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Pineal Gland and Melatonin Influence on Chronic Alcohol Consumption by Hamsters[1]

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RUDEEN, P. K. AND S. K. SYMMES. *Pineal gland and melatonin influence on chronic alcohol consumption by hamsters*. PHARMAC. BIOCHEM. BEHAV. **14**(2) 143–147, 1981.—Male golden hamsters preferentially consume alcohol solution when given a free-choice between water and the alcohol solution. The pineal gland has been implicated as influencing the predilection for the ethanol solution. Melatonin, a pineal hormone, was administered either daily for 11 weeks as a subcutaneous injection (25 μg/animal) or weekly as a subcutaneous beeswax implant (1 mg melatonin/24 mg beeswax) for 5 weeks to hamsters allowed a free-choice between water or a 10% ethanol solution. Food, water and alcohol consumptions were measured on a daily basis. Animals treated by daily injection with melatonin consumed slightly less ethanol than animals not given melatonin. In light-deprived animals given chronic implants of melatonin, alcohol consumption was reduced when compared to alcohol consumption by light-deprived hamsters not receiving melatonin. Melatonin treatment also resulted in reducing daily total fluid intake as well as ethanol consumption in light-deprived hamsters. The results indicate that the pineal gland may influence fluid consumption in the hamster, and indirectly alters the propensity of the hamster to consume alcohol.

Pineal gland Melatonin Hamster Alcohol consumption

THE golden hamster (*Mesocricetus auratus*) normally shows a clear preference for a 10% (v/v) alcohol solution in a free choice experimental condition [1]. Hamsters will drink an average of 88% of their daily fluid intake as alcohol solution. Furthermore, male hamsters will preferentially consume alcohol solutions of up to 25% with the most preferred concentration of alcohol of 15% [2]. The hamster, therefore, is able to discriminate between water and alcohol and show a clear preference for alcohol solutions when given less than a 25% solution.

Another rodent species, the albino rat, willingly consumes about 30% of the total daily fluid intake as alcohol [1]. A situation has been described whereby the propensity of rats to drink an alcohol solution was influenced by the photoperiod in which the animals were maintained [4]. Rats maintained in constant darkness drank more ethanol solution (4% v/v) than water, and under constant light or diurnal photoperiods, ethanol intake was reduced. The response of the drinking preference by the animal to changes in the photoperiod suggests that the pineal gland might be involved in this behavioral response.

Because hamsters exhibit a preference to drink alcohol at the expense of water, the effects of the photoperiod and the pineal gland to further enhance the alcohol preference in the hamster were examined [5,13]. Indeed, surgically blinded (light-deprived, LD) hamsters given a 5% solution of alcohol showed an exaggerated preference for alcohol consumption which was reduced by removal of the pineal gland. The authors [13] reported that administration of a pineal hormone, melatonin, did not alter alcohol preference.

Administration of melatonin to golden hamsters has been shown to have different effects upon the reproductive organs depending on the photoperiod in which the animal is maintained and the mode of administration of the indoleamine [14,15]. Therefore, the effects of melatonin on alcohol consumption as administered by two different modes to hamsters maintained in long photoperiods or that were light deprived were investigated in this report. The data presented herein show that the pineal is involved in the alteration of alcohol consumption and that under appropriate conditions, melatonin administration will reduce alcohol consumption in the golden hamster indirectly by decreasing the total daily fluid intake by the animal.

METHOD

Adult male golden hamsters (Sprague-Dawley, Madison, WI) were maintained in photoperiodic conditions as described below for each experiment. Food was available to all animals ad lib. Alcohol was available in a three-bottle, two-choice method unless otherwise noted [12]. Food, water and alcohol consumptions were measured as noted in each experiment.

Experiment 1

Thirty-six adult male hamsters were used in this experi-

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ment. Twenty of the animals were sham-pinealectomized (SPx) and 16 of the animals were surgically pinealectomized (Px) [8]. Eight of the SPx animals and eight of the Px animals were blinded by orbital enucleation while under ether anesthesia. These animals are hereinafter referred to as light deprived (LD). The combination of treatments resulted in the following groups: (1) Px+EtOH (N=8), (2) SPx+EtOH (N=8), (3) Px+LD+EtOH (N=8), (4) SPx+LD+EtOH (N=8), and (5) SPx+H_2O (N=4).

