ether. Because the free leucine concentration increases, the ratio q_p/q_l, which does not take into account the decrease of precursor specific activity, is not indicative of the rate of protein synthesis. Use of this ratio alone gives overestimates of the magnitude and duration of the ES inhibition of protein synthesis. The largest changes in q_p/q_l were observed for groups in which q_p/q_l was measured immediately after ES (no ether, 12-ma and ether, 30 ma). After correction for free leucine the differences became smaller but remained significant. The significant decrease in q_p/q_l found at 10 minutes after ES in mice given 30-ma ES after ether treatment is due to dilution of the specific activity of the precursor and does not reflect a real change in the rate of protein synthesis. The significance of the decrease in q_p/q_l in the 30-ma group at 30 minutes is difficult to interpret.

These findings indicate that ES administered at an intensity sufficient to produce brain seizures (and RA as indicated by previous findings) has two effects: it produces a significant but short-lasting inhibition of brain protein synthesis and it increases the amount of free leucine in the brain. These results are consistent with other evidence suggesting that ES may produce a short-lasting inhibition of protein synthesis in the brain (*13*). In addition, the increases in free leucine might indicate an increase in protein catabolism at early times.

The most important finding in this study is that the inhibition of protein synthesis is not produced by the passage of current per se. Inhibition was produced only at ES levels above that necessary for eliciting brain seizures. Thus, it appears that the ES effects which produce brain seizures also result in an inhibition of brain protein synthesis. In view of our earlier finding that memory losses (RA) are produced by ES only if the current is at or above the brain seizure threshold intensity, it appears that an inhibition of brain protein synthesis is also found with ES levels which produce RA. It should be noted, however, that the degree of inhibition of protein synthesis produced by ES in this study (a maximum of 24 percent) is considerably less and of shorter duration than that produced by antibiotic drugs such as cycloheximide (*14*). In general, the degree of RA produced by antibiotic drugs is less than that produced by ES. Thus, it does not seem likely that the amnesia produced by ES is due to inhibitiion of protein synthesis per se. Rather, it seems more likely that brain seizures and inhibition of protein synthesis are signs that the coordinated function of the cells of the brain is altered to such a degree that the processes involved in memory storage are disrupted.

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$$K_{1p} = \frac{Q_p \, da_p/dt}{a_1 - a_p}$$

where, for the case under consideration, K_{1p} is the rate of protein synthesis, a_p and a_1 are the specific activities of the protein and precursor, respectively, and Q_p is the total quantity of protein present. We have previously shown (*8*) that under similar conditions the increase in a_p is linear with time during the first 8 minutes after the administration of precursor and that a_p is negligible as compared to a_1. In this case

$$da_p/dt = ca_p$$

and

$$K_{1p} = \frac{cQ_p a_p}{a_1}$$

where c is a constant. In a pilot study it was verified that Q_p remains constant after ES so that

