Pharmacology Biochemistry & Behavior, Vol. 3, pp. 909–913.

BRIEF COMMUNICATION

Retention of Functional Tolerance to Ethanol in Rhesus Monkeys (*Macaca mulatta*)[1]

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(Received 12 December 1974)

PIEPER, W. A. AND M. J. SKEEN. *Retention of functional tolerance to ethanol in rhesus monkeys* (Macaca mulatta). PHARMAC. BIOCHEM. BEHAV. 3(5) 909–913, 1975. – Tolerance to ethanol (3 g/kg 90 min prior to testing) was assessed in a group of 4 rhesus monkeys in which tolerance development had been observed using the same behavioral task one year prior to the present study. Although some decrements in performance on a two-choice discrimination-reversal learning task were observed, these changes were transient and statistically insignificant. Results indicate that functional tolerance persisted throughout a one year abstinence period.

Ethanol Tolerance Rhesus monkeys Discrimination-reversal learning

IN an earlier report from this laboratory the development of functional tolerance to ethanol in a group of four rhesus monkeys was described [4]. The animals were tested using a two-choice discrimination-reversal learning task and tolerance was defined as a recovery of baseline levels of performance following an initial decrement despite the continued daily administration of the same dose of ethanol. Following a 24 day period in which testing was continued but no drug was administered, tolerance was still evident upon a reinitiation of ethanol administration, thus suggesting a need for the investigation of tolerance retention over longer periods of time. Consequently this report describes a follow-up study in which the same dose of ethanol was administered to this group of rhesus monkeys after a one year period during which no drug testing was carried out. Prior to reinitiation of drug administration, baseline performance on the behavioral task was re-established.

METHOD

Animals

One female and 3 male rhesus monkeys (*Macaca mulatta*) were readapted to the test apparatus and their behavioral baselines were re-established 11 months following the termination of a previous study in which the development of tolerance to ethanol had been investigated in these same animals. In the intervening months none of these animals was used for behavioral studies nor were any psychoactive drugs administered to them. They weighed 4.0–5.5 kg and were individually caged in an artificially

[1]Supported by USPHS Grants AA 00217 and RR 00165. We thank Rebecca Haynie, Robert Walker and Carol Watters for technical assistance.

lighted room with a 12 hr light-dark cycle and with water available ad lib.

Procedure

Animals were tested for 21 days, the last 12 of which were used as a baseline for comparison to performance during 12 days of ethanol administration. A placebo solution of lactose, which was isocaloric to 3 g/kg of ethanol, was then administered to each animal 90 min prior to the beginning of the test session for the subsequent 6 days to control for the possible effects of the nasogastric intubation procedure and caloric supplementation on performance. Ethanol (3 g/kg) was then substituted for lactose and was administered 90 min prior to testing for the following 12 days. Ethanol administration began exactly one year from the day on which the animals were given the final dose of ethanol in the previous tolerance study. A second series of 6 days of placebo administration completed the study. Thus, the experiment was divided into 4 treatment periods: (1) Baseline (Days 1–12); (2) Placebo 1 (Days 13–18); (3) Ethanol (Days 19–30); and (4) Placebo 2 (Days 31–36).

Ethanol (as a 20 percent v/v aqueous solution) or isocaloric lactose solutions were administered 90 min prior to the behavioral test sessions because ethanol absorption curves obtained in this laboratory indicated that blood ethanol concentrations had reached a plateau by this time and were therefore relatively stable throughout a 15 min test session. After the solutions were injected nasogastrically through a No. 8 French infant feeding tube, the animals were returned to their home cages until the beginning of the test session. Each session continued until 10 reversals had been completed or until 15 min had elapsed, and all animals were tested daily between 2:30 and 4:00 p.m. Immediately following each test session during the ethanol administration phase, 50 μl of blood was obtained from the heel of each monkey and analyzed using gas chromatography [5] to determine blood ethanol concentration. The entire daily food ration of Purina Monkey Chow 25 was fed to the animals within 1 hr following the test sessions.

