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Relationship Between Heterosynaptic Reflex Facilitation and Acquisition of the Nictitating Membrane Response in Control and Scopolamine-injected Rabbits[1]

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Abstract

Classical conditioning of the rabbit nictitating membrane response was accomplished by presenting a 100-msec tone conditioned stimulus at intervals of 0, 100, 200, 400, and 800 msec before the presentation of a 100-msec shock unconditioned stimulus. In addition, tone-alone and shock-alone trials were interspersed during conditioning. On the first day of conditioning, during which there was no evidence of acquisition of conditioned responses to the tone conditioned stimulus, the amplitudes of the nictitating membrane response evoked on paired tone-shock trials were compared with the amplitudes obtained on shock-alone trials to provide a measure of reflex facilitation. There was a significant correlation (+0.86) in control animals between the degree of reflex facilitation and the degree of learning demonstrated at the various tone-shock intervals. Both reflex facilitation and learning were absent at the 0-msec tone-shock interval, increased at the 100-msec interval, reached a maximum at the 200-msec interval, and then declined at the longer intervals. Scopolamine (0.4 mg/kg) did not affect the amplitude of the nictitating membrane response elicited on shock-alone trials but eliminated any evidence of reflex facilitation or learning at the 100- and 800-msec intervals and significantly reduced both reflex facilitation and learning at the 200- and 400-msec intervals. The comparable effects of scopolamine on both reflex facilitation and learning were reflected by a significant correlation (+0.95) between these two measures. It was concluded that reflex facilitation provides a measure of the temporal course of excitation induced by the tone stimulus within the unconditioned reflex arc and that maximal learning occurs when the shock stimulus is delivered at the maximal point of this tone-induced excitation. Scopolamine, therefore, appears to retard learning by blocking the unconditioned excitatory effects of the tone within the unconditioned reflex arc.

Classical conditioning research with the rabbit's nictitating membrane response (NMR) has provided a substantial and robust set of data on associative learning (Gormezano et al., 1983) due to the virtual absence of nonassociative contributors to conditioned response (CR) measurement. For example, the base line rate of NMR occurrence is low (<2%), there is no unconditioned NMR to the presentation of tone or light stimuli, and there is no evidence of sensitization or psuedoconditioning during the unpaired presentations of tone and light stimuli with an unconditioned stimulus (UCS). Consequently, acquisition of CRs to tone and light conditioned stimuli (CSs) provides a fairly unambiguous measure of associative learning. An additional advantage of the rabbit NMR preparation is that both the CR and the unconditioned response (UCR) consist of the same response, extension of the nictitating membrane (the NMR), and are expressed via the same final common pathway, the VIth nerve (Prince, 1964; Cegavske et al., 1976; Gray et al., 1981; Harvey et al., 1983b, 1984; Marek et al., 1984).

Recent studies have shown that the NMR preparation can serve as a model for delineating the mode of action of drugs on learning (Gormezano and Harvey, 1980; Harvey and Gormezano, 1981; Harvey et al., 1983a; Schindler et al., 1984). These studies revealed that drugs which altered the rate of associative learning also produced a change in the conditioned excitatory properties of the CS. For example, D-lysergic acid diethylamide enhanced the rate of learning and, in previously trained animals, lowered the CS intensity threshold for elicitation of CRs (Gormezano and Harvey, 1980). Haloperidol (Harvey and Gormezano, 1981), morphine (Schindler et al., 1984) and scopolamine (Harvey et al., 1983a) retarded the rate of associative learning and raised the CS intensity threshold for elicitation of CRs. Moreover, the degree of enhancement or retardation of learning was highly correlated with the degree of change in the CS intensity threshold (Schindler et al., 1984), suggesting that the change in rate of associative learning produced by these drugs might be due to their ability to alter the excitatory properties of the tone CS in a manner analogous to an increase or decrease in its nominal intensity. For example, in normal animals, changes in the intensity of a tone CS produce a highly correlated change in the rate of CR acquisition and, once learning has occurred, in the ability of the CS to elicit CRs (Scavio and Gormezano, 1974). Such outcomes are accounted for by stimulus trace formulations of conditioning (Hull, 1943, 1952; Anderson, 1959; Gormezano, 1972; Gormezano and Kehoe, 1981) which postulate that the intensity of the CS affects both its rate of entry into associative learning by altering the intensity and duration of an underlying neural trace (i.e., the unconditioned excitatory properties of the stimulus used as the CS) and its ability to energize (i.e., elicit) CRs once conditioning has occurred. This formulation would predict that the degree to which a drug blocks the unconditioned excitatory properties of a stimulus used as the CS it would also decrease the rate at which the CS enters into associative learning, while the reduction in the conditioned excitatory properties of the CS would block the ability of the CS to elicit CRs once learning had occurred.

