit's NM response and partially blocked the retention of previously acquired CRs. They suggested that these effects might be due to a harmaline-induced activation of the inferior olive.

Although the results cited above indicate that either inactivation or activation of the inferior olive can block the performance of CRs, it is not clear whether these effects are due to deficits in learning and memory or to a general impairment in sensory–motor function. For example, it is well known that electrolytic (Soechting et al., 1976) or reversible lesions (Kennedy et al., 1982) of the inferior olive prevent the optimal performance of arm movements in the primate. Rabacchi et al. (1986) reported that 3-acetylpyridine–induced lesions of the inferior olive in the rat produced a 17% decrease in the threshold of a nociceptive, unconditioned stimulus (US) for eliciting the flexor reflex in the hind paw, whereas activation of the inferior olive by means of harmaline produced a 27% increase in the threshold of the flexor reflex. Moreover, harmaline produced a dose-dependent decrease in the amplitude of the acoustic startle response and pinna response in the guinea pig (Pellet et al., 1983). These results suggest that alterations in the normal activity of the olivocerebellar system due to either inactivation or activation of the inferior olive can alter an animal's response to the unconditioned properties of auditory and tactual stimuli, which might in turn be responsible for the observed impairment in the performance of CRs.

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Our study further examined the effects of harmaline on Pavlovian conditioning of the rabbit's NM response in order to identify the behavioral processes through which harmaline might be affecting the performance of CRs. Because tolerance to the effects of harmaline on the inferior olive develops rapidly (Lutes et al., 1988), drug effects were examined after a single acute injection. Four experiments were carried out. The first experiment determined the effects of a single injection of 5, 10, or 20 mg/kg of harmaline on the acquisition of CRs during a conditioning session carried out immediately after drug injection and the subsequent retention and acquisition of CRs 48 hr later when no drug was administered. In the second experiment, we examined the effects of harmaline on responding during the explicitly unpaired presentations of the conditioned stimulus (CS) and US at intensities employed during conditioning to determine (1) whether harmaline affected nonassociative determinants of responding such as baseline levels of responding or sensitization to the CS, and (2) whether harmaline affected the characteristics of the unconditioned response (UR) elicited by the US. A third experiment examined whether harmaline altered the US threshold for eliciting URs by employing a range of US intensities. Finally, in the fourth experiment we employed previously trained animals to determine whether harmaline affected the threshold of the CS for eliciting CRs.

Materials and Methods

Subjects. Rabbits of both sexes (New Zealand white albino), weighing 2.5 kg on arrival, were obtained from Hazelton Research Animals (Denver, PA). They were housed individually under a 12 hr/12 hr light/dark cycle, and maintained on rabbit chow and water.

Apparatus and general procedure. The apparatus, including the IBM PC-AT and ASYST software for stimulus control and data acquisition, have been described in detail (Romano et al., 1991). Briefly, each animal was placed in a Plexiglas restrainer and fitted with a headmount that supported a potentiometer directly coupled to a suture placed in the right NM. Movements of the NM were transduced to DC voltages and digitized every 5 msec with a resolution of 0.03 mm of NM movement per analog-to-digital count. A response was defined as a 0.5 mm or greater extension of the NM, and its onset latency was calculated from the time at which the response first deviated from baseline by at least 0.03 mm. The headmount also supported a 2-mm-diameter metal tube positioned 6 ± 1 mm from the center of the right cornea for delivery of a 100 msec air puff US. Unless otherwise noted, the pressure of the US was 200 gm/cm^2 as measured at the end of the metal tube. Experiments were conducted in sound-attenuated chambers containing a house light. A speaker mounted in front and above the animal was used to deliver a 300 msec, 1 kHz tone CS. Unless otherwise noted, the tone intensity was 90 dB.

Harmaline hydrochloride dihydrate (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile water to give doses of 5, 10, or 20 mg/kg as the base. Harmaline or saline vehicle was injected subcutaneously in a volume of 2 ml/kg body weight, 25 min (range, 20–30 min) before behavioral testing. The subcutaneous route was chosen because preliminary experiments indicated that the duration of drug action was longer after subcutaneous than after intravenous injections. Four experimental procedures were employed as described below. One day prior to each of these procedures, animals were given one 60 min adaptation session during which no stimuli were presented or drugs administered; however, to obtain a measure of baseline rates of NM extension, responses were recorded at the intervals to be used during the experimental sessions.

