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Brief Communication

Epinephrine-Induced Learning Under Anesthesia: Retention Performance at Several Training–Testing Intervals

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While under deep barbiturate anesthesia, rats received a series of 10 classical conditioning trials in which white noise was paired with intramuscular shock. The anesthetized animals received either saline or epinephrine injections prior to the training trials. Independent sets of animals were tested for retention performance 2, 7, or 15 days after training. In these test trials, a conditioned suppression measure was used in which the white noise was turned on while the animals were drinking. The results indicated that the animals that had received saline while trained under anesthesia exhibited no evidence of later retention. Animals that had received epinephrine injections prior to training under anesthesia suppressed their drinking in the presence of the white noise when tested 2 or 7, but not 15, days later. Thus, the results indicate that epinephrine can enable learning under anesthesia and, in addition, forgetting occurs within 15 days.

When injected near the time of training, epinephrine can enhance later retention performance for a variety of learned responses (cf. Gold, 1984; McGaugh, 1983). In addition, epinephrine can facilitate memory storage in several situations in which memory storage is deficient; for example, a posttraining epinephrine injection allows juvenile rats to acquire and later to perform learned responses at an age in which they otherwise exhibit infantile amnesia (Gold, Murphy, & Cooley, 1982). Also, in aged rats in which new memories are rapidly forgotten, epinephrine significantly retards the rate of forgetting (Sternberg, Martinez, Gold, & McGaugh, in press). Epinephrine also enhances memory in animals that have poor memory storage after pretreatment with diethyldithiocarbamate, a norepinephrine synthesis inhibitor (McGaugh et al., 1979), or amygdala damage after electrode implantation and adrenal demedullation (Liang, Bennett, & McGaugh, in press).

Recently, Weinberger, Gold, and Sternberg (1984) reported that an injection of epinephrine also enables learning to occur in rats under deep general anesthesia. In that study, animals were anesthetized with sodium pentobarbital and chloral hydrate. During anesthesia, the animals received an injection of saline or one of several doses of epinephrine prior to the start of 10 classical conditioning trials in which white noise was paired with shock to a hindlimb. After recovery, the animals were deprived of water and were tested, 10 days after training, for conditioned suppression of drinking behavior during presentation of the white noise. The animals that received saline injections showed no evidence of Pavlovian conditioning on the test trial. However, the animals that had received epi-

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nephrine while trained under anesthesia exhibited significant suppression of drinking upon presentation of the auditory conditioned stimulus. Analyses of heart rates and reflex scores indicated that this effect of epinephrine was not due to a lessening of the depth of anesthesia.

A major rationale for this study was to determine whether it is possible to train two groups of rats identically (except for drug treatment), yet have one group (epinephrine) learn, and the other group (saline) fail to learn. This would permit neurobiological studies in which differences between the two groups might be related to learning per se rather than to differences in the degree of learning, or the learning of different information, as in the case of groups exposed to stimulus pairing (conditioning) versus random stimulus presentation (sensitization controls). In the Weinberger et al. (1984) study, animals receiving saline did not differ in the retention test from untrained controls. This suggested that the saline group did not learn the conditioned stimulus/unconditioned stimulus association at all rather than that they learned it at a lower level than did the animals that received epinephrine. However, because the retention test was given 10 days after training, it is possible that the animals receiving saline also acquired the association but forgot it prior to the retention test. The present experiment was designed to evaluate this hypothesis by testing for retention at various intervals following training.

Thus, this experiment is designed to replicate and to extend the earlier findings by evaluating the possibility that all animals, epinephrine- and saline-treated animals alike, acquire the learned response but that the saline animals forget that response during the 10 days before testing.

Method

The subjects were 58 male Sprague-Dawley rats, 80–120 days of age at the start of the experiment. The rats were maintained in individual cages with free access to food and water for at least 1 week prior to the start of the experiment.