It has recently been found that one can obtain a measure of the unconditioned excitatory properties of a CS and, thus, presumably

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those of the previously hypothetical neural trace, by measuring heterosynaptic reflex facilitation of the NMR. Prior to conditioning, auditory stimuli are reflexively subliminal in that they produce detectable neural excitation within the accessory abducens nucleus and the VIth nerve (Berthier and Moore, 1983) but not an overt NMR (Gormezano, 1972). However, an auditory stimulus will increase the amplitude of the NMR elicited by a UCS (Young et al., 1976; Harvey and Gormezano, 1981), and this provides a measure of the amount of excitation produced by the auditory stimulus within the unconditioned reflex arc. Thompson et al. (1976) have noted that there appears to be a direct correlation between the ability of a tone to produce reflex facilitation of the NMR as a function of the tone-UCS interval (Young et al., 1976) and the rate of acquisition of conditioned NMRs at those intervals during conditioning (Smith et al., 1969). In addition, it has been found that haloperidol, which retards the acquisition of CRs to a tone CS, also blocks reflex facilitation of the NMR by a tone stimulus (Harvey and Gormezano, 1981). These data suggest that heterosynaptic reflex facilitation of the NMR may represent the basis for the plastic neuronal changes that lead to learning (Thompson et al., 1976), and that drugs may alter the rate of learning by enhancing or depressing reflex facilitation (Harvey and Gormezano, 1981). In particular, behavioral theories of conditioning based on stimulus trace formulations assume that associative strength accrues at the point of contiguity between the unconditioned neural trace produced by the CS and UCS-UCR occurrence. Thus, the largest increment in associative strength would result from training at a CS-UCS interval for which the CS trace is at a maximum intensity at the time of UCS-UCR occurrence. At progressively shorter or longer CS-UCS intervals, the intensity of the CS trace is presumed to decline and, thus, is less able to produce appreciable increments in associative strength. Therefore, differences in the acquisition of CRs at different CS-UCS intervals are postulated to reflect the variations in the intensity of the CS-neural trace at the point of UCS-UCR occurrence. In this context, changes in the effects of the CS on the amplitude of the UCR at different CS-UCS intervals, as measured by reflex facilitation, should provide an independent measure of the intensity of the CS-neural trace at the time of UCS-UCR occurrence (Gormezano et al., 1983). From this, one would expect that the CS-UCS interval yielding maximum reflex facilitation should also support the most rapid rate of CR acquisition.

The present study sought to determine whether the function relating reflex facilitation of the NMR to CS-UCS interval could serve to anchor the form of the neural trace, independently of conditioning, and, therefore, to predict the rate of CR acquisition at those CS-UCS intervals. This was accomplished by employing several CS-UCS intervals to compare reflex facilitation of the NMR by a tone CS during the first day of conditioning, when no learning had yet occurred, with the subsequent rate of CR acquisition to the tone CS at the same CS-UCS intervals. In addition, we examined whether the effects of scopolamine (0.4 mg/kg) on CR acquisition could be predicted from its effects on reflex facilitation at these CS-UCS intervals.

Materials and Methods

Subjects. This study utilized a total of 112 male and female rabbits (New Zealand white albino) weighing approximately 2 kg on arrival from local suppliers. The rabbits were housed singly with free access to Purina Rabbit Chow and water.

Apparatus and general procedure. The apparatus and procedure have been described in detail (Gormezano, 1966, Gormezano et al., 1983; Harvey et al., 1983a, b). Rabbits were placed in Plexiglas restrainers, fitted with headmounts, and then positioned in ventilated and sound-attenuated experimental chambers. A phototransistor assembly, positioned on the headmount and coupled to a loop of nylon sutured into the right nictitating membrane, converted membrane movements into electrical signals that were subjected, by an Apple II/FIRST computer system (Scandrett and Gormezano, 1980), to an analogue-to-digital analysis using a 2-msec sampling rate and having a resolution of 0.06 mm of actual membrane movement. The digitized values were logged on a Corvus (IMI-7710) for subsequent statistical analysis. Two stimuli were used: a 100-msec, 84-dB (2×10^{-4} dynes/cm^2 reference), 1-kHz tone delivered by an audio-oscillator (Hewlett-Packard, model 201CR) through an 11.4-cm speaker positioned above and in front of the animal; and a 100-msec, 2-mA, 60-Hz shock delivered through two woundclips attached to the skin over the paraorbital region of the head at a distance 10 mm posterior to the canthus and 15 mm apart in the vertical direction.

Drugs. Scopolamine hydrobromide dihydrate (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile nonpyrogenic phsyiological saline just prior to injection. Scopolamine (0.4 mg/kg, calculated as the salt) or saline was injected via a Harvard infusion pump (model 975) into the marginal ear vein in a volume of 1 ml/kg and at a rate of 1.2 ml/min.