$$K_{1p} = c' a_p/a_1$$

where $c' = cQ_p$. The use of these quantities as a measurement of the rate of protein synthesis is based on the assumption that precursor and product compartments are homogeneous. When a variety of products are made at different rates, the measured rate becomes less accurate with increasing incorporation time [C. W. Sheppard and A. S. Householder, *J. Appl. Phys.* **22**, 510 (1951)]. Although this seems to be the case for brain proteins [A. Lajtha, *J. Neurochem.* **3**, 358 (1959)], measurement of the rate over a short, constant incorporation time should allow meaningful comparison of control and ES-treated animals.
10. Control animals were etherized and treated in the same manner as animals in the other groups but did not receive ES. The incorporation of amino acid into protein was measured immediately after and 2 hours after ether treatment. The two control groups were identical. Further, S. Roberts and B. S. Morelos [*J. Neurochem.* **12**, 373 (1965)] have reported that ether does not affect brain protein synthesis. In view of this finding, a more detailed analysis of the effect of ether was not undertaken.
11. At 120 minutes after ES (12 ma with ether) a small increase was seen which was statistically significant ($P < .01$). This effect appears to represent a delayed stimulation of the rate of protein synthesis, although alterations of the precursor pool cannot be ruled out.
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14. The percentage of inhibition may not represent maximum inhibition since a 4-minute pulse was used and in the "immediate" group the ES was given 2 minutes after the injection of the isotope.
15. We thank C. Green, K. Domoto, and B. Longacre for technical assistance. Supported by research grants MH 12526, training grant MH 11095-04, and predoctoral fellowships MH 36372-03 and MH 50166-01 from the National Institute of Mental Health; research grant NS 08597 and biomedical sciences support grant RR-07008-05 from the National Institutes of Health; and research grant NSF-GB-14491 from the National Science Foundation.
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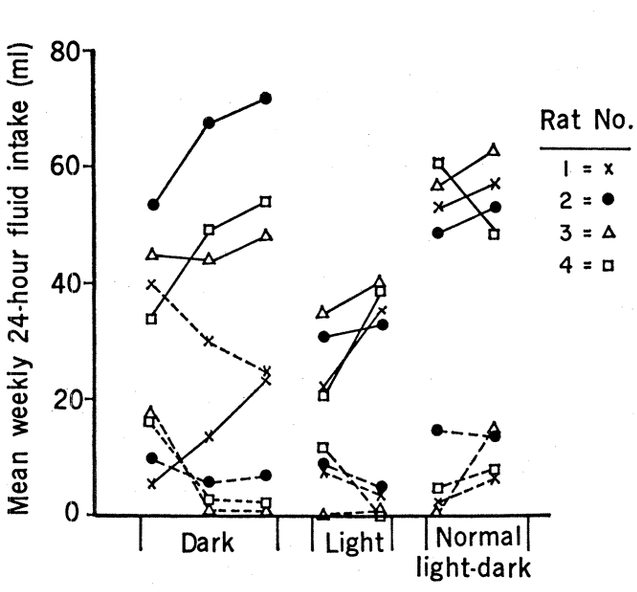
10 March 1971; revised 17 May 1971

Ethanol Preference in the Rat as a Function of Photoperiod

Abstract. *Ethanol-drinking was induced in laboratory rats that were maintained in total darkness. The established preference for ethanol was not reversed under conditions of constant illumination although a decrease in ethanol intake occurred. Administration of melatonin to rats maintained under "normal" photoperiods (9 hours of darkness during a 24-hour day) also induced ethanol-drinking.*

The search for an experimental model of alcoholism has generated considerable research dealing with those variables that are critical for establishing ethanol preference or self-selection of ethanol by laboratory animals. Richter and Campbell (*1*) accomplished this through a gradual day-to-day increase of the percentage concentrations of ethanol offered their rats. Other methods for inducing ethanol preference in laboratory rats include caloric restriction (*2*), intraventricular administration of minute quantities of ethanol (*3*), and exposure of animals to ethanol as their sole drinking fluid over a period of time (*4*). Induction of ethanol preference also has been attempted by subjecting animals to stressful or anxiety-provoking situations (*5*). The present investigation evolved from an unsuccess-



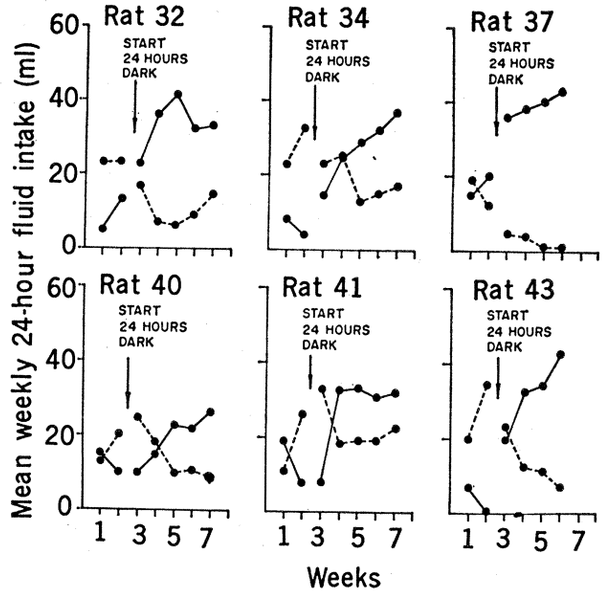


Fig. 1 (left). Ethanol intake in rats as a function of variations in photoperiods. Solid lines show ethanol intake; broken lines, water intake. Fig. 2 (right). Ethanol intake in rats as a function of photoperiods. Solid lines show ethanol intake; broken lines, water intake.

ful attempt to induce home-cage drinking of ethanol in four laboratory rats trained in a conflict-behavior paradigm (*6*). Since conflict behavior is selectively affected by clinically active "anti-anxiety" agents (*7*), it is presumed to involve "anxiety." During a 14-week course of daily conflict-behavior sessions, water and a 4 percent solution of ethanol were available to four rats in their home cages at all times. Amounts of ethanol consumed were recorded on each week day. Readings taken on Tuesday through Friday represented amounts of ethanol the rats drank during 24-hour periods, while Monday readings showed the amounts they drank during the weekend.

