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

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PIEPER, W. A. AND M. J. SKEEN. *Development of functional tolerance to ethanol in rhesus monkeys (Macaca mulatta).* PHARMAC. BIOCHEM. BEHAV. 1(3) 289–294, 1973.–Four rhesus monkeys were extensively trained until performance reached asymptote on a two-choice discrimination-reversal task. Doses of ethanol (3 g/kg) or placebo (aqueous lactose solutions isocaloric to 3 g/kg of ethanol) were then administered by gavage 90 min prior to testing. Following an initial decrement when ethanol was first administered, performance gradually returned on subsequent days to levels which were equivalent to those found under placebo conditions despite continued drug administration. Ethanol treatment affected accuracy of responding during both the acquisition and reversal phases of the task as well as the quantity of behavior emitted by the animals. Changes in performance levels were independent of fluctuations in blood ethanol concentrations. Functional tolerance developed within approximately 18 days as indicated by the recovery of performance on the discrimination-reversal task. Furthermore, this tolerance was retained during a 24 day period during which no ethanol was administered.

Rhesus monkeys Tolerance to ethanol

ALTHOUGH functional tolerance has been reported to occur following repeated doses of ethanol in humans [7, 16, 27], rabbits [1], dogs [19,20] and rats [2, 3, 4, 8, 10, 12, 13, 18, 20, 25, 26], limited information regarding tolerance in nonhuman primates is presently available, despite the recent emphasis placed upon developing this animal as a model for studying the problems associated with alcoholism [14]. In an earlier report Hogans, Moreno and Brodie [6] measured the effects of repeated doses of ethanol (2 g/kg/day) on EEG activity and avoidance behavior in rhesus monkeys *(Macaca mulatta)*. Although the EEG pattern continued to demonstrate ethanol-induced high-voltage, slow-wave activity, performance on the avoidance task returned to normal after several days which suggested the development of tolerance.

Following the suggestion of Kalant, LeBlanc and Gibbins [9] the term functional tolerance is used in describing the present experiment in preference to other terms which tend to imply a dichotomy between biological and behavioral processes. Tolerance is viewed as an adaptive change of the organism to the functional disturbance produced by ethanol [13]; and consequently, behavioral manifestations of tolerance are observable concomitants of this adaptive change. A two-choice discrimination-reversal learning task [23] was used in the present experiment to behaviorally examine the development of functional tolerance in rhesus monkeys. Three dependent measures were obtained: (1) number of trials required to obtain the prereversal criterion of 84% correct; (2) percent correct in reversal (Trials 2–10); and, (3) response latency. On the basis of the results from a preliminary experiment, it was predicted that a 3 g/kg dose of ethanol given by gavage 90 min prior to testing would initially impair the performance of rhesus monkeys on this task. However, functional tolerance was expected to develop with repeated exposure such that performance would subsequently return to the predrug baseline. Not only were both of these expectations confirmed by the results of this experiment, but the tolerance which devel-

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oped was found to persist following a 24-day period during which ethanol was not administered.

METHOD

Animals

One female and three male rehsus monkeys *(Macaca mulatta)* were reduced to approximately 90% of their free-feeding weight by food deprivation and maintained at this level throughout the experiment. These young adult animals were 3–4 years old with weights ranging between 3.3–3.7 kilograms prior to the experimental treatment. They were individually housed with water continuously available and artificial illumination provided on a 12-hr light-dark cycle. All animals had been extensively pretrained and tested in a two-choice, discrimination-reversal learning experiment [23], but had not received any previous experimental treatment with alcohol or other drugs.