Paired CS-UCS training. This procedure utilized 88 experimentally naive rabbits. One day after placement of sutures into the nictitating membrane, animals were placed into each of 12 individual, experimental chambers for a 72-min adaptation session during which no stimuli were presented and no drug or saline was injected. However, in order to obtain a measure of base line responding, NMRs were recorded during the observation intervals to be used during training. On the day after adaptation, animals were assigned at random to one of five training conditions consisting of CS-UCS intervals of 0, 100, 200, 400, or 800 msec. A CS-UCS interval was defined as the time between onset of the 100-msec tone CS and the onset of the 100-msec shock UCS. Rabbits in each of the five CS-UCS training groups were further subdivided into two injection conditions and received either saline or scopolamine (0.4 mg/kg), 30 to 45 min before each of 10 daily conditioning sessions. Thus, at each of the CS-UCS intervals, 9 rabbits received saline and 9 received scopolamine, except for the group at the 200-msec CS-UCS interval receiving scopolamine and the group at the 400-msec CS-UCS interval receiving saline, each of which contained 8 rabbits. Each daily (72-min) training session consisted of 72 trials composed of 60 tone CS-shock UCS pairings at the designated CS-UCS intervals, as well as 6 CS-alone (test) trials and 6 UCS-alone trials. The 72 trials were divided into six 12-trial blocks. Within each 12-trial block, the 6th was always a tone-alone (test) trial and the 12th was always a shock-alone trial. The remaining 10 trials consisted of CS-UCS pairings at the designated CS-UCS interval. The intertrial intervals were a mean of 60 sec (range, 50 to 70 sec). Throughout the experiment a response was defined as an extension of the nictitating membrane of at least 0.5 mm. On paired CS-UCS trials, NMRs were scored as CRs if they occurred during the time between CS and UCS onset and as UCRs if they occurred after UCS onset. It should be noted that the occurrence of CRs in the 0-msec CS-UCS interval group could not, of course, be determined on paired trials since there was a simultaneous onset of CS and UCS, and, therefore, since all NMRs occurred after UCS onset, they were scored as UCRs. On CS-alone (test) trials, NMRs were scored as CRs if they occurred within 900 msec of CS onset. NMRs occurring after UCS onset on UCS-alone trials were scored as UCRs. The amplitude and latency of each CR and UCR were also recorded.

The amplitudes of UCRs obtained on the first day of training were used to assess reflex facilitation of the UCR by the tone CS, in the following manner. Because the amplitude of the UCR demonstrated substantial habituation across the first two blocks of trials (see "Results"), the amplitude of the UCR elicited on the shock-alone trials in the 3rd, 4th, and 5th blocks of trials (trial numbers 36, 48 and 60) served as a base line measure of the UCR. The amplitudes of the UCR obtained on each CS-US trial, preceding and following a UCS-alone trial, was then expressed for each rabbit as a percentage of change from the amplitude of the UCR on the UCS-alone trial. For example, the amplitude of the UCR on CS-UCS trials 35 and 37 was calculated separately as a percentage of change from the amplitude of the UCR on the 36th UCS-alone trial, and these two values were then averaged. Therefore, there were three such mean values per rabbit representing the calculations around the 36th, 48th, and 60th UCS-alone trials. Trials on which a CR occurred during the CS-UCS interval were not used for these calculations. The occurrence of CRs in the 0-msec CS-UCS interval could not, of course, be determined since the CS and UCS occur simultaneously. At the remaining CS-UCS intervals only 4 of the 420 trials had to be eliminated. The frequency of these CRs (0.95%) was comparable to the base line rate of responding (0.9%) that occurred during adaptation when no stimuli were presented, indicating that acquisition of CRs had not yet occurred during the first day of training. Accordingly, there was no evidence that CRs might have affected the measure of reflex facilitation.

Unpaired CS and UCS training. This experiment utilized 24 rabbits that received a 66-min adaptation session carried out as described above and that, 1 day later, were divided into two groups with 12 rabbits receiving saline and 12 receiving scopolamine (0.4 mg/kg), 30 to 45 min before each of 10 daily sessions. Each daily (66-min) session consisted of 132 trials composed of 66 tone-alone and 66 shock-alone trials. The tone and shock stimuli were

identical to those used in experiment 1. The 132 trials were divided into six 22-trial blocks. Within each block equal numbers of tone and shock stimuli were presented in a random fashion with the restriction that the 11th and 22nd trial always consisted of a tone and shock stimulus, respectively. The 11th (tone) trial and the 22nd (shock) trial were considered to be comparable to the tone-alone (test) trial and the shock-alone trial of the paired CS-UCS training procedure. The intertrial interval was 30 sec (range, 25 to 35 sec). NMRs were recorded if they occurred within 800 msec of tone onset or after shock onset. In addition, NMRs occurring during the 800 msec preceding shock onset were recorded as base line responses. All responses were also recorded in terms of onset latency and peak amplitude. As a control for the measurement of reflex facilitation in the paired CS-UCS training procedure, the amplitude of the UCR elicited by the last shock-alone trial in blocks 3, 4, and 5 (trials 66, 88, and 110) was used as a reference UCR. The amplitudes of the UCRs occurring on the shock-alone trial preceding and following trials 66, 88, and 110 were then calculated separately, for each rabbit, as a percentage of change from the reference UCR as described above for the paired CS-UCS training procedure. Finally, responses occurring to the tone on the 11th trial of each block served as a control for the test (CS-alone) trials of the paired CS-UCS procedure.

