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Effects of Frontal Polar Cortical Ablation and Cycloheximide on Ethanol Tolerance in Rats

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LEBLANC, A. E., M. MATSUNAGA AND H. KALANT. *Effects of frontal polar cortical ablation and cycloheximide on ethanol tolerance in rats.* PHARMAC. BIOCHEM. BEHAV. 4(2) 175–179, 1976. – Thirty adult male Wistar rats were pretrained to criterion on the moving belt test, and then made tolerant to ethanol by daily administration of increasing doses over a period of 3 weeks. After a one-month recovery period, they were divided into 3 groups, subjected to bilateral frontal polar cortical ablations, sham-operation and no operation respectively. After postoperative recovery, the cycle of ethanol treatment and testing was repeated. Only the lesioned group failed to reacquire tolerance. A pilot experiment showed that occipital cortical ablations also prevented tolerance. In a second experiment 32 rats, which had similarly undergone and then recovered from an initial period of ethanol tolerance, were divided into 4 groups which received daily treatment with sucrose plus cycloheximide (0.3 mg/kg), sucrose plus saline, ethanol plus cycloheximide, and ethanol plus saline respectively. Only the ethanol plus saline group re-acquired tolerance. It is concluded that frontal polar cortical lesions and cycloheximide can both block the development of tolerance to ethanol in animals previously shown to be capable of developing such tolerance.

Ethanol tolerance Cycloheximide Cortical ablation Motor performance

MANY investigators have been concerned with the factors influencing the rate and extent of development of tolerance to ethanol and other drugs. A number of points of striking similarity have been shown between alcohol and drug tolerance, physiological adaptation, and some types of learning [12]. It might, therefore, be expected that manipulations which alter the ability to undergo adaptation or learning would have a similar influence on the development of ethanol tolerance.

Various frontal cortical lesions in several species have been reported to impair the development of physiological adaptation to heat [8] and the acquisition of certain delayed-response and sensory discrimination tasks [9]. Human subjects who had suffered various unilateral or bilateral lesions of the frontal, parietal or occipital cortex were found to have subnormal habituation to sustained auditory stimuli [11]. Other work [18] has suggested that the development of tolerance to opiates in animals could be slowed or prevented by the administration of an inhibitor of protein synthesis.

The present paper reports the effect of bilateral frontal polar cortical ablations and of cycloheximide, an inhibitor of protein synthesis in brain and other organs, on the ability of rats to become tolerant to ethanol. To ensure that any change in this ability could be attributed only to the experimental treatments, both experiments were carried out on animals with a demonstrated capacity to develop tolerance.

METHOD

Animals

The animals used in these experiments were all male Wistar rats (300 g) purchased from Woodlyn Farms, Guelph, Ontario. They were housed singly and fed standard laboratory chow in a ration which was individually adjusted to hold the body weight constant. Drinking water was available ad lib.

Apparatus

The testing apparatus employed in these experiments was the moving belt test described previously [7]. In this test, animals are obliged to remain on a motor-driven belt which moves continuously over a shock grid. If the animal puts one or more paws on the grid it receives shock and activates a cumulative timer. The effect of ethanol, pentobarbital and other depressant drugs is seen as a monotonic dose-dependent increase in time off belt. Slight modifications of the apparatus, for convenience of training and

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maintenance, had no significant effect on the dose-response or blood level-response curves. Details of these changes are available on request from the Addiction Research Foundation.

All animals were trained to a criterion of no more than 1% error on each run [14]. The training procedure occupied several weeks, and led to a highly stable level of performance.

Production of Tolerance

For each of the main experiments, fully trained rats were tested on the moving belt test after a dose of ethanol (2 g/kg) IP. For this purpose, each rat was given six 2 min test trials, beginning 2.5 min after the ethanol injection, and separated from each other by 2.5 min intervals. The highest time off belt during any of the 6 trials (most commonly during the second) was taken as the measure of impairment by ethanol. The animals were then treated with ethanol by daily gavage for 3 weeks. The dose was 4 g/kg daily for the first 4 days, 5 g/kg for Days 5–8, and 6 g/kg for the rest of the 3 weeks. A control group of animals was treated with daily gavage of sucrose solution, of volume and caloric value equal to the corresponding ethanol doses.

During this 3 week period, the rats were tested every fourth day on the moving belt apparatus, under the influence of ethanol (2 g/kg IP); on these test days, the remaining alcohol needed to complete the prescribed daily dose (or the corresponding amount of sucrose for the control animals) was given by gavage immediately after completion of the test. Tolerance was complete for some animals by the fourth or fifth test day, but it was clearly maximal for all animals by the sixth test day. For each of the experiments, the difference between test scores on Days 1 and 6 was highly significant ($p<0.001$).

