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Effects of Photoperiod, Melatonin and Pinealectomy on Ethanol Consumption in Rats

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BURKE, L. P. AND S. Z. KRAMER. *Effects of photoperiod, melatonin and pinealectomy on ethanol consumption in rats.* PHARMAC. BIOCHEM. BEHAV. 2(4) 459–463, 1974. – Rats offered a choice of water or 4% ethanol solution preferred ethanol. The amount of ethanol consumed was influenced by environmental lighting, increasing during constant darkness. In rats which showed the least ethanol preference, administration of melatonin resulted in significant increases in ethanol consumption and reciprocal decreases in water intake. Pinealectomy had no significant effects on ethanol consumption under various environmental lighting conditions. Long-term observation revealed no inherent cyclic changes in ethanol consumption in pinealectomized or sham operated animals.

Environmental lighting Ethanol preference Melatonin Pinealectomy

WORK in several laboratories has implied involvement of the pineal gland in the regulation of ethanol consumption in the rat. Geller [7] reported an increase in preferential ethanol intake during periods of constant darkness and a decrease during constant light. He inferred pineal involvement, and produced increases in ethanol consumption with melatonin treatment. Pineal involvement in ethanol preference has been reported by Blum *et al.* [3] and Reiter *et al.* [17]. Sinclair [18] has criticized Geller's conclusions about the effects of ambient lighting conditions on the grounds of the existence of an inherent cyclic pattern of ethanol consumption unrelated to external conditions of light or darkness. We have studied the effects of environmental lighting, melatonin, and pinealectomy on patterns of ethanol consumption in an attempt to clarify the possible role of the pineal gland in this behavior. We have also looked for evidence from which to consider Sinclair's assertion that the changes in ethanol consumption Geller associated with photic conditions were a coincidental expression of inherent cyclic drives.

METHOD

Ten Sprague-Dawley rats were housed (one to a cage) in a temperature regulated room. Cages were arranged to allow illumination of 290 Lux on the center of the floor. The animals were fed ad lib on Wayne Lab Blox. Fluids, offered in a two tube choice, were placed in Kimax graduated drinking tubes, one on each side of the rectangular cage, the same distance (7.3 cm) from the floor. Ethanol solutions were prepared from 95% alcohol [6]. Fluid consumption was recorded each night (9:00 p.m.) after which the tubes were washed, refilled and exchanged. Readings in the dark were made under a dim red light. Pinealectomy was performed under pentobarbital anesthesia according to the method of Hoffman and Reiter [9]. Successful pineal extraction was verified by gross examination of the intact removed gland and its stalk. Postmortem gross and histological verification was also obtained. Sham operations were carried out in control animals by duplicating the procedure to the point of removing the bone plug without penetrating the dura and replacing the plug. Following surgery the animals were given 150,000 units of penicillin G intramuscularly. Melatonin (Calbiochem) was administered subcutaneously, as were control injections of aqueous vehicle [5]. Lighting conditions included: diurnal (D), 15 hr light, 9 hr dark, constant light (CL), and constant darkness (CD).

The first group of 10 animals were offered only water in both tubes for a period of three weeks to acclimate them to the environment and to determine levels of fluid consumption and the influence of various lighting conditions on total fluid consumption. Following this period the animals were offered a choice of 4% ethanol or water for a period of 4 weeks, with one week periods of varying environmental lighting in the sequence D, CL, CD, CL. The effects of lighting on consumption of an aversive solution of ethanol (12%) was then observed for a 3 week period in the sequence D, CL, CD, after which only water was offered.

The effect of melatonin was studied in the same group of animals because they were already acclimated. Five animals which showed the lowest ethanol preference were

selected to receive melatonin, the remaining five were given vehicle. Melatonin was administered subcutaneously over a 7 week period in increasing doses of 0.5–2.5 mg/kg.