Apparatus

The testing apparatus which has previously been described [24] consisted of a metal cage (approximatley 50 x 50 x 50 cm) that was placed inside a sound attenuating room. One wall of the cage was modified to accept a sheet metal panel for mounting the response manipulanda, stimulus projectors and pellet dispenser. The two projectors were mounted through the frontispiece such that the two stimulus images (7.6 cm by 5.0 cm wide) could be viewed from the interior of the cage when the projectors were activated. When the animal responded by reaching toward one of the projected images (two-dimensional colored patterns), the light beam to a photo cell placed directly above each of the projectors was interrupted and the response was recorded and both projectors turned off for a 2-sec intertrial-interval. If the response was correct, a 45 mg sucrose pellet was dispensed and a 1-sec tone (conditioned reinforcer) sounded. The left-right position of the correct (+) stimulus was automatically varied from trial to trial following a random sequence with the restriction that a stimulus could not appear on the same side more than three times consecutively. Responses and response latencies were automatically recorded on a printing counter. A masking noise was used inside the sound attenuating room to further acoustically isolate the animal from the programming and data recording apparatus located outside the chamber.

Procedures

The experiment was divided into seven time periods: (1) Baseline 1 (Days 1–12); (2) Placebo 1 (Days 13–18); (3) Ethanol 1 (Days 19–54); (4) Placebo 2 (Days 55–60); (5) Baseline 2 (Days 61–72); (6) Placebo 3 (Days 73–78); and, (7) Ethanol 2 (Days 79–90). The behavioral test conditions of the two-choice discrimination-reversal learning task remained constant throughout the 90 day experimental period. When an animal had achieved a level of performance such that the correct stimulus image was chosen 84% of the time, the cue values were reversed and the previously incorrect stimulus was then defined as correct and vice versa (for a discussion of the selection of the 84% criterion see Rumbaugh [23] p. 60). Following reestablishment of the 84% level of performance, the cues were again reversed. In this paradigm reversal learning performance is evaluated by examining Trials 2–10 following the reversal of cue values; Trial 1 is considered an information trial and is not used in the analysis. The same pair of stimulus images was used for all animals throughout the entire experimental period to eliminate that source of variability. Using this procedure the acquisition and reversal phases of the problem were alternated as usual in a discrimination-reversal learning task, but since the stimulus pairs remained unchanged, Trials 2–10 following cue reversal were used not only for analysis of reversal performance but were also considered as a portion of the subsequent acquisition phase and as such were used for evaluation of the number of trials required to reach the 84% criterion. A minimum of 12 trials were completed prior to a given cue reversal, even though Trials 2–10 following the previous reversal were each completed correctly. However, if the animal had not achieved the 84% criterion by Trial 12, testing was continued (for procedural details see Rumbaugh [22] p. 268). Dependent measures included the following: (1) percentage of correct responses during reversal trials 2–10; (2) the number of trials required to reach the 84% criterion during acquisition; and, (3) the sum of the latencies to respond during each successive reversal (Trials 2–10 following reversal of cue values). Animals were tested between 12:30 and 2:00 p.m. daily, seven days per week and were fed their entire daily food ration of Purina Monkey Chow 25 within one hour following the test sessions. A test session continued until 10 reversals had been completed or until 15 min had elapsed, whichever occurred first.

Drug or placebo solutions were administered by nasogastric intubation 90 min prior to the beginning of each animal's test session. This 90 min interval was selected because previous data from our laboratory as well as data reported by Mello [15] indicated that blood ethanol concentrations had reached a plateau by this time and were therefore relatively stable throughout the 15 min test period. During the two ethanol administration periods (36 days and 12 days respectively) 3 g/kg of ethanol was administered daily as a 20% (v/v) aqueous solution via a No. 8 French infant feeding tube. During the three placebo periods (six days each) aqueous lactose solutions which were isocaloric to 3 g/kg of ethanol were similarly administered as a control procedure to evaluate the effects of intubation and/or caloric supplementation on performance levels. On drug administration days, a 50 μl blood sample was collected from the heel of each animal immediately following the test session and analyzed gas chromatographically [21] to determine the blood ethanol concentration.

Statistical Analysis

Individual scores from each animal were transformed to normalize their distributions as follows: (1) an arcsin transformation was used for percent correct in reversal scores; (2) a reciprocal transformation was performed on the number of trials to criterion measure; and, (3) a $\log_{10}$ transformation was used for the latency measure. The transformed scores were then subjected to analysis of variance procedures using randomized block designs with repeated measures for each animal [11]. These analyses were used to test for trends within each of the seven treatment periods as well as to examine performance differences between treatment periods.

