1979

Trials distribution and Ur excitability in conditioned inhibition of the rabbit's nictitating membrane response.

Anthony G. Romano
University of Massachusetts Amherst

TRIALS DISTRIBUTION AND UR EXCITABILITY IN CONDITIONED INHIBITION OF THE RABBIT'S NICTITATING MEMBRANE RESPONSE

A Thesis Presented

By

Anthony G. Romano

Submitted to the Graduate School of the
University of Massachusetts in partial
fulfillment of the requirements for the degree of

MASTER OF SCIENCE

February 1979

Psychology

TRIALS DISTRIBUTION AND UR EXCITABILITY IN CONDITIONED INHIBITION OF THE RABBIT'S NICTITATING MEMBRANE RESPONSE

A Thesis

By

Anthony G. Romano

Approved as to style and content by:

John W Moore 19 Dec 78

John W. Moore, Chairman of Committee

John J. B. Ayres, Member

Rachel Keen Clifton, Member

Bonnie Strickland, Department Head
Psychology

February 1979

Abstract

The rabbit NMR preparation was employed in two studies of Pavlovian conditioned inhibition. Experiment 1 was a parametric study designed to determine the effects of 10, 20, 40, and 80 trials per session at 30 and 60 second ITIs on the rate of development of conditioned inhibition. In contrast to similar studies of the rate of acquisition in excitatory conditioning, the results indicated that the rate of development of conditioned inhibition was not affected by manipulations of trials per session and ITI. However, 40 trials per session at a 30 sec ITI was judged to be a more efficient set of parameters for producing conditioned inhibition than other sets. Experiment 2 tested the hypothesis that a conditioned inhibitor would attenuate the UR by raising the threshold of excitability in a "US-center." Other hypotheses regarding the locus of action of a conditioned inhibitor were also examined. Following training under the Pavlovian conditioned inhibition paradigm (A+/AX-), the amplitude of the UR was measured on three trial-types: a) US-alone trials, b) X-US trials, and c) AX-US trials. The results indicated that the conditioned inhibitor (X) did not attenuate the UR while the compound consisting of the conditioned inhibitor and a conditioned excitor (AX) significantly augmented the UR relative to control conditions. The latter result was interpreted as a verification of the Rescorla-Wagner model's prediction of superasymptotic excitation. The results of both experiments were regarded as further evidence that conditioned excitation and conditioned inhibition are not symmetrical opposites.

Table of Contents

List of Tables

List of Figures

According to Rescorla (1969), a stimulus may be called a conditioned inhibitor if, as a result of experience with some operation relating that stimulus to the US, the stimulus comes to control a tendency opposite to that of a conditioned excitor. The procedure used in establishing a stimulus as a conditioned inhibitor was first outlined by Pavlov (1927). Briefly, the conditioned inhibition paradigm (A+/AX-) involves reinforced presentations of one CS (A) interspersed with nonreinforced presentations of that same stimulus plus another CS (X) which is the potential conditioned inhibitor. Such a procedure usually results in the formation of a discrimination between the excitatory CS (A) and the inhibitory compound (AX).

Rescorla and Wagner (1972) have proposed a model of conditioning whereby conditioned excitation and conditioned inhibition are treated as symmetrical opposites based on the same fundamental mechanism through which a CS is either positively or negatively associated with a particular US. According to the Rescorla-Wagner model, the strength of conditioned stimulus control is indicated by the theoretical dependent variable V (for value). When a particular CS, A, is repeatedly paired with a US, the associative strength of that CS, V_A, will eventually approach some asymptotic value (λ). Thus, the increments in V_A are taken to be a decreasing linear function of the difference between V_A and the asymptotic value towards which it may grow. The model further states that when several CSs are concurrently present on a trial, the strength of the individual components is modified only until the collective value of the compound reaches asymptote.

It is therefore possible for individual elements of a compound to have associative strengths greater than or less than the asymptotic value of the compound. An example of this occurs in conditioned inhibition where the excitatory CS (A) is associated with a V of positive value and the inhibitory CS (X) is associated with a V of negative value. As applied to the conditioned inhibition paradigm (A+/AX-), the model specifies that reinforcement changes V_A according to the formula

$$\Delta V_A = \alpha_A \beta_1 (\lambda_1 - V_A),$$

that nonreinforcement changes V_A and V_X according to the formulae

$$\Delta V_A = \alpha_A \beta_2 (\lambda_2 - V_{AX})$$

$$\Delta V_X = \alpha_X \beta_2 (\lambda_2 - V_{AX})$$

where

$$V_{AX} = V_A + V_X.$$

The rate parameters α and β are bounded by 0 and 1; α varies directly with stimulus salience and β_1 varies directly with the strength of the US. The other rate parameter, β_2, is associated with nonreinforcement and is typically assumed to be equal to β_1. Finally, the excitatory asymptote, λ_1, is a direct function of US intensity while the inhibitory asymptote, λ_2, is usually assumed to be equal to zero.

As the preceding equations indicate, training under the A+/AX- paradigm maintains the positive associative strength of stimulus A while allowing the AX compound to undergo decrements in associative strength, and therefore, conditioned responding, as it approaches a net associative value of zero. Furthermore, these decrements endow stimulus X with negative associative strength. However, as Rescorla (1969) has indicated, the continued failure of a CS to evoke a CR is not sufficient grounds for designating that CS a conditioned inhibitor since, for example, nonreinforced presentations of a neutral stimulus will produce the same results (Lubow & Moore, 1959). Thus, Rescorla has suggested that special testing procedures be employed before designating a stimulus as a conditioned inhibitor: summation and retardation. A summation test consists of pairing the suspected inhibitor with a CS which has undergone excitatory conditioning. Conditioned inhibition is indicated when this combination reduces the strength or likelihood of a CR below the level of the excitatory CS presented alone. A retardation test consists of pairing the suspected inhibitor with the US and observing the rate of acquisition. Conditioned inhibitors show retarded acquisition relative to a control procedure in which the subject has had no previous experience with the CS.

Although the aforementioned summation test employs a conditioned excitor against which inhibition is assessed, Rescorla (1969, p.80) indicates that alternative procedures are available. One such procedure involves determining the threshold value of a US required to

elicit a UR in the presence of an inhibitory stimulus. Thus, Wagner, Thomas, and Norton (1967), using electrical stimulation of motor cortex in dogs as a US, found that the threshold intensity of the US for eliciting a UR was higher when the US was preceded by a CS- than when it was preceded by a CS+ or when the US was presented in isolation. In a related study, Thomas and Basbaum (1972) employed hypothalamic stimulation in cats as a US in discriminative conditioning where the UR was either a fear or rage reaction. For one group of animals (intramodal group), the required discrimination was between two tones while for a second group of animals (intermodal group), the required discrimination was between a tone and a light. Once discrimination was achieved, Thomas and Basbaum evaluated the effects of CS+ and CS- on US-elicited URs. In both groups, the greatest amount of unconditioned responding occurred in the presence of CS+. However, in the intermodal group, fewer URs occurred in the presence of CS- than when the US was presented alone. In the intramodal group, by contrast, more URs were elicited when the threshold US was coupled with CS- than when the US was presented alone.

Although these studies were not concerned with conditioned inhibition per se, the finding that certain inhibitory stimuli increase the threshold value of a US required to elicit a UR may be related to Konorski's (1948) suggestion regarding the mode and locus of action of a conditioned inhibitor. Konorski proposed that a conditioned inhibitor acts by raising the threshold of excitability in a "US-center" which controls both conditioned and unconditioned responding. Other

potential loci and actions of a conditioned inhibitor have been outlined by Rescorla (1973, in press) and Rescorla and Holland (1977). Konorski's proposal implies that the UR would be attenuated in the presence of the conditioned inhibitor. In Experiment 2 of the present study, the magnitude of the UR to a weak US in the presence of the conditioned inhibitor was contrasted with the magnitude of the UR to the US presented alone. A weak US was necessary in order to avoid any ceiling effects on the amplitude of the UR and to prevent rapid conditioning as CRs would artifactually contaminate measurement of UR amplitude.

The following experiments were concerned with conditioned inhibition in the rabbit nictitating membrane preparation. Since the topography of the rabbit's unconditioned nictitating membrane response can be precisely measured and conditioned inhibition has been demonstrated in this preparation on previous occassions (Marchant, Mis, & Moore, 1972; Marchant & Moore, 1974), it was deemed to be an ideal preparation for testing Konorski's (1948) proposal. Experiment 1 was designed to determine the most efficient trials distribution parameters for producing a rapid rate of development of conditioned inhibition. Aside from enhancing the rate at which conditioned inhibition develops, the use of more efficient trials distribution parameters would also be expected to produce a stronger conditioned inhibitor. Once these parameters were determined, they were applied to Experiment 2 which was designed to test Konorski's proposal that conditioned inhibitors act on a "US-center."

Experiment 1

The trials-per-session variable in conditioning the rabbit's nictitating membrane response (NMR) has received considerable attention since Gormezano, Schneiderman, Deaux, and Fuentes (1962) first introduced this preparation. Hupka, Massaro, and Moore (1968), using 15, 50, 65, or 90 trials per session, found an inverse relationship between the rate of acquisition and the number of trials per session. This finding has since been corroborated by Kehoe and Gormezano (1974) employing 1, 5, 10, and 50 trials per session, and Salafia, Terry, and Daston (1975) who sampled 5, 15, 30, 60, and 120 trials per session.