Procedure, Experiment 1

The cortical ablation experiment was carried out in 2 main phases. As a preliminary experiment, surgery was performed on ten animals full trained on the moving belt test. The animals were anesthetized with pentobarbital (40 mg/kg IP, as a 10 mg/ml solution in saline). Four were subjected to bilateral frontal polar cortical ablations, 4 to bilateral occipital cortical ablations and 2 to sham operations. Following surgery, half of the animals were brought back with difficulty to criterion performance on the moving belt test. Of these, 4 (2 with frontal and 2 with occipital lesions) were chosen to be used in the experiment.

Time off belt was first determined after a standard dose of ethanol (2 g/kg IP). The animals were then made tolerant to ethanol by daily gavage with increasing doses, as described above. This regimen had been shown previously to produce tolerance [14]. On the day following the last intubation, the effect of ethanol (2 g/kg IP) on the moving belt test performance was re-examined. No change in effect was found from the pretreatment to the posttreatment test day. This applied equally to the animals with frontal and with occipital cortical lesions. When an attempt was made to increase the daily ethanol intake further by an increment of 1 g/kg every third day, 2 of the animals died at a level of 8 g/kg and the experiment was terminated.

For the second and main phase of the cortical ablation experiment, only frontal polar lesions were used, since the pilot study had indicated no difference in effects of frontal and occipital lesions. Trained rats were made tolerant to ethanol over a 3 week period as described in "Production of Tolerance," while 8 other animals were used as sucrose controls. All animals were then left without alcohol or sucrose for a period of 1 month. The ethanol-treated animals from the first cycle were then subdivided into 3 groups. Group 1 contained 10 animals, designated as the lesion group. Group 2 consisted of 10 sham-operated animals and Group 3 contained 8 unoperated controls. The unequal numbers constituted provision for possible deaths in Groups 1 and 2.

The surgery on Groups 1 and 2 was carried out over a 2-day period in balanced order. The rats in Group 1 were anesthetized with pentobarbital and, under visual inspection, bilateral 3 mm × 5 mm ablations were performed by suction with a fine-tipped glass aspirator connected to a Gomco vacuum pump. The ablated area began 1–2 mm from the midline and extended rostrally from the line of the coronal suture. The opening in the skull was filled with Gelfoam and both the muscle layer and skin were sutured. The sham-operated animals had the same area exposed down to the cortex, but no lesion was made; closure was carried out as in Group 1. The unoperated controls were only anesthetized. Within 5 days all animals had recovered criterion performance on the moving belt test. One animal in the surgical group had a bizarre orientation of the head, and was removed from the experiment. A second animal was designated as a spare, but did not have to be used. The 2 animals designated as spares for the sham-operated group were also not used.

After a further 9 days of postoperative recovery, the cycle of ethanol treatment and testing, as described above, was repeated. At the end of 21 days the animals were killed and the brains were removed and stored in formaldehyde.

Procedure, Experiment 2

Since cycloheximide has known toxic effects on the gastrointestinal mucosa, which might possibly be aggravated by conjoint administration of ethanol, a toxicity study was performed as a preliminary to Experiment 2.

Doses of cycloheximide, ranging from 0 to 1 mg/kg in 0.1 mg increments, were given to 66 rats distributed among 11 groups of 6 animals each. The drug was given by IP injection, dissolved in isotonic saline. Rats receiving doses in excess of 0.5 mg/kg became flaccid and scruffy, and at the higher doses some animals died in 5 to 10 days.

On this basis, 0.3 mg/kg was taken as a reasonable dose. This dose is much less than that required to inhibit RNA synthesis, but Farber and Farmar [4] have shown that a single dose of 0.5 mg/kg caused about 45% inhibition of protein synthesis in the rat liver. For the joint chronic toxicity study, alcohol was given by daily gavage on the same dosage schedule as used in the main portion of Experiment 1. However, the same dosage was given in the form of solutions of 20, 25 and 30% (w/v) respectively to 3 groups of 10 animals each, to find the highest concentration of alcohol which would be tolerated by the GI mucosa when given together with the 0.3 mg/kg dose of cycloheximide. By the end of 3 weeks of daily treatment, all of the 30% group and 3 of 10 animals in the 25% group had developed intestinal obstruction and died. Autopsy revealed a full stomach and atrophied lower bowel. The animals in the 20% group all maintained normal appearance and weight; this concentration of ethanol was therefore used in the subsequent experiments.

In the main phase of Experiment 2, 32 animals were given daily gavage with 20% ethanol over a 3 week period on the same dosage schedule as in Experiment 1, while 8 controls received equicaloric doses of sucrose. Development of tolerance was followed on the moving belt test. The rats were then left without ethanol or sucrose for 1 month.