Paired CS–US conditioning procedure. This experiment was carried out in two phases. In phase 1, 35 animals were injected with saline (n = 11) or one of three doses of harmaline (5, 10, or 20 mg/kg; n = 8 at each dose) before a single Pavlovian conditioning session using the procedures and parameters described by Welsh and Harvey (1991). The 60 min acquisition session consisted of 120 paired presentations of the 300 msec tone CS and 100 msec air puff US at an intertrial interval of 30 sec (range, 25–35 sec). Each conditioning trial consisted of a 200 msec baseline period followed by onset of the 300 msec tone CS and then onset of the 100 msec US 275 msec after CS onset. Membrane movement during the 200 msec baseline period did not prevent the pairing of CS and US; however, the trial itself was not included in the behavioral analyses. Responses were scored as CRs if they occurred within 275 msec after CS onset and as URs if they occurred after US onset. At the completion of the phase 1 conditioning session, all animals were allowed 2 d of rest in their home cages before they entered phase 2. In phase 2, all animals were given another conditioning session exactly as before except that no drug or vehicle was injected.

Unpaired CS/US procedure. Eight animals were injected with harmaline (5 mg/kg; n = 4) or saline (n = 4) before a single 60 min session consisting of the explicitly unpaired presentations of the 300 msec tone CS and the 100 msec air puff US. The session consisted of 120 tone-alone and 120 air puff–alone trials presented, on the average, every 15 sec (range, 10–20 sec). Thus, the number of presentations of tone and air puff stimuli and the duration of the session were identical with the paired CS–US procedure. Trials were presented in a semirandomized order with the restriction that there were no more than three consecutive tone or air puff trials within any of the 10 blocks of 24 trials. In addition to measuring the frequency of NM responses on tone-alone and air puff–alone trials, responses occurring in the 275 msec interval before US onset were scored as baseline responses. Responses occurring on US-alone trials were also scored in terms of their onset latency, peak amplitude, latency to peak amplitude, and rise time (time between UR onset and attainment of peak amplitude).

Determination of US thresholds for eliciting URs. Fifteen animals were injected with harmaline (5 mg/kg; n = 7) or saline (n = 8) before a single 60 min session consisting of 60 trials presented every 60 sec (range, 55–65 sec). The 100 msec air puff US was presented on each trial at one of three intensities of 65, 98, and 134 gm/cm^2 as measured at the end of the tube. Each air puff intensity was presented in blocks of 10 trials, first in descending and then in ascending order. The frequency of URs was recorded at each US intensity.

Determination of CS threshold for eliciting CRs. Twenty-three animals received two consecutive daily conditioning sessions using the paired CS–US procedure described above, except that no drug or saline was injected. To ensure stable levels of performance, animals had to achieve the criterion of at least 80% CRs on the second day of conditioning to be included in the study. Fifteen of the 23 animals reached this criterion. These 15 animals were then injected with either harmaline (5 mg/kg; n = 8) or saline (n = 7) before a third conditioning session. In this session, the tone CS continued to be paired with the US, but the intensity of the tone was varied from trial to trial. Six tone intensities were employed: 0 (no tone), 50, 60, 70, 80, and 90 dB. Each intensity was presented once within each of 20 six-trial blocks. The order of tone intensity presented in each six-trial block was randomized. Responses were recorded as CRs if they occurred within 275 msec of CS onset. Responses occurring in the 275 msec interval before US onset when no tone (0 dB) was presented were scored as baseline responses.

Data analysis. A repeated-measure analysis of variance (ANOVA) using the SYSTAT statistical package (version 4.1; Wilkinson, 1988) was carried out on the various response measures. Follow-up tests of simple main effects were performed with the method of Dunnett to allow comparison of all harmaline-injected groups with the vehicle controls (Winer, 1971). Significance for all statistical comparisons was set at $p < 0.05$, two-tailed test.

Results

Harmaline blocks the acquisition of CRs. Each dose of harmaline produced some evidence of resting and/or intention (movement-associated) tremor that persisted throughout the duration of the behavioral testing. After the 5 mg/kg dose, animals demonstrated a mild intention tremor when allowed to move freely. However, these animals demonstrated no resting tremor at any time after injection, including the time that they were in their restrainers for behavioral testing. Animals receiving 10 mg/kg demonstrated a resting tremor that was intensified during movement. Finally, the highest dose of harmaline (20 mg/kg) produced intense resting and intention tremors. The resting tremor produced by doses of 10 and 20 mg/kg was responsible for an increase in baseline levels of membrane movement in phase 1, resulting in an increased probability of responding during the

