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Amnesia Induced by Short-Term Treatment With Ethanol: Attenuation by Pretest Oxotremorine

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BRIONI, J. D., J. L. McGAUGH AND I. IZQUIERDO. *Amnesia induced by short-term treatment with ethanol: Attenuation by pretest oxotremorine*. PHARMACOL BIOCHEM BEHAV **33**(1) 27–29, 1989.—CD-1 mice were administered a series of tones paired with footshock in the closed arm of a Y maze. On a test session 8 days later the animals were tested for retention of the conditioned emotional response (CER). On the 2-min test session, the three arms of the maze were open and the number of entries into the arms was counted. Retention of the CER was measured by the decrease in the number of entries in comparison with animals trained with no footshock. Starting 24 hr after training, and continuing for the 7 days between training and testing, the animals in different groups received a daily IP injection of saline, 3.6 g/kg of ethanol, 150 μg/kg of the cholinergic muscarinic agonist oxotremorine, or ethanol plus oxotremorine. Retention was evaluated 24 hr after the last injection. Ethanol reduced retention of the conditioned emotional response. This effect was attenuated by oxotremorine (150 μg/kg) given IP 6 min prior to testing, but not by the same dose of oxotremorine given daily together with the ethanol treatment. Oxotremorine injections administered prior to the retention test also enhanced the retention performance of the control group. Daily oxotremorine administration had no effect. These findings suggest that ethanol weakened retention of the conditioned emotional response, that this effect was unrelated to acquisition or consolidation, and that the deleterious effect of the ethanol treatment can be attenuated by oxotremorine administered prior to the retention test.

Ethanol Alcoholic blackouts Amnesia Oxotremorine Retrieval Conditioned emotional response

CHRONIC alcohol intake may lead to persistent memory deficits in humans (12,15). Furthermore, short periods (hours, days) of very intense consumption (over 2 g/kg/day) may lead to amnesia for experiences or events that occurred during or prior to the intoxication [alcoholic "blackouts" (9,12)]. It is not known whether these two types of memory disorder are related. Experiments examining the effects of alcohol on memory in rats or mice have often succeeded only partially (6) or not at all (14) in reproducing the impaired memory seen in humans. Attempts to reproduce the chronic effects are usually hindered by the lack of tolerance of animals to high concentrations of alcohol over an extended period of time; at some point the animals typically begin to lose weight and become generally deteriorated (6,14). Such effects preclude interpretations of behavioral effects in terms of alcohol influences on learning and memory. Findings of studies attempting to reproduce the "blackout" effect using the single posttraining injection model are also difficult to interpret because ethanol itself or its consequences [i.e., attack of the intoxicated animal by conspecifics (5)] can act as an aversive stimulus and therefore add to the punishment used for training (11). Furthermore, "blackouts" in humans are rarely, if ever, seen following ingestion of a large quantitity of alcohol over a short period of time. "Blackouts" typically develop after the repeated ingestion of high amounts of alcohol over several hours or days (9,12).

Recently, Arendt *et al.* (1) succeeded in getting Sprague-Dawley rats to consume 20% ethanol v/v as the sole drinking fluid over a period of 28 weeks. Following this treatment the animals lowered levels of choline acetyltransferase, acetylcholine, and acetylcholinesterase levels in the neocortex and hippocampus (1), as well as a pronounced memory deficit in a radial maze task. Both the biochemical and the behavioral deficits were counteracted by the implant of cholinergic-rich fetal septal cells into the hippocampus, parietal cortex, or both (1). These data are of great potential clinical interest in view of the fact that lesions in brain cholinergic nuclei (2) and low brain choline acetyltransferase (3), have been described in human alcoholic patients with memory deficits.

The experiment reported here was an attempt to obtain a

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"blackout" effect in mice, and to determine whether the effect can be reversed by cholinergic stimulation. Hunt and Brady (10) observed, in 1951, that 7 or more daily electroconvulsive shocks interpolated between training and testing disrupted retention of a conditioned emotional response in rats. In the present paper we used the Hunt and Brady model, using 7 daily 3.6 g/kg IP ethanol injections instead of the electroconvulsive shock, and studied the reversal of their effect by oxotremorine given either together with the ethanol, or at the time of testing. Oxotremorine is a well known cholinergic muscarinic agonist which has been found to enhance memory in mice and rats when given after training (4,8) or prior to testing (3).