The animals were re-examined on the moving belt test, with an ethanol dose of 2 g/kg IP, and assigned on the basis of their performance to 4 matched groups. These were immediately started on daily treatment with either ethanol or equicaloric sucrose on the same schedule as before, combined with either cycloheximide (0.3 mg/kg) or saline (Table 2). The respective treatments were continued for 3 weeks, during which the moving belt test under the standard 2 g/kg dose of ethanol was repeated every 7 days. On each test day, the remainder of the required daily dose of ethanol (or the caloric equivalent as sucrose, for the controls) was given by stomach tube immediately after the test. Cycloheximide was given 1 hr before alcohol on nontest days and before the supplemental dose on test days.

RESULTS

Experiment 1

During the first cycle of chronic treatment, preceding surgery, the ethanol group showed a steady decline in error score from 72.64 ± 3.71 sec on the first test day to 40.94 ± 7.96 sec on the sixth test day. In contrast, the sucrose controls showed no change (76.12 ± 6.14 and 75.31 ± 6.4 on the first and sixth test days respectively). The degree of tolerance produced in the ethanol group was consistent with that found in previous studies [14].

By the end of the following 4 week period, which included the surgery and postoperative recovery, ethanol tolerance had returned to normal. In other words, at the beginning of the second ethanol/sucrose cycle (postoperative) the impairment produced by the standard dose of ethanol (Table 1) was at least as high as it had been at the beginning of the first (preoperative) tolerance cycle.

The maximum scores for motor impairment on successive test days during the second cycle of chronic treatment are given for the three groups in Table 1. An analysis of variance revealed a significant interaction between the groups and test days, $F(10,105) = 6.22$, $p<0.01$. This interaction is due to the fact that the lesioned group failed to re-aquire tolerance during the second cycle (level of impairment did not change over the 6 test days; $p>0.05$), whereas the other 2 groups did re-acquire tolerance, showing significantly less impairment as early as the second test day ($p<0.01$). The rate and extent of tolerance development in Groups 2 and 3 were again similar to that noted in earlier experiments [14].

The extent of the lesions can be seen in Fig. 1 for seven of the eight animals. The eighth brain was damaged during removal from the skull and is not shown.

Experiment 2

The results are summarized in Table 2. As already mentioned, all ethanol-treated animals developed tolerance during the first alcohol cycle, $t = 30.45$, $df = 31$, $p<0.001$. Again, as in Experiment 1, the response to ethanol had apparently reverted fully to normal before the start of the second cycle of chronic alcohol treatment. Mean impairment scores at the beginning of the second cycle were above 80.0 for all groups, compared to a mean of 79.4 at the start of the first tolerance cycle.

An analysis of variance showed significant interaction of treatments × days. Tukey's test showed that the ethanol plus saline treatment group had a significantly lower impairment score than the other groups by test Day 2 ($q = 13.76$, $p<0.01$). None of the other 3 treatment groups showed any significant change in performance during the 3 week period.

TABLE 1

EFFECT OF CORTICAL LESIONS ON RE-ACQUISITION OF ETHANOL TOLERANCE*

Group		Test Days 1	2	3	4	5	6
Lesioned	$\bar{x}$	80.3	76.2	83.1	80.2	85.3	74.2
	SE	2.79	1.98	2.30	2.82	3.44	2.41
Sham-Operated	$\bar{x}$	86.2	65.7	51.3	48.2	39.3	40.7
	SE	2.95	4.51	3.55	2.55	2.72	3.01
Unoperated Controls	$\bar{x}$	85.4	60.2	41.3	50.0	42.5	37.6
	SE	3.53	7.23	2.50	3.22	2.94	2.13

*All animals were first made tolerant to ethanol, then allowed to recover, before being subjected to brain lesioning or sham operation. Values shown are the maximum error scores on the moving belt test under a 2 g/kg dose of ethanol during a second cycle of chronic ethanol treatment after cortical lesioning or sham operation (Experiment 1).

TABLE 2

EFFECT OF CYCLOHEXIMIDE ON RE-ACQUISITION OF ETHANOL TOLERANCE*

Treatment		Test Days 1	2	3	4
Sucrose & Cycloheximide	$\bar{x}$	81.3	79.3	78.2	76.4
	SE	2.53	2.42	2.24	3.10
Sucrose & Saline	$\bar{x}$	82.0	79.7	76.2	77.3
	SE	3.67	3.44	2.65	2.76
Ethanol & Cycloheximide	$\bar{x}$	83.2	83.1	85.2	79.3
	SE	2.62	2.69	3.34	3.50
Ethanol & Saline	$\bar{x}$	83.3	57.3	43.2	39.1
	SE	4.84	3.34	3.13	2.04

*All animals were first made tolerant to ethanol, then allowed to recover. Values shown are maximum error scores on the moving belt test under a 2 g/kg dose of ethanol during a second cycle involving the treatments indicated (Experiment 2). For details of procedure and dosage, see text.