A parameter which is related to the number of trials per session is the intertrial interval (ITI). This parameter also affects the rate of acquisition with superior performance being found under longer ITIs (cf. Gormezano & Moore, 1969). For example, in human eyelid conditioning, Spence and Norris (1950) found increased conditioned response frequency as ITIs were increased from 9 to 90 seconds. Salafia, Mis, Terry, Bartosiak, and Daston (1973) reported a similar function for the rabbit NMR preparation using a range of ITI values from 5 to 120 seconds. Additional evidence for improvements in conditioning over time has been reported by Frey and Gavin (1975). Using the rabbit eyeblink preparation, these investigators found that CR strength increased over "retention" intervals ranging from 5 minutes to 24 hours.

Several interpretations of the foregoing results are possible. It may be that massed trials and multiple trials within a session retroactively interfere with consolidation of the memory trace thus delaying the formation or stabilization of those memories which are necessary for the development of the CR (cf. Glickman, 1961). Wagner, Rudy, and Whitlow (1973) have proposed a similar interpretation although these investigators have preferred to use the information processing term "rehearsal" rather than consolidation. According to Wagner (1976), the degree to which an event is learned is determined by the degree to which that event is rehearsed. Furthermore, the amount of rehearsal an event receives is dependent upon whether or not that event is prerepresented (primed) in short-term memory (STM). Events which are already represented or primed in STM are assumed to command less rehearsal than events which are not primed in STM. That is to say, surprising events command more rehearsal than expected events.

Wagner (1976) distinguishes between two types of priming mechanisms: (a) self-generated priming and (b) retrieval-generated priming. Self-generated priming of a target event in STM is accomplished by the recent presentation of that same event whereas retrieval-generated priming of a target event is accomplished by the presentation of other cues with which the target event has been associated. These associated cues are said to initiate retrieval of the target event from long-term memory (LTM).

With regard to acquisition training, Wagner's formulation indicates that massed trials and multiple trials within a session command less rehearsal than more distributed trials as a result of being primed in STM. Thus, the acquisition process is retarded with massed practice relative to distributed practice due to differential amounts of rehearsal. A question of interest then, is whether or not a similar effect can be obtained with conditioned inhibition training. At the present time, the development of conditioned inhibition using the rabbit NMR preparation is a rather arduous process. For example, with 100 trials per daily session at an ITI of 30 seconds, conditioned inhibition, as measured by suppression of conditioned responding on nonreinforced trials, frequently does not emerge until after several hundred trials (eg. Marchant, Mis & Moore, 1972; Marchant & Moore, 1974). Any procedure which would accelerate this process would be most helpful. To this end, two parameters, trials per session and ITI, were manipulated in the conditioned inhibition paradigm.

Method

Subjects and Apparatus

The subjects were 64 experimentally naive albino rabbits weighing approximately 2.2 kg. A detailed description of the apparatus has been described elsewhere (Marchant *et al.*, 1972). Briefly, a small nylon loop sutured into the animal's right NM was connected to the shaft of a Minitorque potentiometer. Lateral movement of the NM was then transduced to a dc signal and recorded on a polygraph located in

an adjacent room. A conditioned response (CR) was defined as a pen deflection of at least one mm (corresponding to an NMR of less than one mm) occurring within the 450 msec CS-US interval.

The excitatory stimulus (CS+) consisted of the onset of two 4.5 V incandescent lights located in front of the animal and mounted behind white translucent screens. The inhibitory compound (CS-) was composed of the light CS and a 1200 Hz, 90 db (re: .0002 dynes/cm^2) tone delivered over a speaker mounted directly in front of the animal. Previous studies in our laboratory have shown that the tone is more salient (ie. leads to more rapid conditioning) than the light. No attempt was made to counterbalance the roles of light and tone as unpublished observations have suggested that conditioned inhibition is most easily obtained when the conditioned inhibitor is the more salient of the two stimuli. Furthermore, most of the previous investigations of conditioned inhibition in the rabbit NMR preparation have employed a tone as the conditioned inhibitor (eg. Marchant et al., 1972; Marchant & Moore, 1974). The US was a 2 ma ac shock of 50 msec duration delivered via two stainless steel wound clip (Clay-Adams, 9 mm) electrodes affixed to the skin of the infraorbital region of the right eye. The CS and US ended simultaneously.

Procedure

Following suturing of the right NM, all animals were habituated to the apparatus for a period of 50 minutes. Acquisition training to the light CS began on the next day. In this phase, all animals received 100 training trials per session at an ITI of 30 sec until a

criterion of 90% CRs in one conditioning session was achieved. Conditioned inhibition training began on the next day and continued for a period of 14 days. Rabbits were randomly assigned to eight experimental conditions with a total of 10, 20, 40, or 80 trials per session at ITIs of 30 or 60 seconds. Each session consisted of an equal number of CS+ and CS- presentations.

Results and Discussion

Figures 1 and 2 depict the percentage of CRs occurring to CS+ and CS- for each combination of ITI and trials per session (T/S).

See Figures 1 and 2, pp. 36-37

Since all of the groups had acquisition training to CS+ prior to conditioned inhibition training, the percentage of CRs made to CS+ remained fairly stable over the 14 sessions with all of the groups showing a 14 sessions average of better than 90% CRs to CS+. However, the percentage of CRs to CS- appears to vary over sessions for each group of animals. For all animals, the decrement in CS- responses relative to CS+ responses in the initial sessions is most likely the result of external inhibition brought about by the introduction of the tone into the CS- compound. Moreover, it appears that this external inhibition effect was more pronounced in the 60 sec ITI groups than in the 30 sec ITI groups. This difference is probably due to the more rapid habituation of external inhibition allowed by the shorter ITI. There also appears to be a trade-off between habituation of external

inhibition and the development of conditioned inhibition which would account for the apparent flatness of some of the CS- curves.

The difference in the development of discriminative performance between groups can be seen most clearly in Figures 3 and 4 where the ordinate represents the mean percentage of CRs to CS+ minus the mean percentage of CRs to CS-. Given the high level of responding to CS+, the difference between responses to CS+ and CS- was regarded as a

See Figures 3 and 4, pp. 38-39

good index of discriminative performance. As Figures 3 and 4 indicate, the groups experiencing 20 T/S at a 60 sec ITI and 40 T/S at a 30 sec ITI showed an improvement in discriminative responding over sessions while the remaining groups discriminated at a fairly stable level throughout conditioned inhibition training. Although the 80 T/S, 30 sec ITI group also appears to improve over sessions, reference to Figure 5 indicates that that group's overall performance was inferior

See Figure 5, p. 40

to that of the 40 T/S, 30 sec group ant the 20 T/S, 60 sec group. Figure 5 also indicates that the best overall performance was obtained with 40 T/S, at a 30 sec ITI followed by 80 T/T, 40 T/T, and 20 T/S at a 60 sec ITI.

A 2 x 4 analysis of variance was performed of the data depicted in Figure 5 in order to determine if there were any significant effects of ITI and T/S on overall performance. No significant effects were found for ITI ($\underline{F}$ (1, 56) = 1.46), T/S ($\underline{F}$ (3, 56) = 1.00), or ITI x T/S ($\underline{F}$

(3, 56) = 1.01). The ITI x T/S source of variation was further analyzed for differences between trends for the simple effects of T/S at each ITI. No differences were found in the linear trend (F (1, 56) = 0.39), quadratic trend (F (1, 56) = 0.08), or cubic trend (F (1, 56) = 2.55). Thus, various combinations of ITI and T/S do not seem to affect overall performance in a conditioned inhibition paradigm.

In order to determine how ITI and T/S affected discriminative performance over sessions, a 2 x 4 x 14 mixed factorial analysis of variance was conducted on the per cent CR difference data. Since the main effects for ITI, T/S, and the ITI x T/S interaction are reported above, only those sources of variation involving sessions are reported here. No significant effects were found for sessions (F (13, 728) = 1.30) or the interactions between sessions and ITI (F (13, 728) = 1.25), sessions and T/S (F (39, 728) = 0.73), or sessions, ITI, and T/S (F (39, 728) = 1.41). Thus, rates of development of conditioned inhibition remained constant over the combinations of ITI and T/S. These results are inconsistent with the effects of similar manipulations on the rate of acquisition of the excitatory CR (Hupka, et al., 1968; Kehoe & Gormezano, 1974; Salafia et al., 1975).

Although the rate of development of conditioned inhibition was unaffected by manipulations of trials distribution parameters, the results of the present study suggest that one set of parameters was more efficient in producing conditioned inhibition than others. Specifically, if efficiency is assessed in terms of overall performance and the length of the conditioning session, then the most efficient parameters for producing conditioned inhibition are 40 T/S at a 30 sec ITI.

Experiment 2

According to Rescorla and Wagner (1972), training under the Pavlovian conditioned inhibition paradigm (A+/AX-), where reinforcement is administered on A trials and witheld on AX trials, results in the acquisition of excitatory associative strength to stimulus A and inhibitory associative strength to X. The excitatory associative strength of A is indicated by its ability to evoke CRs while the inhibitory strength of X is reflected by its ability to reduce or suppress CRs when compounded with A. Thus, A is commonly referred to as a conditioned excitor and X, a conditioned inhibitor.

