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Effect of Propofol on Memory in Mice

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PANG, R., D. QUARTERMAIN, E. ROSMAN AND H. TURNDORF. *Effect of propofol on memory in mice.* PHARMACOL BIOCHEM BEHAV 44(1) 145-151, 1993.—The amnestic effects of the intravenous hypnotic anesthetic agent 2,6-diisopropylphenol (propofol; Diprivan) were studied in a single-trial passive avoidance task. Mice were injected with propofol 10 min before or immediately after training. Memory was impaired in a dose-dependent fashion when the anesthetic was administered before learning, but no amnesia was apparent with posttraining injections. Examination of the acquisition of passive avoidance using a multitrial task showed that propofol-treated mice learned the response normally but forgot the learning significantly faster than vehicle-treated controls. The anterograde amnesia was not the result of state-dependent learning. Propofol also disrupted extinction of fear conditioning when the anesthetic was given during the extinction session. Propofol-induced amnesia could be attenuated by amphetamine (1 mg/kg) injected 30 min before the retention test.

Propofol Anesthesia Memory Anterograde amnesia Passive avoidance learning

SINCE its introduction into anesthesiology in the 1980s, 2,6-diisopropylphenol (propofol; Diprivan) has become widely used as an intravenous agent for induction and maintenance of anesthesia. The popularity of propofol as an anesthetic is due to its fast, smooth induction and rapid recovery after either a single bolus or prolonged infusion lasting many days (19). Although the neurochemical mechanisms of action of propofol have not been identified, recent studies indicate that the $GABA_A$ receptor complex may play an important role in mediating its pharmacological effects (9).

Little is known about the effects of propofol on memory. A few informal experiments have been carried out using surgical patients as subjects. The results indicate that the anesthetic causes amnesia for intraoperative events (8,15,17), but few attempts have been made to examine possible anterograde or retrograde amnestic effects of propofol. In one study, patients had retrograde amnesia when tested 1 h after surgery but retention was normal 1 h later (8). Another experiment reported that anterograde amnesia occurred in only 4% of subjects (17) but these results may underestimate the amnestic effects of propofol because the patients were trained several hours after recovery from anesthesia. One of the objectives of the present study was to obtain more information on the effects of propofol on memory processes using an animal model of retrograde and anterograde amnesia.

We have recently shown (13) that the memory loss produced by the inhalation anesthetic halothane is the result of a retrieval failure caused by state-dependent learning. A second objective of this research was to determine if propofol, chemically unrelated to halothane, has similar effects on memory processes.

METHOD

Male Swiss Webster mice (Harlan; hsd: ND4) 10 weeks of age between 20 and 30 g body weight were subjects for these experiments. Mice were housed five per cage with food and water available ad lib.

Behavioral Task and Apparatus

Single-trial inhibitory avoidance learning was used to investigate the effects of propofol on memory processing. Mice were trained in a two-compartment shuttle chamber (LVE MSC-002). The dark compartment was 23 × 9 × 11 cm and constructed from black Plexiglas. The floor was made from stainless steel rods (0.3 cm diam. 7 cm between rods) through which a scrambled shock could be delivered from a Coulbourn (Coulbourne Instruments, Lehigh Valley, PA) constant-current shocker. The safe side of the chamber, which was black, was the same size as the white shock side, had a solid floor, and was covered by a lid in the center. The white side was illuminated by a 28-V lamp during training and testing. The two compartments were separated by a wall that contained a guillotine door 8 cm high and 4 cm wide.

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Procedure

A training trial was begun by placing a mouse in the dark side and opening the door. When the animal entered the white compartment, the door was lowered and a 0.2-mA shock was administered for 1.2 s. A latency timer automatically recorded the time to cross into the dark side. Retention of this learning was tested by returning the mouse to the dark compartment and recording the time to reenter the white compartment. Mice failing to cross within 300 s were given the maximum latency as the test score.

Drug Administration

Drug doses for these experiments were prepared from 20-ml ampules of Diprivan (Stuart Pharmaceuticals, Wilmington, DE) containing 10 mg/ml propofol. The drug was diluted with lactated Ringers solution and injected intraperitoneally at a volume of 10 ml/kg body weight. Control animals were injected with the lactated Ringers solution.