Statistics. A repeated measures analysis of variance was performed on the data of each experiment with follow-up-analyses of significant effects carried out by the method of Tukey (Winer, 1971).

Results

Reflex facilitation is a function of CS-UCS interval and is blocked by scopolamine. The amplitude of the UCR on UCS-alone trials demonstrated habituation on the first day of the paired CS-UCS procedure, from a mean of 4.1 mm in the first block of trials to 2.4 mm in the last (sixth) block (Fig. 1, *left*) as indicated by a significant effect of trial blocks ($p < 0.001$). The amplitude and habituation of these UCRs were not affected by the injection of scopolamine (Fig. 1, *left*) or by the CS-UCS interval, as indicated by the absence of any significant main effects of these factors or of their interactions with blocks of trials. A similar habituation in UCR amplitude occurred under the unpaired CS,UCS procedure ($p < 0.001$), which also was not affected by scopolamine (Fig. 1, *right*). Because of the large amount of habituation occurring across the first two blocks of trials (Fig. 1), these were not used for calculations of reflex facilitation. In addition, we took the conservative approach of estimating reflex facilitation for the trials immediately preceding and following the reference UCS-alone trial in blocks 3, 4, and 5 in order to minimize any influence of habituation on this measure (see "Materials and Methods" for details).

Facilitation of the nictitating membrane reflex by the tone CS (as measured by the percentage of change in UCR amplitudes) was a significant ($p < 0.05$) function of the CS-UCS interval (Fig. 2). The *points* above the symbol *C* in Figure 2 represent the equivalent calculations of percentage of change in UCR amplitude for the unpaired CS,UCS procedure, the actual values being 0.8% for saline- and 1.5% for scopolamine-injected animals. These low values confirm that the reflex facilitation obtained in the paired CS-UCS procedure was due to the temporal proximity of the tone stimulus to the UCS. For saline-injected animals, there was no reflex facilitation at the 0-msec CS-UCS interval, the actual value 0.5% not being significantly different from 0.8% obtained in the unpaired CS,UCS procedure. The facilitation of the reflex by the tone CS then increased, reaching a maximum of 66% at the CS-UCS interval of 200 msec, and then declined at longer CS-UCS intervals. Scopolamine significantly ($p < 0.001$) reduced reflex facilitation produced by the tone CS (Fig. 2). This effect of scopolamine was also dependent on the CS-UCS interval. As for saline-injected animals, reflex facilitation was absent at the 0-msec CS-UCS interval (0.1%). In addition, scopolamine also eliminated the reflex facilitation seen in saline-injected animals at the 100- and 800-msec intervals, the actual values being −1.6 and 5.3% respectively, as compared with the value of 1.5% obtained in the unpaired CS,UCS procedure. Finally, reflex facilitation was greatly reduced at the 200- and 400-msec intervals as compared with saline-injected controls. Nevertheless, as for control animals, the maximum reflex facilitation demonstrated by scopolamine-injected animals (22%) occurred at the 200-msec CS-UCS interval. It should be noted that the analysis of variance failed to reveal any significant effect of blocks of trials ($p > 0.80$) on reflex facilitation of the NMR, further suggesting that these measures were little affected by processes of habituation.

Acquisition of CRs is a function of CS-UCS interval and is blocked by scopolamine. Figure 3 presents the percentage of occurrence of CRs on the tone-alone (test) trials across the 10 days of the paired CS-UCS procedure. The acquisition of CRs was a significant function of the CS-UCS interval ($p < 0.001$) as was the rate of CR acquisition as reflected by a significant days × interval interaction ($p < 0.001$). Scopolamine significantly reduced acquisition of CRs as compared with vehicle-injected controls ($p < 0.001$), and the reduction in CR occurrence and rate of CR acquisition was a function of the CS-UCS interval as reflected by a significant drug × interval and drug × interval × days interaction ($p < 0.001$, for both interactions). The joint effect of CS-UCS interval and scopolamine on acquisition of CRs is more clearly seen in Figure 4, which plots the mean percentage of CRs, occurring across all 10 days training, as a function of CS-UCS interval. Saline-injected animals failed to demonstrate any evidence of CR acquisition at the 0-msec CS-UCS interval (Fig. 3), and the overall percentage of CRs (2.1%, Fig. 4) was not significantly different from the percentage responding to the tone CS demonstrated by control animals receiving explicitly unpaired CS,UCS presentations (2.2%, *point* above *C* in Fig. 4). Control animals did demonstrate significant acquisition of CRs at the remaining CS-UCS intervals ($p < 0.001$, Fig. 3), with maximum acquisition occurring at the 200-msec interval (Fig. 4). Scopolamine produced a significant retardation in CR acquisition ($p < 0.001$, Fig. 3) which was a significant function of CS-UCS interval ($p < 0.001$,