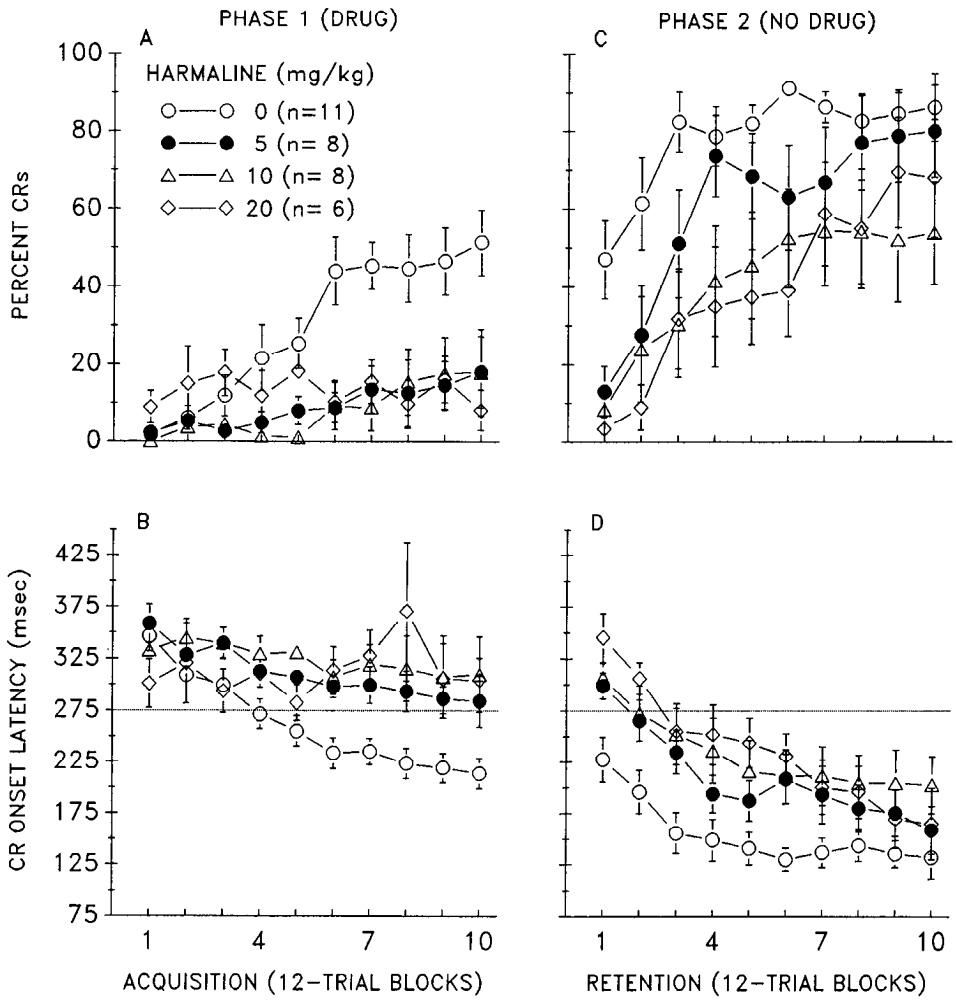

Figure 1. *A* and *B* present the acquisition of CRs in phase 1 after subcutaneous injection of harmaline at doses of 0 (vehicle control), 5, 10, or 20 mg/kg, as measured by the percentage of CRs (*A*) and NM onset latencies (*B*). The retention and subsequent acquisition of CRs in phase 2 when no drug was injected are presented in *C* for percentage of CRs and *D* for NM onset latencies. Phase 2 occurred 48 hr after phase 1. Data are plotted in 10 blocks of 12 trials each during the paired CS–US procedure. Error bars represent 1 SEM.

200 msec period of baseline measurement that immediately preceded each conditioning trial. Because the occurrence of membrane movement during baseline measurement led to discarding that trial, animals injected with 10 or 20 mg/kg harmaline had a greater number of such aborted trials. The percentages of aborted trials for saline controls and animals receiving the lowest dose of harmaline (5 mg/kg) were (mean ± SEM) 9 ± 2 and 10 ± 2, respectively. At the 10 and 20 mg/kg doses the percentages of discarded trials were significantly increased to 17 ± 2 and 48 ± 8, respectively. Two animals were eliminated from the 20 mg/kg dose group because 98% and 61% of their trials had to be discarded. Thus, there were only six animals in the 20 mg/kg group. Nevertheless, the number of discarded trials for these six animals (36 ± 3%; range, 24–42%) remained significantly ($p < 0.01$) higher than that for controls. Because resting tremor after the 10 and 20 mg/kg doses of harmaline increased the baseline level of responding, thus reducing the number of usable trials, only the 5 mg/kg dose was used in subsequent experiments.

