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# Chronic Ethanol Consumption Impairs Spatial Remote Memory in Rats But Does Not Affect Cortical Cholinergic Parameters

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PEREIRA, S. R. C., G. A. MENEZES, G. C. FRANCO, A. E. B. COSTA AND A. M. RIBEIRO. *Chronic ethanol consumption impairs spatial remote memory in rats but does not affect cortical cholinergic parameters.* PHARMACOL BIOCHEM BEHAV **60**(2) 305–311, 1998.—We have studied learning, memory and cortical cholinergic parameters after oral administration of 20% v/v ethanol solution to male Fisher rats for 6 months. A group of rats were trained to behave efficiently in an eight-arm radial maze and after that split into two subgroups submitted to ethanol or control treatment. Ethanol-treated rats had more difficulty in relearning the same task 1 year later, compared to ethanol-untreated rats (control). Differences in working memory performance were found, but only in the first 10 training sessions. Another group of rats, which had not been pretrained, was also split into two subgroups submitted to ethanol or control treatment. After that, these rats were trained in the radial maze task for the first time. No significant difference was found between the reference memory performance of the untreated subgroup and the treated one. These two subgroups did not significantly differ in their working memory performance either. Moreover, there were no significant differences between treated and control subjects in the following biochemical brain cortical parameters: *in vitro* acetylcholinesterase (AChE) activity, and stimulated acetylcholine (ACh) release. This work presents an experimental design that allows assessment of remote memory performance after ethanol chronic consumption and shows that the experimental subject is able to retain the behaviors learned 1 year before. It was concluded that chronic ethanol treatment may cause retrograde amnesia, which does not seem to be linked with a cortical cholinergic deficit. © 1998 Elsevier Science Inc.

Chronic ethanol treatment  Reference memory  Working memory  Acetylcholinesterase activity
Acetylcholine release  Fisher rats

SEVERAL studies have shown that chronic ethanol intake has effects on cognitive performance in both humans and laboratory animals (1,30,36). Mild cognitive impairment can be demonstrated by neuropsychological testing in 50–70% of detoxified alcoholics. Abstinence leads to a partial recovery of function, particularly in the first weeks after the cessation of drinking. However, approximately 10% of alcoholics exhibit stable and severe cognitive dysfunction ranging from selective anterograde and retrograde amnesia to dementia (14).

Long-term alcohol drinking has been correlated with retrograde amnesia (15) which is a difficulty in evoking information acquired before the cerebral lesion. However, unlike the traumatic and surgical cerebral lesions, the development of the alcohol cerebral damage is gradual and progressive, and it is difficult to determine when it starts. Therefore, it is hard to know the exact nature of the detected amnesia. What is seen as a retrograde amnesia might be just a consolidation memory impairment (anterograde amnesia). In studying how ethanol affects specific memory aspects, it is important to make sure that the learned task to be evoked was perfectly consolidated in memory before the beginning of the ethanol intake. This is more easily achieved in experiments with animals. Some aspects of animal memory have been studied and, in many cases, the results are similar to those obtained in experiments

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with humans (26,42). Some experimental studies with animal subjects have shown that prolonged ethanol intake affects learning of both appetitive (17) and aversive (18,44) tasks. It has been shown that the impairment found is not due to any malnutrition, incidentally provoked by ethanol treatment (18,44).

Arendt et al. (3,5) found that chronic ethanol consumption affects subject's performance in two forms of memory: the episodic memory, involved in holding information that is pertinent only within a short period of time (working memory), and that involved in holding information that remains constant over time (reference memory).

The brain cholinergic neurons appear to be particularly vulnerable to ethanol (3,4,12,23,39,41). After intake interruption, the cholinergic hypofunction and behavioral impairment induced by ethanol in rodents are either partially or fully reversible, depending on the duration of the treatment and of the abstinence (3,13). For instance, there are evidences that cholinergic parameters assessed after a 1-week withdrawal were significantly lower in ethanol-treated rats, while after a period of 4 ethanol-free weeks both nonsignificant (13) and significant (3) differences in these parameters between treated and untreated rats were found. Some experimental results suggest a causal link between cognitive dysfunction and damage to the cholinergic system (6,8,23,37), while others showed evidence that changes in this system seem not to be related to learning and memory impairments (9,22). The specific relationship between the ethanol-related changes in the cholinergic system and possible behavioral impairments is still obscure.