All animals were maintained in 14 hours of light daily (on at 0600 hr). At the end of a one-week acclimation period, the animals were separated according to treatment and maintained in polycarbonate cages with four animals per cage. Ethyl alcohol (EtOH) (5% by volume) was replaced for water in all cages except in the cage containing the sham-operated control group, which received only water. Water consumption by the sham-operated control animals and alcohol consumption by the experimental animals were measured every second day. Fluid consumption was calculated as milliliters consumed per hamster per day. Spillage was monitored by placing a full bottle on an empty cage and fluid consumption was corrected as necessary.

After 72 days the animals were killed by decapitation between 0600 hrs and 0900 hrs. Blood was collected, centrifuged at 3000 rpm for 30 min, and the serum was harvested and stored at −20°C until analysis for alcohol content by a modification of the spectrophotometric method [3].

The daily consumption of fluids by each group was analyzed by linear regression and an analysis of covariance. The differences between the total average daily fluid consumption were examined by the 2-tailed Student's *t*-test. Significance was accepted at the 5% level of confidence.

Experiment 2

Thirty adult male golden hamsters were used in this study. All animals were housed in polycarbonate cages at 5 animals per cage and allowed food and tap water ad lib. The animals were maintained in 14 hr of light daily (on at 0600 hr) and allowed to acclimate to these conditions for one week. Alcohol (10% w/v) was given by the three-bottle, two-choice method, and the bottles were rotated daily to prevent position preference. The animals were assigned to the following groups: (1) ethanol and water (N=10), (2) ethanol, water and a daily injection of peanut oil (N=10), and (3) ethanol, water and a daily injection of melatonin in peanut oil (25 μg/animal) (N=10). Melatonin was given 3 hours prior to the onset of the dark phase. Water, alcohol and food consumption were measured daily. Body weights were recorded weekly. Fluid and food consumptions were expressed as the amount consumed per hamster per day. The consumption of fluids and food was monitored for 77 days. The total average fluid intake for each solution was determined as well as the ratio of the volume of alcohol consumed to the volume of total fluid consumed. The data were analyzed by one way analysis of variance (ANOVA) for each respective group. When significance was indicated, a *t*-test for differences between two means was used to determine the level of significance between two groups.

Experiment 3

Thirty-five adult male golden hamsters were used in this study. Fourteen animals were subjected to surgical optic enucleation (light-deprived, LD). All of the animals were allowed to acclimate as described above, and at the end of this period, the animals were given a subcutaneous implant of either beeswax (25 mg) or beeswax containing melatonin (1 mg melatonin/24 mg beeswax). These pellets were renewed weekly. The animals were then presented with an alcohol solution (10% w/v) or water as described above. This treatment resulted in an equal number of animals (N=7) in each of the following groups: (1) visually intact+beeswax implant having only water, (2) visually intact+melatonin-beeswax implant, (3) visually intact+beeswax implant, (4) LD+melatonin-beeswax implant and, (5) LD+beeswax implant. Water, alcohol and food consumptions were monitored on a daily basis and the animal weights were recorded weekly. The daily fluid and food consumptions were recorded for 6 weeks. The total average fluid intake for each solution was determined as well as the ratio of the volume of alcohol solution consumed to the volume of total fluid consumed. Statistical analysis was done as described in Experiment 2.

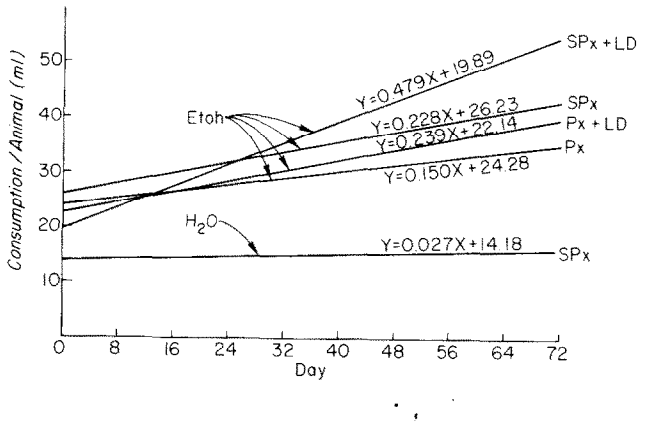

FIG. 1. Line of best-fit of daily consumption of alcohol or water by hamsters. Each line indicates the line of best fit as determined by linear regression of either alcohol (5% v/v) or water given to hamsters for 72 days. SPx=sham pinealectomy; Px=pinealectomy; LD=light deprived. Equation indicates slope and y-intercept of each line.