Details of the apparatus and specifics of the two-choice discrimination-reversal task, which remained constant throughout the 4 phases of the study, have been reported elsewhere [7]. Briefly, the animal was required to discriminate between two projected images (two-dimensional colored patterns) by reaching toward them and thereby breaking a photo cell beam. A correct choice was followed by delivery of a 45 mg sucrose pellet and a 1 sec tone. When the animal reached a criterion of 84 percent correct, the cue values were reversed such that the previously incorrect stimulus became correct and vice versa. Following re-establishment of the 84 percent level of performance, the cues were again reversed. The two stimulus images remained unchanged throughout the experiment; only their cue values were varied. Furthermore, these same images had been used throughout the previous tolerance experiment and thus discriminability of stimulus pairs was not a variable.

Three primary dependent measures were assessed and scores from each animal were transformed for statistical purposes as follows: (1) an arcsin transformation was used for the percentage of correct responses during reversal (Trails 2–10 following cue reversal); (2) a reciprocal transformation was performed on the number of trials required to reach the 84 percent criterion; and (3) a $\log_{10}$ transformation was used for the mean latency per reversal measure. As in the earlier experiment [4], these transformed scores were then subjected to analysis of variance to test for trends within each phase of the experiment as well as to examine performance differences between treatment periods.

RESULTS

Group median performance during the four phases of the experiment is illustrated in Figs. 1, 2, and 3. Although apparent performance decrements were reflected in all 3 dependent measures when ethanol administration was begun, none of these apparent changes was statistically significant and baseline performance was recovered rapidly despite continued drug administration on subsequent days. Analyses of variance indicated that the differences in performance across days during the ethanol administration period were not significant for any of the dependent measures (see Table 1). Similarly, there were no statistically significant trends in performance over time during any of the 3 control periods, with the exception of a slight increase in latency in the reversal trials during the baseline period. Comparison of performance following ethanol administration with performance under either baseline or placebo conditions also showed no significant differences (Table 1); nor were there any significant interactions between treatments and trends across days in any of these analyses ($F < 1.15$ in all cases). The observed changes in performance on Day 19, the first day of ethanol administration, were largely due to the failure of 2 (Nos. 34 and 44) of the 4 animals to reach the initial acquisition criterion; percent correct in reversal was thus scored as zero for these two monkeys. Performance decrements on Day 2 of Placebo 1 were similarly due to the failure of 2 animals (Nos. 33 and 44) to achieve acquisition criterion. A large increase in mean latency was observed on Day 1 of Ethanol and not on Day 1 of Placebo, however, because a greater number of responses was made under the placebo condition than under the drug condition.

DISCUSSION

The results suggest that once functional tolerance to ethanol is developed in rhesus monkeys, the phenomenon persists over protracted periods of time. Although some apparent performance decrements were observed upon reinitiation of ethanol administration following the one year abstinence period, recovery was rapid and the decrements were not statistically significant. In contrast, when the same dose of ethanol was initially administered to this group of monkeys as naive animals, performance decrements were statistically significant and recovery of baseline performance levles required 18–20 days [4].

These findings differ from some reports from other laboratories. Newman and Card [3] found that 7 months of abstinence was sufficient to result in a loss of tolerance in dogs as assessed by a rating scale for evaluating degree of intoxication. More recently, it was reported that ethanol tolerance as measured by the rat's ability to stay on a motor-driven belt over an electrified grid was lost 14 days following cessation of ethanol treatment [2]. Species differences may account in part for discrepancies between

