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# Memory Deficits Following Chronic Alcohol Consumption in Mice: Relationships With Hippocampal and Cortical Cholinergic Activities

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BERACOCHEA, D., J. MICHEAU AND R. JAFFARD. *Memory deficits following chronic alcohol consumption in mice: Relationships with hippocampal and cortical cholinergic activities.* PHARMACOL BIOCHEM BEHAV **42**(4) 749–753, 1992.—Chronic ethanol consumption (12% v/v for 12 months) produced an accelerated decay of T-maze spontaneous alternation (SA) rates as the interval that elapsed between forced trials, used as acquisition, and a free test trial, used as a retention test, increased. Thus, alcohol-treated mice that exhibited normal SA rates at a short interval (5 min) were impaired at the longer one (6 h) relative to controls. This alcohol-induced deficit was almost completely reversed by physostigmine (0.05 mg/kg, IP) given only before the test trial. Parallel neurochemical analysis showed that chronic alcohol intake produced a significant decrease in hippocampal and cortical sodium-dependent high-affinity choline uptake. In particular, the significant cholinergic activation produced by a T-maze exploration in controls was attenuated in experimental subjects so that the between-groups differences already present in the quiet condition were amplified in the active (exploration) state. These findings suggest that the memory deficits induced by chronic ethanol consumption stem from a failure of some cholinergic-dependent retrieval processes. An attempt is made to relate the present results with our previous ones that emphasized the importance of diencephalic damage in alcohol-induced retrieval deficits.

Acetylcholine High-affinity choline uptake Hippocampus Cortex Alcohol Memory
Physostigmine Mice

CHRONIC ethanol consumption in man is associated with a loss of memory that has been most often attributed to gross neuropathological changes observed in diencephalic structures [e.g., mammillary bodies (MM) and/or the dorsomedial nucleus of the thalamus]. More recently, however, it has been suggested that two kinds of biochemical disorders could be involved in the memory deficits of Korsakoff subjects. Thus, on one hand, the idea that a noradrenergic dysfunction, caused by locus coeruleus lesions, can contribute to amnesia has received support from the work of Mair and McEntee (14); on the other hand, other studies have shown cholinergic dysfunctions in chronic alcoholics (1,3). More recently, Butters proposed that cholinergic disorders resulting from a reduction of neurons in the basal forebrain of amnesic alcoholics could be responsible for more severe memory problems than diencephalic lesions alone (8).

Animal models have succeeded in showing that chronic ethanol administration produced both learning and memory impairments together with regional neuronal losses and changes in several neurotransmitter systems. Thus, on one hand, there is evidence that the alcohol-induced damage to the forebrain cholinergic system plays an important part in the radial maze deficit of alcohol-treated rats since these animals showed improvement after treatments with cholinergic drugs and cholinergic-rich brain transplants (2,12); on the other hand, we have argued that the alcohol-induced neuronal loss in MM is mainly responsible for increased sensitivity to proactive interference and faster forgetting rates displayed by alcohol-treated mice since both of these deficits were observed following experimental lesions of the MM (6,17). In addition, however, we have shown that alcohol treatment did induce a slight but significant decrease in hippocampal and cortical choline uptake and that the anterograde amnesia displayed by experimental subjects was alleviated by physostigmine (but not by neostigmine) given before both acquisition and retention test trials (5).

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The present experiment further investigated the contribution of acetylcholine to the alcohol-induced memory deficit in mice. Indeed, our previous investigations ignored two potentially important factors. The first concerns the type of memory process that is actually impaired in alcohol-treated mice. Previous experiment have provided evidence that the alternation deficit observed in both sequential and delayed alternation procedures resulted from an impairment of retrieval processes. However, in both paradigms we found that a context variation provided on retention trials alone was sufficient to completely alleviate the alcohol-induced deficit. The second problem concerns the real significance of differences in cholinergic activity measured in quiet animals seeing that, as indicated in our previous experiments, these differences were very weak. It has been shown that memory testing induced significant changes in hippocampal and cortical choline uptake and that this activated state is presumably a more important marker of cholinergic dysfunction than the basal (quiet) state. Moreover, a specific loss of test-induced changes can be related to a hypofunction of phasically active transsynaptic mechanisms that mediate these changes.