The effects of pinealectomy were studied in a second group of 10 animals (5 pinealectomized, 5 sham operated). Fifteen days were allowed for recovery and acclimatization, after which all animals were offered a choice of 4% ethanol or water. They were observed for a period of 6 weeks in the following lighting sequence: D, CD, D (2 weeks), CL, CD.

RESULTS

The Mann-Whitney U test and Wilcoxon Matched-Pairs Sign Rank test were used to analyze the data statistically. Fluid intake was averaged per rat and per group over weekly intervals. During the first three weeks, when only water was available (Fig. 1), the animals drank significantly ($p<0.005$) more during CD but not significantly less during CL than during the initial diurnal period. Distribution of fluid consumption during the water, 4% ethanol choice showed a preference for ethanol through all lighting conditions. During the week of diurnal lighting, water intake was lower than that drunk during the same environmental condition when only water was available. The ethanol solution consumed, comprised 70% of the total. Total fluid intake (ethanol plus water) was significantly ($p<0.005$) higher than total water only, drunk earlier. During the week of CL, the water consumption dropped further and ethanol consumption increased slightly. During the week of CD that followed, there was a significant increase in ethanol consumption ($p<0.005$) with a slight decrease in water intake. Total fluid intake increased during this period ($p<0.005$) which reflected the increase in ethanol consumption. Return to CL resulted in a significant ($p<0.005$) decrease in ethanol and total fluid consumption. Water intake increased so that the decrease in total fluids reflected the decrease in ethanol consumption.

The presentation of an aversive ethanol concentration (12%) resulted in complete reversal in the proportionate consumption of each fluid. During the first (D) week of this choice, ethanol intake decreased significantly ($p<0.005$) while water intake increased significantly ($p<0.005$) when compared to diurnal conditions with a 4% ethanol choice. There was some variation in both 12% ethanol and water consumption with CL and CD conditions. During the week of CL, ethanol consumption increased and water consumption decreased slightly. With CD there were small increases in both ethanol and water intake. None of these changes were significant. When the animals were returned to water only, with diurnal lighting, intake was the same as that seen during the same condition earlier.

Although total fluid consumption throughout these experiments varied with environmental lighting, there was a