METHOD

Subjects

Male CD-1 mice (medium weight, 27 g) from Charles River Laboratories were housed six per cage and acclimatized to laboratory conditions for 1 week prior to the experiments. They were maintained on a 12-hr light cycle (lights on at 07:00) with food and water ad lib.

Apparatus and Procedures

The mice were trained on a conditioned emotional response (CER) task (3,10). The training procedure was as follows (3). The mice were placed in one of the arms of a trough-shaped Y maze. Each arm of the maze was 13.5 cm high, 15.5 cm long, 11.5 cm wide at the top and 2.5 cm wide at the base. The door to the other alleys was closed throughout training. The animal was given 20 trials on which tone was paired with footshock. On each trial, a 5-sec, 1 kHz tone was delivered through a loudspeaker located 20 cm from the training apparatus. The last 2 sec of each tone overlapped with a 0.75-mA scrambled footshock delivered to the floor of the closed compartment. The intertrial interval was 15 sec. Training was completed within 400 sec. Control groups were presented with tones but no footshock. Retention was tested 8 days later. On the test session, the animals were again placed in one arm of the maze, as in the training session, but the doors of the three compartments were left open, and the number of entries into each arm of the radial Y maze during a 2-min test session was counted. The tone was presented continuously starting 3 sec after the animals were placed in the apparatus.

Starting 24 hr after training, the animals received 7 daily IP injections of saline (n = 36), ethanol (3.6 mg/kg) (n = 36), oxotremorine sesquifumarate (150 μg/kg) (n = 18), or ethanol plus oxotremorine (n = 18). Testing was carried out 24 hr after the seventh injection. The experimental conditions are summarized in Table 1. Half of the animals in the saline and the ethanol groups received an injection of oxotremorine 6 min prior to the retention test; all other animals received saline prior to the retention test. Twelve animals in each treatment group received tone-footshock-training as described above, and six received tone but no footshock. Three animals died: 2 in the daily ethanol/pretest saline groups, and 1 in the daily ethanol plus oxotremorine/pretest saline groups.

Ethanol was administered from a 20% v/v solution of absolute ethanol in saline. Oxotremorine was also dissolved in saline solution. Statistical analysis was by a one-way ANOVA followed by individual Duncan multiple range tests (7).

TABLE 1

EXPERIMENTAL GROUPS

Daily Treatment	Pretest	Group	n
1. Saline	Saline	S-S	18
2. Saline	Oxotremorine	S-O	18
3. Alcohol	Saline	A-S	16
4. Alcohol	Oxotremorine	A-O	18
5. Oxotremorine	Saline	O-S	18
6. Oxotremorine + Alcohol	Saline	OA-S	17

RESULTS

The results are shown in Fig. 1. A one-way ANOVA revealed a significant group effect: $F(11,93) = 33.51$, $p<0.0001$. In groups given daily saline injections (S-S and S-O), the tone-footshock pairing significantly reduced responses on the retention test session. For these groups the entries into the arms of the maze during the retention test session were lower than those of the no-footshock groups ($p<0.001$). However, the responses of mice given daily ethanol either with (OA-S) or without (A-S) daily oxotremorine and tested under saline did not differ from those of the tone-only controls ($p>0.20$). Thus, it is clear that the training conditions produced a CER and that the daily ethanol treatment impaired retention of the CER. Further, the retention impairment was not attenuated by concomitant daily administration of oxotremorine. Also, daily injections of oxotremorine administered alone did not affect performance on the retention test; the scores of the O-S group were comparable to those of the S-S controls.

Administration of oxotremorine prior to the test session enhanced retention both in the group that received daily saline injections (S-O) as well as in the group given ethanol (A-O) ($p<0.001$ in both cases). In the former, in confirmation of a previous report (3), oxotremorine enhanced retention performance; the test scores were lower ($p<0.001$) than those of mice