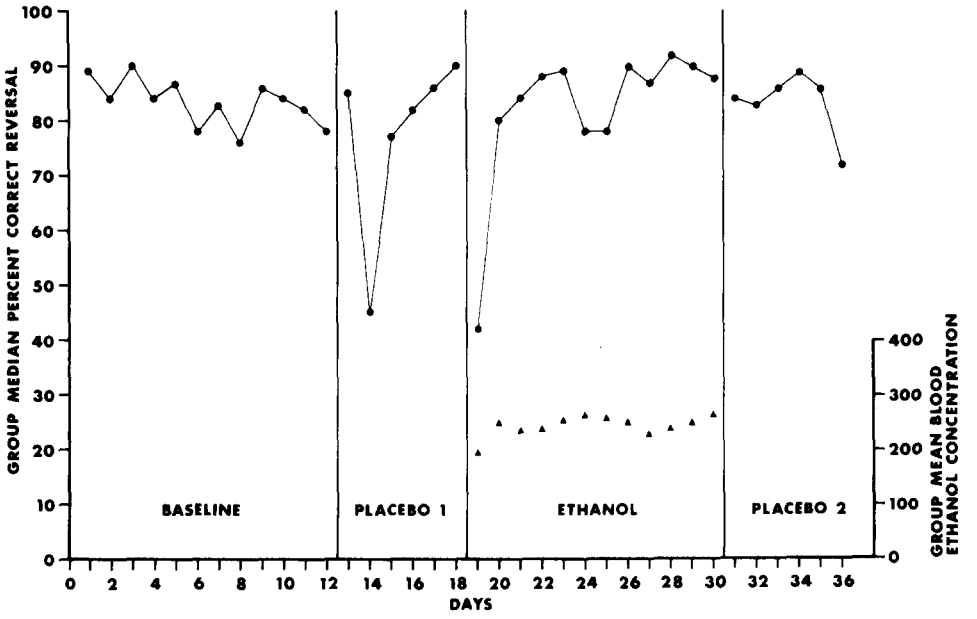

FIG. 1. Group median percentage of correct responses during Trials 2–10 following cue reversal during the 4 treatment periods. Scores for individual animals were obtained by calculating the mean percentage of correct responses during reversal for each daily test session. Group mean blood ethanol concentrations (▲) are shown for days on which ethanol was administered.

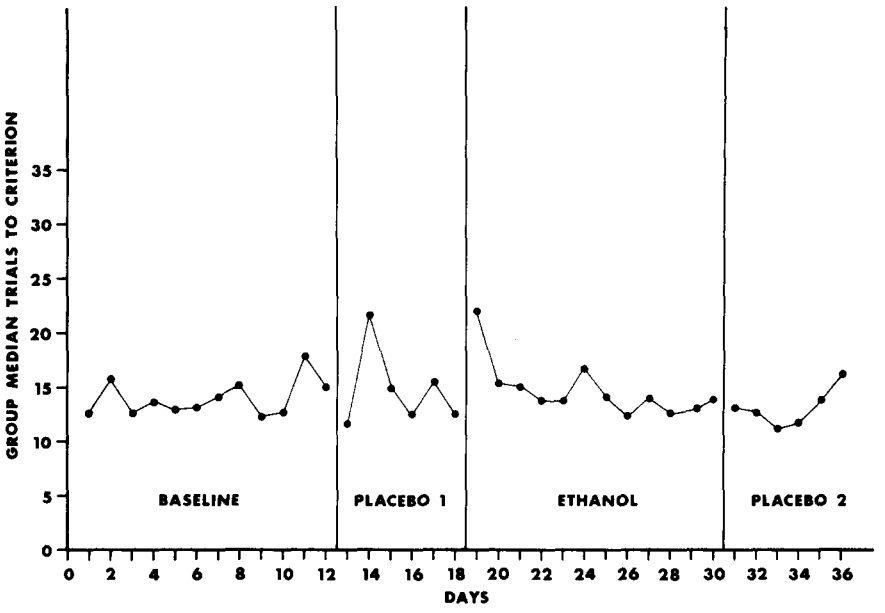

FIG. 2. Group median scores of the number of trials to reach the 84 percent correct criterion prior to cue reversal during the 4 treatment periods. Scores for individual animals were obtained by calculating the mean number of trials required to reach criterion during each daily test session.

the present study and those involving rats and dogs. However, the measures used to evaluate tolerance development may also be important. Both the rating scale for intoxication and the motor-driven belt task are assessments of motor impairment; whereas the discrimination-reversal task used in the present study presumably reflects higher order integration and problem-solving capacities of the organism. Chen [1] found that tolerance to ethanol which was assessed using a temporal circular maze task was retained 7 days following termination of ethanol administration. He suggested that different underlying processes might be involved in the development of tolerance in this