RESULTS

It can be seen from Figs. 1, 2 and 3 that an apparent

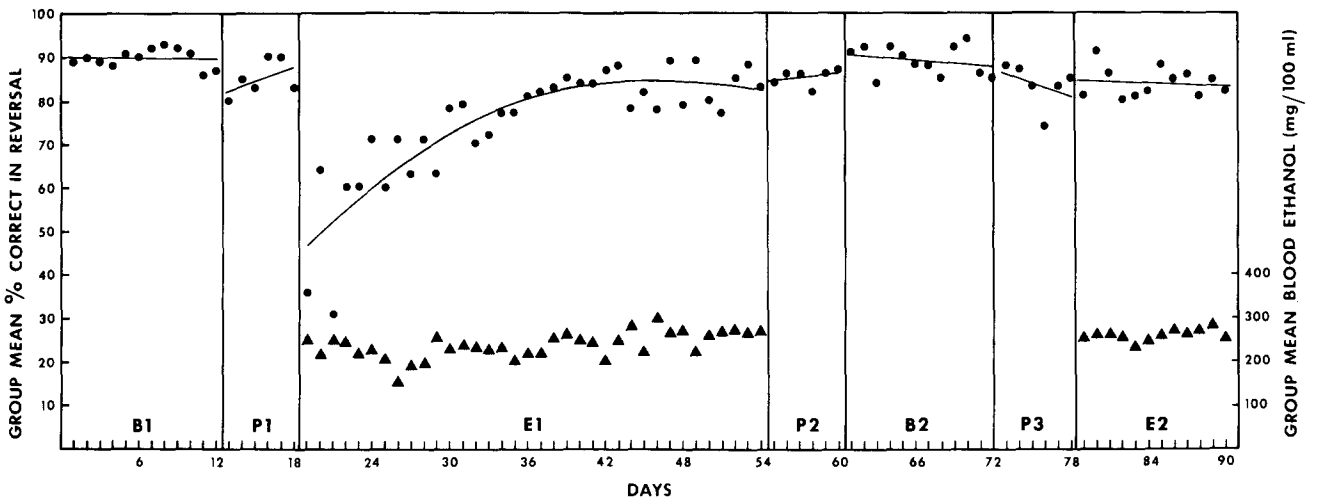

FIG. 1. Group mean percentage of correct responses during Trials 2–10 following cue reversal. Scores for individual animals were obtained by calculating the mean percentage of correct responses during reversal for each daily test session; equations for the lines were calculated using the method of least squares. Group mean blood ethanol concentrations (▲) are shown for days on which ethanol was administered. The various treatment periods are designated as follows: (1) B1 = Baseline 1, (2) Pl = Placebo 1, (3) El = Ethanol 1, (4) P2 = Placebo 2, (5) B2 = Baseline 2, (6) P3 = Placebo 3, and (7) E2 = Ethanol 2.