Accordingly, the present experiments examined a) the effects of physostigmine given only before the test trial of a delayed T-maze spontaneous alternation task and b) hippocampal and cortical sodium-dependent high-affinity choline (SDHACU) uptake in animals either maintained in quiet conditions or following T-maze exploration (active condition).

## METHOD

### *Subjects*

Subjects were male mice of the Balb/c strain, approximately 6 weeks old at the time of receipt. They were housed in cages of 15-20 animals matched for weight and placed in a colony room (ambient temperature 22°C) with an automatic light cycle (8:00 a.m.-8:00 p.m.). Free access to food and water was provided for 3 weeks before the beginning of ethanol administration.

### *Ethanol Administration*

Animals were randomly assigned to one of three groups. Subjects in the experimental group were given, as their only source of fluid, an increasing progression of ethanol solutions as follows: 5% v/v solution for the first week, 10% for the second week, and 12% for the remaining time (12 months). The solutions were mixed from 95% ethanol and supplemented with sucrose (30 g/l). They were available in two 500-ml bottles in each cage. Dry food was freely available throughout the experiment. Mice in the first control group were pair fed. They received an isocaloric solution of dextrimaltose and dry food that was equivalent to the quantity consumed by the experimental group. Animals assigned to the second control group had ad lib access to dry food and tapwater. After 12 months of treatment, tapwater was progressively substituted for ethanol-sucrose (or dextrimaltose) solution in steps of 5% per week. Behavioral testing began 3 weeks later.

### *Behavioral Testing*

Memory was assessed by a delayed response task based upon the innate alternation behavior. The task has been described in full elsewhere (5). Basically, all testing was conducted in a T-maze constructed of grey Plexiglas. The stem and arms were 35 cm long, 10 cm wide, and 25 cm high. The starting box (10 × 12 cm) was separated from the stem by a vertical sliding door. Horizontal sliding doors were placed at the entrance of each arm. Memory was tested using a discrete trial procedure that consisted of two forced trials (acquisition) followed by a free test trial, with varying acquisition-test intervals (ATI). During acquisition, subjects were twice forced to enter one of the arms, the other being blocked by the sliding door. On the test for retention, animals had free access to both arms. The innate tendency of rodents is to enter the arm that has not been visited during the acquisition trials (SA). The decay of SA rates as a function of ATIs is used as an index of memory (26). Before being tested with the discrete trial procedure (memory testing), mice were submitted to a sequential trial procedure (three successive trials separated by a 30-s intertrial interval over 3 consecutive days). This procedure was used to determine whether subjects or groups differed in their ability to alternate spontaneously.

### *Experimental Design for Behavioral Analysis*

All subjects were submitted to daily handling for 5 days. They were then given two free exploration sessions of 5 min in the apparatus on each of 3 days before being tested for SA with the sequential procedure. Then, groups were constituted and matched for performance according to the individual SA rates observed in the sequential procedure. Half the experimental subjects received a saline solution 20 min before the acquisition trials and were under the influence of physostigmine (P) (0.05 mg/kg) administered 20 min before the test trial (group Sal-P, $n = 10$); the remaining experimental subjects received a saline solution administered 20 min before both the acquisition and test trials (group Sal-Sal, $n = 9$). The same experimental design was used with control mice with half the subjects belonging to the pair-fed condition in each group (group Sal-P, $n = 8$; group Sal-Sal, $n = 8$). All subjects were tested six times with a 6-h ATI and four times with a 5-min ATI. Sessions involving a 5-min ATI were run for all subjects in a Sal-Sal condition. Successive tests were run every 3 days and ATIs (5 min and 6 h) were mixed throughout the experiment.