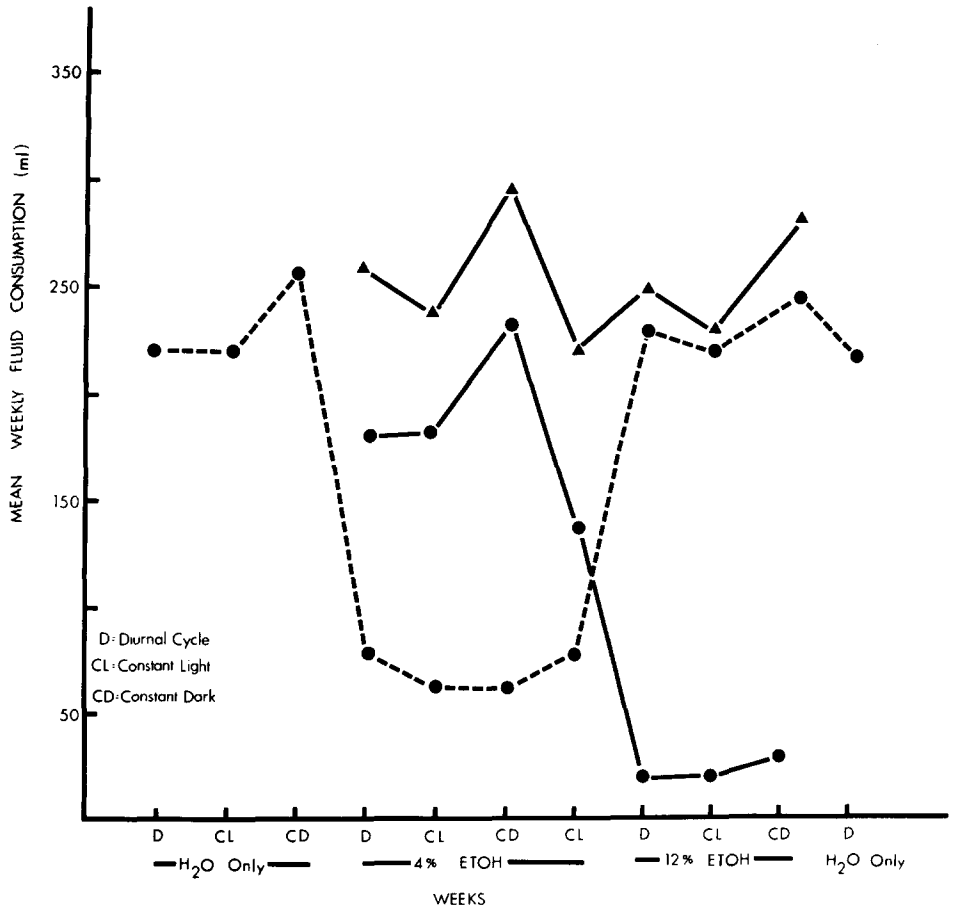

FIG. 1. Mean weekly ethanol (●——●) water (●– – –●) and total fluid (▲——▲) intake plotted over successive weeks for various conditions of fluid choice and lighting (abscissa).

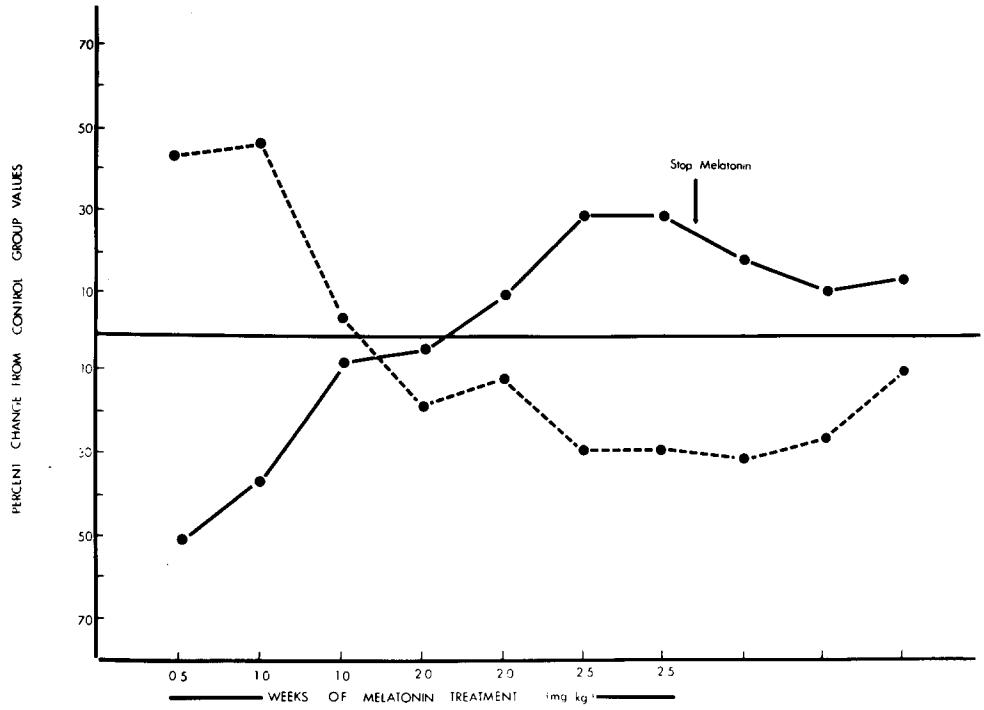

FIG. 2. Ethanol (●——●) and water (●– – –●) consumption of rats treated with melatonin as percent change from the control group (ordinate). Absicissa shows the melatonin dose administered for successive weeks of treatment. Points plotted for the last 3 weeks show post treatment effects.

tendency for it to remain relatively constant in the face of large variations in the ethanol/water ratio.