One commonly accepted view of conditioning assumes that a stimulus which has been repeatedly paired with a US, such as A, becomes capable of evoking CRs by developing an excitatory association with an internal representation or memory of the US (Rescorla, 1973, in press). With this view in mind, Rescorla and Holland (1977) have delineated four potential loci for the action of a conditioned inhibitor, X. First, X may be acting at the peripheral response level by preventing the exhibition of the CR evoked by A. Second, X may develop an inhibitory association with A, thus neutralizing the excitatory strength of A. Third, the inhibitor may act on the excitatory association existing between A and the internal representation of the US. Finally, the view favored by Konorski (1948) and Rescorla (1973, in press) is that X acts on the internal representation of the US by raising its threshold for activation.

Although Konorski (1948) and Rescorla (1973, in press) have very similar models of excitatory conditioning, they differ in one important respect. According to Konorski, a conditioned excitor evokes a CR by weakly activating a "US-center" which the US itself activates more strongly. Conversely, a conditioned inhibitor prevents the execution of a CR by raising the threshold of excitability in the US-center. In Rescorla's model, a conditioned excitor effects conditioned responding by activating a US representation while a conditioned inhibitor attenuates conditioned responding by raising the threshold for activation of the US representation. For Rescorla then, the US representation controls only conditioned responding while for Konorski, the US-center controls both conditioned and unconditioned responding. Thus, with regard to conditioned inhibition, both views call for attenuation of conditioned responding in the presence of a conditioned inhibitor while only Konorski's view predicts a concomitant attenuation of unconditioned responding.

Ison and Leonard (1971) and, more recently Young, Cegavske, and Thompson (1976) demonstrated an augmentation of the rabbit's unconditioned NMR when a pure tone preceded US presentations. Ison and Leonard reported that the degree of augmentation was dependent upon the interstimulus interval, the intertrial interval, the intensity of the tone, and, finally, the intensity of the shock US. Young et al., in a partial replication of the Ison and Leonard experiment, varied the interstimulus interval as well as the type of US. In one US condition, the NMR was elicited by means of a corneal airpuff. The

second US condition involved electrical stimulation of the abducens nucleus which has been shown to be the efferent center controlling the NMR (Cegavske, Thompson, Patterson, & Gormezano, 1976). Tone-induced excitability under these two conditions was essentially identical.

The present study was designed to determine the locus of action of a conditioned inhibitor by examining the effects of a conditioned inhibitor on UR excitability. Briefly, the design of the experiment involved training under the A+/AX- paradigm followed by evaluation of UR amplitude where the UR was elicited on the following trial-types: AX, X, and US alone. Attenuation of the UR on AX trials relative to X trials would provide support for those views of conditioned inhibition which demand the presence of an excitatory association in order for a conditioned inhibitor to exert its effect. Alternatively, attenuation of the UR on X trials relative to US alone trials would provide support for Konorski's (1948) proposal that conditioned inhibitors act by raising the threshold of excitability in a US-center which controls both conditioned and unconditioned responding. However, since the present study employed a tone as the conditioned inhibitor, X, a straightforward attenuation of the UR on X trials would not be anticipated in light of studies demonstrating tone-induced augmentation of the UR (cf. Ison & Leonard, 1971; Young, et al., 1976). Despite the tone's facilitating effect on the UR, a tone was selected for the role of conditioned inhibitor since previous studies of conditioned inhibition in the rabbit NMR preparation succeeded in

establishing a tone as a reliable conditioned inhibitor (eg. Marchant, et al., 1972; Marchant & Moore, 1974).

In order to assess the effects of a tone as a conditioned inhibitor on UR excitability, the experimental group was compared with several control groups. All of the groups, including the experimental group, were expected to exhibit facilitated URs on tone trials. However, based on Konorski's (1948) suggestion that a conditioned inhibitor would attenuate the UR, the experimental group was expected to show less tone facilitation than the control groups.

Method

Subjects and Apparatus

The subjects were 28 experimentally naive albino rabbits weighing approximately 2.2 kg. The apparatus was the same as in Experiment 1.

For all conditions, the excitatory CS (A) consisted of the onset of two 4.5 V incandescent lights while the inhibitory compound (AX) consisted of the light CS (A) in conjunction with a 1200 Hz, 90 db (re: .0002 dynes/cm^2) tone (X). During acquisition and conditioned inhibition training, the US was a 2 ma ac shock of 50 msec duration delivered via two stainless steel wound clip (Clay-Adams, 9 mm) electrodes affixed to the skin of the infraorbital region of the right eye. The CS-US interval was 450 msec where the CS and US terminated together.

Procedure

Twelve subjects were randomly selected for the experimental group and four subjects were randomly assigned to each of four control groups. Following suturing of the nictitating membrane, all subjects were habituated to the apparatus for a period of 50 minutes. The experimental design is summarized in Table 1. Stage 1 acqui-

See Table 1, p. 41

sition training to A began 24 hours later. All animals received 100 training trials daily at a 30 sec ITI until a criterion of 90% CRs in one conditioning session was achieved. Stage 2 training began on the next day and continued for a period of 14 days. In this stage, Group CI, the experimental group, received conditioned inhibition training daily with 20 reinforced A trials (A+) interspersed in a quasirandom order with 20 nonreinforced AX trials (AX-) at a 30 sec ITI. Experiment 1 determined that these parameters efficiently produced the most robust conditioned inhibition. Nevertheless, a discrimination criterion was established for Group CI such that the percentage of CRs occurring to AX had to be at least 70% less than the percentage of CRs occurring to A on at least two consecutive days. Since four subjects did not meet this criterion, their data did not enter into any of the analyses.

Group LI received nonreinforced presentations of X as a control for the effects of a latent inhibitor on UR excitability. The number and distribution of X presentations paralleled the number and

distribution of AX presentations in Group CI. Although latent inhibitors do not suppress conditioned responding when compounded with an excitatory CS (Reiss & Wagner, 1972), they do show retarded acquisition when subsequently paired with a US (Lubow & Moore, 1959). Hence, it was conceivable that nonreinforced presentations of a tone might have a nonspecific effect on UR excitability which would obscure the effects of a tone on the UR when that tone has been nonreinforced in a conditioned inhibition paradigm.

Group US received only US presentations as a control for US habituation. Hupka, Kwaterski, and Moore (1970) found that, shortly after the emergence of CRs, there is a between- and within-session decrement in the amplitude of the UR on US alone trials relative to CS-US trials. This finding suggested to the authors that US habituation probably occurs on early CS-US trials but is interrupted with the start of conditioning as the CS begins to acquire excitatory control over the UR. In light of this possibility, the present study required a control for US habituation since UR amplitude on US alone trials served as the baseline response for determining UR excitability. The number and distribution of US trials in Group US paralleled the number and distribution of reinforced trials in Group CI.

Group SD received simple discrimination training with 20 reinforced A trials and 20 nonreinforced X trials according to the same trials distribution parameters as Group CI. Group SD was run at a later date than the other groups. Moore (1974) has presented data which suggest that such a procedure does not endow X with conditioned inhibitory properties unless A and X are in the same modality. Thus,

Group SD served as a control for experience with CSs and the US while maintaining the excitatory strength of A and the essentially neutral value of X. Group SD also served as a control for any interaction between US presentations and nonreinforced tone presentations.

Finally, Group Sit was naive with respect to Stage 2 training but spent the same amount of time in the conditioning apparatus as the other groups.

Stage 3 was a testing phase in which the UR was elicited and measured on AX, X, and US alone trials. Each trial was presented five times in an unsystematic order. This procedure was repeated on a second day but evidence of anticipatory CRs precluded the use of that data in the subsequent analysis (see appendix for the data from test day 2). Although Stage 1 and Stage 2 training employed a 2 ma US, the intensity of the US was decreased to .50 ma for Stage 3 in order to avoid any ceiling effects on the amplitude of the UR and to prevent rapid conditioning as CRs could artifactually contaminate measurement of UR amplitude. The ISI and ITI were the same as employed during training. A retardation test was conducted on the day following completion of Stage 3. One hundred reinforced X trials were presented at the original 2 ma shock level.

Results and Discussion

Figure 6 shows the percentage of CRs made by Group CI during conditioned inhibition training. Averaged over the 14 sessions, there was a significant difference between the percentage of CRs to A+ and the percentage of CRs to AX- ($\underline{t}$ (7) = 9.85, $\underline{p} < .001$) thus

indicating that a reliable discrimination was formed. Furthermore, a retardation test conducted subsequent to the Stage 3 testing phase

See Figure 6, p. 42

indicated that X was a reliable conditioned inhibitor. Analysis of of the retardation test is reported at the end of this section.

Mean UR amplitudes for each trial-type in the testing phase are depicted in Table 2. Although Group SD's URs are larger than the

See Table 2, p. 43

other groups', their large scores do not represent a ceiling effect because Group SD was selected from a different shipment of rabbits. An individual Friedman two-way analysis of variance was conducted for each group in order to determine if there were any significant differences in mean UR amplitudes across trial-types. No significant differences were found for Group LI ($\chi^2_r(\underline{k} = 3, \underline{n} = 4) = 3.5$, $\underline{p}<.273$), Group US ($\chi^2_r(\underline{k} = 3, \underline{n} = 4) = 1.625$, $\underline{p}<.653$), Group SD ($\chi^2_r(\underline{k} = 3, \underline{n} = 4) = .5$, $\underline{p}<.931$), or Group Sit ($\chi^2_r(\underline{k} = 3, \underline{n} = 4) = 3.5$, $\underline{p}<.273$). However, Group CI, the experimental group, did show a significant difference in UR amplitude across trial-types ($\chi^2_r(\underline{k} = 4, \underline{n} = 8) = 11.81$, $p<.0024$). Mean UR amplitude for each trial type for Group CI is depicted in Figure 7 along with the standard error of each mean. Separate comparisons be-

See Figure 7, p. 44

tween trial-types for Group CI were made using the Wilcoxon matched-pairs signed ranks test. As Figure 7 indicates, UR amplitude in the

presence of the AX compound was enhanced relative to UR amplitude on US alone trials ($T = 0$, $p < .01$), and relative to UR amplitude on X trials ($T = 0$, $p < .01$). However, UR amplitude in the presence of the conditioned inhibitor, X, was not attenuated relative to the UR elicited by the isolated US ($T = 9$, $p > .05$).