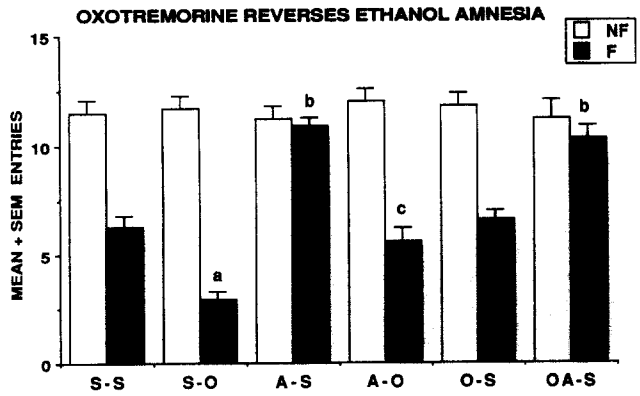

FIG. 1. Mean+SEM number of entries into the arms of a Y maze in the CER test session 8 days following CER training consisting of tones paired with footshocks (striped columns), or control training consisting of tones alone, i.e., without footshocks (white columns). In the 7 days between the training and the testing the animals received a daily IP injection of saline (S), 3.6 g/kg alcohol (A), 150 μg/kg oxotremorine sesquifumarate (O), or the alcohol plus oxotremorine (OA). The daily injection schedules started 24 hr after training and stopped 24 hr before testing. Six min prior to testing S or A groups received either saline or oxotremorine (groups S-S, S-O, A-S and A-O), and the other groups received saline (O-S and OA-S). Pretest oxotremorine enhanced retention (a) and attenuated (c) the amnestic effect of alcohol (b). a) Significant difference from S-S group at $p<0.001$ level in Duncan test; b) same, and in addition not different from no-footshock controls ($p<0.2$); c) significant difference both from A-S and from S-O groups at $p<0.001$ level.

given saline (S-S) prior to the test. In the latter, oxotremorine attenuated the ethanol effect; the retention scores were comparable to those of the S-S controls but significantly lower than those of the A-S animals and significantly higher than those of the S-O group.

Each daily injection of ethanol or of ethanol plus oxotremorine was followed within 5 min by a pronounced ataxia, which lasted 3 to 6 hr. During that time most of the ethanol-injected animals appeared to be asleep most of the time, and the ataxia showed whenever they walked about the cage. These effects subsided in all ethanol animals within about 6 or 7 hr, and no effect was observed 24 hr later; the responses in the no footshock groups given daily ethanol injections (A-S, A-O or OA-S) were comparable to those of the no footshock saline group (S-S).

DISCUSSION

The present results indicate that a 7-day ethanol treatment given between training and testing produces amnesia for a conditioned emotional response in mice. The effect is similar to the alcoholic "blackout" seen in humans (9,12). The effect of ethanol cannot be attributed to an influence on acquisition, consolidation or performance, since the treatment started 24 hr after training and was stopped 24 hr before testing. Further, the ethanol treatments did not affect test session performance in the groups that did not receive footshock. Ethanol obviously did not completely block memory processes as the retention performance of ethanol-treated animals was enhanced by oxotremorine. Ethanol either made the memory trace weaker, or made it less accessible to retrieval, as oxotremorine administered prior to the retention test did not improve the retention performance of the ethanol group above that of controls, as it did in the saline-treated animals.

It is unlikely that the ethanol-induced retention impairment was due to the daily repeated inhibition of cholinergic mechanisms, as concomitant daily oxotremorine administration was unable to antagonize the impairment. The present findings are comparable to those of a previous report (3) indicating that oxotremorine induces a scopolamine-reversible enhancement of retrieval in mice (3). Our finding of cholinergic reversal of ethanol amnesia also fits with the observation of Arendt *et al.* (1), that a memory deficit produced in rats by a much longer treatment with alcohol was reversed by the intrahippocampal and/or cortical implants of functional cholinergic cells.

Our finding that oxotremorine enhanced retention in controls and attenuated the amnestic effect of the ethanol treatments argues that the effects may be due to enhanced retrieval. If this is the case, it might also be that the attenuation of amnesia induced by the cholinergic transplants is due simply to a retrieval-enhancement effect against a background of impaired memory functioning rather than to a specific replacement-therapy effect. However, it would seem advisable to refrain from such a generalization on two counts. First, it is not known whether the memory deficit reported here, and the one Arendt *et al.* (1) obtained in chronically-treated animals have the same neurobiological basis (or bases). Second, Arendt *et al.* (1) reported finding very clear anatomical and biochemical lesions in central cholinergic structures in chronically-treated animals. We do not know whether there were any such lesions in our mice and, in any case, it seems unlikely that ethanol-induced cholinergic lesions could have developed in a period as short as one week (1,14).

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