EXPERIMENT 1

The objective of this experiment was to investigate the anterograde and retrograde effects of propofol on retention. Different groups of mice (n = 10/group) were injected with propofol (0, 5, 25, 50, and 75 mg/kg) 10 min before the training trial. Other groups were injected immediately after training (0, 50, and 100 mg/kg). Retention was tested 24 h after training. These concentrations did not result in full loss of consciousness and would therefore be classified as subanesthetic doses.

Results

Anterograde effects. The results of administering different doses of propofol prior to training are shown in Fig. 1. A one-way analysis of variance (ANOVA) applied to these data indicated a significant difference among the five groups, $F(4, 45) = 10.21$, $p < 0.001$). Posthoc Bonferroni t-tests revealed that 50 and 75 mg/kg produced significant amnesia when compared with the vehicle control group, $t(18) = 4.02$ and 4.99, respectively. Propofol at 50 and 100 mg/kg given immediately after training did not result in amnesia. Mean test latencies were 206.3 ± 35.2 and 219.6 ± 27.6 s, respectively. These results indicate that propofol produces anterograde but not retrograde amnesia.

EXPERIMENT 2

When a drug is administered before the training trial, it is often difficult to determine whether poor performance on the retention test is due to impairment of learning or to true anterograde amnesia. One way to distinguish between these two possibilities is to examine the performance of drugged and control animals during acquisition of the task to determine whether the drug alters the rate of learning. This experiment examined the effect of propofol on rate of acquisition in a multitrial passive avoidance task.

Procedure

Twenty-four mice were subjects for this experiment. Twelve were injected with 50 mg/kg propofol and 12 control animals were given vehicle. The training session began 10 min later by placing a mouse in the dark compartment and raising the door. When the mouse entered the light compartment, the shock (0.2 mA) was initiated and remained on for the remainder of the training session. The mouse could escape the shock by running back into the safe, dark compartment. The door remained raised and mice received foot-shocks each time they stepped into the white compartment. Training was continued until mice remained in the dark compartment for 100 s without a foot-shock. The number of shocks taken until the 100-s learning criterion was attained and recorded for all animals. Retention was tested 24 h later.

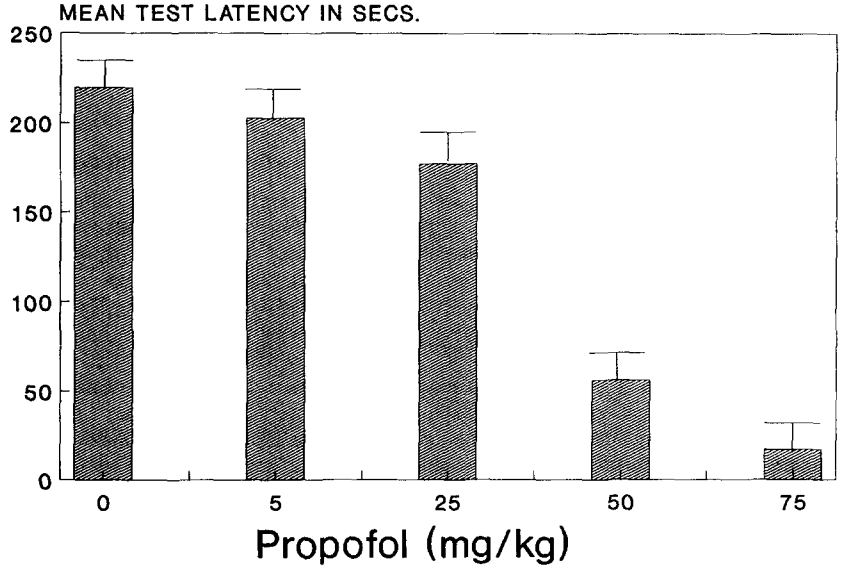

FIG. 1. Effects of different doses of propofol on amnesia. Groups of mice were given vehicle (0) or propofol 5, 25, 50, and 75 mg/kg 10 min prior to training. Retention was tested 24 h after training.

Results

Mean number of shocks taken to attain criterion was 11.6 ± 1.7 for mice injected with vehicle and 11.5 ± 1.17 for the propofol 50-mg/kg group. Twenty-four hours later, the mean test latency for the control group was 206.2 ± 24.1 and 96.2 ± 19.6 s, respectively, for animals treated with propofol 50 mg/kg. This difference was statistically significant, $t(22) = 3.52, p = 0.001$. This finding indicates that mice given propofol before the training session learned the avoidance response normally but forgot that learning significantly faster than control subjects.