The data in part (a) of Table 1 were drawn from the last 5 weeks of the 14-week period. Average ethanol intake during the weekend was always at least twice the intake during any 24-hour weekday. Since the laboratory was in complete darkness from Friday afternoon until Monday morning, a subsequent control experiment was conducted in which normal photoperiods were maintained in the laboratory. The data in part (b) of Table 1, averaged for four rats, show that ethanol intake did not increase during weekends when the laboratory was illuminated for 9 hours of each 24-hour day. These findings suggested that increased ethanol intake might be induced by darkness and provided the basis for the following study.

Four male Sprague-Dawley rats, 75 days old, were housed individually in cages 9 by 15 by 18 inches (Wahmann LC-28) (1 inch = 2.54 cm). They were kept in a laboratory with ambient temperatures of 21° to 24°C and were maintained on an unrestricted diet of Wayne Lab Blox. Water or a 4 percent ethanol solution were available at all times in 100-ml, Kimax drinking tubes mounted on the back or on either side of the cages so that the drinking spouts protruded into the cages approximately 1¼ inches above floor level. The two-choice, three-bottle method as previously described (*8*) was used to prevent the rats from selecting a fluid based on a position preference. The cages contained a tube of water, a tube of ethanol, and an empty tube. At 10:30 each morning the experimenter recorded the amounts of fluids consumed during the preceding 24 hours. The drinking tubes were washed, refilled, and put back on the cages, and their positions were rotated randomly from day to day.

For the first 2½ weeks of the experiment, the laboratory was maintained in total darkness except for the short period of time required to take readings and fill drinking tubes. During the next 2 weeks the laboratory was illuminated constantly, while during the final 2 weeks the lights were programed through a timer so that the rats lived in a "normal" light-dark cycle consisting of 9 hours of darkness during each 24-hour day. Figure 1 shows the 24-hour intake of fluids, averaged for weekly periods under each photoperiod condition (*9*).

Three of the rats drank excessive amounts of ethanol solution during the first week in darkness. During the next 1½ weeks intake of ethanol increased progressively in all four rats. Switching from total darkness to constant illumination in the laboratory resulted in a drop in ethanol intake, while water intake remained at the same low levels. When the rats were maintained in a "normal" light-dark photoperiod, ethanol intake again increased to levels approximating those obtained for three of the rats in total darkness.

Since these rats were not tested first during a "normal" light-dark photoperiod, one might argue that a high preference for ethanol would have occurred initially irrespective of ambient lighting conditions. Therefore, six male Sprague-Dawley rats, 75 days old, that showed no ethanol preference during a 2-week period in a "normal" light-dark environment, were placed in total darkness. A precipitous increase in ethanol intake was observed for all rats. Data for the individual animals are shown in Fig. 2. During the first 2 weeks, under normal photoperiod conditions, ethanol intake was generally less than water intake. Placing the rats in an environment of total darkness resulted in a progressive increase in ethanol intake during a 4- or 5-week period. Increased ethanol intake was accompanied by a concomitant decrease in water intake.

It has been reported that rats kept in darkness have larger pineal glands than rats kept in constant illumination and that the activity of the pineal melatonin-forming enzyme, hydroxy-*O*-methyl transferase (HIOMT) is increased (*10*). The HIOMT activity is also subject to diurnal variations in the monkey, the highest activity occurring during the period of darkness (*11*). The possibility that darkness-induced ethanol preference might be related in some way to increased HIOMT activity and melatonin formation provided the basis for a further experiment. Two male Sprague-Dawley rats that showed no ethanol preference when maintained in a "normal" light-dark environment were given prolonged treatment with melatonin. Results of this experiment are shown in Fig. 3. Rat No. 58 received daily subcutaneous administrations of 1 mg of melatonin per kilogram of body weight during a 2-week period, while for rat No. 48 the doses were increased progressively from 0.2 to 1.5 mg/kg during a 4-week period. The data clearly show that melatonin effectively increased ethanol intake and decreased water intake in these rats.