In the present work, we had two purposes: 1) studying the nature (anterograde and/or retrograde) of the amnesia induced by ethanol chronic treatment, and 2) assessing the acetylcholinesterase (AChE) activity and release of acetylcholine (ACh) from the cerebral cortex as a first step to study whether there is any brain cortical cholinergic parameters change related with a specific cognitive deficit. This was done using a sensitive experimental design (savings design) in which a group of subjects (Fisher rats) initially learned a task, had time to consolidate it, then were submitted to an ethanol treatment, and finally, after an abstinence period, were retested for that task. And their performance was compared both to: (a) that of control subjects that had learned the task but had not been treated with ethanol, and (b) that of control and ethanol-treated subjects that were learning the task for the first time. The underlying rationale is that if the subject has some remembrance of the original learning, relearning the task will be quicker when compared to the initial acquisition. When the time intervening between learning and relearning is long, as was the case of the present work, this comparison is complicated by the fact that the subjects are much older at the time of relearning, and thus, the relative ease induced by prior learning is counteracted by the effect of aging. To avoid the aging effects, we compared the subjects relearning to that of an age-matched control group of naive subjects rather than using repeated measures. After that, all animals were sacrificed and biochemical parameters were evaluated.

## METHOD

### *Subjects*

Twenty male Fisher rats, 23 days old upon arrival in the laboratory, were divided into four groups paired for weight: 1) trained and ethanol treated (TE); 2) untrained and ethanol treated (NE); 3) trained and untreated = control (TC); and d) untrained and untreated = control (NC). They were housed individually in standard rat cages.

### *Treatment*

Ethanol was added to an 8.75% sucrose solution and administered to rats from TE and NE groups as the sole source of fluids available. The ethanol concentration was initially 2% and was increased progressively by 2% every 2 days, until a 20% concentration was reached and set constant for 32 weeks. After that, there was a withdrawal period of 20 days, in which the ethanol concentration was gradually decreased to 0%. All rats had free access to chow during the treatment. Body weight, food and fluid intake were regularly recorded along the treatment and withdrawal periods. At the start of treatment the mean body weights were 301.34 ± 15.32 (treated group) and 288.80 ± 17.82 (control group), and the difference between them was not statistically significant. There was no significant difference in amount of chow consumed between ethanol-treated and control groups. The average consumption of the ethanol solution was 20 ml/rat/day. Control rats were given 40 ml of the 8.75% sucrose solution daily and consumed about 2 g/day more chow than the ethanol-treated groups, so that the average daily caloric intake was almost identical. Blood ethanol concentrations and thiamine level were assessed in a different set of animals that were maintained under the same conditions as the ethanol-treated rats. Tail blood samples for ethanol concentration assay were collected at 10:00–12:00 h, and the ethanol levels were determined by spectrophotometric enzymatic procedures (Sigma 330-1 kit). The thiamine level was assessed at the end of the treatment by measuring the transketolase activity in the erythrocyte (46), and no significant difference was found between ethanol-treated and control groups. Only one animal was lost during the experiment. During behavioral training, all rats were given chow pellets in a sufficient amount to maintain their body weight at about 85% of their free-feeding level adjusted for growth.

### *Behavioral Testing*

*Apparatus.* The apparatus was a radial eight-arm maze with an octogonal center platform that was 60 cm in diameter. Each of the eight arms was 90 cm long and 10 cm wide. The maze was elevated above the floor with good visibility for room cues (posters, chairs, etc). The eight arms remained in the same location with respect to extramaze cues, and the same four arms were consistently baited for any particular animal.