The results of the testing phase for all groups are summarized in Table 3 where the mean amplitude of the UR on AX and X trials is

See Table 3, p. 45

expressed as a percentage of the UR on US alone trials. Table 3 indicates that the tone, X, facilitated the UR in all of the groups with the exception of Group US whose mean was reduced due to one animal who responded below the 100% US alone level. These results are therefore consistent with reports of tone-induced augmentation of the rabbit's unconditioned NMR (Ison & Leonard, 1971; Young et al., 1976).

As was stated earlier, a straightforward attenuation of the UR in the presence of the inhibitory tone, X, was not anticipated for Group CI due to the tone's facilitatory effect on the UR. Therefore, Konorski's (1948) suggestion that a conditioned inhibitor would attenuate the UR was assessed by comparing the amount of tone facilitation exhibited by Group CI with the amount of tone facilitation occurring in each of the control groups. Tone facilitation is represented by the column labelled X in Table 3. On the basis of Konorski's suggestion, Group CI was expected to show less tone facilitation than

each of the control groups. However, one-tailed Mann-Whitney U tests indicated that the amount of tone facilitation exhibited by Group CI did not differ from that exhibited by Group SD (U =12, p = .285), Group LI (U = 26, p = .055), Group US (U = 11, p = .23), or Group Sit (U = 16, p = .533). Thus, there is no evidence that a conditioned inhibitor attenuates the UR.

One interesting facet of the data depicted in Table 3 is Group CI's response to the AX compound. It appears as though the presence of the conditioned inhibitor in the AX compound amplified the CI group's response to the compound relative to the control groups. In order to determine the magnitude of this amplification effect, a difference score was computed for each animal by subtracting mean UR amplitude on X trials from mean UR amplitude on AX trials and expressing this difference as a percentage of the mean UR amplitude on US alone trials. The mean difference score for each group appears in the column labelled AX-X in Table 3. The difference scores were subjected to a one-way Kruskal-Wallis analysis of variance which indicated a significant difference among the groups (H (4) = 12.03, $p<.02$). In light of this difference, individual Mann-Whitney U tests were conducted on the difference scores in order to compare Group CI with each of the control groups. Group CI was found to be significantly different from Group SD (U= 4, p = .048), Group US (U = 2, p = .016), and Group Sit (U = 0, p = .004). However, the difference between Group CI and Group LI was not significant (U = 6, p = .110).

In retardation testing, the mean percentage of CRs for Group CI was 60.75 and for Group Sit, 90.5. Thus, Group CI demonstrated retarded acquisition relative to Group Sit ($\underline{U}$ = 3.5, $\underline{p} < .024$, one-tailed).

General Discussion

The major findings of Experiment 2 were as follows. (a) Training under the A+/AX- paradigm resulted in successful discriminative performance with suppression of conditioned responding on AX trials. A subsequent retardation test established that X was a reliable conditioned inhibitor. These results are consistent with previous studies demonstrating conditioned inhibition in the rabbit NMR preparation (Marchant et al., 1972; Marchant & Moore, 1974). (b) When X and AX were coupled with a low-level US, X produced UR amplitudes comparable to those produced by an isolated US while AX produced greater UR amplitudes than either X or the isolated US.

Since the conditioned inhibitor attenuated CRs but not URs, the present results argue against Konorski's (1948) view that conditioned inhibitors act on a "US-center" common to both CRs and URs. These results may be related to a finding reported by Mis (1975) that electrical stimulation of brain sites capable of attenuating the CR were less than optimal in attenuating the UR.

The present results provide support for Rescorla's (1973, in press) position that conditioned inhibitors act on an internal representation of the US whose arousal is responsible for the CR. However, a modification of this view would have to be made in order to account for the observed augmentation of the UR in the presence of the AX compound; that is, that presentations of AX produced a subthreshold arousal of the US representation which then facilitated the effects of the subsequent US. Thus, in addition to controlling CRs, the US

representation appears to exert excitatory control over the UR. Such a view is consistent with the finding that CS-US trials produce higher amplitude URs that US alone trials once the CS acquires an excitatory tendency (Hupka et al., 1970).

The present study also has a bearing on other suggested loci for the action of a conditioned inhibitor. It is unlikely that conditioned inhibitors act at the peripheral response level since such an action would have attenuated URs in the presence of X relative to URs elicited by the isolated US. Furthermore, the lack of attenuated URs in the presence of AX relative to X is a result opposite to that predicted by those views of conditioned inhibition which demand the presence of excitatory cues for conditioned inhibitors to be effective.

The finding that the presence of the conditioned inhibitor in the AX compound amplified Group CI's unconditioned response to the compound relative to Group SD may be related to the phenomenon of "superconditioning." Rescorla (1971) demonstrated that reinforcement of a neutral stimulus in the presence of a conditioned inhibitor enhanced the effectiveness of reinforcement relative to similar treatments in which a conditioned inhibitor was absent. The Rescorla-Wagner model predicts a similar enhancement in the effectiveness of reinforcement when an excitatory stimulus, A, is reinforced in the presence of X, a conditioned inhibitor (Rescorla & Wagner, 1972). According to the model, such a procedure would initially endow the AX compound with superasymptotic excitatory strength. It seems likely,

therefore, that Group CI's amplified URs on AX trials reflects the acquisition of superasymptotic excitation.

As indicated earlier, tone-induced facilitation of the unconditioned NMR occurred in all groups. This finding underscores the view that tone-facilitation is not a learned effect (Ison & Leonard, 1972; Young et al., 1976) since facilitation occurred even in the presence of a tone which reliably attenuated CRs.

In summary, the present study suggests that two more additions may be made to the list of asymmetries between excitation and inhibition. Experiment 1 revealed that, unlike excitatory conditioning, the development of conditioned inhibition is not subject to manipulations of trials distribution parameters. Thus, there is no evidence that time-dependent processes, such as consolidation or rehearsal, play a role in the development of inhibitory associations. Experiment 2 indicated that, although conditioned excitors and conditioned inhibitors have symmetrically opposite effects on the CR, these stimuli have asymmetric effects on the UR in that conditioned excitors tend to augment the UR (cf. Hupka et al., 1970) while conditioned inhibitors do not have the symmetrically opposite, attenuating effect on the UR.

The results of Experiment 2 have implications for future studies of neural substrates of conditioning in the rabbit NM preparation. These results may also be applicable to similar studies employing the cat NM preparation recently introduced by Patterson, Olah, and Clement (1977). With regard to conditioning, the neural sites of

particular interest are the hippocampus and the neural centers controlling the NM.

The neural mechanisms responsible for reciprocal control of both the rabbit and cat NM have been discussed at length by Cegavske et al. (1976). In both species, NM extension is controlled by the abducens nerve which innervates the retractor bulbi muscles. In rabbit, these muscles mediate eyeball retraction thus producing a passive extension of the NM across the eyeball. In cat, however, abducens control of the NM is more direct as slips of the retractor bulbi muscles are attached directly to the NM. Retraction of the rabbit NM is primarily a passive response although a small active component is present due to innervation of striated muscle fibers in the NM by the oculomotor nerve. By contrast, the cat NM is actively retracted due to autonomic innervation of smooth muscle fibers in the NM by the superior cervical ganglion.

Patterson et al. (1977) have indicated that conditioning of the cat NMR closely parallels that of the rabbit in terms of rate of acquisition and response topography. However, extinction of the cat NMR proceeds more rapidly than extinction of the rabbit NMR under similar conditioning parameters. The authors suggested that such rapid extinction may reflect strong autonomic inhibitory activity brought on by the extinction procedure. Since extinction presumably involves an inhibitory process, these results suggest that the superior cervical ganglion may be the efferent neural substrate of inhibitory conditioning of the cat NMR.

One method used in identifying potential neural substrates of conditioning involves the correlation of neural activity in the suspected substrate with the learned, behavioral response. Using this procedure, Thompson, Cegavske, and Patterson (1973) successfully demonstrated that the abducens nucleus is the motoneuron substrate of excitatory conditioning of the rabbit NMR. This same procedure may be used as a first approximation in identifying the efferent neural substrates of inhibitory conditioning of the rabbit and cat NMRs. Applying this procedure to the conditioned inhibition paradigm, one would expect to find high correlations between differential responding and activity in suspected neural substrates of inhibitory conditioning. Since extension and retraction of the cat NM are active responses, one would expect to find a positive correlation between activity in the abducens nucleus and responding on A+ trials and an inverse correlation between activity in the superior cervical ganglion and responding on AX- trials. In rabbit, however, it may be more difficult to identify the potential efferent substrate of conditioned inhibition. The most likely candidate would appear to be the oculomotor nucleus in light of its role in NM retraction; however, since NM retraction is primarily a passive response, activity in this nucleus may show only small increases above its background level in the presence of a conditioned inhibitor. Such a possibility may be congruent with the results of Experiment 2 if it is assumed that the conditioned inhibitor engendered weak activity in the oculomotor nucleus which was insufficient for attenuating the UR. Clearly, the

role of the oculomotor nucleus in conditioned inhibition of the rabbit NMR merits investigation.