RESULTS

Experiment 1

In a single choice situation, hamsters given an alcohol solution drank more fluid per day than the hamsters given water. From day 1 to day 72, the hamsters given alcohol drank increasingly more alcohol daily regardless of treatment. Figure 1 indicates the milliliters of alcohol consumed per day per animal in each group throughout the 72-day period as established by the line of best fit derived by linear regression. SPx-LD hamsters increasingly consumed the greatest amounts of alcohol solution ($r=0.945$, $p<0.001$). SPx-visually intact hamsters showed the next greatest increase in the propensity to drink alcohol ($r=0.812$, $p<0.001$), followed by Px-LD hamsters ($r=0.867$, $p<0.001$). Px-visually intact animals exhibited the lowest propensity to drink the alcohol solution ($r=0.760$, $p<0.001$).

The average total alcohol or water consumption per day per animal for the 72 days are shown for each group in Fig. 2. SPx-LD hamsters drank the most alcohol (38 ± 2 ml/

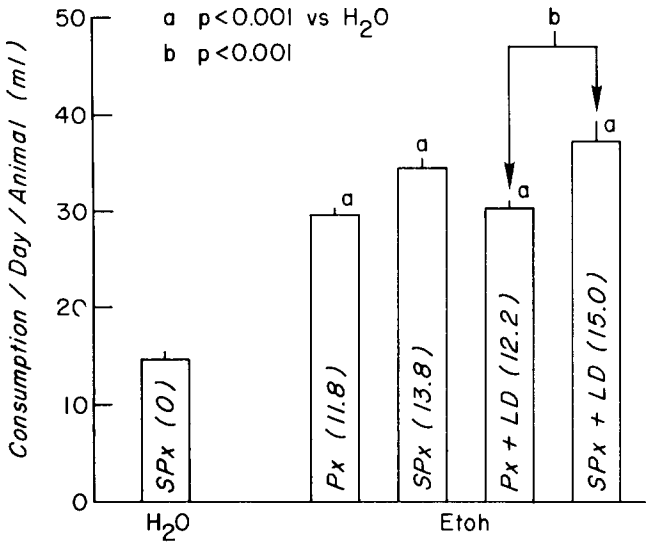

FIG. 2. Average daily consumption of alcohol or water by hamsters. Each bar represents the average daily fluid consumed by each hamster over a 72-day period. The vertical line indicates the S.E.M.. The number in parentheses indicates the average daily intake of alcohol represented as grams of EtOH over the 72-day period. SPx=sham pinealectomy; Px=pinealectomy; LD=light deprivation.

animal/day; 15 gm EtOH/kg b.w./day) followed by SPx-visually intact animals (35 ± 1 ml/animal/day; 13.8 gm EtOH/kg b.w./day, $p<0.05$). Px hamsters drank less alcohol than SPx animals regardless of whether they were LD or visually intact (31 ± 1 ml/animal/day; 12.2 gm EtOH/kg b.w./day, and 30 ± 1 ml/animal/day; 11.8 gm EtOH/kg b.w./day), respectively. Px decreased alcohol consumption by LD hamsters when compared to that by SPx light deprived animals, $F(4,165)=64.3$, $p<0.001$. However, all hamsters that were given alcohol to drink consumed significantly more fluid (>30 ml/animal/day) than those that were given water (15 ± 1 ml/animal/day, $F(4,165)=64.3$, $p<0.001$. Serum alcohol levels in animals in all groups were relatively low (<60 mg%) being greater in animals which were allowed alcohol to drink (40–60 mg%) than in animals receiving only water to drink (15 mg%).