EXPERIMENT 3

We have previously shown that the amnesia induced by the inhalation anesthetic halothane is the result of a retrieval failure caused by state-dependent learning (13). Experiment 3 was designed to evaluate possible state-dependent effects of propofol.

Procedure

One group of 24 mice was injected with propofol (50 mg/kg) and a second group of 24 injected with vehicle 10 min before the training trial. Before the retention test, half of each group was reinjected with propofol (50 mg/kg) and the other half with vehicle. The resultant four groups (Veh-Veh; Veh-Prop; Prop-Prop; and Prop-Veh) were tested 24 h after training.

Results

The test latencies of the four groups are shown in Fig. 2. The results of a one-way ANOVA show a significant difference among the four groups, $F(3, 44) = 5.73, p = 0.003$. Posthoc comparisons using Bonferroni t-tests showed: a) that the latencies of the Prop-Prop group were significantly shorter than those of the Veh-Veh group, $t(22) = 3.571$, indicating that a second injection of propofol does not alleviate the anterograde amnesia; and b) that the Veh-Prop latencies were not significantly shorter than those of the Veh-Veh group, $t(22) = 2.230$. Together, these findings show that the anterograde amnesia is not the result of state-dependent retrieval failure.

EXPERIMENT 4

It is well established that *d*-amphetamine can alleviate memory deficits caused by many amnestic agents (7,10,14,16). For example, we have shown (11) that amnesia for inhibitory avoidance learning induced by such diverse treatments as protein synthesis inhibition, cholinergic receptor blockade, inhibition of norepinephrine biosynthesis, and spontaneous forgetting can be ameliorated by amphetamine administered 30 min prior to the retention test. The purpose of this experiment was to determine if propofol-induced amnesia could also be reversed by amphetamine.

Procedure

A group of 24 mice was injected with propofol (50 mg/kg) prior to training as previously described. Thirty minutes before the retention test, half the mice were treated with *d*-amphetamine sulphate (1 mg/kg) and half with saline.

To evaluate the influence of side effects of acute amphetamine on performance at the time of testing, we trained another group of 12 propofol-treated mice in a distinctly different apparatus (V-trough; 12 × 12 × 18 cm, made of black lucite with an aluminum plate floor bent at a 45° angle) and tested them in the regular training apparatus after amphetamine injection. We reasoned that if amphetamine was increasing test latencies because it was reactivating the training memory, mice trained in the V-trough (sham trained) should not exhibit avoidance (increased test latencies), while mice trained and tested in the regular apparatus should demonstrate improved avoidance. On the other hand, if amphetamine was

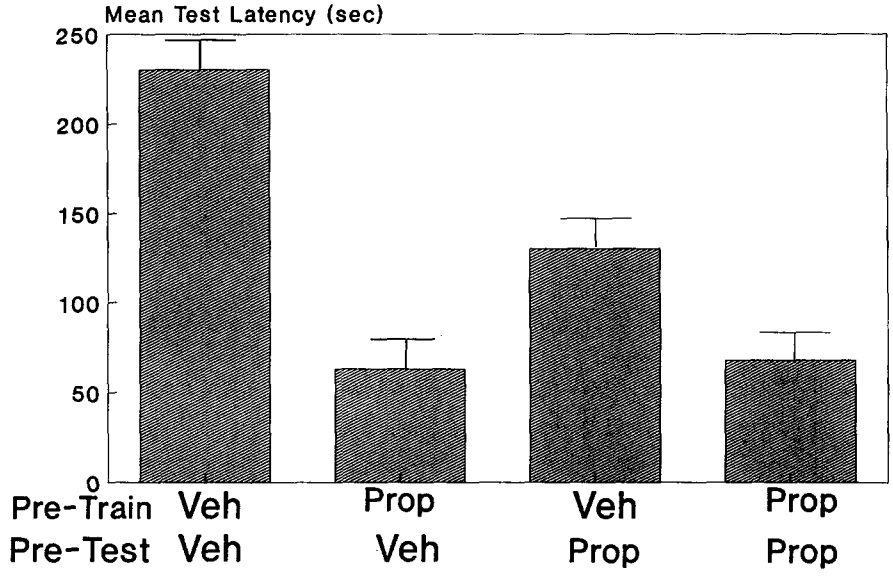

FIG. 2. Absence of state-dependent learning. Mice were treated with a subanesthetic dose of propofol (50 mg/kg) or vehicle and trained 10 min later. Half of each group was retreated with propofol or vehicle before the 24-h retention test.

increasing latencies by nonspecific means (e.g., by increasing behavioral stereotopy) both groups should show similar levels of avoidance.