Harmaline produced a significant ($p < 0.001$) retardation in CR acquisition during phase 1 as measured by both the percentages of CRs (Fig. 1*A*) and NM onset latencies (Fig. 1*B*). Control rabbits demonstrated a significant ($p < 0.001$) increase in CR frequency during phase 1, from 1.8% on the first block of trials to 51% by the last block of 12 trials, and decrease in NM onset latency from 347 msec to 214 msec. Animals injected with the lowest dose of harmaline, 5 mg/kg, demonstrated only marginal evidence of CR acquisition across the 10 blocks of trials in phase 1. Thus, the percentage of CRs for the 5 mg/kg group increased from 2.2% in the first block to 18.0% in the last block of trials (Fig. 1*A*), but this increase just failed to achieve significance ($p = 0.054$). Although the decrease in response onset latencies from the first to the last block of trials (from 358 to 284 msec; Fig. 1*B*) was highly significant ($p < 0.001$) for animals receiving the 5 mg/kg dose, it should be noted that, even in the last block of trials, the average initiation of responses was still occurring 9 msec after US onset (i.e., 284 minus 275 msec). In contrast, animals receiving the two higher doses of harmaline (10 and 20 mg/kg) failed to demonstrate significant changes in either percentage of CRs ($p > 0.07$) or NM onset latencies ($p > 0.50$) across the 10 blocks of trials in phase 1. It should be noted that animals receiving the 20 mg/kg dose of harmaline demonstrated a higher percentage of CRs than controls across the first three blocks of trials in phase 1 (Fig. 1*A*). This increased responding most likely resulted from the tremor-associated increase in baseline activity since, as noted above, there was no systematic change in this level of responding across the 10 blocks of trials.

Two days later, in phase 2, all animals were given another conditioning session except that no drug was injected (Fig. 1*C*,

D). For all groups, the percentage of CRs in the first block of trials in phase 2 was essentially equal to, and not significantly different from, the percentage of CRs in the last block of trials in phase 1. Control rabbits showed a good retention of CRs and continued acquisition reaching 86% CRs and 133 msec NM onset latency by the last block of trials. Animals that had received the two highest doses of harmaline (10 and 20 mg/kg) demonstrated significantly ($p < 0.01$) lower percentages of CRs in phase 2 (Fig. 1C) and significantly ($p < 0.001$) longer NM onset latencies than vehicle controls (Fig. 1D). More importantly, CR acquisition in phase 2 by animals that had received the 10 and 20 mg/kg doses of harmaline during phase 1 was not significantly different from the initial acquisition demonstrated by vehicle controls in phase 1 as measured by either percentages of CRs or NM onset latencies ($p > 0.3$ for each comparison). Animals that had been injected with the 5 mg/kg dose of harmaline during phase 1 also demonstrated a significantly ($p < 0.02$) lower percentage of CRs and longer NM onset latencies during the first block of trials in phase 2 than vehicle controls. However, the percentage of CRs rapidly increased in this group of animals and reached the level of controls by the last three blocks. Consequently, the overall percentage of CRs, collapsed across all 10 blocks of trials in phase 2, did not differ significantly ($p > 0.10$) between control animals and those that had received the 5 mg/kg dose of harmaline in phase 1; however, the NM onset latencies were significantly longer than that of controls ($p < 0.02$).

Harmaline does not affect nonassociative responding or amplitude and topography of URs. Baseline responding during the unpaired procedure (i.e., responding during the 275 msec prior to US onset when no other stimuli were presented) was low and did not demonstrate any systematic changes across the 10 blocks of trials (Fig. 2A). However, when calculated across all 10 blocks of trials, the baseline responding for animals receiving the 5 mg/kg dose of harmaline (3.4 ± 0.7%) was significantly ($p < 0.01$) higher than that of controls (0.5 ± 0.3%), due solely to an increased responding by the harmaline-injected group in blocks 3 and 4 (Fig. 2A). The average percentage of responding to the tone during the explicitly unpaired presentations of tone and air puff was also low and not significantly ($p > 0.75$) different between animals injected with the 5.0 mg/kg dose of harmaline (10.1 ± 5.2%) and vehicle controls (8.5 ± 2.5%). Both groups demonstrated a significant ($p < 0.02$) increase in percentage of responding to the tone across the 10 blocks of trials (Fig. 2B); however, this increase was not significantly different between control and harmaline-injected animals ($p > 0.50$).

Harmaline (5 mg/kg) had no significant ($p > 0.75$) effect on URs elicited by the US as measured by their peak amplitudes (Fig. 2C). Both the saline- and harmaline-injected groups demonstrated habituation of the UR as reflected by a significant ($p < 0.001$) reduction in its peak amplitude across the 10 blocks of trials (Fig. 2C), which was equivalent for both groups. There was no significant ($p > 0.50$) effect of harmaline on the onset latency of the UR, the actual values being, for control, 56.7 ± 3.6 msec, and for harmaline, 65.2 ± 14.3 msec. Similarly, the latency to peak amplitude was not significantly ($p > 0.25$) different between controls (250 ± 34 msec) and harmaline-injected animals (301 ± 33 msec).