Experiment 2

Hamsters given a two-choice, three-bottle situation, drank significantly more alcohol than water (Fig. 3). Hamsters that were maintained in 14 hr of light daily drank an average of 90.5% of their total average fluid per day as alcohol. Animals that were given daily injections of peanut oil consumed 88.3% of their total daily fluid as alcohol. The average daily intake of alcohol in vehicle injected animals (16.9 ± 0.5

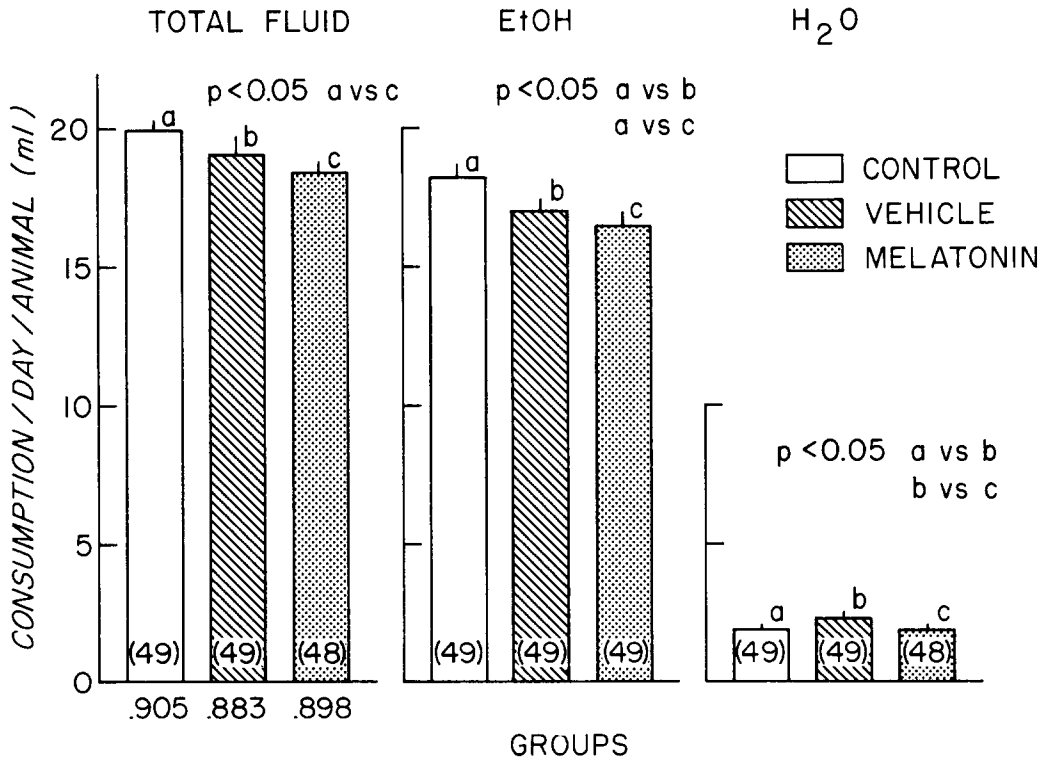

FIG. 3. Average daily consumption of fluids during daily melatonin administration by subcutaneous injections. Resultant data are grouped according to the fluids consumed and analyzed with respect to type of treatment. Vertical bars indicate the average daily consumption of respective fluids by hamsters for 77 days. Animals were given daily subcutaneous injections (25 μg) of melatonin. All animals were maintained in a long photoperiod. Numbers in parentheses indicate the number of observations in each group. The numerical values located below the horizontal axis indicate the ratio of the average amount of alcohol consumed relative to the total average amount of fluids consumed per day per animal over the total time period. Total Fluid=average daily alcohol consumed plus the average daily water consumed; EtOH=average daily alcohol consumed; H_2O=average daily water consumed.