### *Neurochemical Analysis*

Neurochemical analysis was carried out with subjects treated in exactly the same way as mice used for behavioral analysis (rearing conditions, age, treatments, and withdrawal). As for behavioral experiments, all subjects were withdrawn 3 weeks before neurochemical studies were performed. Sixteen experimental and 11 control mice were used and assigned either the "active" or the "quiet" condition. In the active condition, subjects were removed from their cages and placed in the T-maze used for behavioral testing. They were allowed to freely explore the apparatus for 30 min. Then they were killed and brains removed within a period of 30-60 s following the exploration session. Animals assigned to the quiet condition did not explore the T-maze but were simply removed from the colony room and killed.

SDHACU kinetics were measured in aliquots of resuspended crude synaptosomal (P2) pellets of hippocampus and frontal cortex from the different groups. The procedure, based upon that of Atweh et al. (4), consisted of measuring the difference in the amount of methyl-[$^3$H]choline (0.30 $\mu$M) taken up by the synaptosomal aliquots over a 4-min period in parallel incubations in sodium-free and normal sodium Krebs-Ringer solution.

*Statistical Analysis*

Statistical analyses were performed using an analysis of variance (ANOVA) followed by an appropriate posthoc test (Scheffe's $F$-test).

## RESULTS

*Behavior*

*Effects of physostigmine.* There were no significant between-groups differences in the rate of SA in the sequential test procedure (three successive trials separated by a 30-s interval): 75.4 and 69.7% for, respectively, the experimental and control subjects, $F(1, 66) = 0.029$.

No between-groups differences were observed at the 5-min ATI, performances for all groups being above 75%, $F(3, 30) = 0.17$. The effects of physostigmine are summarized in Fig. 1. A significant difference was observed between experimental and control mice in the Sal-Sal condition at the 6-h ATI [46.2 ± 6.0% and 71.8 ± 4.85%, respectively; $F(1, 15) = 11.4$, $p = 0.004$]. However, experimental subjects in the Sal-P condition exhibited a significantly different performance than experimental subjects in the Sal-Sal one [68.2 ± 5.8% and 46.2 ± 6.0% at the 6-h ATI, respectively; $F(1, 17) = 6.88$, $p = 0.017$]. Experimental subjects in the Sal-P condition did not differ significantly at the 6-h ATI from either control subjects in the Sal-Sal condition, $F(1, 16) = 0.22$, or Sal-P condition, $F(1, 16) = 0.29$. No significant difference was observed between controls of the Sal-P and Sal-Sal conditions at the 6-h ATI [77.05 ± 5.4% and 71.8 ± 4.1%, respectively; $F(1, 14) = 0.58$].

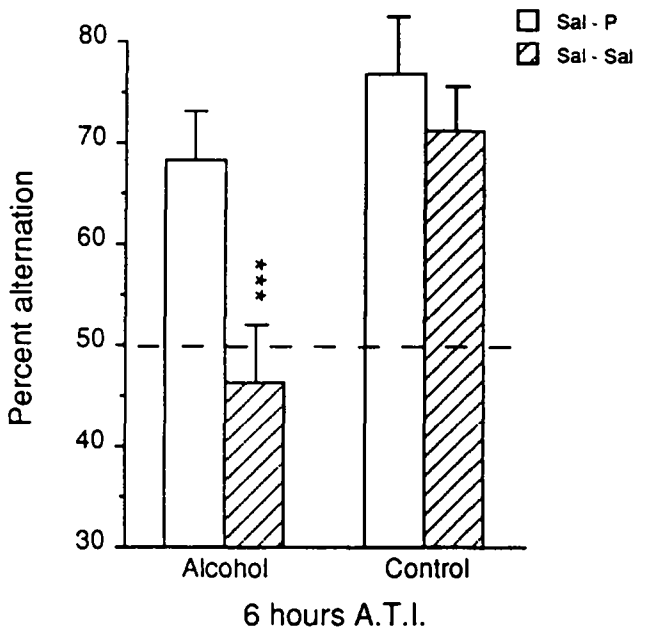


FIG. 1. Percentage of delayed alternation observed with a 6-h ATI in experimental (alcohol) and control groups. As can be observed, the deficit of alcohol-treated mice in the saline-saline (Sal-Sal) condition as compared to controls (Sal-Sal) is alleviated following the administration of physostigmine (Sal-P) delivered IP 20 min before testing (***, $p < 0.01$). Physostigmine administered to controls did not significantly improve performances.

