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BRIEF COMMUNICATION

Intake of Water and Ethanol by C57BL Mice: Effect of an Altered Light-Dark Schedule

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MILLARD, W. J. AND V. P. DOLE. *Intake of water and ethanol by C57BL mice: Effect of an altered light-dark schedule.* PHARMACOL BIOCHEM BEHAV **18**(2) 281–284, 1983.—Mice of the C57BL/6J strain were given unrestricted access to food, water, and ethanol-water solutions, and intake was studied under different light-dark (LD) conditions. The instrumentation for recording licks permitted the accurate desrciption of the temporal distribution of drinking over the 24-hr period. The 12–12 LD schedule resulted in a consistent pattern of licking for the three solutions, and this pattern was altered by the subsequent change to a 6–6 LD condition. The intake of ethanol, and the preference for ethanol relative to water, were reduced during the 6–6 condition. The distribution of drinking was circadian to period, though not sinusoidal in form.

C57BL mice Circadian rhythm Drinking Ethanol Light-dark schedule

THE self-administration of ethanol by animals has been studied in an effort to develop an analog of human addiction. Despite extensive research a preparation has not been described in which tolerance and dependence occur as a result of the selection of ethanol independent of food and water consumption [4,10]. Ethanol intake is usually measured for 24-hr intervals and therefore cannot describe periodic variation within the interval. Because the effects [1,8] and disposition [1, 6, 14, 15] of ethanol may be periodic, and because these processes are likely to be related to self-administration, a more precise analysis of ethanol intake and preference is indicated.

The objectives of this experiment were to study ethanol intake and preference within the 24-hr period, and to examine the effect of an altered light-dark (LD) schedule. Mice were given a choice of water and ethanol-water solutions and observed under a 12–12 LD schedule before and following exposure to a 6–6 LD schedule.

METHOD

Subjects and Apparatus

Eight male C57BL/6J mice, approximately eight-weeks old, were obtained from The Jackson Laboratory, Bar Harbor, Maine. The animals were individually housed in polycarbonate cages and given Purina Mouse Chow (No. 5015). Acidified tap water (W), and 10% (E10) and 20% (E20) ethanol-water solutions (v/v, absolute ethanol, U.S.I.) were presented in graduated glass bottles. Details of the procedure and the method of measuring drinking have been previously described [2]. The solutions, bottles, and the hardwood shavings were replaced at seven-day intervals between 1000 and 1100 hr EST. Body weight (±0.1 g), food weight (±0.1 g), and fluid volumes (±0.5 ml) were measured at these times.

Procedure

The mice were initially maintained for eight weeks under a 12–12 LD schedule (on: 0500; off: 1700 hr EST) in the main vivarium, and subsequently removed to a second room with similar environmental conditions. Data collected during a 10-week period in this room were used as the baseline for studying the effects of the subsequent change to a 6–6 schedule for a 10-week period (on: 1100, 2300; off: 1700, 0500 hr EST). The original 12–12 LD schedule was established for a final 10-week period after the second condition.

The statistical analyses were based on means computed for each mouse during the last six days of each 10-week condition. A repeated measures analysis of variance was

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TABLE 1

MEAN (SE) 24-HR MEASURES OF WATER AND ETHANOL INTAKE, FOOD INTAKE, AND BODY WEIGHT AS A FUNCTION OF LIGHT-DARK SCHEDULE

	Light-Dark Schedule						
Measure	12–12 (1) (a)		6–6 (b)		12–12 (2) (c)		
Lick Frequency							
W	1249	(377)	3107	(423)	2418	(417)	*
E10	1921	(509)	974	(388)	1562	(375)	‡
E20	689	(310)	66	(18)	513	(63)	‡
Total	3860	(389)	4147	(189)	4493	(237)	
% in dark							
W	73	(6)	50	(2)	70	(4)	‡
E10	79	(10)	48	(10)	81	(6)	‡
E20	89	(3)	62	(10)	79	(5)	‡
Total	88	(2)	55	(3)	79	(3)	‡
Ethanol (mg)	208	(33)	70	(21)	163	(26)	‡
Water (g)	2.8	(0.3)	3.2	(0.1)	3.4	(0.2)	
Mg ethanol/g water	73	(7.7)	22	(8.2)	48	(13.9)	†
Food intake (g)	4.2	(1.1)	4.4	(0.9)	4.4	(1.1)	
Body weight (g)	34.1	(1.1)	36.7	(1.3)	36.8	(1.5)	‡

Values are based on the last six days in each condition.
*a < c < b, $p<0.05$.
†b < a and c, $p<0.05$.
‡a < b and c, $p<0.05$.

used with the LD schedule as the within-subjects factor of three levels: 12–12(1), 6–6, and 12–12(2). A significant effect was studied further by multiple comparisons using the Bonferroni t statistic [11].

RESULTS

The mean lick frequencies for each condition and fluid are presented in Table 1. Lick frequencies in the 12–12(1) period were highest for E10 and intermediate for W. This order was reversed in the 6–6 period and the 12–12(2) period. The mean lick frequency for the E20 solution was consistently the lowest. Significant effects were found for the LD Schedule, $F(2,14)=15.96$, Fluid, $F(2,14)=22.96$, and the Fluid × LD Schedule interaction, $F(4,28)=9.27$. Pairwise comparisons of the means for E10 and E20 indicated that the 6–6 schedule resulted in a significantly reduced licking rate, relative to the two determinations of the 12–12 schedule. A similar analysis for W, however, showed that the mean lick frequency was highest in the 6–6 condition and lowest during the initial 12–12 schedule. The mean number of licks within a 24-hr period did not significantly change over conditions, $F(2,14)=1.66$.