*Procedure.* There were two behavioral experiments with identical procedures: 1) a pretreatment training, to which only TE and TC groups were submitted, and 2) a posttreatment training for all groups. Pretreatment training started as soon as rats reached 85% of their ad lib weight. TE and TC groups received pretreatment training while rats of the NE and NC groups were not trained, but were submitted to identical food deprivation. Rats were shaped by placing them in the maze and allowing them, during 20 min, to eat reinforcements, which consisted of one-quarter of a Froot Loop cereal piece put into holes drilled at the ends of the arms and also scattered through the maze. Shaping ended when the subject learned to retrieve the food from the holes of all baited arms. This procedure required four to five sessions. The training followed a slightly modified version of the Olton and Papas (35) procedure where only half of the maze arms were baited. Unlike Olton and Papas, who used a 17-arm maze, we used an eight-arm maze, and for all groups the baited arms were ran-

domly distributed around the maze. For each rat, these four arms were selected at random but remained the same throughout the experiment. The animals were trained in squads of four rats, in 20 daily sessions of four trials/subject, five sessions/week. At the beginning of a trial, a rat was placed in the center of the maze and allowed to explore until all four reinforcements had been eaten, or until 10 min had elapsed, whichever came first. Then the rat was removed from maze and another animal was given its trial. When all rats of the squad were given the first trial, there was another round of trials, and so on, until all of the rats had four trials. The intertrial interval for each rat varied around 15 min in the initial sessions and tended to become shorter (around 5 min) as the animals spent less time to retrieve the reinforcements. Two types of errors were scored: 1) reference memory errors, when a rat entered a nonrewarded arm for the first time in each trial; and 2) working memory errors, when an arm was revisited. Returns to unbaited arms were scored as working memory errors. In short, rats had to learn: 1) which arms were baited; and 2) a "win-shift" strategy, that is, they had to learn not to return to a previously visited arm, from which food had already been taken. The 20 sessions of pretreatment training were followed by a 3-month period in which all rats had ad lib access to food and water. After that, TE and NE groups were submitted to an ethanol treatment (described above) similar to that used by Arendt et al. (3) while TC and NC groups underwent a control treatment. This lasted for 224 days, followed by an ethanol detoxification period (30 days of free access to water and food), after which all animals were submitted to a posttreatment training. Following this, all rats were sacrificed for biochemical experiments.

### *Biochemical Experiments*

The AChE activity and the ACh release were assessed in cortex slices from brains of control and ethanol-treated rats. All determinations were in triplicate. Rats were killed by decapitation and the brain was rapidly removed and kept on ice. Cortex from one of the hemispheres was imediatly dissected and sliced in prisms of 400 μm in a McIlwain Tissue Chopper. The tissue prisms from the whole cortex area were mixed with a spatule and approximately 10 mg of the homogeneous tissue were transferred to tubes containing 0.6 ml of incubation medium, and the following steps for the ACh release study were carried out as described below. For the AChE activity, aliquots of tissue (aproximatly 5 mg) were transferred to Eppendorf tubes containing 50 μl of borate buffer and frozen at −20°C until the day of the assay.

### *Acetylcholine Release*

Aliquots of 10 mg of cortical slices were preincubated under shaking for 5 min at 37°C in 600 μl of incubation medium containing (mM): NaCl 136.0, KCl 2.7, $CaCl_2$ 1.35, $NaHCO_3$ 12.0, $NaH_2PO_4$ O.36; $MgCl_2$ 0.49, glucose 5.5, eserine 0.01. After 30 min of incubation in the presence and absence of potassium 50 mM, 500 μl of the incubation medium from each sample were transfered to a tube containing 50 μl of trichloroacetic acid 50%. All tubes were kept at −20°C until the day of the assay (maximum of 2 days). The ACh released from the cortical slices was extracted using the method of Prado et al. (38) and measured by chemiluminescence using the method described by Israel and Lebats (25). The stimulated ACh release is expressed as percent of unstimulated released for 30 min (13.6 ± 3.4 pmol/mg of tissue) from brain cortical control rats, which was taken as 100%.

### *Acetylcholinesterase Activity Assay*

Samples (5 mg of cortical tissue) in 50 μl of 0.1 M borate buffer pH 8.2 were homogenized and assayed for AChE activity by the method of Ellman et al. (16). A volume of 5 μl of homogenate was added to a cuvette containing the following: 5 μl of 5 mM dithiobisnitrobenzoic acid (DTNB), 5 μl of 75 mM acetylthiocholine (ATCh), 0.1 M borate buffer, pH 8.2 was added to make a final volume of 620 μl. The development of color was analyzed at 412 nm, using a recording spectrophotometer (UV-160A Shimadzu). The AChE activity was expressed in mol of ATCh hydrolyzed per min per g of tissue.