*Neurochemistry*

Results are summarized in Fig. 1. ANOVA with treatment (alcohol vs. control) and behavior (active vs. quiet) as between-groups factors and structure (hippocampus vs. frontal cortex) as the within-group factor showed that, globally, alcohol treatment significantly reduced high-affinity choline uptake (−17.6%) [treatment: $F(1, 44) = 22.6$, $p < 0.001$]. The observed exploration-induced increase in cholinergic activity (+26.9%) [behavior: $F(1, 44) = 34.9$, $p < 0.001$] was consistently reduced in alcohol as compared to control subjects [20.0 vs. 35.8%: interaction treatment × behavior: $F(1, 44) = 4.1$, $p < 0.05$]. However, no significant differential effects of alcohol were observed in hippocampus compared to frontal cortex ($F < 1$ for all interactions). Finally, as compared to controls alcohol-intoxicated animals displayed a nonsignificant lower cholinergic activity in quiet conditions [−12,4%; $F(1, 27) = 2.63$, $p = 0.11$], but this difference was amplified in the active condition [−22.6%; $F(1, 23) = 20.14$, $p = 0.0002$]. Separate comparisons for each structure are summarized in Fig. 2.

## DISCUSSION

At first sight, the ability of physostigmine to reverse alcohol-induced memory impairment seems in accordance with results from neurochemical analysis. Indeed, although measures of high-affinity choline uptake in alcohol-intoxicated animals showed a modest decrease (−12.4%) in cholinergic activity in the quiet condition this decrease was about twice more important in the activated state (−22.6%), that is, 30 s following exploration of a T-maze. Taken as a whole, these results are congruent with previous findings showing that long-term ethanol consumption induced cholinergic hypofunction in rats as shown by a reduction of cholinergic markers (5) and that this hypofunction would be causal to memory impairments as suggested by the benefits of treatments with cholinergic agonists and cholinergic grafts (2,12). Moreover, the specific reduction of the testing-induced cholinergic activation presently observed in alcohol-treated mice would be indicative of an attenuation of the phasically active transynaptic inputs thought to be responsible for this activation (10). More precisely, it has been suggested that the excitatory noradrenergic input to the basal forebrain complex might be involved in these training-induced increases in cholinergic activity [(11,15), see (18)]. Thus, together with the Mair and McEntee hypothesis relating memory deficits in Korsakoff patients to noradrenergic dysfunction (14), our present findings might indicate that the presently observed presynaptic cholinergic hypofunction in alcohol-treated mice stems in part from an impairment of noradrenergic mechanisms. However, one must be cautious in assuming both that behavioral pharmacological and neurochemical observations prove that the cholinergic dysfunction is responsible for the observed memory impairments of alcohol-treated animals and, more importantly, that this would be the sole factor involved in these behavioral deficits. On the first point, one cannot exclude that physostigmine may be compensating for a functional deficit relating to alcohol-induced damage in brain systems unrelated to the cholinergic basal forebrain complex, which, moreover, presently exhibits a quite limited hypofunction [see (9)]. Concerning the second point, it remains that experimental lesions of the mammillary bodies that we initially performed to mimic the neuronal losses produced by alcohol intoxication on this brain structure (13) induced memory impairments very similar to those displayed by alcohol-treated mice (6,7). Finally, as