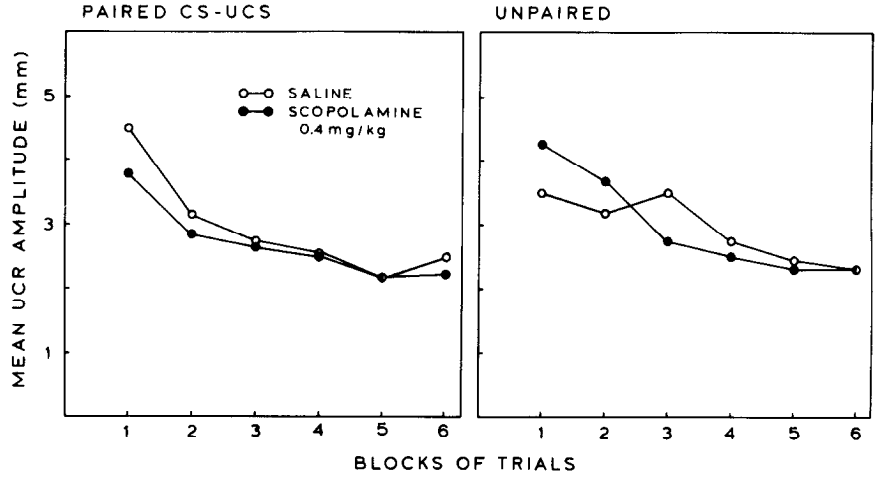

Figure 1. Habituation of the UCR during the first day of training. Data are presented as the mean amplitude of the NMR, in millimeters of actual membrane extension, elicited on each of the six shock-alone trials during the first day of paired CS-UCS training, irrespective of CS-UCS interval (*left*) and on each of the comparable shock-alone trials during the frist day of the unpaired stimulus procedure (right) for saline- and scopolamine-injected animals.

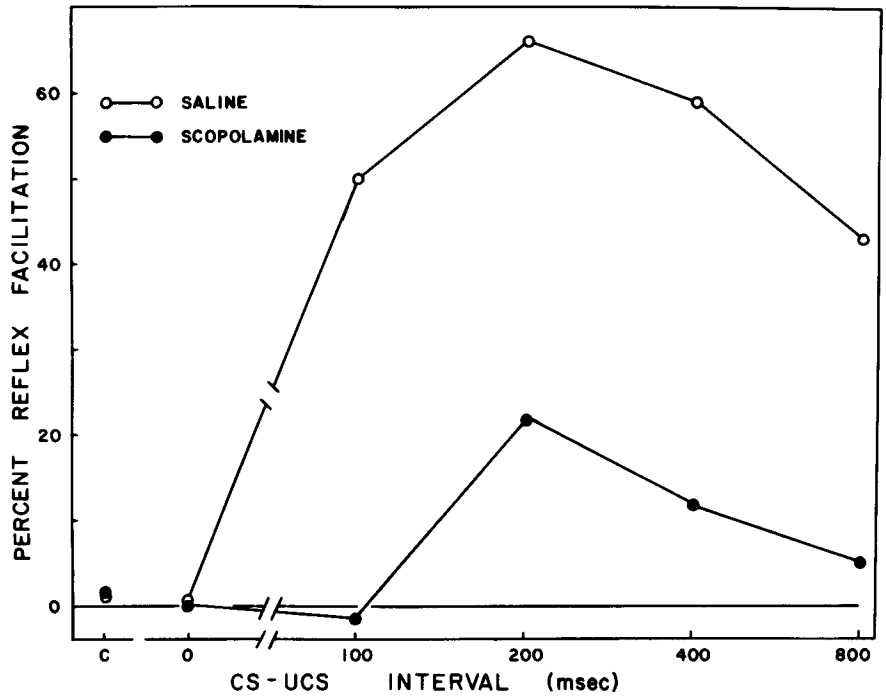

Figure 2. Tone-induced reflex facilitation of the NMR during the first day of training as a function of CS-UCS interval in saline- and scopolamine (0.4 mg/kg)-injected animals. Data are expressed as a mean percentage of change in the amplitude of the NMR on a CS-UCS trial from the amplitude of the NMR on a UCS-alone trial (see "Materials and Methods" for exact details). The *points* above *C* represent comparable calculations for animals in the unpaired stimulus procedure.