### *Statistical Analysis*

The statistical analysis of cholinergic parameters data was done through the test of Student (20). In the behavioral part, a variance analysis for longitudinal data was used to verify if there was any treatment (ethanol vs. control) or time effect (20 sessions) on the working and reference memories. When the interaction term group × session was stastistically significant, we compared the groups at each session and also assess each group performance along the sessions. This study followed the methodology of Milleken and Johnson (32) and Singer and Andrade (40), where the multiple comparisons were done using the Least Square Distance values. The results were considered statistically significant at the level of 0.05.

## RESULTS

### *Body Weight and Ethanol Blood Levels*

Body weight differences at the end of the treatment between control (370.35 ± 20.39) and ethanol (374.83 ± 19.47) groups were not statistically significant ($p > 0.05$). The average ethanol consumption was around 9.0 g/kg body weight, resulting in a mean blood ethanol levels around 45 mg % (daytime).

### *Behavioral Testing*

*Pretreatment training.* Performance of groups TE and TC at pretreatment training is shown in Fig. 1. A and B present mean reference and working memory errors/four sessions, respectively. The difference between groups TE and TC along the 20 sessions was not statistically significant for both kinds of memory (reference memory: $F = 0.004$, $p > 0.05$; working memory: $F = 0.07$, $p > 0.05$).

*Posttreatment training.* Reference memory. Mean reference memory error scores/four sessions of NC, NE, TC, and TE groups are shown in Fig. 1C. The comparison between TC and NC groups showed a significant group × session interaction, $F(19, 133) = 3.39$, $p < 0.01$, so multiple comparisons were done to evaluate the group effect. A significant difference of performance was found for sessions 12–20 ($F = 32.89$, $p < 0.01$), showing that the pretreatment training affects the posttreatment performance of subjects. The comparison between TE and NE groups showed no significant group × session interaction ($F = 1.17$ $p > 0.05$) and no significant group effect, $F(1, 7) = 3.62$, $p > 0.05$, showing that for ethanol-treated groups the pretreatment training did not affect the posttreatment performance. The comparison between TE and TC groups showed no significant group × session interaction ($F = 2.35$, $p > 0.05$), and a significant group effect in sessions 12–20 ($F = 6.18$, $p < 0.05$), showing that pretreatment training effect is smaller for ethanol-treated groups when compared with untreated groups. The comparison between NE and NC

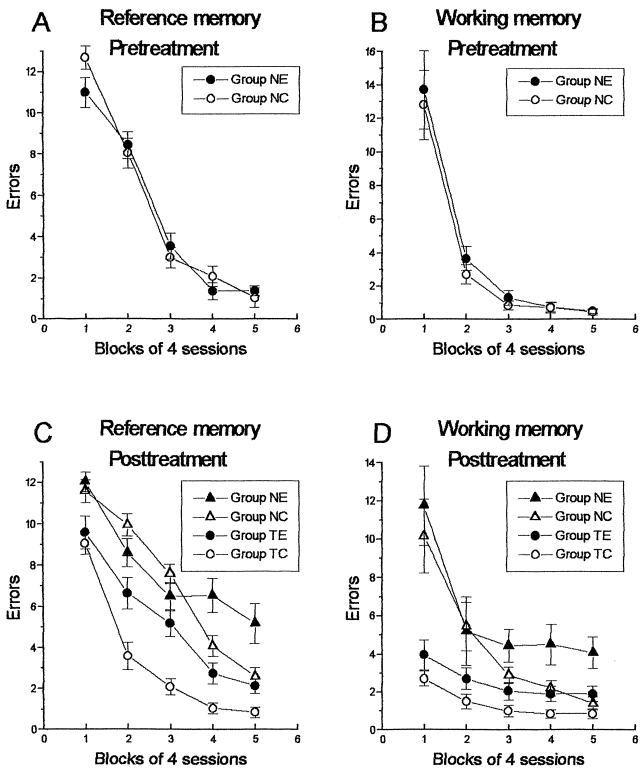


FIG. 1. Radial maze performance of subjects in behavioral tests. Mean errors/four sessions (±SE). Pretreatment training of (A) reference memory and (B) working memory. Posttreatment training of (C) reference memory and (D) working memory. Groups TE and TC learned the radial maze task 1 year before while groups NE and NC were learning the task for the first time. Groups TE and NE were submitted to ethanol treatment for 32 weeks (20% v/v) while group TC and NC had a control treatment.

groups showed no significant group × session interaction ($F = 1.65$, $p > 0.05$), and no significant group effect ($F = 0.44$, $p > 0.05$), showing that ethanol treatment has no effect on performance for subjects with no previous training.