Harmaline does not affect the threshold for elicitation of URs. Both saline controls and animals injected with 5 mg/kg harmaline demonstrated a significant ($p < 0.001$) increase in percentage of URs with increasing US intensity (Fig. 3). There was

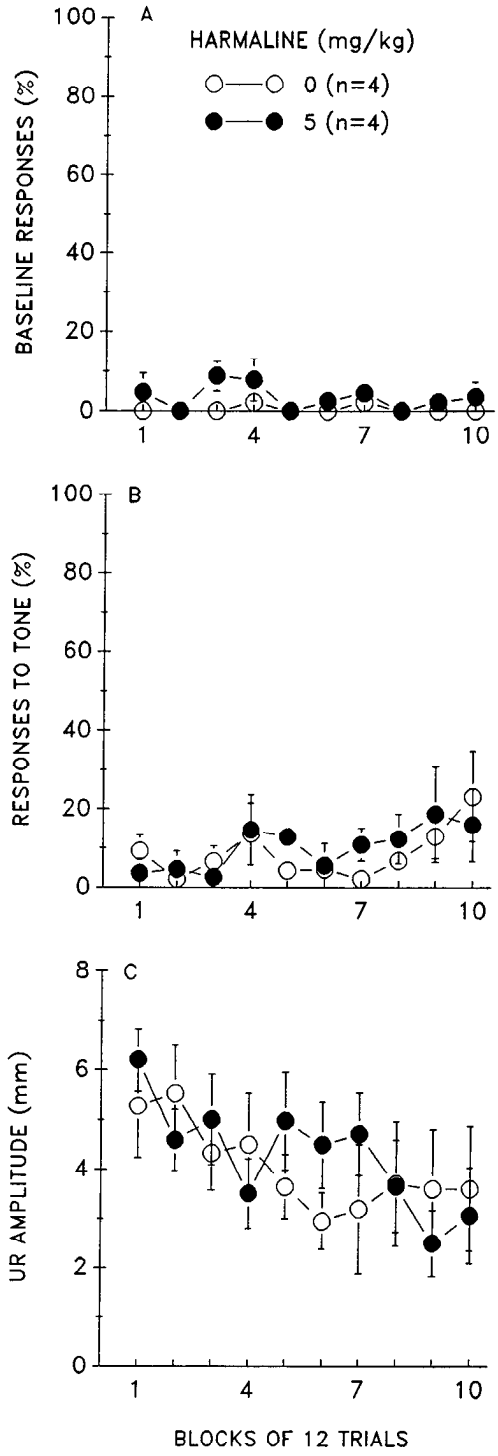

Figure 2. Responding during the explicitly unpaired presentations of tone and air puff stimuli after either saline or harmaline (5 mg/kg): *A*, percentage of baseline responding as measured during the 275 msec interval prior to US onset; *B*, percentage of responding on the tone alone trials; and *C*, the amplitude of the UR on US-alone trials. Responding is presented in 10 blocks of 12 trials, each during the explicitly unpaired CS/US procedure. Error bars represent 1 SEM.

no significant ($p > 0.75$) difference between the percentage of URs demonstrated by control and harmaline-injected animals or in the threshold for elicitation of URs.

Harmaline increases the threshold for elicitation of CRs. Before the determination of CS intensity thresholds, all animals

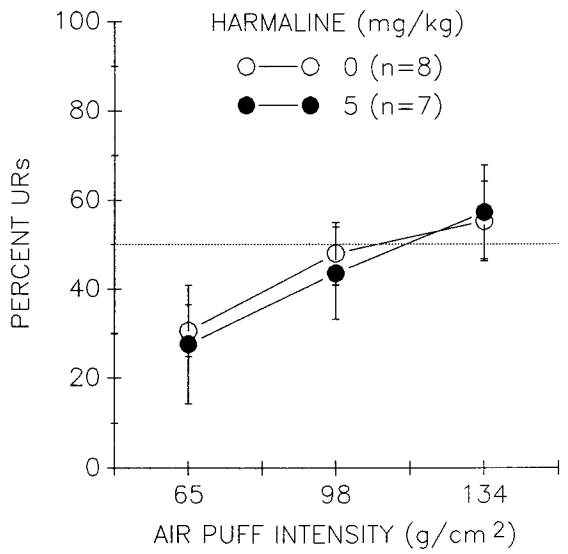

Figure 3. Frequency of URs as a function of three intensities of the air puff US. Each US intensity was presented 20 times. Error bars represent 1 SEM.