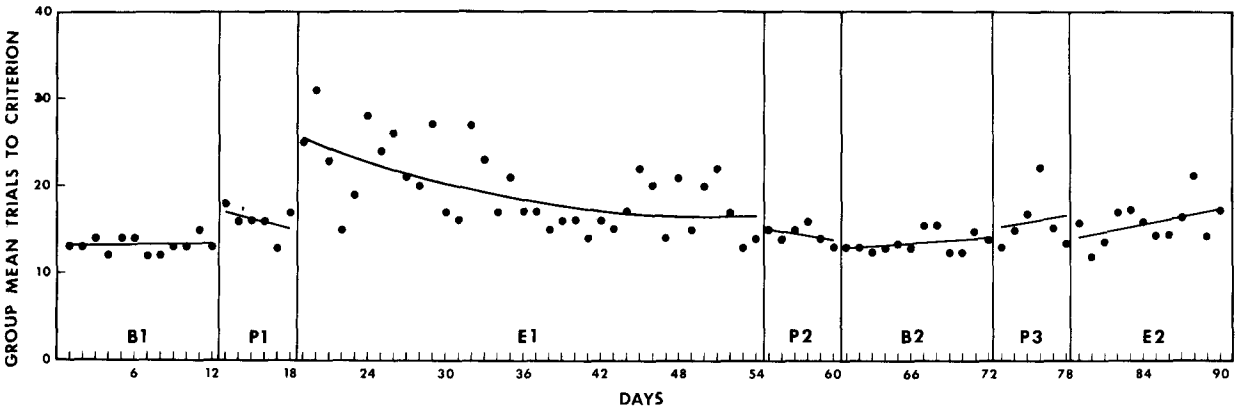

FIG. 2. Group mean scores of the number of trials to reach the 84% correct criterion prior to cue reversal. Scores for individual animals were obtained by calculating the mean number of trials required to reach criterion during each daily test session. Equations for the lines were calculated by the method of least squares, and treatment periods are designated as above.

decrement in performance, as evaluated by all three dependent measures, occurred when ethanol was first administered but that a gradual recovery tended to follow this initial decrement (see Days 19–54). When ethanol was again administered following a 24-day period during which no drug was given, however, no decrements in performance were evident with the exception that latency to respond in reversal was exceptionally high on the first day of Ethanol 2. When performance across days in each of the seven treatment periods was examined statistically, the changes observed during Ethanol 1 were found to be significant for all three measures (see Table 1) but no significant trends (95% confidence level) across days were found during any of the other periods, including Ethanol 2, with one exception. If the exceptionally high latency scores on the first day of Ethanol 2 were included in the analysis, the trend across days during Ethanol 2 with respect to latency was statistically reliable (see Table 1); but if these values were omitted from the analysis the change during days 2–12 was not significant (df = 10,30; F = 1.75). Thus a transient but significant change in latency to respond occurred during the Ethanol 2 period, although no significant changes as assessed by the other two dependent variables were observed during this treatment period. Blood ethanol concentrations determined from samples obtained immediately following each test session remained relatively constant throughout the two drug administration periods (see Fig. 1), indicating that the observed changes in performance on the discrimination task were not simply a reflection of metabolic changes which could have resulted in reduced blood ethanol concentrations at the time of testing.

Differences in performance between individual animals were detected with respect to all three dependent measures during Baseline 1. These differences remained statistically significant throughout the subsequent experimental treatment periods with the exception that the trials to criterion measure did not differentiate individual animals during three of these periods (Placebo 2, Baseline 2, and Ethanol