Working memory. The posttreatment performance on working memory is shown in Fig. 1D, where the mean number of errors/four sessions/group is plotted. There is a significant decrease of errors over sessions for NC and NE groups, which received no pretreatment training ($F = 7.2$, $p < 0.01$). TC and TE groups, which received pretreatment training, show this trend too, but while it is statistically significant when all sessions are considered ($F = 9.63$, $p < 0.01$), it is not statistically significant in the last 10 sessions ($F = 1.02$, $p > 0.05$). This indicates that NE and NC groups learned the win-shift strategy demanded by the task all over the 20 sessions, while TE and TC groups relearned it in the first 10 sessions. These results show that TE and TC groups partially retained the win-shift strategy learned in the pretreatment training. Indeed, Fig. 1D shows that the differences between pretrained and nonpretrained groups were larger in the first block of sessions.

The comparison between TC and NC groups showed a significant group × session interaction, $F(19, 152) = 2.85$, $p < 0.01$, so each session was separately examined. Significant differences between groups were found only in sessions 1, 2, 3, and 7. There was a significant group effect favoring the performance of TC group ($F = 5.59$, $p < 0.05$), with no significant group × session interaction ($F = 1.00$, $p > 0.05$) when only sessions 13–20 were considered. The comparison between TE and NE groups also showed a significant group × session interaction, $F(19, 133) = 2.03$, $p < 0.01$). When we made comparisons separately for each session, we found significant differences for sessions 1 and 4. No significant group effect was found when we considered only sessions 13–20. The comparison between TE and TC groups showed no significant group × session interaction ($F = 0.71$, $p > 0.05$) and a significant group effect favoring TC performance ($F = 7.27$, $p < 0.05$). This is probably due to initial differences in mastering the win-shift strategy, because this effect was not significant when considered only sessions 12–20 ($F = 3.9$, $p > 0.05$). The comparison between NE and NC groups showed no group × session interaction ($F = 0.62$, $p > 0.05$) and no group effect ($F = 0.57$, $p > 0.05$). There was also no significant group effect when only sessions 13–20 are considered ($F = 1.85$, $p > 0.05$).

### *Biochemical Experiments*

Table 1 shows the cholinergic parameters (ACh release and AChE activity) assessed in vitro in the cortex slices from the brain of control and ethanol-treated rats. The average basal ACh output was 13.6 ± 3.4 pmol/mg of tissue for 30 min taken as the 100% reference value. There were no significant differences on any biochemical measure between the pretrained and nonpretrained groups, whose data were therefore combined. The differences of ACh release between unstimulated and stimulated subgroups from both control and ethanol-treated groups were significant (results not shown). Although we observed a tendency of decrease in stimulated ACh release by tissues of treated rats compared to controls, this difference was not significant ($p = 0.14$). Moreover, there

TABLE 1

MEAN ± SE ACETYLCHOLINE RELEASE AND ACETYLCHOLINESTERASE ACTIVITY FROM CORTICAL BRAIN OF CONTROL AND CHRONIC ETHANOL-TREATED RATS

| | ACh Stimulated Release* % of unstimulated control | AChE Activity† moles ATCh hydrolysed/min g of tissue |
|---|---|---|
| Control | 454.5 ± 56.8 ($n = 8$) | $3.43 \times 10^{-6} \pm 0.20 \times 10^{-6}$ ($n = 10$) |
| Ethanol-treated | 342.8 ± 29.4 ($n = 9$) | $3.52 \times 10^{-6} \pm 0.27 \times 10^{-6}$ ($n = 9$) |

Unstimulated control is taken as 100%. There were no significant differences between control and treated groups.

*$p = 0.14$.