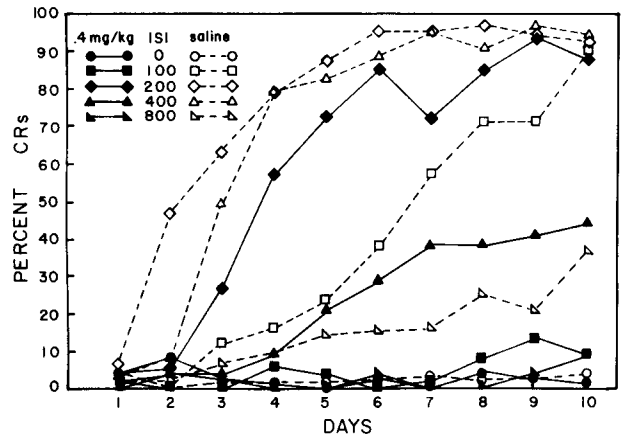

Figure 3. Acquisition of CRs to the tone CS across 10 days of training for animals injected with saline (*dashed lines*) and scopolamine (*solid lines*). Data are expressed as mean percentage of CRs calculated for the six daily tone-alone test trials during paired CS-UCS training at the indicated CS-UCS intervals.

Fig. 4). As for controls, there was no evidence of CR acquisition by scopolamine injected animals at the 0-msec CS-UCS interval (Fig. 3). In addition, scopolamine blocked the acquisition of CRs at both the 100- and 800-msec CS-UCS intervals (Fig. 3). The overall percentage of CRs for scopolamine-injected animals at the 0-, 100-, and 800-msec intervals were 3.5, 3.5, and 2.0%, respectively (Fig. 4), and these values were not significantly different from the percentage responding demonstrated by scopolamine-injected animals in the unpaired CS,UCS procedure (3.8%, *point* above *C* in Fig. 4) or from base line responding (2.4%). Scopolamine-injected animals did demonstrate a significant acquisition of CRs at both the 200- and 400-msec CS-UCS intervals (Fig. 3), but this was significantly slower than that demonstrated by controls ($p < 0.001$). However, as in the case of saline controls, animals given scopolamine demonstrated the most rapid acquisition (Fig. 3) and the greatest percentage of CRs (57%, Fig. 4) at the 200-msec CS-UCS interval.

Reflex facilitation is related to CR acquisition. The shape of the reflex facilitation curve (Fig. 2) and the shape of the CR acquisition curve (Fig. 4), as a function of CS-UCS interval, were quite similar for control rabbits. Moreover, the effects of scopolamine on reflex facilitation as a function of CS-UCS interval (Fig. 2) were quite similar to its effects on CR acquisition (Fig. 4). These relationships are presented in Figure 5, which plots the percentage of CRs (across all 10 days of conditioning) as a function of the percentage of reflex facilitation obtained on day 1. It can be seen that the percentage of reflex facilitation obtained at each CS-UCS interval on day 1, is highly correlated with the percentage of CRs obtained across the 10 days of conditioning at those CS-UCS intervals. The Pearson product moment coefficient of correlation for this relationship in saline-injected animals was ±0.86 and for scopolamine-injected animals was ±0.95 ($p < 0.01$ for each correlation). However, it should be noted that the animals injected with scopolamine demonstrated a greater reduction in reflex facilitation than in percentage of CRs as compared with saline-injected controls. This is reflected in the shift of the scopolamine curve to the left of controls. Thus, although scopolamine-injected animals demonstrated the same relationship between reflex facilitation and percentage of CRs as did controls, the quantitative aspects of this relationship differed.

Discussion

The results of this study indicated a high degree of correlation (+0.86) in control rabbits between the degree of reflex facilitation produced by a tone at different tone-shock intervals and the degree of CR acquisition demonstrated at those intervals. These findings support the general view that heterosynaptic reflex facilitation may represent the plastic changes that lead to learning (Thompson et al., 1976; Kandel, 1979; Harvey and Gormezano, 1981). These results also provide additional data in support of the stimulus trace interpretation of conditioning as formulated by Hull (1943, 1952). He assumed that a stimulus used as a CS would have an unconditioned excitatory effect in the CNS which would increase with time and then decay. Optimum conditioning would then occur when the UCS was presented at the point of maximum excitation produced by the CS. In agreement with this view, the data on reflex facilitation obtained in this study suggest that the presentation of a tone stimulus leads to an excitation within the unconditioned reflex arc that increases with time, reaching a maximum at approximately 200 msec after tone onset, and it is at this point in time that delivery of the UCS produces maximum learning. Learning at a particular CS-UCS