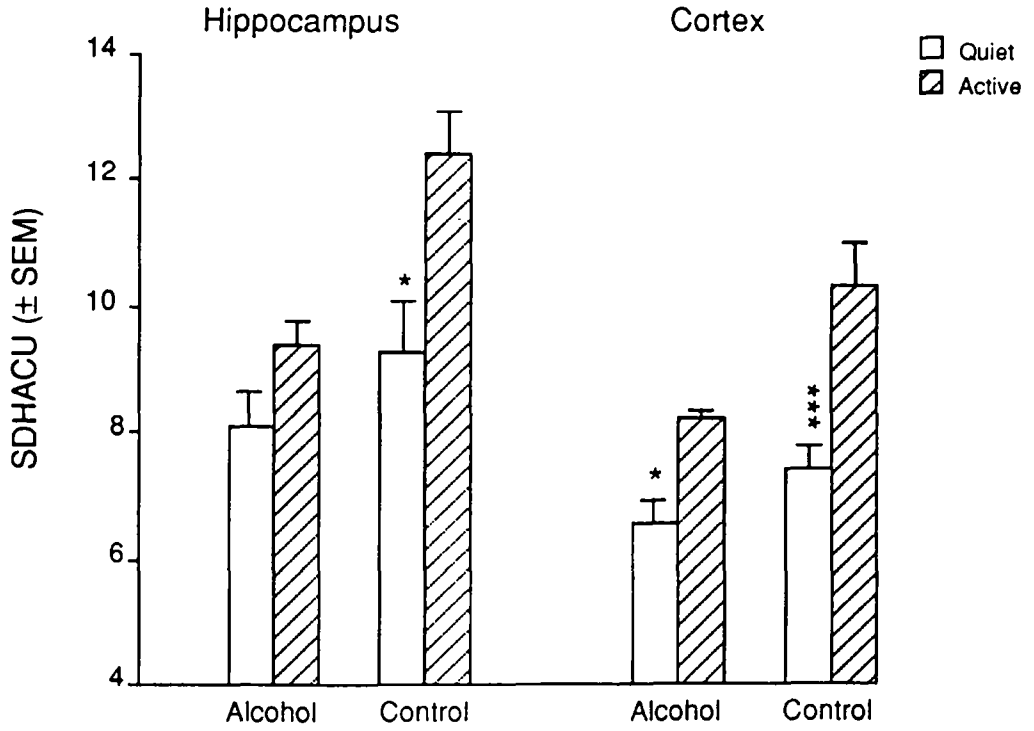


FIG. 2. Mean velocity of SDHACU (pmol/4 min/mg protein ± SEM) in the hippocampus and cortex of alcohol-treated and control mice either in quiet or active conditions. Within-groups comparisons in the active and quiet conditions are represented. $*p < 0.05$, $***p < 0.01$.

previously assumed by Butters (8) for Korsakoff patients, it may be postulated that cholinergic hypofunction superimposed to diencephalic damage is responsible for the observed memory impairments. This conclusion, however, clearly does not completely account for both the observations that physostigmine quite completely reversed the delayed alternation deficit in animals that exhibits alcohol-induced diencephalic damage and inversely that mammillary bodies alone produced the same behavioral deficit as the alcohol treatment. The current lack of studies investigating the effects of systemic cholinergic agonists on the memory deficits produced by MM lesion would certainly help shed light on this problem. However, some data are suggestive of the possibility that the facilitative effect of physostigmine could be explained in a more unitary way. More precisely, it has been shown that the anterior thalamic nuclei receives a cholinergic projection from the lateral tegmentum together with afferents from mammillary bodies; moreover, data from neurophysiological and behavioral experiments suggest that mammillary bodies would act as an "amplifier" of anterior thalamic neuronal responses to the ascendant cholinergic afferents that, in turn, would be specifically involved in the discrimination aspects of avoidance learning performance (16). Thus, it cannot be excluded that one of the mechanisms by which physostigmine improved performance of alcohol-treated subjects is through the facilitation of cholinergic transmission at thalamic synapses, thereby compensating for an attenuation of excitatory inputs provided by mammillary bodies to its thalamic target sites. Finally, without underestimating the postulated contribution of basal forebrain cholinergic neurons to memory impairments induced by alcohol intoxication, this indicates that at least for the behavioral task and animal species we used alternative hypotheses must be considered.

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