Administration of melatonin with diurnal lighting resulted in a progressive increase in ethanol and decrease in water consumption. Results expressed as percent change from control group values (Fig. 2), show that during the first week of treatment with the lowest dose (0.5 mg/kg), the treated animals drank less ethanol and more water than the controls. During the fourth week this was reversed. Ethanol (4%) consumption in the pinealectomized animals did not differ significantly from that of the controls (Fig. 3) over a six week period during which the lighting conditions were varied. During the first 4 weeks the pinealectomized animals drank more ethanol than the controls. This reversed during the last 2 weeks. The general tendency for ethanol consumption by both groups to increase during this period (with small variations correlated with lighting) was accompanied by a reciprocal decrease in water intake. The increase in ethanol consumption by both groups in the second week (CD) was significant ($p = 0.05$), but the decrease in the fifth week (CL) was significant only for the pinealectomized group ($p = 0.05$). Return to CD from CL resulted in significant ($p = 0.05$) increases in ethanol consumption for both groups. During the 13 weeks in which the pinealectomized and sham operated animals were observed under diurnal lighting conditions (Fig. 4), there were no significant or consistent differences in ethanol consumption between the two groups. During all but one week of this period, the sham operated group drank more ethanol than the other. Water intake was consistently higher in the pinealectomized animals through the entire period. There was no evidence of uniform cyclic variations in either ethanol or water consumption.

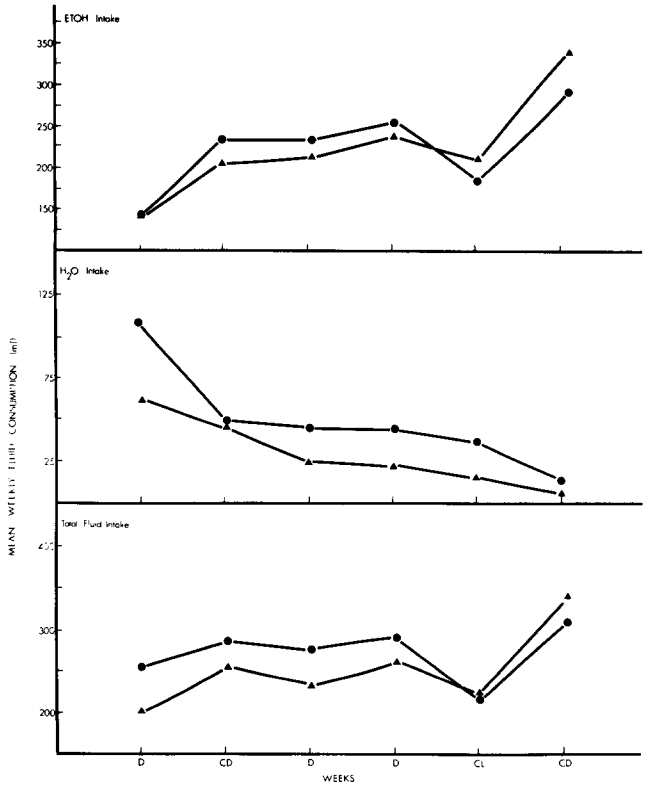

FIG. 3. Mean weekly ethanol, water and total fluid intake for pinealectomized (●——●) and sham operated (▲——▲) animals plotted over successive weeks for various lighting conditions (abscissa).