One further point may be made regarding conditioned inhibition in the cat NM preparation. The rapid rate of extinction of the cat NMR reported by Patterson et al. (1977) suggests that differential responding in a conditioned inhibition paradigm would develop more rapidly in the cat than in the rabbit under identical conditioning parameters. Moreover, conditioned inhibition in the cat preparation may prove to be a more robust phenomenon than in the rabbit.

The conditioned inhibition paradigm may also be used to clarify the role of the hippocampus as a neural substrate of conditioning. After relatively few CS-US pairings, neural activity in the hippocampus closely parallels and precedes the behavioral NMR in both rabbit (Berger, Alger, & Thompson, 1976) and cat (Patterson, Berger, & Thompson, in press). Since this activity is dependent upon CS-US pairings, Berger et al. suggested that it may be regarded as a neuronal indication that learning is occurring. However, since Berger et al. examined hippocampal activity only in the presence of an excitatory association, this activity may be specific to excitatory associations rather than learning in general. This issue may be resolved by examining hippocampal activity during the development of conditioned inhibition. If the hippocampus is a neural substrate of learning in general, then hippocampal activity should be evident on A+ trials as well as AX- trials. However, if the hippocampus is a neural substrate of excitatory associations only, then hippocampal

activity on AX- trials would show a progressive decline over successive AX- presentations. Thus, the conditioned inhibition paradigm may prove to be most useful for studies of neural substrates of conditioning.

References

Berger, T. W., Alger, B., & Thompson, R. F. Neuronal substrate of classical conditioning in the hippocampus. Science, 1976, 192, 483-485.

Cegavske, C. F., Thompson, R. F., Patterson, M. M., & Gormezano, I. Mechanisms of efferent neuronal control of the reflex nictitating membrane response in rabbit (Oryctolagus cuniculus). Journal of Comparative and Physiological Psychology, 1976, 90, 411-423.

Frey, P. W., & Gavin, W. Overnight incubation of a partially conditioned eyeblink response in rabbits. Animal Learning and Behavior, 1975, 3, 114-118.

Glickman, S. E. Perseverative neural processes and consolidation of the memory trace. Psychological Bulletin, 1961, 58, 218-223.

Gormezano, I., & Moore, J. W. Classical conditioning. In M. H. Marx (Ed.), Learning: Processes. Toronto: Collier-MacMillan, 1969.

Gormezano, I., Schneiderman, N., Deaux, E. B., & Fuentes, I. Nictitating membrane: Classical conditioning and extinction in the albino rabbit. Science, 1962, 138, 33-34.

Hupka, R. B., Kwaterski, S. E., & Moore, J. W. Conditioned diminution of the UCR: Differences between the human eyeblink and the rabbit nictitating membrane response. Journal of Experimental Psychology, 1970, 83, 45-51.

Hupka, R. B., Massaro, D. W., & Moore, J. W. Yoked comparisons of instrumental-avoidance and classical conditioning of the rabbit

nictitating membrane response as a function of interstimulus interval and number of trials per day. Psychonomic Science, 1968, 12, 93-94.

Ison, J. R., & Leonard, D. W. Effect of auditory stimuli on the amplitude of the nictitating membrane reflex of the rabbit (Oryctolagus cuniculus). Journal of Comparative and Physiological Psychology, 1971, 75, 157-164.

Kehoe, E. J., & Gormezano, I. Effects of trials per session on conditioning of the rabbit's nictitating membrane response. Bulletin of the Psychonomic Society, 1974, 4, 434-436.

Konorski, J. Conditioned reflexes and neuron organization. Cambridge: Cambridge University Press, 1948.

Lubow, R. E., & Moore, A. U. Latent inhibition: The effect of non-reinforced preexposure to the conditioned stimulus. Journal of Comparative and Physiological Psychology, 1959, 52, 415-419.

Marchant, H. G., III, Mis, F. W., & Moore, J. W. Conditioned inhibition of the rabbit's nictitating membrane response. Journal of Experimental Psychology, 1972, 95, 408-411.

Marchant, H. G., III, & Moore, J. W. Below-zero conditioned inhibition of the rabbit's nictitating membrane response. Journal of Experimental Psychology, 1974, 102, 350-352.

Mis, F. W. Midbrain and brain stem mechanisms of conditioned inhibition of the rabbit's nictitating membrane response. Unpublished doctoral dissertation, University of Massachusetts, 1975.

Moore, J. W. Contextual constraints on Pavlovian inhibitory control. In Conditioning context and stimulus control. Symposium presented at the meeting of the American Psychological Association, New Orleans, September, 1974.

Patterson, M. M., Berger, T. W., & Thompson, R. F. Neuronal plasticity recorded from cat hippocampus during classical conditioning. Brain Research, in press.

Patterson, M. M., Olah, J., & Clement, J. Classical nictitating membrane conditioning in the awake, normal, restrained cat. Science, 1977, 196, 1124-1126.

Pavlov, I. P. Conditioned reflexes. London: Oxford University Press, 1927.

Reiss, S., & Wagner, A. R. CS habituation produces a "latent inhibition effect" but no active "conditioned inhibition." Learning and Motivation, 1972, 3, 237-245.

Rescorla, R. A. Pavlovian conditioned inhibition. Psychological Bulletin, 1969, 72, 71-94.

Rescorla, R. A. Variation in the effectiveness of reinforcement following prior inhibitory conditioning. Learning and Motivation, 1971, 2, 113-123.

Rescorla, R. A. A model of Pavlovian conditioning. In V. S. Rusinov (Ed.) Mechanisms of formation and inhibition of conditional reflex. Moscow: "Nauka" Academy of Sciences of the U.S.S.R., 1973.

Rescorla, R.A. Conditioned inhibition and extinction. In B. A. Boakes & A. Dickinson (Eds.), Mechanisms of learning and memory.

A memorial to Jerzy Konorski. Hillsdale, N.J.: Erlbaum, in press.

Rescorla, R. A., & Holland, P. C. Associations in Pavlovian conditioned inhibition. Learning and Motivation, 1977, 8, 429-447.

Rescorla, R. A., & Wagner, A. R. A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black and W. F. Prokasy (Eds.), Classical conditioning II: Current theory and research. N.Y.: Appleton-Century-Crofts, 1972.

Salafia, W.R., Mis, F.W., Terry, W. S., Bartosiak, R. S., & Daston, A. P. Conditioning of the nictitating membrane response of the rabbit (Oryctolagus cuniculus) as a function of length and degree of variation of intertrial interval. Animal Learning and Behavior, 1973, 1, 109-115.

Salafia, W. R., Terry, W. S., & Daston, A. P. Conditioning of the rabbit (Oryctolagus cuniculus) nictitating membrane response as a function of trials per session, ISI, and ITI. Bulletin of the Psychonomic Society, 1975, 6, 505-508.

Spence, K. W., & Norris, E. B. Eyelid conditioning as a function of the intertrial interval. Journal of Experimental Psychology, 1950, 40, 716-720.

Thomas, E., & Basbaum, C. Excitatory and inhibitory processes in hypothalamic conditioning in cats: Role of the history of the negative stimulus. Journal of Comparative and Physiological Psychology, 1972, 79, 419-424.

Thompson, R. F., Cegavske, C., & Patterson, M. M. Efferent control of the classically conditioned nictitating membrane response in the rabbit. Paper presented at the meeting of the Psychonomic Society, St. Louis, November, 1973.