Results

Figure 3 shows the results of this experiment. A one-way ANOVA indicated a significant difference in test latencies among the four treatment groups, $F(3, 44) = 12.5$, $p = 0.001$. Posthoc Bonferroni t-tests ($p = <0.05$) revealed that the Prop-Amp group had significantly longer test latencies than the Prop group, $t(22) = 4.71$, and that the Amp-Sham group exhibited significantly shorter test latencies than the regularly trained Prop-Amp group, $t(22) = 6.16$. These data indicate that propofol-induced anterograde amnesia can be alleviated by *d*-amphetamine administered prior to testing.

EXPERIMENT 5

We observed (13) that the anterograde amnesia induced by halothane administration spontaneously dissipates 48 h after training. The intention of this experiment was to determine if propofol-induced anterograde amnesia has similar temporal characteristics.

Procedure

Thirty mice were injected with 50 mg/kg propofol and 30 given vehicle 10 min before training as previously described. Animals in each group were randomly assigned to one of three groups and tested 1, 3, or 7 days after training.

Results

Figure 4 shows the results of this experiment. A 3 × 2 ANOVA carried out on the data indicated that there was a significant drug effect, $F(1, 54) = 13.85$, $p = 0.001$, and a significant effect of time of testing, $F(2, 54) = 7.09$, $p = 0.002$, but no interaction between these two variables. These results show that propofol amnesia does not spontaneously dissipate in a 7-day period after training.

EXPERIMENT 6

The issue of awareness during anesthesia has been the subject of considerable interest in anesthesiology (2). Several studies have attempted to determine whether information presented during clinical anesthesia can be recalled in the awake state (1,3,4–6,18). The results from most of the studies are negative but there are some positive findings that indicate that under certain circumstances some learning may be possible (1,4,5).

Using extinction and latent inhibition paradigms, we found that animals do not show evidence of associative learning when auditory stimuli are presented while they are anesthetized with halothane (in preparation). The purpose of Experiment 6 was to investigate the effects of propofol on the extinction of auditory stimuli previously paired with shock in a fear conditioning paradigm.

Behavioral Task

The experimental procedure used to examine learning during anesthesia is conducted as follows. Thirsty mice are trained to drink from a water tube in a test chamber. Following adaptation, animals are given three conditioning trials in which a brief tone is followed by foot-shock. On the following day, mice are given an extinction session in which the tone is presented 40 times without the shock. Drug or anesthetic treatments are administered before this session. Strength of conditioning is tested on day 5. Mice are returned to the drinking chamber and the amount of suppression of drinking induced by the tone is measured. Undrugged mice given the extinction session have less fear of the tone at the time of testing and as a consequence exhibit significantly reduced suppression of drinking in its presence. Failure to learn during

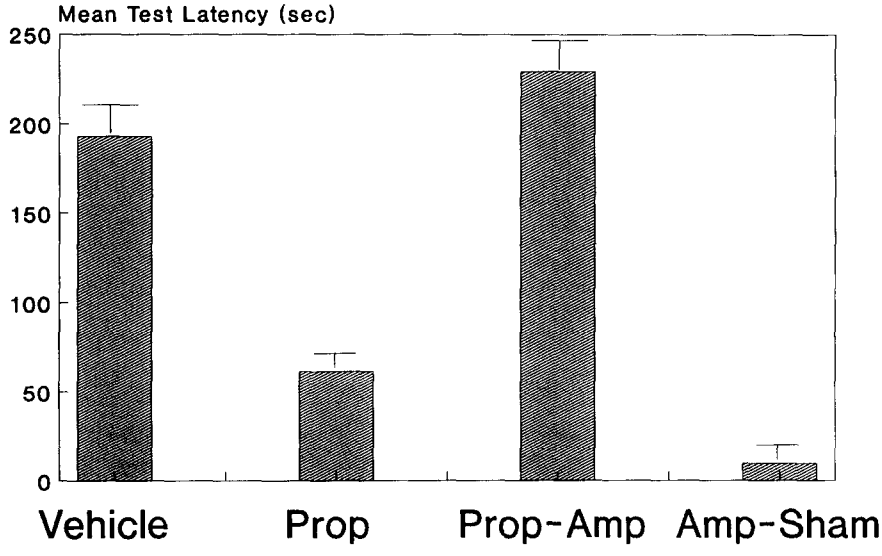

FIG. 3. *d*-Amphetamine reversal of propofol anterograde amnesia. Animals were given propofol 10 min before training and treated with *d*-amphetamine (1 mg/kg) 30 min before the 24-h retention test. The amphetamine–sham group was treated similarly but trained in a V-trough and tested in the regular apparatus.