DISCUSSION

Experiment 1 shows that dorsolateral frontal cortical (frontal polar) lesions, of a type previously reported to impair physiological adaptation to altered ambient temperature [8], also prevent the re-acquisition of tolerance to ethanol in rats which had previously been able to develop such tolerance. The preliminary portion of the experiment indicated that occipital cortical lesions were equally effective. This appears consistent with the observation [11] that physiological habituation to a constant tone was also impaired in patients with occipital or parietal lesions. It is therefore tempting to speculate that cortical lesions, in some as yet unexplained manner, impair a non-specific adaptive mechanism which is common to habituation and to alcohol (and possibly other drug) tolerance.

It is not permissible, however, to extend the speculation to learning in general. The frontal cortex is neither cytologically nor functionally homogeneous [2], and the topographic localization of functionally equivalent portions differs in different species [9,16]. The lesions shown in Fig. 1 are probably not equivalent to prefrontal cortical lesions in cats, dogs and primates [9,16], and are therefore better described by the purely topographic term frontal polar lesions [10]. It has long been recognized that such lesions have diverse effects on different types of learning [13]. Recent studies have confirmed that they do not affect the rat's learning of a conditioned avoidance response in the shuttle box [1] or of a delayed response food-rewarded task [10].

In earlier work [3], the imposition of a requirement for task performance under the influence of ethanol was found

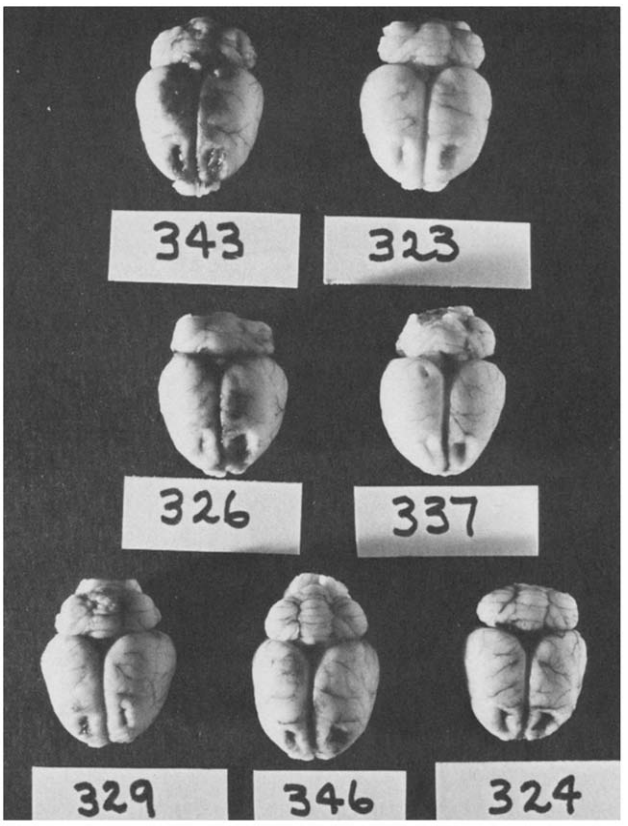

FIG. 1. Photographs of frontal polar cortical lesions which prevented the re-acquisition of ethanol tolerance.

to produce an ethanol-tolerant state which was considered to be a learned compensation for the impairment caused by ethanol. However, this state proved to be indistinguishable from the tolerance produced by conventional pharmacological means, except that its rate of development was greatly increased [15]. This finding, together with various formal resemblances between the kinetics of acquisition, loss and re-acquisition of learned behaviours and of tolerance, raised the possibility of possible common mechanisms in the two processes [12]. The prevention of tolerance by frontal polar or occipital lesions, though it can not yet be explained, appears to argue against this possibility.

Synthesis of RNA and protein is believed to be essential for the retention of newly acquired responses [18], as well as for a wide variety of physiological and metabolic adaptations. Indeed, the duration of impairment of retention of shock avoidance learning in mice treated with cycloheximide has been reported to be proportional to the duration of inhibition of cerebral protein synthesis [6]. Rats are very much more sensitive to cycloheximide than mice are: hepatic protein synthesis in rats was reduced by 45% at 3 hr after a single dose of only 0.5 mg/kg [4]. This is quite close to the daily dose used in the present study. Morphine tolerance was inhibited in mice by a daily dose of 20 mg/kg cycloheximide [17], while in rats as little as 1 mg/kg weekly was effective [5]. It seems reasonable to assume that prevention of ethanol tolerance in the present study was related to inhibition of cerebral protein synthesis, although other mechanisms of action of cycloheximide can not be ruled out.

However, the very fact that protein synthesis is essential to such a wide range of adaptive changes makes the effect of cycloheximide of little use in clarifying the relationship between ethanol tolerance and learning. Further exploration of the effects of lesions or stimulation at different loci would appear to be a more promising approach for this purpose.

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