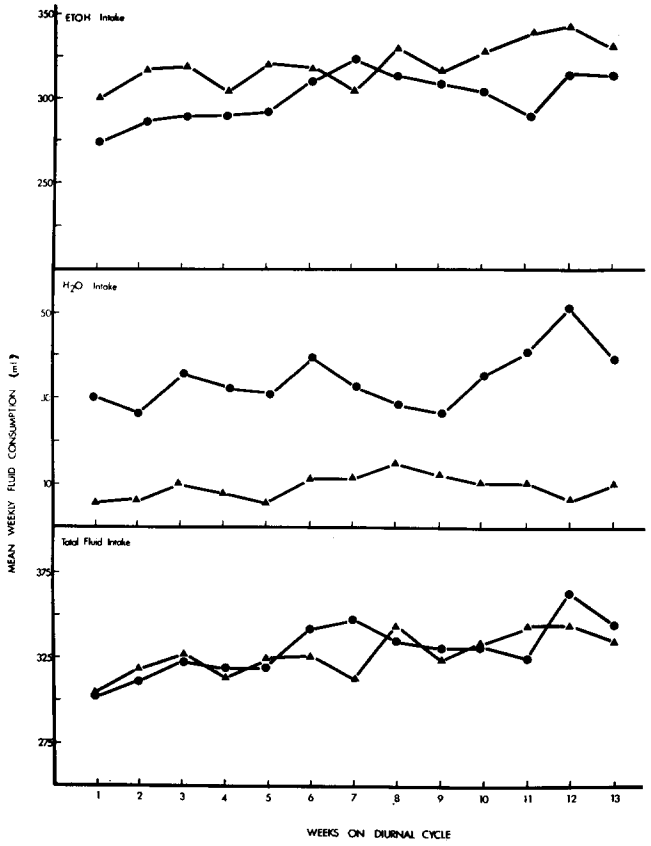

FIG. 4. Mean weekly ethanol, water and total fluid intake for pinealectomized (●——●) and sham operated (▲——▲) animals plotted over a 13 week period with diurnal lighting conditions.

DISCUSSION

The results of this study confirm the effects of environmental lighting on drinking behavior in these animals. Because it was important to observe the effect of continuous light on ethanol preference, we risked the possibility of the retinal degeneration reported by O'Steen [14] and Reiter and Klein [16] to occur in albino rats exposed to continual light. Although out animals may have sustained retinal damage, they retained the ability to discriminate between light and darkness. Zucker [20] found that the rhythmic consumption of food and water correlated with photoperiod was eliminated by exposure to constant light for 21 days. Blind rats showed no rhythmicity in consummatory behavior and were unresponsive to photoperiod. Our rats exhibited a normal rhythm in food and water intake before and after periods of continuous light exposure. In addition, ethanol consumption continued to vary with photoperiod after continuous light exposure. It is therefore unlikely that our rats were blind. Although extraretinal photoreception has been proposed for very young rats, it seems unlikely to be the route of light detection in our animals. When only water was available, increased water intake was clearly associated with constant darkness following diurnal conditions. Constant light did not however produce the expected reduction. It is possible that this novel environmental condition had a complex effect on behavior or that constant light following diurnal conditions did not alter the level of activity of the animals. The increased drinking seen during constant darkness is consistent with the normal feeding and drinking behavior of the rat [20]. It is presently difficult to explain the preference for 4% ethanol to water. Among the factors which may be involved are caloric needs and the formation of alkaloids from ethanol and amine metabolities which may stimulate ethanol preference. Changes in ethanol and water consumption during the week of constant light are not significant and imply that the effects of this change in lighting condition is minimal. The change to constant darkness however had a marked effect on ethanol preference. This is reflected in the increase in the ethanol/water ratio from 2.3 during the diurnal period to 3.7 during the week of constant darkness and supports the finding of Geller [7]. The significant depression of ethanol consumption during the week of constant light which followed, (ethanol/water ratio 1.8) suggests a specific effect of this condition on ethanol consumption.

The refusal of the animals to drink significant quantities of 12% ethanol probably reflects the effect of palatability factors on the intake of this fluid. When the animals were offered both water and ethanol (4 and 12%), total fluid consumption was somewhat greater than during periods when only water was available. This finding may be explained by division of total caloric intake between food and ethanol when the latter was available [13].