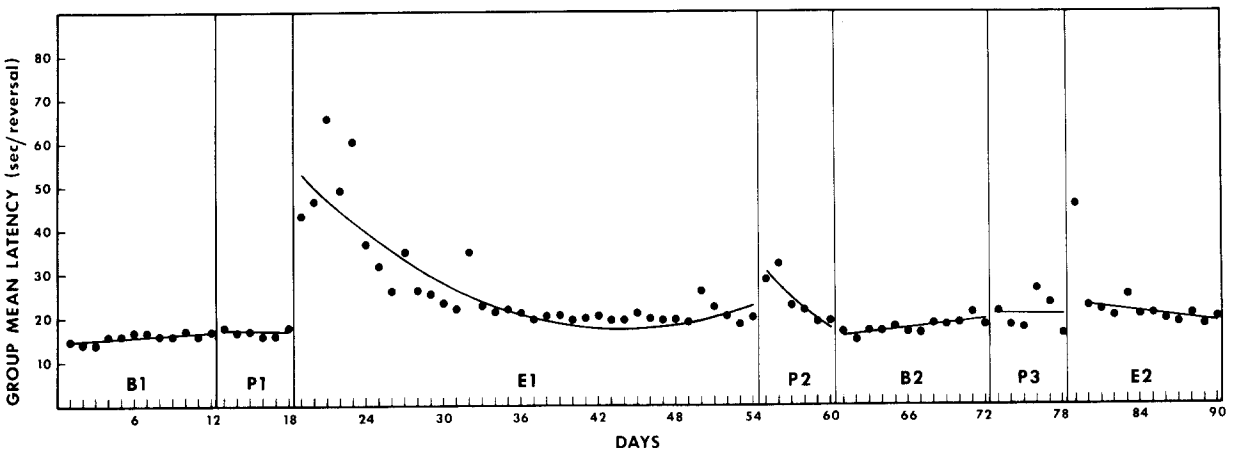

FIG. 3. Group mean response latencies during Trials 2–10 following cue reversal. Scores for individual subjects were obtained by summing the latencies during the nine trials of each reversal period and then calculating the mean of these sums for each daily test session. Equations for the lines were obtained using the method of least squares and treatment periods are designated as above.

TABLE 1

F RATIOS FROM ANALYSES OF VARIANCE

	df	Dependent Measure		
		% correct in reversal	trials to criterion	latency to respond
TREND ANALYSES				
change over days during Ethanol 1	35,105	3.38‡	1.59*	3.85†
change over days during Ethanol 2	11,33	0.50 NS	0.96 NS	5.69†
TREATMENT COMPARISONS				
first 12 days of Ethanol 1 vs. 12 days of Ethanol 2	1,69	41.04‡	8.27†	31.47†
last 12 days of Ethanol 1 vs. Ethanol 2	1,69	0.82 NS	3.10 NS	7.19†
Baseline 1 vs. Baseline 2	1,69	0.54 NS	0.36 NS	8.40†
last 12 days of Ethanol 1 vs. Baseline 1	1,69	15.58‡	30.93‡	71.95‡
Ethanol 2 vs. Baseline 2	1,69	5.02*	8.68†	35.56‡
Ethanol 2 vs. Placebo 2 + Placebo 3	1,69	0.01 NS	0.74 NS	3.60 NS

*$p<0.05$ †$p<0.01$ ‡$p<0.001$

2). Thus although the animals were generally not equivalent in absolute performance level, they demonstrated similar repsonse decrements following initial doses of ethanol as well as comparable patterns of tolerance development. Inasmuch as an analysis of individual response patterns was consistent with the group data presented in Figs. 1, 2, and 3 this information is not presented in detail.

Results of the factorial analyses of variance which were used to compare performance levels between various treatment periods are also summarized in Table 1. A significant difference in performance with respect to all three dependent variables was found between the first 12 days of the initial ethanol administration (Ethanol 1) and the 12 days comprising the second period of ethanol administration (Ethanol 2). However, performance measured during the last 12 days of Ethanol 1 was not statistically distinguishable from performance during Ethanol 2, except with respect to the latency measure (see Table 1). Once again this distinction was no longer significant if the marked increase in latency observed on Day 1 of Ethanol 2 was omitted from the analysis and the last 11 days of Ethanol 1 were compared with the last 11 days of Ethanol 2. With this exception the gradual improvement in ability to perform the two-choice discrimination-reversal task under the influence of relatively high blood ethanol concentrations was retained during a 24 day no-drug period. During this abstinence period baseline performance levels were recovered as indicated by the finding that Baseline 1 and Baseline 2 performance scores were statistically indistinguishable (see Table 1) except with respect to the latency measure. However, the statistically significant differences found between the last 12 days of Ethanol 1 and Baseline 1 as well as the differences between Ethanol 2 and Baseline 2 (see Table 1) suggest that asymptotic performance during periods of ethanol administration is not equivalent to normal baseline performance; examination of the data (see Fig. 1) showed that this difference was in the direction of lower performance levels when subjects were under the influence of ethanol. Further analysis of the data, however, indicates that performance during Ethanol 2 was not reliably different from performance under placebo conditions (see Table 1) which suggests that the observed differences were probably due to the effects of intubation and/or caloric supplementation, common to both placebo and ethanol treatment periods, rather than to the ethanol *per se*. These results indicate that asymptotic performance during ethanol administration periods reached a level which was not distinguishable from performance under placebo conditions.