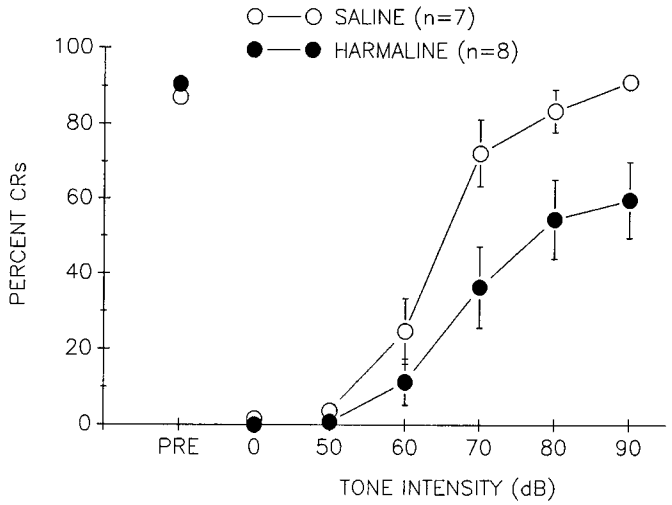

Figure 4. The frequency of CRs as a function of CS intensity. Animals received 2 d of conditioning (120 trials per day) and were then injected with vehicle or harmaline (5 mg/kg) prior to the presentation of various CS intensities. Each CS intensity, including the 0 db (no tone) intensity, was presented 20 times. The *points above PRE* indicate the percentage of CRs to the 90 dB tone CS on the day prior to CS intensity testing. The *points above 0 dB* represent baseline responding, that is, responding in the 275 msec prior to US onset in trials in which no tone was presented. Error bars represent 1 SEM.

had received 2 d of conditioning exactly as described for the paired CS–US procedure and had achieved asymptotic levels of CRs. The percentages of CRs elicited by the 90 dB tone on the day prior to threshold testing was 87.1 ± 2.2% and 90.6 ± 2.0% for animals that were injected with saline and harmaline, respectively, during the third session (see points above PRE in Fig. 4). For control animals, decreasing the CS intensity from 90 to 50 dB produced a concomitant decrease in the elicitation of CRs from 91% to 4%. Harmaline (5 mg/kg) produced a systematic and significant ($p < 0.01$) reduction in the ability of the CS to elicit CRs, as compared with controls. We calculated for each animal the threshold intensity of the CS for elicitation of CRs. Threshold was defined as the CS intensity at which the animal would have exhibited 50% CRs. These values were obtained by interpolation from the CS–CR function of each animal. The mean threshold value for control animals was calculated to be 65.3 ± 5 dB. Harmaline significantly ($p < 0.05$) increased the CS intensity threshold for CR elicitation to 77.5 ± 9 dB. In contrast with the results obtained during the unpaired CS/US procedure (see Fig. 2*A*), harmaline had no effect on baseline responding, responding during the 275 msec before US onset when no tone was delivered (see points above 0 dB in Fig. 4).

Discussion

Harmaline blocks acquisition and not performance of CRs. In agreement with Türker and Miles (1984), harmaline blocked the acquisition of CRs. The block of CR acquisition was dose dependent, with the threshold dose for this effect being 5 mg/kg when injected subcutaneously. The results of this study also confirm the previous conclusions of Türker and Miles (1984) that the block in acquisition produced by harmaline was not due to a motor performance deficit. If acquisition had occurred in phase 1 under harmaline, but could not be expressed in performance, one would expect to observe a large increase in the occurrence of CRs during phase 2 when no drug was injected, such as was obtained by Welsh and Harvey (1991) during reversible inactivation of the interpositus nucleus. At the least, one might expect to observe a more rapid acquisition of CRs than that demonstrated by vehicle controls during initial acquisition in phase 1 (i.e., a savings effect). However, this was not the case for the 10 and 20 mg/kg doses of harmaline. In each case, the percentage of CRs during the first block of trials in phase 2 was low (3.3% and 8.3%, respectively) and subsequent acquisition was not significantly different from initial acquisition by vehicle controls in phase 1, indicating the absence of any savings due to acquistion. As noted above, the 5 mg/kg dose of harmaline was the threshold dose for these effects. Thus, animals at this dose did demonstrate a small and marginally significant acquisition of CRs in phase 1 and consequently some marginal savings in phase 2 acquisition.

Harmaline specifically blocks associative learning. The effects of harmaline on CR acquisition appeared to be due to a block in associative learning since nonassociative learning and other nonassociative determinants of responding were not affected. For example, both sensitization and habituation are considered to be nonassociative forms of learning. Harmaline (5 mg/kg) had no effect on the development of sensitization to the tone stimulus (Fig. 2*B*) or habituation to the air puff (Fig. 2*C*) during the explicitly unpaired presentations of these two stimuli. Harmaline also had no consistent or systematic effect on baseline responding. Thus, while harmaline produced a significant increase in baseline responding during the unpaired CS/US procedure, the increase was quite small (2.9% above controls) and due solely to an increase occurring in 2 of the 10 blocks of trials (Fig. 2*A*). Moreover, even this small effect was not reliable since harmaline had no effect on baseline responding measured during the determination of CS intensity thresholds (see points above 0 dB in Fig. 4). In summary, the absence of any depressant effect of the 5 mg/kg dose of harmaline on baseline responding or responding to the tone during the unpaired procedure further confirms that its depression of CR acquisition during paired conditioning of phase 1 was due to a block of associative learning.