The significant increase in ethanol consumption and concomitant decrease in water intake produced by melatonin is difficult to explain. This finding confirms that of Geller [7]. Melatonin was selectively administered to those animals which showed the lowest ethanol preference in order to make the expected effect more striking. Figure 3 illustrates the increase in ethanol consumption relative to pretreatment levels. When the ethanol consumption of the treated group is compared to that of the controls, the effect of melatonin is seen as an increase in ethanol consumed from volumes considerably below the control group during the first week of treatment, to exceed and remain above the controls by the fifth week. Our finding differs from those of Blum *et al.* [3,4] who report that melatonin had no significant effect on the consumption of 5% ethanol in rats. The difference in our results may be due to differences in procedure. We administered melatonin subcutaneously in increasing doses over a longer time period to intact animals which showed the least preference for 4% ethanol, whereas Blum *et al.* administered melatonin in subcutaneous wax implants to pinealectomized animals which were already drinking large amounts of 5% ethanol. The possibility that melatonin increased ethanol consumption through general effects on the level of excitability and activity in our animals is inconsistent with the finding that melatonin decreases wheel running activity of rats [15,19]. The effect of exogenous melatonin on ethanol preference may be produced indirectly through its effect on brain serotonin levels. Elevation of serotonin in midbrain and hypothalamus following melatonin administration has been reported by Anton–Tay *et al.* [2]. Various investigators have described correlations between serotonin levels in the brains of rats which showed ethanol preference. Myers *et al.* [10, 11, 12] have reported a reduction in ethanol consumption in rats with serotonin depletion produced by para-chlorophenylalanine. On the other hand, Geller [8] found increased ethanol consumption after parachlorophenylalanine administration. Because

of the conflicting reports and uncertainty about the relationship of brain serotonin levels to ethanol preference, it is difficult to explain the melatonin effect on the basis of its effect on indole metabolism.

The results of our experiments with pinealectomized animals indicate that this organ is not significantly involved in ethanol preferences. Both pinealectomized and sham operated animals preferred ethanol to water. Pinealectomy did not eliminate variations in consumption associated with various lighting conditions. The nonsignificant differences between the two groups when subjected to various lighting conditions are not consistent with the proposal that the pineal plays a role in the observed variation of ethanol consumption with environmental lighting. Although, during the 13 week period of diurnal lighting, with the exception of one week in the middle of the period, the pinealectomized animals drank less ethanol than the sham operated ones, none of the differences were statistically significant. The contribution of the pineal in our experiments appears to be nonexistent or minimal. Our findings differ from those of Blum *et al.* [3,4] and Reiter *et al.* [17] who have reported a significant effect of pinealectomy on ethanol consumption in sighted and congenitally blind rats. We cannot account for the difference in these results. In our study, 15 days were allowed for postoperative recovery. In the study of Reiter *et al.* [17] no statistical evaluation of the effect of pinealectomy is presented and the asserted pinealectomy effect disappeared eighteen days after the operation. Blum *et al.* [3] present data which show no statistically significant differences in the amount of ethanol consumed by pinealectomized and sham operated rats. When however, ethanol to total fluid ratios were compared, significant differences appeared in five scattered weeks out of the eleven in which data were obtained.

Our observations of ethanol consumption under diurnal lighting conditions for both pinealectomized and sham operated rats over an extended time period (13 weeks) shows no evidence of the inherent cycles proposed by Sinclair [18] to explain the finding of Geller [7] that ethanol preference was related to environmental lighting conditions. However, some of the animals that we observed (pinealectomized and sham operated) had previously received melatonin. While this may have altered the inherent cyclic changes in ethanol consumption proposed by Sinclair, it is unlikely that it would do so for so long a period of time. Although it is possible that the sham operations could disrupt such an internal rhythm, this seems an even less likely explanation for the failure to observe it. During the period of observation, with the exception of one week in the middle of the period, the sham operated animals drank more ethanol than the pinealectomized group. Water intake was lower in the sham operated animals through the entire period. The differences in ethanol consumption between the two groups were not significant. Consideration of all the data in this study which bears on the question, does not allow the conclusion that the pineal is involved in regulation of ethanol consumption or that the effects of melatonin which we observed mimic those of endogenous release.

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