possibly, a difference in sensitivity levels between strains.

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Chlordiazepoxide and aversive conditioning: Effects of acquisition and performance of the conditioned nictitating membrane response in the rabbit*

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A 12-mg/kg dosage of chlordiazepoxide was shown to disrupt acquisition and postasymptotic performance of the classically conditioned nictitating membrane response of rabbits induced by pairing a tone with eyeshock. This result makes more plausible the hypothesis that chlordiazepoxide interferes with avoidance learning in rabbits by suppressing the conditioned fear component of avoidance learning.

Although chlordiazepoxide (CDP) reduces shuttle-avoidance performance in the rabbit (Chisholm & Moore, 1970a, b), it is not apparent how the drug affects avoidance behavior. Rescorla & Solomon (1967) have analyzed avoidance by studying the effects of classically conditioned cues associated with shock upon the instrumental avoidance behavior. Their two-process learning theory emphasizes that the classically conditioned cues play a major role in controlling the instrumental avoidance responses. Chisholm & Moore (1970) showed that CDP does not interfere with formation of a conditioned association between a tone and inescapable footshock. In that study, rabbits learned a shuttle-avoidance response to a light signal. Some rabbits then experienced tone-shock pairings under a 12-mg/kg dosage of CDP. Saline-injected control animals

received the same treatment. Rabbits, which experienced the tone-shock pairings in the drugged state, showed transfer of this fear conditioning when subsequently tested in the nondrugged state for avoidance to the tone. Transfer in this case did not differ from that demonstrated by the control animals. A differential conditioning procedure precluded the possibility that nonassociative factors (e.g., sensitization) were responsible for this transfer. Thus, there were actually two tones of equal intensity in the fear conditioning phase of the experiment, but only one was systematically paired with shock. In the transfer phase, the tone paired with shock elevated the speed of avoidance over that to the original avoidance cue (the light), whereas the other nonpaired tone systematically increased response latency in a similar, but opposite, fashion.

Since CDP in the dosage employed in the previous experiments does not seem to impair learning, it would seem most likely that the drug affects the

avoidance response by removing the motivational substrate for the response. This could come about either by blocking the expression of fear (without fear there might be no "reason" for the animal to shuttle on cue) or by reducing whatever reward value a successful avoidance might have. It is our hypothesis that CDP suppresses the expression of conditioned fear: that it is the "performance" of the classically conditioned component of the avoidance task, not the actual learning, which is affected by the drug. As a more direct test of whether or not CDP does suppress aversive conditioning in rabbits, we thought it appropriate to investigate another independent classical conditioning situation. Accordingly, the present experiment was designed to investigate the effect of 12 mg/kg of CDP on the conditioned nictitating membrane response (NMR). This is a widely used aversive conditioning preparation adapted for rabbits by Gormezano and his associates (Gormezano, 1966) and is known to be suitable for pharmacological investigation (e.g., Elliott & Schneiderman, 1968).

SUBJECTS

The Ss were 20 New Zealand albino rabbits, approximately 110 days old.

APPARATUS

The conditioning apparatus and the recording equipment have been described elsewhere (O'Malley, Hupka, & Moore, 1969). Essentially, four rabbits were run simultaneously in a four-drawer fireproof filing cabinet. Each drawer was illuminated by two 4-V dc lights located behind translucent white plastic. Independent ventilation for each S was provided by centrifugal blowers. Continuous white noise of 70 dB SPL and the tones were presented via speakers located directly in front of each animal. Clear Plexiglas boxes, similar to those developed by Gormezano (1966), were used to restrain the rabbits. The movement of the nictitating membrane was recorded by having one end of a thread hooked to a nylon loop sutured in the membrane and the other end attached to a Giannini minitorque potentiometer. Movements of the right nictitating membrane were amplified and recorded on a Grass 5D oscillograph. A CR was defined as a 1-mm deflection of the recording pen within the interstimulus interval.

PROCEDURE

The rabbits were assigned randomly to one of two groups. Each group received intraperitoneal injections of CDP (12 mg/kg) or saline (1 cc/kg) 30 min before the training session and were run using an E-blind procedure. On the days before the first conditioning session, the nictitating

*This research was supported by NSF Grant GB 8634. Chlordiazepoxide (Librium) was provided by Hoffman-La Roche, Inc.

Fig. 1. Mean percentage of CRs for each group as a function of days.

membrane was sutured and the S was placed in the apparatus for 30 min of adaptation (no injection). The adaptation was followed by 4 days of classical conditioning. Each animal received 60 trials per day with an 800-Hz tone at 80 dB SPL as the CS and an interstimulus interval of 300 msec. The tonal CS remained on at the end of the interval and overlapped the 50-msec shock that served as the US. A shock intensity of 2 mA was delivered via two stainless steel electrodes attached to the infraorbital region of the right eye. The intertrial interval ranged from 20 to 40 sec, with an average of 30 sec. Following 4 days of acquisition conditioning, half of the animals in the drug group were switched to saline and half the saline group were switched to drug. The remaining half of the rabbits in each group were not switched, thereby forming Groups SS, SD, DS, and DD.

RESULTS

Figure 1 depicts the percentage of conditioned responses over the 7 training days for the four experimental conditions. It is evident from the figure that animals receiving CDP during the first 4 days of conditioning responded at a much lower level than animals receiving saline. An analysis of variance on the number of conditioned responses on Days 1-4 showed that animals receiving CDP (DD and DS) responded significantly less than animals receiving saline (SS and SD) ($F = 29.56$, $df = 1/18$, $p < .001$).

Other preshift differences were not statistically significant.

Figure 1 also shows that the response level was decreased when CDP was administered for the first time during the 3 postshift days. The significant postasymptotic decrease is reflected in the Mann-Whitney U between Group SS and Group SD ($U = 4$, $p < .05$). Group DS showed a steady increase in responding when shifted to saline during the last 3 days. A randomized test for matched pairs (Siegel, 1956) performed on Day 5 vs Day 7 showed that the increase was significant ($p < .04$). Although animals showed a decrease in the conditioned response level when under CDP, the unconditioned response to the shock occurred consistently.

DISCUSSION

The major results of this experiment show that (1) performance during the acquisition of the NMR was decreased by CDP and (2) CDP reduced postasymptotic performance of the NMR. This suggests that CDP disrupts postasymptotic performance of a classical defensive-conditioned response.

The factor learning theory (Rescorla & Solomon, 1967) has maintained that classically conditioned fear is intricately involved in shuttle-avoidance behavior. The results of the present experiment suggest that the disruption of avoidance behavior by CDP (Chisholm & Moore, 1970) probably does not stem exclusively from the interference of the

instrumental conditioning based on fear reduction. Instead, CDP seems to disrupt the expression or retrieval of the underlying classically conditioned fear component.

The relatively moderate dosage of CDP (12 mg/kg) did not have a heavy sedative effect on the animals in this study. This is evident by the fact that the occurrence of the UCR was not disrupted, yet the number of CRs was reduced. Therefore, any hypothesis based on a motor impairment assumption would not explain these data.

The tolerance effect for benzodiazepines that has been previously demonstrated at high dosages for CDP (50 mg/kg) by Goldberg, Manian, & Efron (1967) and for oxazepam (20 mg/kg) by Margules & Stein (1968) in rats may also be present in our drug group during acquisition. However, it is not possible to determine if the drug has simply suppressed the rate of responding during acquisition or if the drug has a diminishing effect with repeated administration.

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