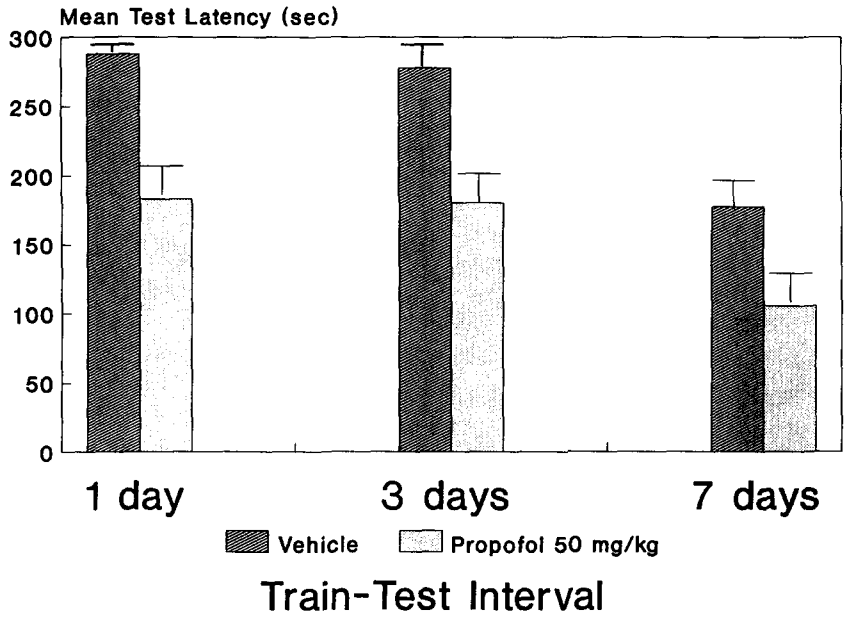

FIG. 4. Effects of varying training to test interval. Animals were treated with propofol and trained 10 min later. Groups were tested either 1, 3, or 7 days after training.

the extinction session would be indicated by suppression ratios comparable to animals not exposed to an extinction session.

Procedure

Following 24-h water deprivation, 35 mice were given two daily sessions in which they were permitted to drink from the tube in the test chamber. On day 3, they were given three conditioned stimulus–unconditioned stimulus (CS–UCS) pairings on a fixed-interval 2-min schedule with the water tube removed. The CS was a 10-s tone and the UCS was a 1-s 0.2-mA foot-shock. The UCS was initiated following termination of the CS. The extinction session was given on day 4. Mice were placed in the test chamber with the drinking tube removed and exposed to 40 10-s tone presentations on a fixed-interval 1-min schedule. No shock was given. One group (n = 12) was injected with 100 mg/kg propofol 10 min before the extinction session and a control group (n = 11) was injected with vehicle. The last group of mice (n = 12) were not exposed to the extinction session. Mice were given free access to water for 1 h in the home cage after each daily session. In the test session on day 5, the time taken to complete two 5-s periods of drinking was recorded. After the first 5 s of drinking, the tone was initiated and remained on until mice had completed a further 5 s of drinking or until 300 s had elapsed. Strength of conditioning was measured by a suppression ratio calculated as $A/A + B$, where A is time to complete 5 s of drinking prior to tone onset and B time to complete drinking during tone presentation. A ratio of 0.50 indicates a total absence of suppression.

Results

The results of this experiment are shown in Fig. 5. The results from a group of mice not given an extinction session are included to illustrate the amount of suppression produced by the fear conditioning. The vehicle-treated group given the extinction session exhibited significantly higher suppression ratios than the no-extinction group, $t(21) = 2.31, p = 0.032$, indicating that extinction attenuated the effects of fear conditioning. Propofol-treated mice, however, failed to demonstrate any weakening of conditioning [vehicle vs. propofol, $t(21) = 2.04$, $p = 0.039$], exhibiting suppression ratios almost identical to those observed in the group not given an extinction session.