Harmaline blocks learning by reducing the excitatory properties of the CS. Harmaline could have retarded the acquisition of CRs by reducing the excitatory properties of the tone CS and/or the air puff US and thus retarding their entry into associative learning. This possibility was confirmed for the CS but not for the US. Harmaline had no effect on the threshold of the US for eliciting URs. Moreover, the unpaired CS/US procedure indicated that harmaline had no effect on any parameter of the UR elicited by the suprathreshold (200 gm/cm^2 intensity) air puff, including its peak amplitude, onset latency, or latency to peak amplitude. The absence of any effect of harmaline on UR onset latency confirms the previous observations of Türker and Miles (1984). Thus, any retardation in the acquisition of CRs during phase 1 could not be attributed to an effect of harmaline on the UR. Both the conditioned and unconditioned NM responses are expressed through the activation of motoneurons of the accessory abducens nucleus that innervate the retractor bulbi muscle via the VIth nerve (Harvey et al., 1984; Marek et al., 1984; Holstege et al., 1986). Since harmaline had no effect on any parameter of the UR, one can assume that the motoric expression of the CR within this common pathway was also not affected by harmaline. Although harmaline did not alter the unconditioned NM reflex, it has been reported to increase the US threshold for eliciting the unconditioned flexor reflex in the rat (Rabacchi et al., 1986), suggesting that harmaline is having a differential effect on various reflex pathways.

In contrast with the absence of any effect on the UR, harmaline (5 mg/kg) produced a large, 12 dB, increase in the threshold of the tone CS for eliciting CRs. This result is consistent with previous reports that harmaline produces a decrease in the ability of a tone CS to elicit CRs in previously trained rabbits (Türker and Miles, 1984) and a decrease in the unconditioned excitatory properties of an auditory stimulus to evoke the unconditioned acoustic startle response in the guinea pig (Pellet et al., 1983). Taken together, these results suggest that harmaline can block both the conditioned and unconditioned excitatory properties of an auditory stimulus by its ability to raise the threshold for response evocation.

The pattern of results obtained with harmaline on CR acquisition in this study was identical with that obtained previously with a number of other drugs (Harvey, 1987; Schindler and Harvey, 1990). Thus, a wide variety of drugs block associative learning by decreasing the excitatory properties of the CS (e.g., haloperidol, scopolamine, morphine) or increase associative learning by increasing the excitatory properties of the CS (e.g., *d*-lysergic acid diethylamide). These drugs also block (haloperidol and scopolamine) or enhance (*d*-lysergic acid diethylamide) the unconditioned excitatory properties of the tone stimulus as measured by changes in the ability of the tone to produce facilitation of the NM reflex (Harvey and Gormezano, 1981; Harvey et al., 1985, 1988). It has been proposed that drug-induced alterations in the excitatory properties of the CS would alter its ability to enter into associative learning as well as its ability to elicit CRs once learning had occurred (Harvey, 1987). Given the pattern of results obtained in this study, we conclude that the primary action of harmaline is to block the sensory processing of the CS, thus retarding its entry into associative learning and its ability to elicit CRs.

Locus of harmaline action on learning. Based on a number of lesion and stimulation studies, it has been proposed that the neural changes necessary and sufficient for acquisition of the rabbit's NM response occur within cerebellar circuitry (Thompson, 1989b). However, there is disagreement as to whether the locus for learning and memory resides in hemispheral lobule VI of cerebellar cortex (Yeo et al., 1985; Yeo, 1989) or in the interpositus nucleus (Lavond et al., 1984, 1987; Thompson, 1989a,b). Both Yeo (1989) and Thompson (1989a,b) have proposed that the essential CS pathway for associative learning of the rabbit's NM response involves mossy fiber projections of the pontine nuclei via the middle cerebellar peduncle and that the essential US pathway involves climbing fiber projections of the inferior olive via the restiform body. Since both hemispheral lobule VI of the cerebellar cortex and the interpositus nucleus receive these mossy and climbing fiber projections, either or both areas could be sites for the convergence of CS and US inputs that is necessary for associative learning.

McCormick et al. (1985) reported a retention of CRs after electrolytic destruction of the inferior olive followed by a loss of CRs without further training. They interpreted this effect as due to a loss of US input to the interpositus nucleus (via climbing fibers) and hence an extinction of CRs during subsequent paired trials. In contrast, electrolytic lesions of the inferior olive (Yeo et al., 1986) or knife cuts of the olivary decussation (Türker and Miles, 1986) were reported to produce an immediate and total abolition of CRs and to prevent subsequent CR acquisition, an effect that was attributed to both the loss of US inputs to cerebellar cortex and the disruption of normal cerebellar function. For example, inactivation of the inferior olive results in an increased firing of Purkinje cells through removal of the inhibitory influences of the afterhyperpolarization of the complex spike on simple spike activity (Montarolo et al., 1982). This, in turn, produces a strong depression of the activity of the intracerebellar nuclei (Ito et al., 1964) due to the increased activity in the inhibitory corticonuclear projections of Purkinje cells as well as the withdrawal of the excitatory action exerted by the collaterals of the olivocerebellar pathway (Benedetti et al., 1983).