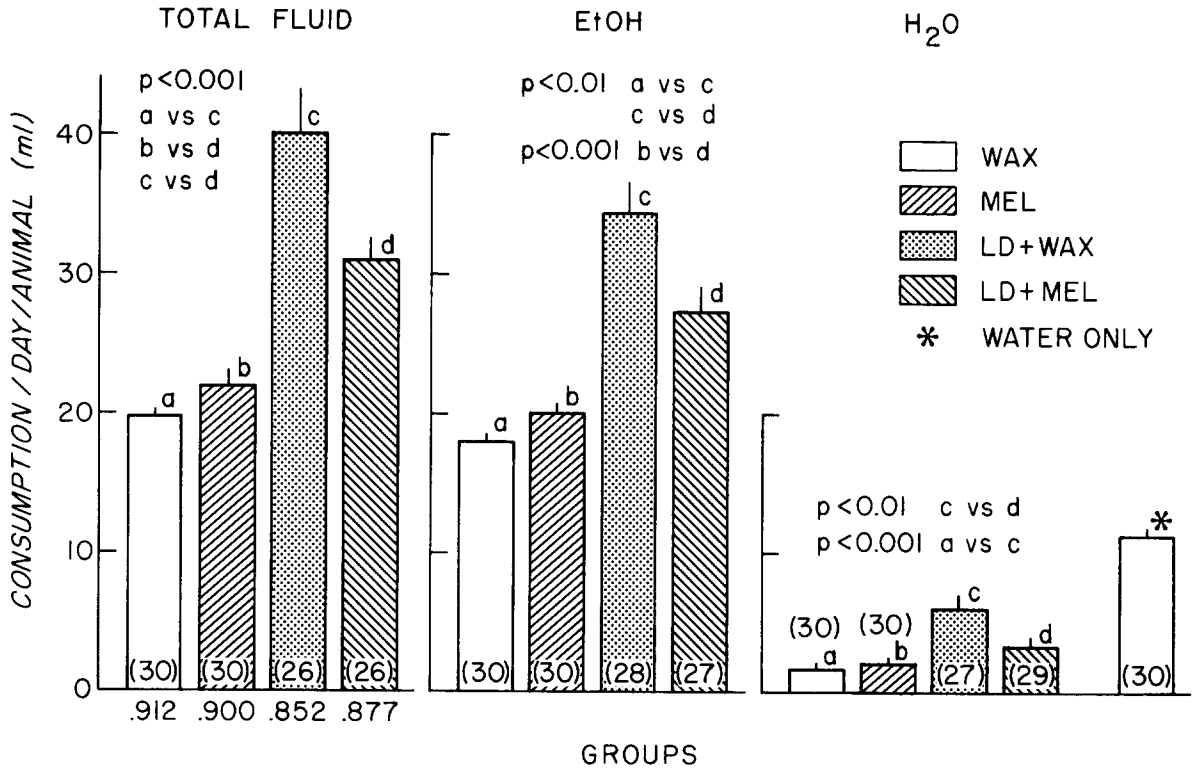

FIG. 4. Average daily consumption of fluids during continuous melatonin administration by subcutaneous deposits. Resultant data are grouped according to the types of fluids consumed and analyzed with respect to type of treatment. Vertical bars indicate the average daily consumption of respective fluids by hamsters for 6 weeks. All animals were maintained in a long photoperiod (14 hours) but some animals were surgically blinded (light deprived, LD). Numbers in parentheses indicate the number of observations in each group. The numerical values located below the horizontal axis indicate the ratio of the average amount of alcohol consumed relative to the total average amount of fluids consumed per day per animal over the total time period. Total Fluid=average daily alcohol consumed plus the average daily water consumed; EtOH=average daily alcohol consumed; H_2O=average daily water consumed; MEL=melatonin (1 mg per animal per week in subcutaneous beeswax depot); WAX=beeswax implant; LD=light-deprivation by surgical blinding; *=group received water only, no alcohol was introduced.

ml) was significantly less than that of animals not receiving a daily injection (18.2 ± 0.5 ml, $F(2,143)=3.91$, $p<0.05$) suggesting a possible effect of the vehicle. Animals that received daily melatonin injections consumed less total fluid, $F(2,143)=2.52$, $p<0.05$, and less alcohol, $F(2,143)=3.91$, $p<0.05$, than did the animals not receiving an injection. However, melatonin treated animals continued to drink approximately 90% of their total average daily fluid intake as alcohol.

Experiment 3

Hamsters allowed a choice between drinking an alcohol solution or water showed a clear preference for the alcohol solution, consuming 90% or more of their total daily fluid intake as an alcohol solution (Fig. 4).

In visually intact animals, a subcutaneous beeswax implant containing 1 mg melatonin had no significant effect on the total daily fluid intake when compared to that of hamsters that received the subcutaneous implant without melatonin. The ratio of alcohol to total fluid consumed remained at about 90% under both conditions.