These factorial analyses also demonstrated performance differences between individual animals as did the trend analyses. Once again animals were significantly differentiated throughout the experimental treatment periods with respect to both percent correct in reversal and latency to respond in reversal scores but were not consistently differentiated by their acquisition performance. Trials to criterion scores for individual animals were statistically distinguishable during only two of the between treatment comparisons (last 12 days of Ethanol 1 vs. Ethanol 2 and Baseline 1 vs. Baseline 2). These findings are an additional demonstration of the consistency of the drug effect despite idiosyncratic differences in performance of the discrimination task.

DISCUSSION

Two-choice, discrimination-reversal learning has previously been demonstrated to discriminate reliably between phylogenetically distinct species of nonhuman primates [5, 17,23]. In this paradigm each animal must always reach a predetermined level of mastery on each problem before reversal takes place and as a consequence all animals are at the same level of acquisition performance immediately prior to cue reversal. Therefore, even during the portions of the present experiment when performance was impaired during the initial days of ethanol administration, each animal was required to master the discrimination at the 84% criterion level before cues were reversed and reversal performance was assessed. During this portion of the study the number of trials required to reach that criterion increased significantly. However, the discrimination was mastered at the 84% level prior to each reversal, indicating that criterional performance of the task was achieved under the influence of the drug. Despite this fact, reversal performance was also significantly impaired, suggesting that the general functional disturbance which underlies the decrement in prereversal performance is also reflected in the impairment of transfer of training necessary for reversal performance. The development of functional tolerance to ethanol was demonstrated by the recovery of performance levels which were equivalent to those during placebo conditions on both the acquisition and reversal portions of the discrimination task despite the continued administration of ethanol. Further, reponse latencies during reversal reliably demonstrated these same changes. Increased response latencies might reflect either sensory-motor impairment and/or a slowing of the decision processes involved in completion of the visual discrimination. In either case, longer response latencies indicate slower rates of responding and thus reflect the quantity of behavior emitted by each animal within a test session. As such, they demonstrate that both the amount of behavior and the accuracy of performance were affected by ethanol administration and that tolerance to ethanol also developed with respect to this aspect of the response pattern.

From Figs. 1, 2, and 3 it may be seen that all response measures appeared to be at asymptotic values after 18 days of exposure to ethanol (Day 36). In fact, the latency measure appears to be at asymptote in 14 days or less. Although this time course for the development of tolerance is somewhat slower than that previously reported in rhesus monkeys which were tested using a shuttle avoidance task [6], it is in general agreement with the results of numerous experiments in which the development of tolerance to ethanol was found to occur in 14–25 days [1, 2, 7, 8, 12, 13, 16, 25, 26, 27]. In two studies using rats specifically designed to evaluate the loss of tolerance, evidence for the complete loss of tolerance in 14–17 days was reported [8,12]. In the present experiment, however, no loss of functional tolerance was evident after an interval of 24 days with the exception of the previously noted marked rise in response latency on the first day of the second alcohol administration period. Further testing over more extended periods of time is clearly indicated and will be included in future experiments.

Even though functional tolerance did develop with respect to the two-choice discrimination-reversal task, behavioral observations of the animals as they were handled daily suggested that some degree of general tranquilization

did persist throughout the periods during which ethanol was administered. For example, these animals were consistently easier to restrain for blood sampling following testing if they had been treated with ethanol. No marked change in this situation was observed during the course of the experiment. General locomotor performance as indicated by the animals' ability to enter and exit from the transport box did, however, appear to improve throughout the first week of the ethanol administration period.

Although the results of this experiment are generally consistent with those previously reported, it is noteworthy that the behavioral task used in the present experiment presumably depends upon the higher-order integrative and problem solving capacities of the animal. In most of the previous investigations behavioral tasks such as the tilted plane test [25], avoidance and/or escape training [6, 18, 26], motor-driven belt tasks [8, 12, 13], or maze tests [3, 8, 13] have been employed to assess sensory-motor functioning following ethanol administration. In contrast, use of the discrimination-reversal paradigm allows for the evaluation of both the ability of the animals to perform a visual discrimination as well as their ability to reverse a previously reinforced response pattern.

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