On the day of training, each rat was anesthetized with sodium pentobarbital (48 mg/kg) followed 10 min later by an injection of chloral hydrate (30 mg/kg). At this time, the animals were placed on a heating pad in a double-walled acoustic chamber (IAC Model 400C), and their temperature was maintained throughout the training period. Needle electrodes were inserted into the left hindlimb for the delivery of the shock. After 10–20 min, each rat was tested for eye-blink, pinna, and tail-pinch reflexes. If any reflex was present, the animal received another injection of chloral hydrate (30 mg/kg) and was tested for reflexes after 10 min. Immediately following verification that reflexes were absent, each animal received three presentations of shock generated by a Grass S-88 constant-current stimulator and isolation unit (50-ms train of 50-Hz, 5.0-ms pulses, 4–6 mA, at intervals of 1 min). Animals were retested to ensure that these pulses had not restored reflex responsiveness, and then each rat received an sc injection of saline or epinephrine bitartarte (Sigma; 0.1 mg/kg). Training began 7 min after this injection.

The conditioned stimulus (CS) was 15 s of white noise (85–90 dB, SPL) provided through a speaker situated approximately 60 cm from the animal. The unconditioned stimulus (US) was identical to the stimulus described above, delivered to the left hindlimb upon the CS offset. Ten paired trials of the CS–US presentations were administered, with an average intertrial interval of 1 min (range = 30–90 s on a variable schedule). After the tenth trial, reflexes were again tested, and the animal was recovered from anesthesia. At each training–testing interval, an additional group of animals was anesthetized as above but did not receive Pavlovian conditioning trials.

The extent of Pavlovian fear conditioning was tested 2, 7, or 15 days after training by using a drink suppression procedure. In order to accomodate the different retention intervals, animals were water deprived at different times with respect to training but always beginning 7 days prior to the test trials. The water deprivation was accomplished by allowing each rat 3 min of access to water in the animal's home cage which was placed daily in the test room. Animals quickly learned to drink almost continuously during this time and maintained themselves at approximately 85%–90% of their original body weight during the experiment.

During testing, the animal's cage was placed in the test room approximately 100 cm from a loudspeaker. Baseline drinking performance was assessed during the first minute with the noise off. At the start of the second minute of drinking, the white noise (75–85 dB) was turned on. During each minute, the experimenter recorded with electrical timers the cumulative time (in seconds) spent drinking. The effects of training were determined by calculating a suppression ratio: the number of seconds spent drinking during Minute 2 divided by the number of seconds spent drinking during Minute 1. With this measure, a score of 0 means total cessation of drinking after the CS onset, and a score of 1.00

indicates continuous drinking throughout the CS presentation.

Thus, this experiment tested retention performance in three groups of animals—saline, epinephrine, untrained—at three training-testing intervals.

Results

As shown in Figure 1, the untrained animals and the saline-treated animals showed little evidence of suppression in the presence of the CS at any retention interval; group suppression score means ranged from 0.83 to 1.01. The scores for the untrained groups and the trained saline-treated animals did not differ significantly (t tests, $p > .01$) at any retention interval. In contrast, when compared with either saline or untrained animals at the same test interval, epinephrine-treated animals showed significant suppression when tested 2 or 7 days after training ($ps < .01$, t tests, two-tailed). However, when tested 15 days after training, the scores in the epinephrine-treated group did not differ significantly from those of the two control groups. The time-dependent change in the scores of the animals that received epinephrine was also evident in a one-way analysis of variance, $F(2, 16) = 15.16$, $p < .01$. Individual t tests indicated that the groups tested 2 or 7 days after training differed significantly from the group tested at 15 days (t tests, $ps < .001$ and .02, respectively).

Reflex tests at the end of training revealed only weak responsiveness in 7/24 saline- and 3/20 epinephrine-treated animals. Of these, 2 saline animals and 1 epinephrine animal also had weak responses to tail pinch.