Estimates of water and ethanol intake, included in Table 1, were derived from the volumetric measures. Ethanol intake was significantly affected by the lighting schedule, $F(2,14)=7.73$, the mean amount being significantly lower during the 6–6 condition than either the first or second 12–12 condition. The mean g water intake did not change significantly over conditions, $F(2,14)=2.79$. Preference for ethanol (mg ethanol per g water) was significantly affected by the schedule of lighting, $F(2,14)=23.65$. The mean value was 73 in the initial 12–12 condition, and the 6–6 schedule resulted in a significant 60% decrease. Preference in the final 12–12 condition was significantly greater than in the 6–6 condition, but did not return to the level observed under the initial 12–12 schedule.

Food intake, measured during the final period of each condition, did not increase reliably over the three conditions (see Table 1). The analysis of variance for body weight indicated a significant effect of the LD Schedule $F(2,14)=26.68$, however, only the mean of the 12–12(1) condition was significantly less than the values for the two subsequent conditions. Therefore this change may be associated with aging rather than the environmental conditions.

Of major interest was the precise characterization of the temporal distribution of drinking. Mean lick frequencies per hour are presented in Fig. 1 for each fluid and condition. The shape of the distributions for the two determinations of the 12–12 condition are similar. Approximately 80% of the drinking occurred during the dark period, with E10 and W showing a distinctive pattern of two or three peaks during the 12 hr of darkness. The 6–6 schedule of lighting resulted in a distribution with two distinct peaks for E10 and E20, with most drinking occurring in the 1700–2300 hr dark period. The amount of W intake for each hour was elevated during the light period, and the three-peak distirbution was maintained with the latest peak occurring in the 0500–1100 hr period of darkness. The percentages of drinking during the dark period for each fluid and each condition are included in Table 1. Significant effects were found for LD Schedule, $F(2,14)=10.21$, Fluid, $F(2,14)=5.82$, and the Fluid × LD Schedule interaction, $F(4,28)=7.31$. Inspection of Fig. 1 suggests the decrease in the mean percentage of drinking during the dark period of the 6–6 schedule was accounted for by the increased intake during the 2300–0500 hr light period.

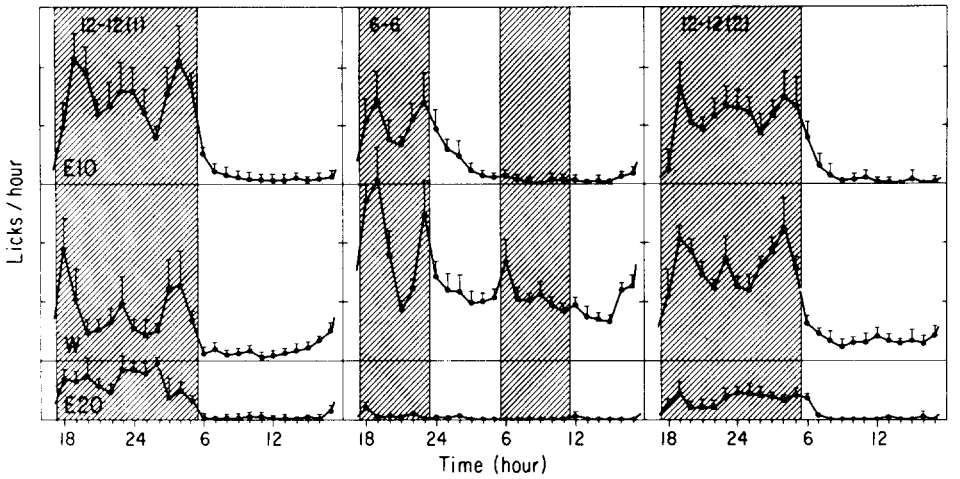

FIG. 1. Mean lick frequency per hour (±SE) for E10, W, and E20 for the three LD conditions: 12–12(1), 6–6, 12–12(2). Means are based on the last six days in each condition. The x-axis represents a 24-hr period, beginning at 1700 hr; each division on the y-axis equals 100 licks. Dark periods are indicated by shading.

That is, drinking in the 6–6 condition continued during the period which was originally a part of the dark period in the 12–12 condition.

DISCUSSION

The consumption of ethanol by mice and rats under different LD conditions has not been studied extensively, though the intake of ethanol combined with food [3], or with the sole source of water [7], is known to occur primarily in the dark period and changes under conditions of continuous light or dark [5, 6, 12].

Under 12–12 LD conditions a distinctive pattern of drinking was observed. The pattern was altered in the 6–6 condition, and re-appeared in the second exposure to the 12–12 condition. Comparison of these data with other descriptions of intake patterns is not possible because ethanol was added to the source of food or water in these other experiments, and the method of monitoring licks resulted in less temporal resolution [3,7].

The mean amount of absolute ethanol consumed in a 24-hr period under the 12–12 LD condition (208 mg, or .26 ml) is consistent with other experiments, as described by McClearn [9]. This amount is equivalent to approximately 6.0 g ethanol/kg body weight per day and less than that required for the development of tolerance or dependence in the C57BL mouse [4,9]. Analyses of the temporal distribution of drinking are pertinent, because such information permits the estimation of blood ethanol concentration and the detection of drinking patterns resulting in significant pharmacological effects. For example, the data presented for the 12–12(1) condition (Fig. 1), and additional observations regarding the rate of clearance in mice (approximately 100 mg/dl·hr) [12], indicate that a maximum and transient blood ethanol concentration of approximately 50–70 mg/dl may occur in the dark period. Blood concentration during the entire light period and most hours of the dark period would be less than 10 mg/dl. Thus, enduring and significant concentrations of ethanol in blood would not result from such a pattern of drinking.

The ability to discern transient changes in consumption may ultimately contribute to the search for factors controlling ethanol intake and preference.

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