Although the findings are derived from only two rats, they strongly suggest an involvement of melatonin in the induction of ethanol drinking by laboratory rats. A plausible speculation to account for these data may be built on the findings of previous investigators. Davis and Walsh (*12*) and Cohen and Collins (*13*) described in vitro studies in which tetrahydroisoquinoline alkaloids were formed from condensation reactions between monoamines and aldehydes. They postulated that the formation of such compounds in vivo could account for the "process of physical dependence and addiction in alcoholism." McIsaac (*14*) reported on the formation of a similar compound by an in vitro reaction of 5-methoxytryptamine and acetaldehyde. The compound, 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline, was also detected in the urine of rats treated with 5-methoxytryptamine and acetaldehyde in conjunction with their respective metabolic inhibitors, iproniazid and disulfiram.

The formation of a similar alkaloid resulting from a darkness-induced melatonin increase and acetaldehyde could account for the increased ethanol intake of rats kept in a dark environment. Support for this conjecture might be gained through experimental manipulation of pineal melatonin or acetaldehyde levels to increase or decrease ethanol intake accordingly. Preparation of 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline for administration to ethanol-drinking and nondrinking rats should also be of value in the further elucidation of the darkness-induced drinking phenomenon.

Blocking of the pineal response to darkness by interruption of sympathetic impulses to the pineal has been accomplished through bilateral cervical sympathetic ganglionectomy (*15*). Similar surgical procedures or pinealectomy might also produce a nondrinking rat. Replacement therapy with exogenous administration of melatonin and subsequent increase in ethanol drinking would implicate further the role of the pineal for the induction of ethanol preference in animals.

The problems involved in establishing a true animal model for alcoholism (*16*) make it difficult to determine whether the results of this study are applicable to the human situation. Nevertheless, the new avenues of research endeavor which evolve from these findings should increase the sphere of knowledge regarding ethanol intake by laboratory animals. It is only through such animal studies that one can hope to attain a clearer understanding and perhaps an ultimate treatment or cure, or both, for alcoholism in humans.

Table 1. Ethanol intake in milliliters.

Day of the week				
Mon.	Tues.	Wed.	Thurs.	Fri.
(a) Total darkness during weekends (N = 4)				
20	3	3	5	4
14	3	3	3	3
12	3	3	2	2
16	4	3	2	5
21	4	5	4	3
(b) Normal light-dark photoperiod during weekends (N = 4)				
			1	1
0	0	0	0	1
2	3	0	5	1
0	3	6	2	5
4				

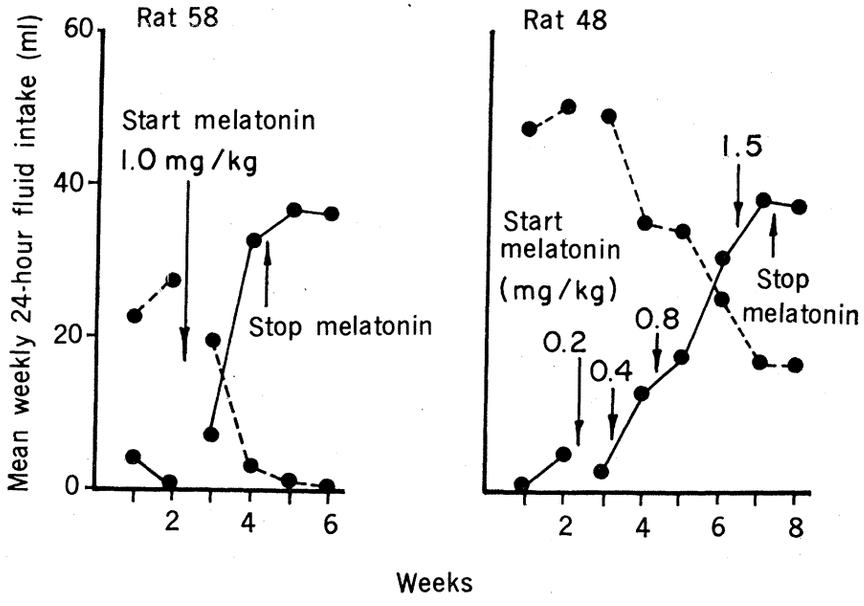


Fig. 3. Effects of melatonin on ethanol intake in rats. Solid lines show ethanol intake; broken lines, water intake.