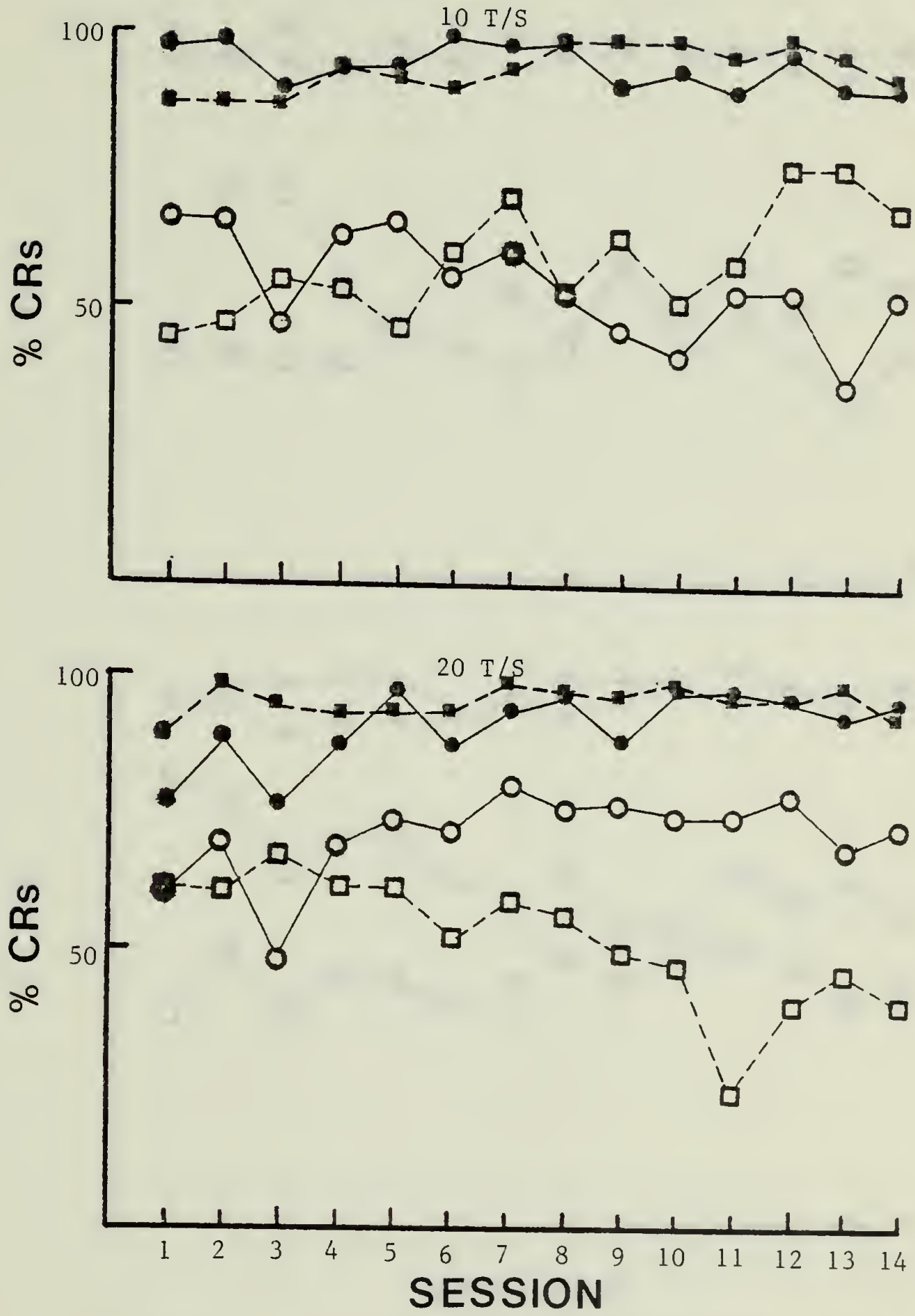
Wagner, A. R. Priming in STM: An information-processing mechanism for self-generated or retrieval-generated depression in performance. In T. J. Tighe and R. N. Leaton (Eds.), Habituation: Perspectives from child development, animal behavior, and neurophysiology. Hillsdale, N.J.: Erlbaum, 1976.

Wagner, A. R., Rudy, J. W., & Whitlow, J. W. Rehearsal in animal conditioning. Journal of Experimental Psychology, 1973, 97, 407-426.

Wagner, A. R., Thomas, E., & Norton, T. Conditioning with electrical stimulation of the motor cortex: Evidence of a possible source of motivation. Journal of Comparative and Physiological Psychology, 1967, 64, 191-199.

Young, R. A., Cegavske, G. F., & Thompson, R. F. Tone-induced changes in excitability of Abducens motoneurons and the reflex path of nictitating membrane in rabbit (Oryctolagus cuniculus). Journal of Comparative and Physiological Psychology, 1976, 90, 424-434.

FIGURE 1



Key: ITI = 30 sec, CS+ ITI = 30 sec, CS-

ITI = 60 sec, CS+ ITI = 60 sec, CS-

FIGURE 2

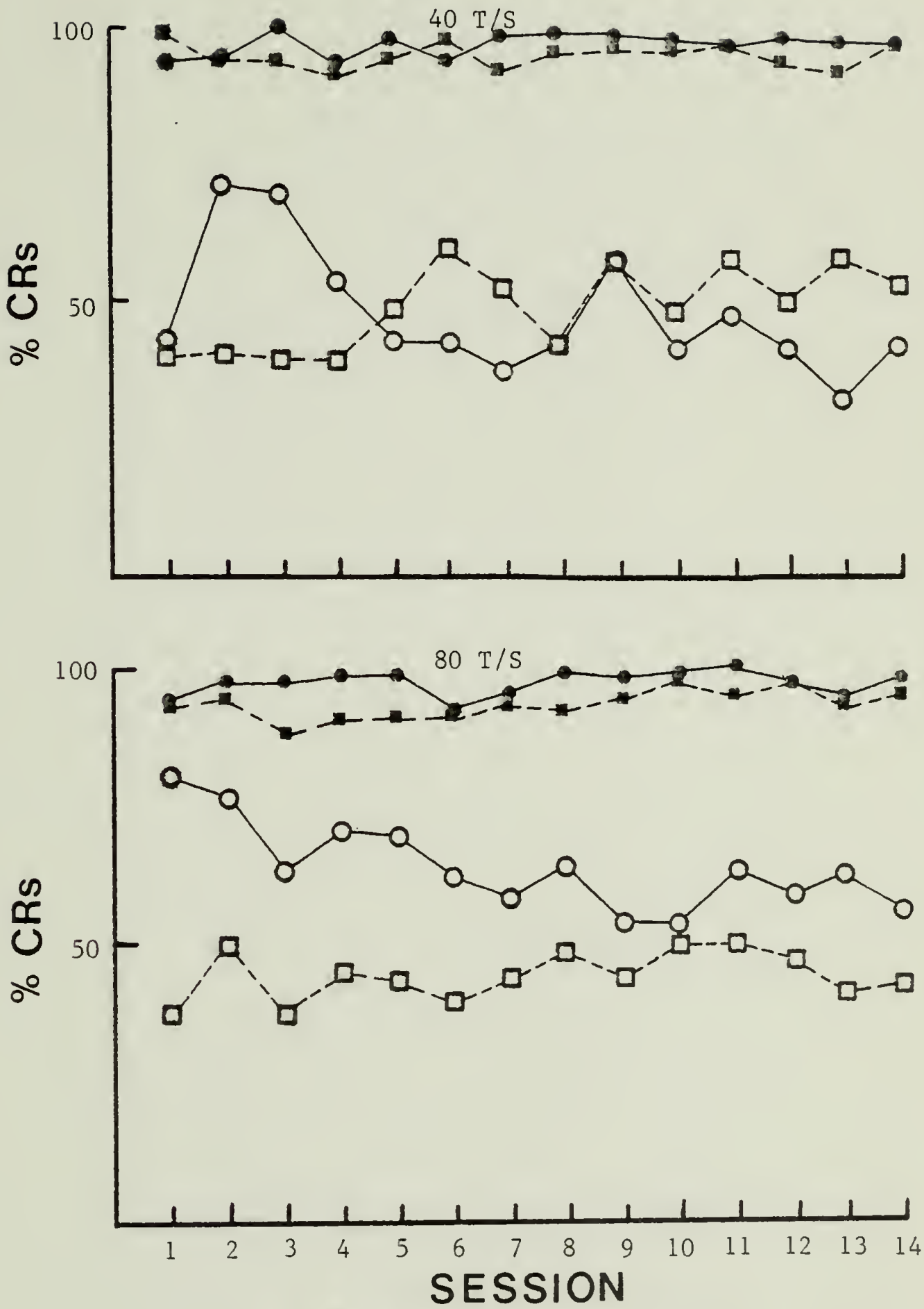

40 T/S
100
50
% CRs
80 T/S
100
50
% CRs
1 2 3 4 5 6 7 8 9 10 11 12 13 14
SESSION
Key: ITI = 30 sec, CS+ ITI = 30 sec, CS-
ITI = 60 sec, CS+ ITI = 60 sec, CS-