†$p = 0.80$.

was no significant difference between the AChE activity from cortical brain of control and ethanol-treated rats ($p = 0.80$).

## DISCUSSION

The significant difference found between the TC and NC groups on reference memory performance shows that the TC group had less difficulty in relearning a radial maze task than the NC group, which was learning that task for the first time. Thus, one can conclude that at least some aspects related to the task were retained by the TC group during the time between pre- and posttreatment training. This means that the experimental subjects used in this study (Fisher rats) are able to retain behaviors learned as long as 1 year before. This is somewhat surprising, considering the life span of rats. Long-term ethanol consumption can interfere with this ability, and this is shown by the lack of statistically significant differences between TE and NC groups. TE group had, in relearning the task, the same difficulty as NE and NC groups, which had no such previous learning. Besides, the TE group had a significantly worse performance than TC group, although the two groups had comparable performances in pretreatment training (Fig. 1A and B). All these results suggest that at least some of the aspects of the task retained by the TC group were forgotten by the TE group. The difference in performance between TE and TC groups cannot be attributed to failure of consolidation mechanisms, because there was a period of 3 months between pretreatment training and the start of the ethanol treatment. Several studies have shown that this period is long enough for that process to take place. It has been demonstrated, for example, that in a period of 5 days, a fear response learned by a group of rats was already consolidated, so that it could not be affected by brain concussion (47). The amnesia found in this study was, thus, one of a retrograde kind.

One of the aspects of radial maze task retained by the TC group, and also by the TE group, was the win-shift strategy demanded by that task. Partial retention of this strategy is shown by the TE and TC groups, whose performances in working memory task were already good in the first block of four sessions, and, unlike that of NE and NC groups, reached an assymptotic level in the third block. It is worth noting that we did not observe the same phenomenon in the reference memory performance of these groups. We assume that the animals retained the general win-shift rule, and this was expressed in their working memory performance. On the other hand, they did not retain the more specific knowledge, such as the exact arms baited with food. Hence, their reference memory performance at the start of posttreatment training sessions did not differ from that of untrained groups. Differences between working memory performances of pretrained groups (TC and TE) occurred only in the first 10 sessions, so these differences may be induced by possible differences in retention of the win-shift strategy. In the last sessions, when the strategy was entirely mastered, this difference was not significant. On the other hand, NE and NC groups had to learn this strategy, and this is detected by statistical analysis, which showed a significant decrease in working memory errors throughout posttreatment sessions. So, in regard to recent memory, the chronic ethanol treatment, as it was used in this work, had no effect on the acquisition of a new task and on working memory performance, because no difference was found between NE and NC groups. However, it can be seen in Fig. 1D that there was an increasing difference between groups NE and NC in the last sessions. Perhaps this difference might become statistically significant with a larger number of subjects, although another group of researchers found no significant difference even with a sample size of 21 rats per group (11). On the other hand, there are reports of significant differences between working memory performances of ethanol-treated and -untreated subjects achieved with larger groups of animals (3,23,24).

Some authors (3,23) found significant differences in recent (working and reference) memory performance in rats chronically treated with ethanol. These differences were found even when the treatment lasted only for 18 weeks, and were much larger when the exposure to ethanol was longer (28 weeks). Perhaps the differences between these and our results were due to differences in subject strains. Here the experimental subjects were Fisher rats, while the other authors used Sprague–Dawley. Using Lewis young and old rats chronically treated with ethanol for 6 months, Blockland et al.(11) failed to observe any impairment in both reference and working spatial memory performances. It is known that rat's strain can influence its performance in spatial memory tasks (2,43), and interact with variables such as aging on this performance (29). Some authors reported evidence that different strains show differences in neuronal systems sensitivity to ethanol (7,33,45). Besides, the decrease of ACh release induced by a long (about 6 months) ethanol treatment reversed after 4 weeks of abstinence in Wistar (13), but lasted at least for 6 months in Sprague–Dawley rats (3).