DISCUSSION

The results of this study indicate that propofol can cause anterograde but not retrograde amnesia for inhibitory avoidance learning in mice and disrupt retention of auditory stimuli in an extinction paradigm. In this respect, propofol appears to have similar amnestic properties to other chemically unrelated anesthetics, such as halothane, which have been shown to induce amnesias for these same learned behaviors (13). These results also show that propofol does not disrupt acquisition processes. Experiment 2 indicated that mice trained 10 min following propofol injection exhibit rates of acquisition of inhibitory avoidance indistinguishable from those seen in vehicle-injected controls. Despite this similarity in the acquisition of avoidance behavior, propofol-treated mice exhibit a marked deficit in the retention of this learning 24 h later. The memory impairment following propofol anesthesia therefore satisfies the requirements of an authentic anterograde amnesia: normal learning accompanied by abnormally rapid forgetting.

The basis of the anterograde amnesia is not clear. Experiment 3 shows that it is not a consequence of state-dependent learning; reexposing mice to propofol before the retention test failed to alleviate the amnesia. In this regard, propofol-induced amnesia differs from halothane amnesia, which has been shown to be the result of retrieval failure produced by state-dependent learning (13). Propofol-induced amnesia does not appear to spontaneously dissipate with time. Experiment 5 showed that amnesia was still present 3 days after learning,

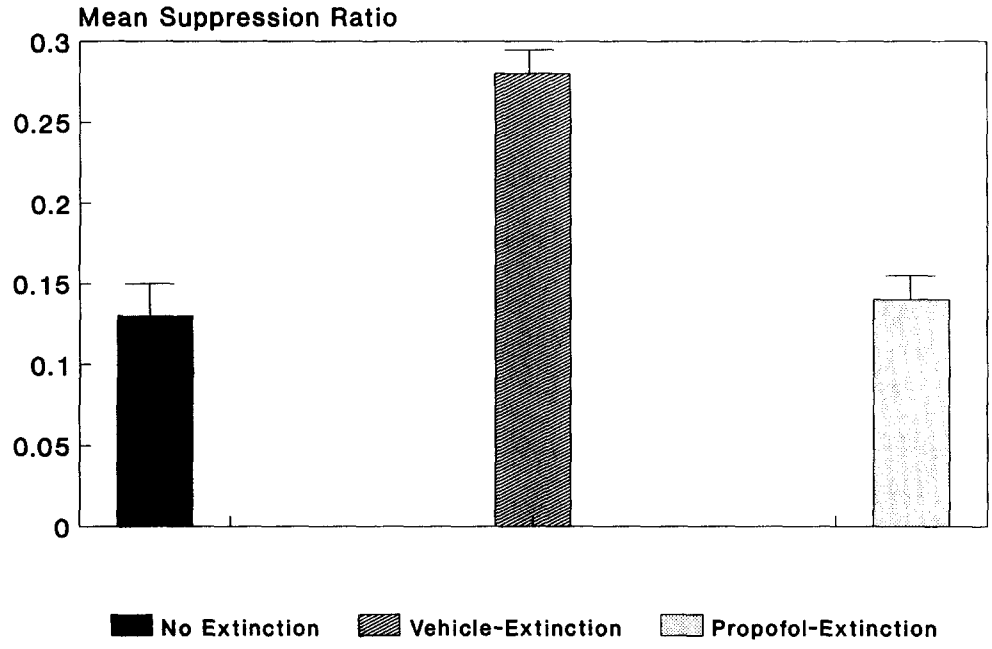

FIG. 5. Absence of an extinction effect under an anesthetic dose of propofol. Twenty-four hours after fear conditioning, propofol- and vehicle-treated mice were given 60 presentations of the conditioned stimulus alone and conditioned suppression was tested 24 h later. High ratios indicate absence of suppression.

a time at which we observed spontaneous recovery from halothane amnesia (13).

The result of Experiment 4 indicated that the amnesia could be alleviated by *d*-amphetamine administered prior to the retention test. The demonstration that memory could be restored implies that propofol either blocks retrieval of an intact memory or impairs consolidation so that only a weak version of the original habit is stored in permanent memory. It is not possible to distinguish between these two alternative interpretations on the basis of these results, but other data suggest that pretraining administration of amnestic agents may impair retention by decreasing the strength of the stored memory (12).

The results of Experiment 6 indicate that propofol can disrupt retention in more than one behavioral task. These results showed that mice treated with propofol during exposure to unpunished tone presentations failed to demonstrate the weakening of conditioned suppression that normally results from extinction. This finding suggests that anterograde amnesia may be a general property of propofol anesthesia.

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