FIGURE 3

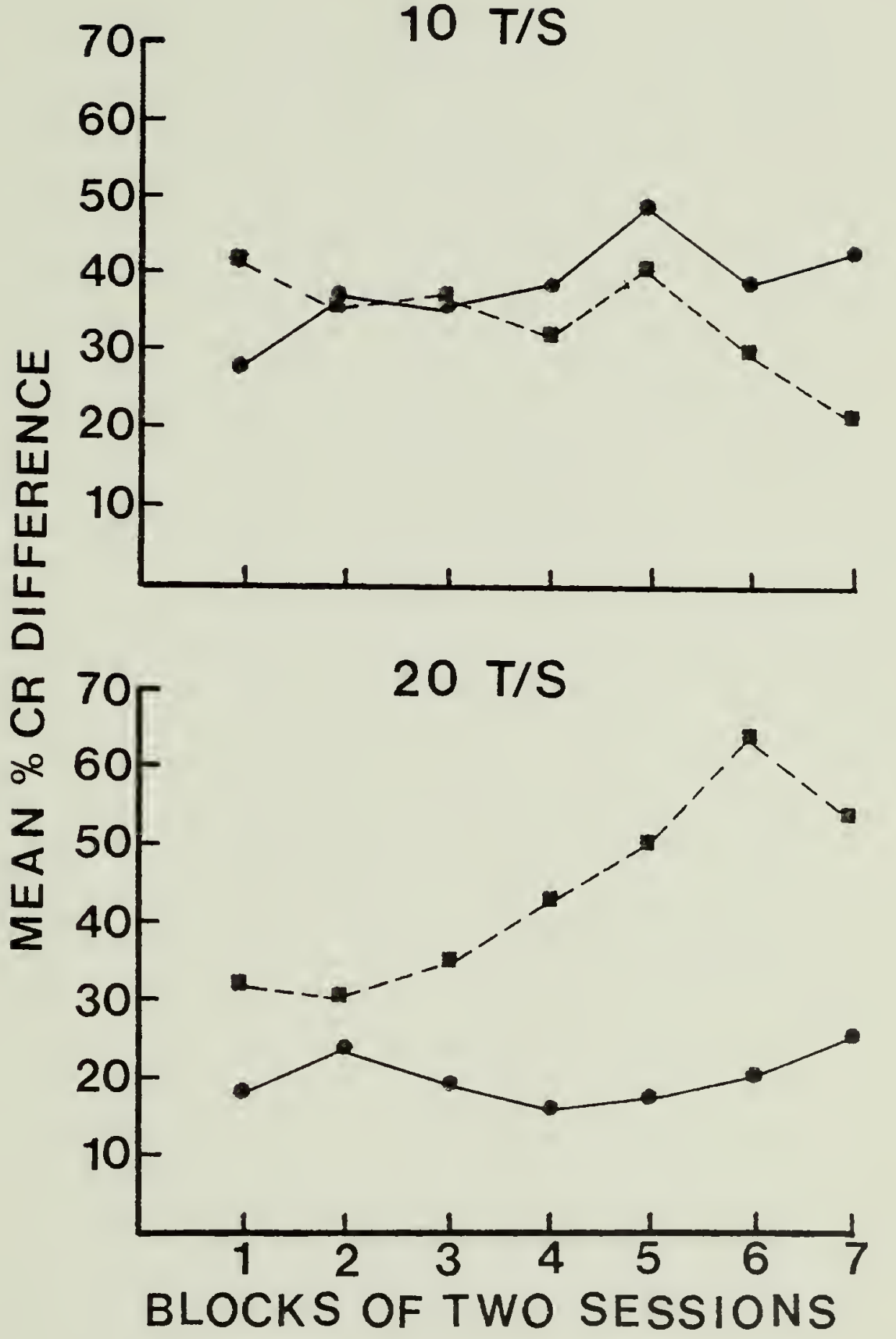

10 T/S
20 T/S
MEAN % CR
70
60
50
40
30
20
10
1
2
3
4
5
6
7
BLOCKS OF TWO SESSIONS
Key:
ITI = 30 sec
ITI = 60 sec

FIGURE 4

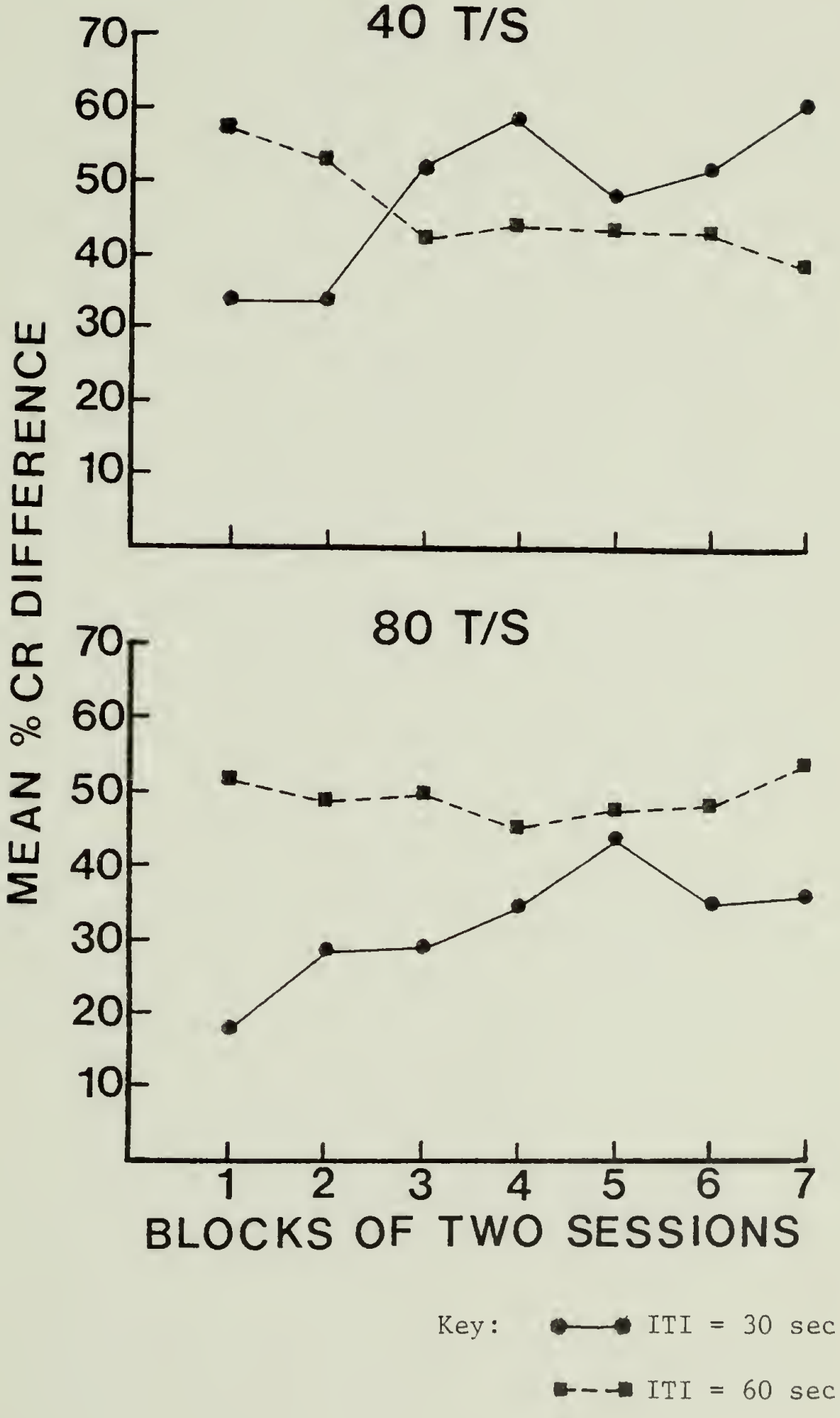

40 T/S
80 T/S
MEAN % CR DIFFERENCE
70
60
50
40
30
20
10
1
2
3
4
5
6
7
BLOCKS OF TWO SESSIONS
Key: ITI = 30 sec
ITI = 60 sec

FIGURE 5

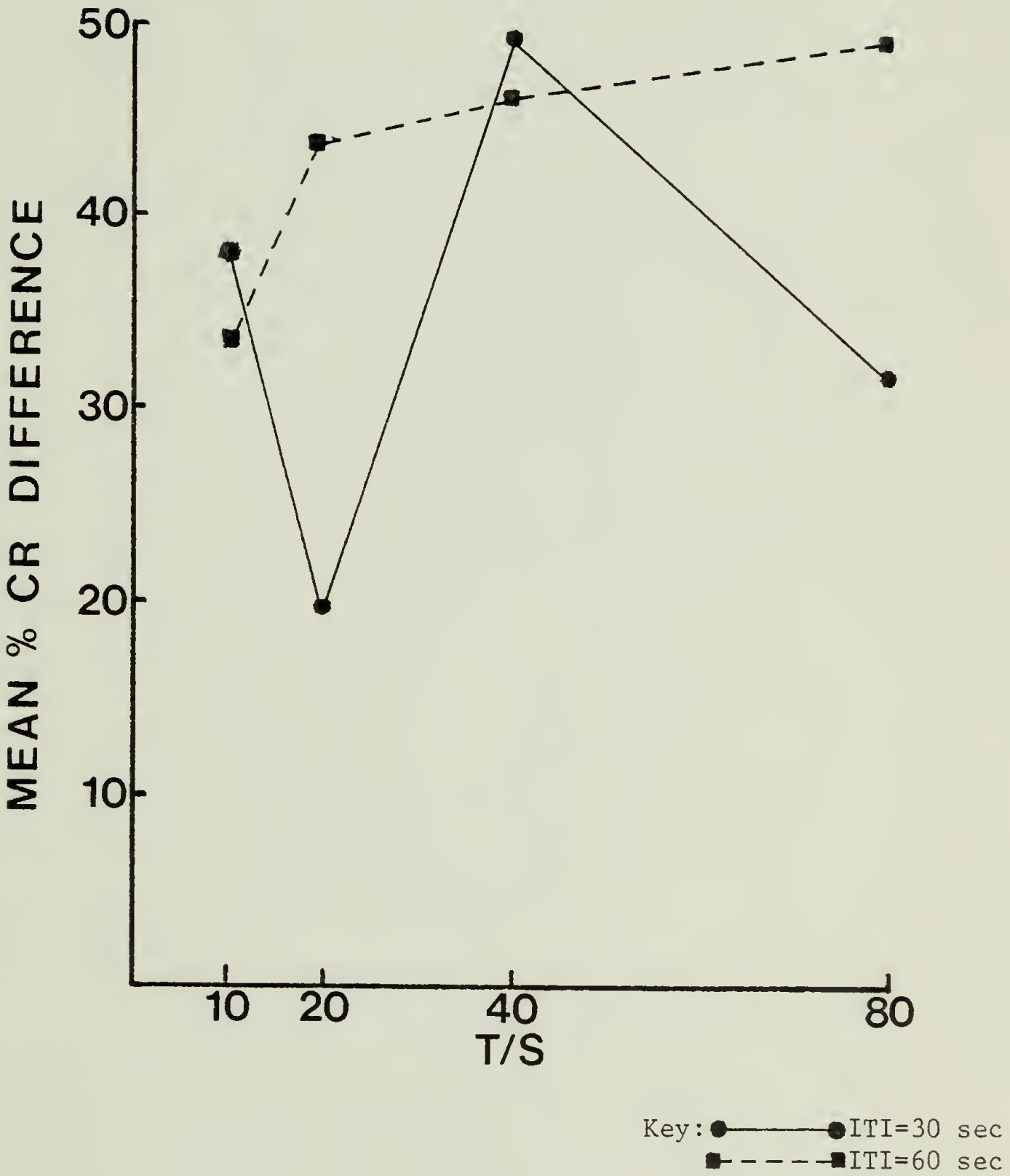

MEAN % CR DIFFERENCE
50
40
30
20
10
10
20
40
80
T/S
Key: ITI=30 sec
ITI=60 sec

Table 1

Group	Stages of Training and Testing		
	1	2	3
CI	A+	A+ AX-	AX+ X+ US
SD	A+	A+ X-	AX+ X+ US
LI	A+	X-	AX+ X+ US
US	A+	US	AX+ X+ US
SIT	A+	SIT	AX+ X+ US