The training schedule may be another explanation of the discrepancies between the present results and those found by Arendt et al. (3). They trained their subjects using a sparser schedule (4–6 trials/week) and a more complicated procedure (two tasks), while our subjects had four trials/day of one single task. This schedule may have benefited the treated subjects, allowing them to reach the performance level of control subjects. Grigoryan et al. (19), using the same task as ours, found that cholinergic lesioned animals do not show much impairment when trained with four trials/day, but are worse than control ones when given only two trials/day. However, an explanation based in schedule effects would predict a progressive disappearance of the initial difference, as the control rats attained a perfect performance. Our data do not show this trend. Possibly, the performance of the treated group is limited to a level beyond which no further improvement is possible. We do not know if that is the case; and the matter deserves futher investigation.

The differences in training schedule cannot explain differences between the cholinergic parameters data shown in the present work and those obtained by Arendt et al.(3). These differences cannot be attributed to daily average amount of ethanol intake and blood ethanol levels, because in the present work these values were similar to those observed by those authors. Our biochemical assays were done approximately 2 months after ethanol withdrawal and only 2 days after the last session of behavioral training. It could be assumed that the biochemical parameters of our subjects were affected by ethanol treatment, but these effects were not detected because they had reversed at the time of our assays, although, as noted, in some cases, these effects can be seen even 6 months after withdrawal (3). Anyway, our results show a deficit in remote memory that seems not to be related to cortical cholinergic system impairment, because the biochemical assays were done only 2 days after the final session, when this remote memory impairment was still detectable.

The relative contribution of ethanol neurotoxicity and thiamine deficiency in the development of alcoholic organic

brain disease remains controversial (27,31). In animal models, it has been demonstrated that thiamine deficiency is accompanied by several changes in intermediary metabolism, which may modify the neurotoxic effects of ethanol (28). As mentioned, we did not find a difference in blood thiamine levels between control and treated groups, suggesting that the thiamine deficiency cannot account for the remote memory impairment observed. Moreover, we have verified that although the chow intake by ethanol-treated animals was slightly lower than that of control rats, the differences were not statistically significant. Thus, a nourishment deficiency could not explain the possible organic brain impairment.

We did not find any significant difference in AChE activity ($p = 0.80$) and stimulated ACh release ($p = 0.14$) between control and treated groups (Table 1). These data suggest that a cortical cholinergic system change is not related to the remote memory ethanol-induced impairment. But it does not imply that this system is not involved in other memory aspects that are sensitive to chronic ethanol consumption, as those described by other authors (3,23). However, our results concur with those obtained by Casamenti et al. (13) who using in vivo microdialysis, an excellent way to measure ACh release, showed that after a period of four ethanol-free weeks, there was no significant difference in cortical and hippocampal ACh release between controls and both groups of rats treated with ethanol for either 3 or 6 months. Some authors have found after a 6-month treatment a decrease in cholineacetyltransferase (ChAT) activity in cerebral cortex and hippocampus of adult subjects (3,10,13). On the other hand, postmortem studies have shown that ChAT activity was either unchanged (21) or decreased (34) in chronic alcoholics. An increase was also found in cortical ChAT activity of adult rats after a 3-month treatment (13). The role of the cholinergic system in learning and memory has been questioned by Baxter et al. (9), who used a selective immunotoxin to lesion the basal forebrain cholinergic cells and did not find amnesic effects on Morris water maze performance in rats. Besides, the cholinergic antagonist scopolamine does not specifically affect working memory in rats (22) and low dose of tetrahydroaminoacridine (THA), a drug that has been reported to be effective in improving the radial maze performance of ethanol-treated rats, does not affect brain ACh or AChE activity (24).

Cadete-Leite et al. (12) showed that prolonged ethanol consumption leads to a substantial reduction in the cholinergic innervation of the hippocampal formation and suggested that these findings may help to explain the cognitive dysfunctions observed after chronic ethanol consumption. So, further studies could be important to investigate a possible relationship between remote memory and hippocampal cholinergic innervation.

In conclusion, this work presents an experimental design that made possible the assessment of remote memory performance after ethanol chronic consumption and shows that the experimental subjects are able to retain the behaviors learned 1 year before. Our data show that chronic intake of ethanol causes retrograde amnesia that cannot be explained as being the result of a cortical cholinergic deficit. Moreover, this treatment does not affect either the acquisition of a new task or a recent memory performance. Fisher rats can be a useful animal model for future investigations on the specific biochemical and morphological changes linked with remote memory impairment.

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