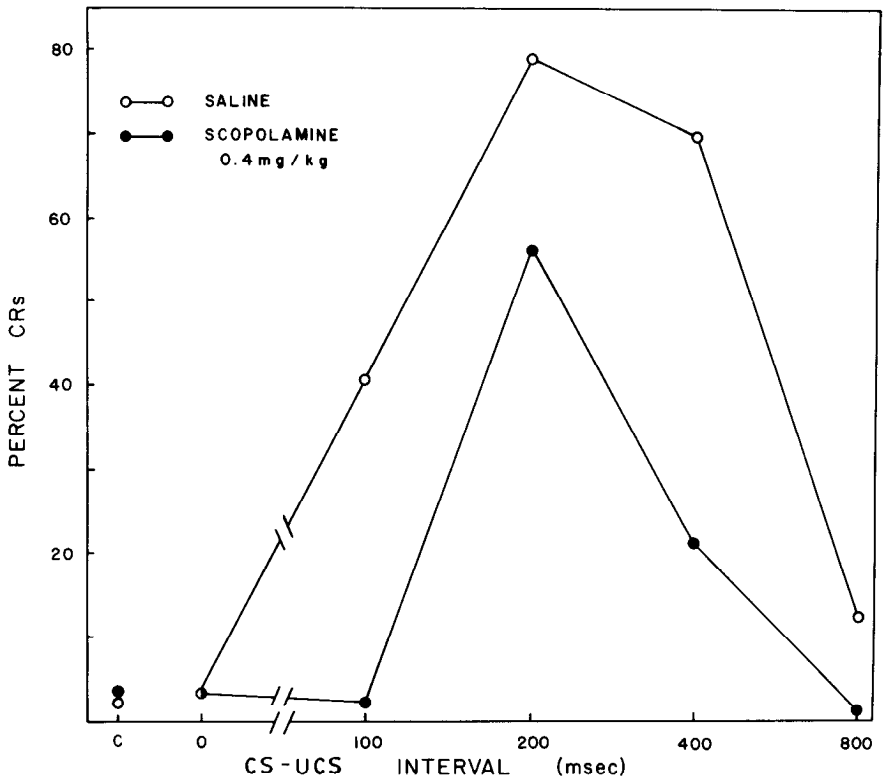

Figure 4. Mean percentage of CRs, calculated across all 10 days of the paired CS-UCS procedure, as a function of CS-UCS interval for saline- and scopolamine-injected rabbits. Data are based on the tone-alone test trials. The *points* above *C* are the mean percentages of NMRs occurring to the tone CS during the unpaired stimulus procedure.

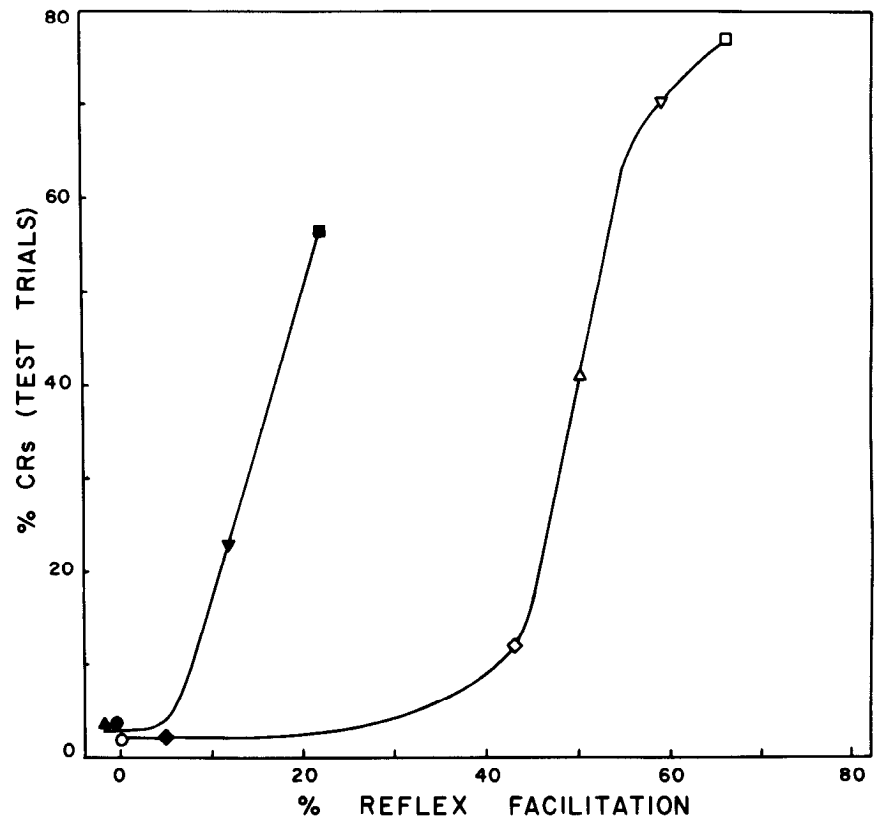

Figure 5. Relationship between reflex facilitation and CR acquisition in animals injected with the saline (*open symbols*) and scopolamine (*solid symbols*). Data are taken from Figures 2 and 4. The *circles*, *triangles*, *squares*, *inverted triangles*, and *diamonds* refer to the 0-, 100-, 200-, 400-, and 800-msec CS-UCS intervals, respectively.

interval thus appears to be a direct function of the intensity of the CS-induced neural excitation at the point of UCS delivery.