When hamsters were light deprived (LD) average total daily consumption of fluids nearly doubled, $F(3,108)=34.0$, $p<0.001$. Interestingly, LD hamsters that received only the beeswax implant consumed 85% of their total daily fluid as alcohol. These data indicate that the LD animals drank greater amounts of alcohol than animals that were not light deprived. However, the LD animals consumed 6% less of the alcohol solution when compared to the ethanol: total daily fluid intake in animals that were not light deprived (LD, 85% EtOH; intact, 91% EtOH). In a comparison of fluids consumed by light deprived animals which received only beeswax implants to that of light deprived melatonin treated hamsters, there is a significant decrease in total fluids consumed, $F(3,108)=34.0$, $p<0.001$, alcohol consumed, $F(3,111)=24.2$, $p<0.01$, and water consumed, $F(3,112)=11.6$, $p<0.001$, in light deprived melatonin-treated hamsters. However, the ratio of alcohol consumed relative to total daily fluid intake increased to 88% in light deprived melatonin treated hamsters approaching the ratio exhibited by animals that were visually intact.

DISCUSSION

Ethanol intake and preferences demonstrated in this report for the male golden hamster are in agreement with val-

ues previously reported [1, 2, 7, 9, 10, 11, 13]. A high ethanol intake (13.8 gm alcohol/kg body weight/day/animal) and preference (91% of total daily fluid intake as alcohol solution) for the alcohol solution allows the hamster to be a model to study parameters which may reduce either alcohol consumption or preference. It was demonstrated in a former study that rats [4] and hamsters [5] would drink more of an alcohol solution when the animals were maintained in a limited light period. The authors suggested that the pineal gland was involved in mediating this behavioral response. The hamster, a more photosensitive animal, was used to investigate the effects of the pineal gland and photoperiod on alcohol consumption [5,13]. Light deprivation of hamsters resulted in an enhancement of alcohol consumption and, indeed, pinealectomy decreased the light deprivation-induced alcohol consumption. However, administration of melatonin, a pineal hormone, did not alter alcohol consumption [13]. The experiments reported herein, confirm the enhancement of alcohol consumption by light deprivation of hamsters, and the resultant attenuation of this consumption by pinealectomy. These data also indicate that chronic melatonin administration to light deprived hamsters results in reducing alcohol intake by the animals. Under conditions of the experiments described herein (Experiment 2) daily melatonin administration was without effect in further inducing alcohol consumption by hamsters as was observed in a previous investigation [13]. It is possible that in the hamster, alcohol consumption cannot be further enhanced by melatonin since the animal is already consuming large amounts of the solution. In contrast, in a species such as the rat which does not exhibit the same propensity to drink alcohol in preference to water, the administration of melatonin to rats has been shown to actually increase alcohol consumption [4]. The reason for the apparent discrepancy between this report and the enhancement of alcohol intake by the rat after melatonin administration is unknown. Since there is a wide variation of preference for alcohol between these species [1], a plausible explanation of the efficacy of melatonin to enhance alcohol consumption by the rat but to reduce intake by the hamster could be a result of species difference.

Under a free choice situation, melatonin administration by daily subcutaneous injections neither enhanced nor attenuated the preference the animals exhibited for alcohol when maintained in conditions of long periods of light. Daily melatonin administration reduced the alcohol and water consumed without significantly altering the ratio of the alcohol consumed to total fluid consumed (Fig. 3). This suggests an effect of melatonin to reduce the total amount of daily fluid intake, including the intake of alcohol.

This relationship between the effects of melatonin administration and the amount of fluids consumed is further demonstrated under conditions of enhanced alcohol consumption and the continuous availability of melatonin by a subcutaneous deposit. Light-deprived hamsters drank 107% more fluid daily than animals maintained in a long photoperiod (Fig. 4). Even though the daily total fluid consumption in light-deprived hamsters was increased, there was a decrease of consumption in the proportionate amount of alcohol solution such that animals that would drink 91% of their daily fluids as alcohol under normal conditions, consumed only 85% of their daily fluids as alcohol. Melatonin administration through the use of a subcutaneous depot not only decreased the amount of fluid consumed, reducing fluid intake by 25%, but restored the proportion of alcohol consumed to 88% of the total daily fluids.

These data are supportive of a hypothesis that the pineal gland and at least one pineal hormone, melatonin, may be involved in regulation of fluid consumption. Another investigator found that melatonin had no affect on water intake [6], but the data presented herein suggest that in regulation of fluid intake, both water and alcohol may be altered according to the animal's needs and that this regulation may be a function of the photoperiod, the pineal gland and/or melatonin. Presumably, the effects of photoperiod on alcohol intake in this species is not necessarily the result of modifying the animals predilection for alcohol, but the amount of alcohol consumed maybe a result of changes which alter total fluid intake by the animal.

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