Key: A = light + = reinforced

X = tone - = nonreinforced

FIGURE 6

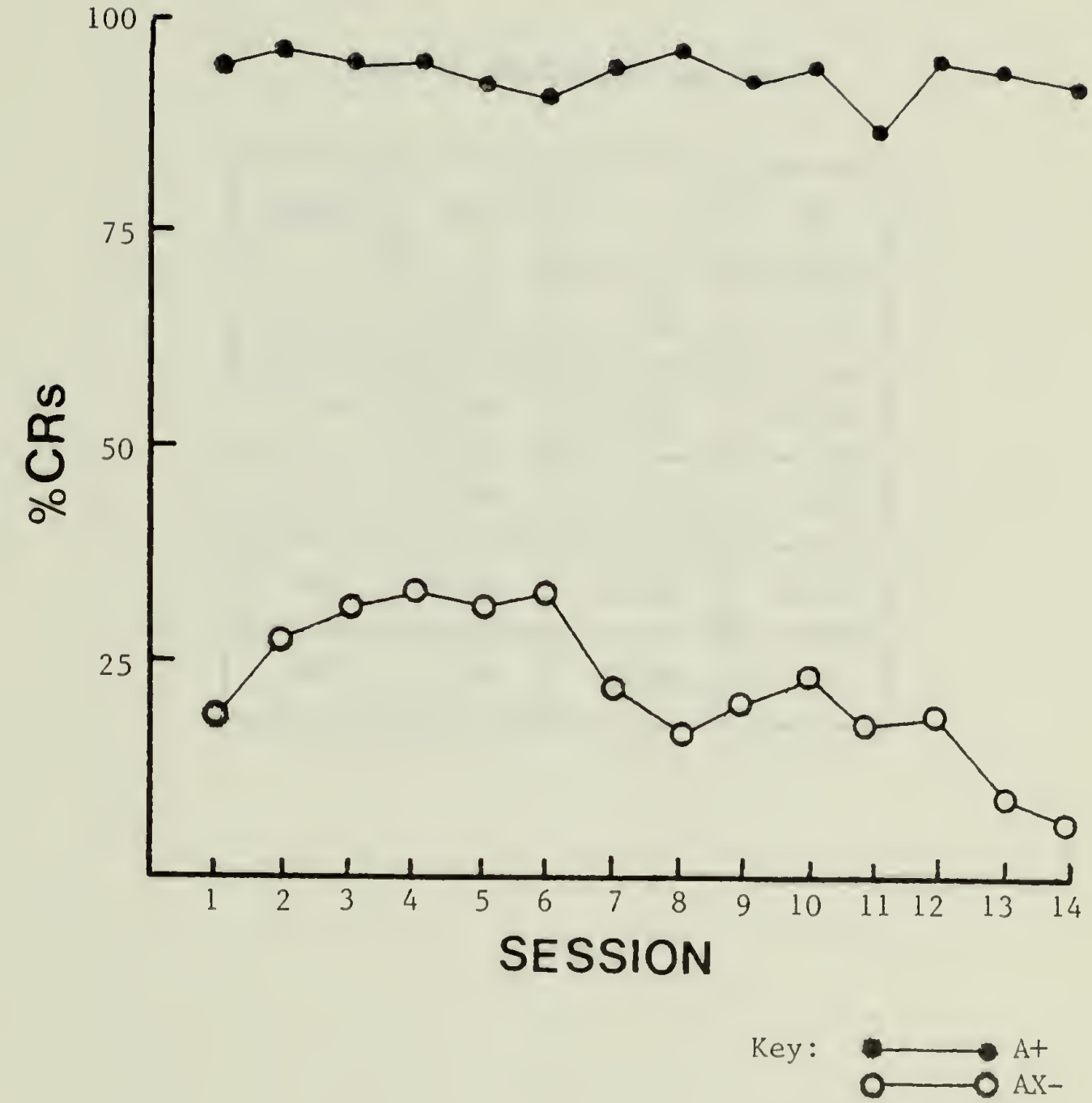


Table 2

Mean UR Amplitudes on Day 1

Group	AX	X	US
CI	8.75	4.60	3.925
SD	15.35	17.30	16.30
LI	6.35	6.35	3.20
US	3.85	3.75	3.70
SIT	9.20	10.60	8.45

FIGURE 7

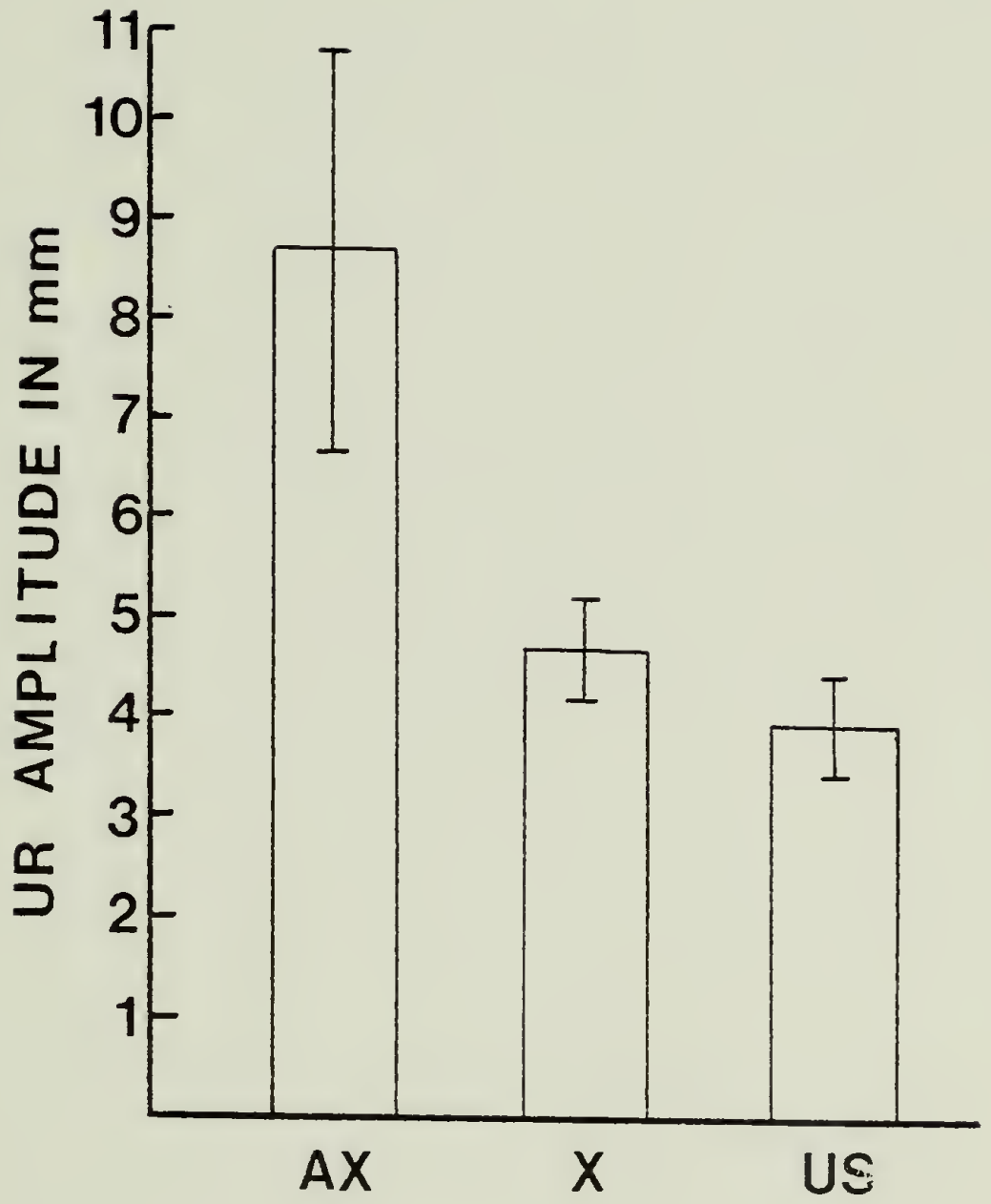


Table 3

Group	Mean % of US-Alone URs		
	AX	X	AX-X
CI	247.54	123.91	123.62
SD	104.17	108.84	-4.67
LI	209.70	210.63	-.93
US	102.63	99.85	2.78
SIT	105.75	121.23	-15.48

Appendix 1

Experiment 2 - Mean UR Amplitudes on day 2

Group	AX	X	US
CI	8.675	7.85	5.80
SD	16.85	18.30	15.65
LI	7.20	5.15	4.20
US	5.00	5.25	5.40
SIT	8.00	9.15	3.80