Harmaline also alters cerebellar activity by producing synchronous activation of inferior olive cells and of their climbing fiber projections to the cerebellum. This, in turn, results in an enhancement of the rhythmic bursting of neurons in the olivocerebellar pathway and thus an increase in complex and decrease in simple spike activity of cerebellar Purkinje cells (de Montigny and Lamarre, 1973; Llinás and Volkind, 1973; Llinás and Sasaki, 1989). Consequently, Türker and Miles (1984) had earlier considered the possibility that harmaline blocked learning by disrupting normal cerebellar function. Based on these data, one might conclude that both lesion-induced inactivation and harmaline-induced activation of the inferior olive might be preventing learning through the disruption of normal activity within hemispheral lobule VI and/or the interpositus nucleus, the two areas proposed to be the locus of the memory trace (Thompson, 1989a,b; Yeo, 1989).

Although the cerebellum is clearly involved in regulation of the NM reflex, its role in learning and memory remains controversial. For example, Welsh and Harvey (1989b) reported that lesions of the interpositus nucleus impaired the unconditioned NM response, an effect that was observed at US intensities equivalent in their response-evoking properties to that of the CS (Welsh and Harvey, 1989a,b; Welsh, 1992). Since both CR and UR are expressed through the same motor pathway consisting of the accessory abducens nucleus, VIth nerve, and retractor bulbi muscle, it was suggested that the deficits in retention and reacquisition of CRs produced by lesions of the interpositus nucleus were due to a deficit in motor function

rather than a deficit in associative learning. Steinmetz et al. (1992) have recently published a rebuttal of the findings of Welsh and Harvey (1989b). They reexamined the effects of interpositus lesions on the NM response and concluded that the effects obtained were due to a deficit in associative learning and not to a deficit in performance. However, it is clear that the permanence of destructive lesions makes it difficult to determine whether the resulting changes in the occurrence of CRs are due to deficits in performance and/or learning. To resolve these issues, Welsh and Harvey (1991) employed reversible lesions of the interpositus nucleus produced by the continuous infusion of lidocaine. Rabbits infused with saline demonstrated a robust acquisition of CRs to a tone CS during a 1 hr conditioning session. Rabbits receiving lidocaine infusions into the interpositus nucleus failed to demonstrate any acquisition of CRs to a tone CS during a 1 hr conditioning session. However, when tested 48 hr later, after the effects of lidocaine had worn off, these rabbits demonstrated a frequency of CRs to the tone CS that was equivalent to that of controls, who had demonstrated acquisition during the 1 hr session. These results clearly demonstrate that the interpositus is essential for the optimal performance of CRs but is not required for associative learning.

Similar problems exist with respect to demonstrating a role for hemispheral lobule VI in associative learning. Thus, Yeo and Hardiman (1992) reported that cortical lesions produced a change in the UR elicited by a range of US intensities, raising the possibility that a performance deficit is responsible for part, if not all, of the deficits in acquisition and retention. Moreover, total destruction of hemispheral lobule VI (Lavond et al., 1987; Harvey et al., 1993; Yeo and Hardiman, 1992) or even complete cerebellectomy (Kelly et al., 1990) did not prevent learning (see also Welsh and Harvey, 1992).

The results obtained with lesions of the cerebellum or deep nuclei cited above make it unlikely that harmaline is disrupting a site of learning restricted to the cerebellum. Türker and Miles (1984) were the first to point out that the effects of harmaline on learning might be due to a disruption of normal neuronal activity within other portions of the brain, including the telencephalon as reported earlier by Fuentes and Longo (1971). One site for activation of the NM reflex by the CS has been suggested to occur at preblink areas that are located in the gigantocellular portion of the reticular formation and that are premotor to the retractor bulbi motoneurons located in the accessory abducens nucleus (Guegan and Horcholle-Bossavit, 1981; Harvey et al., 1984; Holstege et al., 1986). These preblink areas of the reticular formation receive inputs from both CS and US and have been suggested to be one of the important sites for the plastic changes underlying associative learning of the NM reflex (Harvey et al., 1985, 1993; Harvey, 1987). This hypothesis has been supported by recent studies that have employed single-unit recordings (Richards et al., 1991) or lidocaine-induced inactivation of neural activity (Bracha et al., 1991) in the more rostral preblink area. Harmaline could affect these brainstem regions by one of two possible mechanisms. First, given the strong reciprocal connections between cerebellum and brainstem, and the extensive projections of the gigantocellular medullary reticular formation to hemispheral lobule VI, one would expect that the rhythmic activity produced by harmaline within cerebellar circuits would also disrupt the brainstem circuits involved in acquisition of the conditioned NM response. Alternatively, the effect of harmaline on brainstem circuits might be due to some direct pharmacological action on neural activity that was independent of its ability to increase the firing rate of olivary neurons.

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