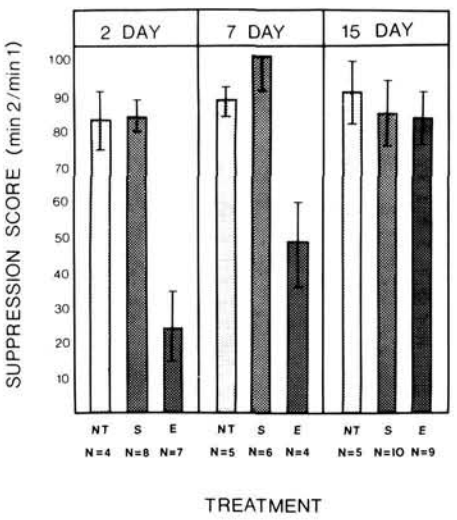

Figure 1. Conditioned suppression scores ($M \pm SE$) for rats trained under anesthesia with saline or epinephrine injections and tested 2, 7, or 15 days later. (Note that, when compared with nontrained controls [NT], the saline-injected animals [S] exhibited no evidence of conditioned suppression at any interval. The epinephrine-treated groups [E] showed significant suppression when tested 2 or 7 days after training but not when tested 15 days after training.)

Discussion

These findings replicate our previous results (Weinberger et al., 1984) and extend them in several important respects. First, the animals that received epinephrine at the time of training under anesthesia demonstrated very good conditioned suppression when tested 2 or 7 days later. However, when tested 15 days later, the epinephrine-treated animals exhibited little evidence of Pavlovian fear conditioning. In our earlier study, the animals were tested 10 days after training and had retention scores intermediate to those in the 7- and 15-day groups here. The respective values are 0.48 (7 days), 0.68 (10 days), and 0.84 (15 days). Thus, it appears that Pavlovian fear conditioning was acquired by the anesthetized rats treated with epinephrine and that the learned response was forgotten within 15 days of training. These results therefore provide further support for the view that learning can occur under anesthesia under appropriate hormonal conditions.

It might be argued that epinephrine enables learning by reducing the depth of anesthesia. This possibility was examined in detail previously (Weinberger et al., 1984) and rejected on the basis of heart rate data, dose-response data, and reflex scores at the end of training. In the present study, reflex scores at the end of training were also unrelated to epinephrine treatment or to later suppression scores. Therefore the present findings replicate and extend the previous finding that epinephrine enables learning under anesthesia by means other than reducing the depth of general anesthesia.

It is possible that the saline animals also learn but rapidly forget the fear conditioned response. However, the present results suggest that if this is the case, the fear conditioning is remembered for less than 2 days. We attempted to test a set of animals 1 day after training. However, the baseline performance (Minute 1) was quite variable in all groups, and all groups showed variable responses when the loud white noise was presented. Presumably, these are proactive effects of the deep anesthesia the day before. Thus, it was not possible to evaluate retention

of fear conditioning on the first day after anesthesia.

As discussed in the introduction, it is of interest to determine whether animals receiving saline learn at all, as opposed to learn less well than animals receiving epinephrine. The groups that were anesthetized but not trained provide a basis for evaluating this possibility. Their suppression scores reflect the effects of noise presentation during drinking unconfounded by training. As noted in Results, there were no significant differences between the suppression scores of these animals and the animals that were trained after saline injections. Consequently, it appears that deeply anesthetized animals that receive saline do not acquire a noise-shock association, or forget it entirely within 2 days.

The present results further show a preparation that may prove to be very useful for understanding the neurobiological processes that underlie learning and memory. Unique to these procedures, the investigator can present identical stimuli to animals during training but only those animals that receive epinephrine later exhibit the learned behavior. Because central nervous system functions are depressed by the anesthetic, it may be possible to determine which brain systems or mechanisms are activated by epinephrine. Such results might reveal both the mechanisms by which epinephrine modulates memory storage and the processes that are enabled by epinephrine and therefore may be responsible for memory storage.

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