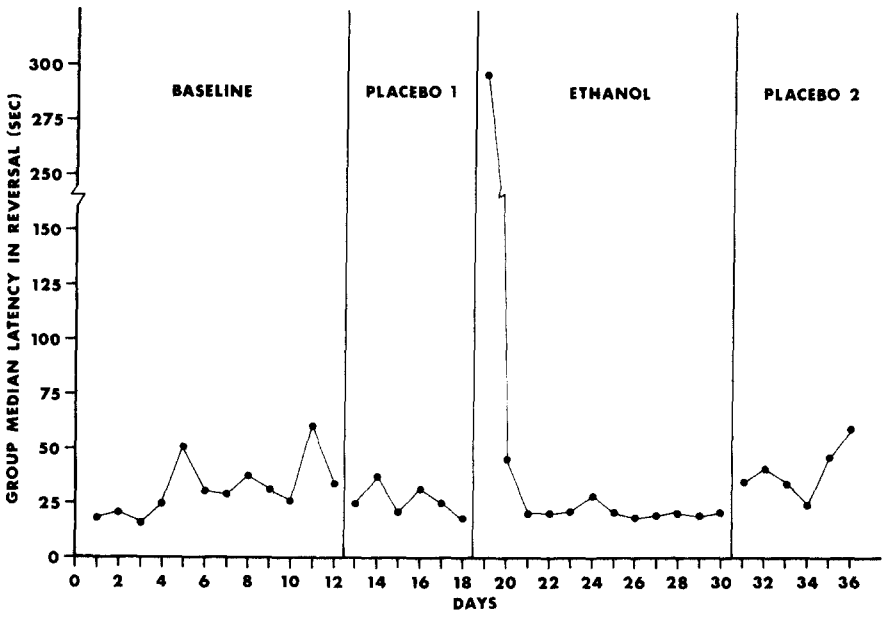

FIG. 3. Group median response latencies during Trials 2–10 following cue reversal during the 4 treatment periods. Scores for individual animals were obtained by summing the latencies during the 9 trials of each reversal period and then calculating the mean of these sums for each daily test session.

TABLE 1

F RATIOS FROM ANALYSES OF VARIANCE

		Dependent Measures		
	df	Percent Correct in Reversal	Trials to Criterion	Latency to Respond
Trend Analyses				
Baseline	11, 33	0.61	0.56	2.27*
Placebo 1	5, 15	2.06	1.69	0.90
Ethanol	11, 33	1.30	0.59	1.10
Placebo 2	5, 15	0.30	1.78	2.56
Treatment Comparisons				
Baseline vs. Ethanol	1, 69	0.99	2.70	0.22
Placebo 1 + Placebo 2 vs. Ethanol	1, 69	0.64	2.90	0.02

*$p<0.05$

learning situation as opposed to the motor-driven belt task in which tolerance was not retained [2]. Unfortunately, Chen did not test for retention after longer periods of abstinence. It is of interest to note that in the present study the greatest magnitude of change in performance associated with the reinitiation of ethanol administration was observed in the latency to respond measure. This reflects the rate of responding and, as such, is perhaps more closely related to motor ability than to higher-order processes. Further investigation concerning the interaction between tolerance

retention time and the nature of the task used to assess that tolerance is clearly necessary.

In the initial tolerance study, these monkeys received 55 days of pretraining in addition to the 90 days of testing during the experiment. For the retention experiment, 9 days of pretraining preceded 18 additional days of baseline and placebo testing prior to the third exposure to alcohol, for a total of 172 days of training. Unfortunately, the degree to which this amount of overtraining might interact with retention of tolerance cannot be determined from the present experiment. Although statistically significant performance decrements were observed when ethanol was initially administered to this group of monkeys [4] after extensive training (73 days), the possibility remains that the tolerance observed in this follow-up experiment is simply due to the nearly 100 days of training following the initial exposure to alcohol.

Although reversible increases in the rate of elimination of ethanol have been demonstrated following chronic ethanol administration [6], changes in disappearance rate *per se* are apparently not important mechanistically in the development and retention of tolerance. The stability of the blood ethanol concentrations across test days in the monkeys and their similarity to those in the previous study [4] indicate that tolerance is not merely a reflection of lower blood ethanol concentrations due to increased elimination rate.

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