The view that the neural events underlying reflex facilitation may be responsible for learning was also supported by the results obtained with scopolamine. Scopolamine had no effect on the unconditioned reflex arc as reflected by the absence of any change in UCR amplitude compared with control rabbits. This result was in agreement with previous studies which also found that scopolamine did not affect the UCS threshold for eliciting UCRs or the amplitude of the UCR across a wide range of UCS intensities (Harvey et al., 1983a). However, scopolamine did reduce both the duration and the magnitude of the tone-induced reflex facilitation at the varying

CS-UCS intervals, and this effect was highly correlated (+0.95) with the degree of learning occurring at those intervals. We conclude, therefore, that scopolamine was blocking the unconditioned excitatory effects of the tone on the unconditioned reflex arc and this was responsible for the decrease in the rate of learning.

Several points should be noted with respect to these correlations as depicted in Figure 5. For controls, reflex facilitation had to exceed 40% before there was an associated increase in percentage of CRs during acquisition. In addition, the percentage of CRs showed a large change (~10 to 80%) across a narrow range of changes in reflex facilitation (approximately 40 to 70%). It is not clear whether this reflects the fact that a stimulus must produce a substantial amount of excitation within the unconditioned reflex arc before it can enter into learning and that small, additional increases in excitation produce large changes in learning, or whether learning would have occurred at lower levels of reflex facilitiation if training had been continued for a longer period of time. It should also be noted that if scopolamine had retarded CR acquisition solely through a decrease in reflex facilitation, then the points for the scopolamine-injected animals would have fallen on the control curve of Figure 5. However, scopolamine produced a far greater reduction in reflex facilitation than it did in CR acquisition as indicated by the shift in the curve to the left. Thus, for the scopolamine treated animals, only small changes in reflex facilitation (10 to 20%) produced large increases in CR acquisition (20 to 60%). Although the data of Figure 5 indicate that reflex facilitation predicts CR acquisition, they also suggest that scopolamine is having some additional actions that also affect learning. For example, it is possible that the decrease in reflex facilitation produced by scopolamine resulted in some compensatory changes that altered the precise relationship between reflex facilitation and learning seen in control animals.

The effects of scopolamine on tone-induced reflex facilitation might well be at the level of the reticular formation. The present study utilized a trace procedure so that maximum reflex facilitation at the 200-msec tone-shock interval was occurring 100 msec after tone offset. Moreover, substantial facilitiation was observed 300 msec after tone offset (the 400-msec interval) and was still detectable at 700 msec after tone offset (the 800—msec interval). Such long-term effects of the tone stimulus are compatible with the time course for the effects of auditory stimuli on the reticular formation (Killam and Killam, 1958). In addition, previous studies have reported that a number of drugs act on the reticular formation to enhance or block the conditioned and unconditioned excitatory effects of auditory stimuli as measured by changes in both behavioral and EEG arousal thresholds (Bradley, 1958; Killam and Killam, 1958; Key and Bradley, 1960). In particular, scopolamine has a depressant effect on these measures of behavioral and EEG arousal (White and Boyajy, 1960; Sadowski and Longo, 1962). Recent electrophysiological and anatomical studies suggest that reticular formation neurons are also involved in the reflex arc of the NMR.

UCSs, such as tactual stimulation of the cornea or electrical stimulation of the skin just lateral to the eye, activate trigeminal inputs to pars oralis of the spinal trigeminal nucleus (Prince, 1964; Spencer et al., 1980; Berthier and Moore, 1983; Harvey et al., 1984) which in turn projects directly to the accessory abducens nucleus (Harvey et al., 1984). Activation of the accessory abducens nucleus leads to contraction of the retractor bulbi muscle (via the VIth nerve) which pulls the eye back into the orbit, and the force of this mechanical action, in turn, forces the nictitating membrane across the cornea (Prince, 1964; Cegavske et al., 1976; Gray et al., 1981; Marek et al., 1984). In addition to this disynaptic reflex arc, cells in pars oralis also project to cells in the reticular formation which are premotor to the accessory abducens nucleus (Harvey et al., 1984). The anatomical evidence for the existence of two pathways for the nictitating membrane reflex is supported by the existence of two components for the retractor bulbi muscle reflex to corneal stimulation, with the longer-latency component appearing to represent a projection through the reticular formation (Guegan and Horcholle-Bossavit, 1981). The multisynaptic component of the nictitating membrane reflex which is mediated by reticular formation neurons may represent the point at which auditory and trigeminal inputs interact to yield both reflex facilitation and the more permanent changes responsible for learning. In addition, the effects of scopolamine in the present study and those of haloperidol in a previous study (Harvey and Gormezano, 1981) suggest that drugs may affect learning by altering the excitatory effects of a CS on these reticular formation neurons as originally proposed by a number of electrophysiological studies (Bradley, 1958; Killam and Killam, 1958).

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