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6 May 1971

Celestial Rotation and Stellar Orientation in Migratory Warblers

In his study of the ontogenetic development of stellar orientation in indigo buntings, Emlen (*1*) develops the hypothesis that fledgling buntings react to the apparent rotational motion of the night sky. By learning the birds establish a reference system for using certain stars or star patterns to guide their nocturnal migrations. Subsequently, through the correlation of stellar and rotational information, experienced birds can locate the polar axis from the specific star patterns alone, so "that celestial motion per se should become a secondary or redundant orientational cue for adult birds."

The results of Emlen's studies seem to support his view that early visual experience is an important factor in the development of stellar orientation in indigo buntings. In support of his hypothesis Emlen cites the accurate orientation of our caged Old World warblers of the family Sylviidae when they were tested under stationary planetarium skies (*2*). Although the migratory orientation of our captured adult warblers, exposed to the stationary sky of a 6-m Zeiss planetarium, may support Emlen's hypothesis, there are notable exceptions. The spontaneously precise and species-specific reactions of inexperienced juvenile and adult migrants, as well as those of birds with very limited exposure to the natural sky, suggest that the warblers utilize a different system of stellar orientation under the same experimental conditions and even under the natural starry sky.

Garden warblers (*Sylvia borin*), blackcaps (*S. atricapilla*), and lesser whitethroats (*S. curruca*) were optically isolated in diffusely lit chambers from the date of hatching, and from the 9th, 14th, and later days of their life. The birds were prevented from viewing the sky until their first orientation tests during their first or later migratory periods. These birds then chose their specific migratory directions within minutes of their first exposure to the natural starry sky [figure 3 in (*2*)] or to the stationary planetarium sky [for example, figure 13a in (*2*)]. These birds were just as reliably and lastingly oriented as their counterparts in the wild who were familiar with the natural sky during day and night and were experienced with previous migrations. Particularly impressive are the spontaneous responses, for example, of the garden warbler kept isolated since hatching and of the lesser whitethroat kept optically isolated for more than 1 year from the 14th day of its life (before it had begun any premigratory wanderings and night activity) until its second autumnal migratory period.

It seems hardly conceivable that the sylviid warblers were able to deduce and establish a knowledge of the location and angular position of the polar axis within a few minutes after their first confrontation with the natural or artificial starry skies. The inexperienced birds were accurately oriented under the stationary planetarium sky even when forced to accept an unnatural zenith (*2–4*); since the azimuth and altitude positions varied from experiment to experiment, this suggests that, in the absence of any visible rotational axis, the birds must have related the key stars or star patterns to a fixed axis, plain, or point in space whose identity is not yet known (*3*).

When the birds were shown a stationary planetarium sky for a length of time, they began to shift their preferred direction of orientation slowly but steadily around the compass opposite to the apparent direction of rotation of the natural night sky. The fact that the visually oriented birds "invented" compensatory angular movements of approximately 15 degrees per hour, thereby "inducing" an otherwise nonexistent motion of the fixed starry sky, strongly suggested the existence of a primary reference system established by other than visual cues. This should not exclude the possibility that the birds may visually recognize the polar axis if they can view a rotating starry sky for a certain time.

Polaris and the circumpolar region of the night sky, seemingly of such importance to indigo buntings (*5*), proved of little significance for the warblers. They made and maintained their precise directional choices when this section of the starry sky was experimentally eliminated in the planetarium or was covered by clouds in nature.

If one accepts the buntings' system of stellar orientation, and for that matter that of the Sylviidae too, the birds' knowledge or experience of the canopy of stars is not sufficient of itself to account for their specifically and seasonally distinct and varying choices of migratory directions. The spontaneously accurate responses of the sylviid warblers without training and experience, their selection of compass bearings in line with the migratory pathways of their species, their upset and compensatory behavior under temporally maladjusted planetarium skies (*2–4*), and their quick adjustments to simulated north-south displacements under latitudinal shifts of the planetarium skies (*2*) provide strong evidence for a genetic substrate. This determines, in the first place, the birds' ability to navigate by the stars and to project their orientation along the lines of specific routes. On the basis of this substrate, learning by association or by imprinting (*6*) can yield new efficiencies and safety mechanisms for their migratory flights. We do not know by what genetically predetermined means the inexperienced Old World warblers are able spontaneously to choose and to follow the long-established migratory routes of their respective species, guided by the starry sky. Contrary to the opinion expressed by Emlen (*1*), we never meant to propose that the young warblers possessed a "genetically predetermined star map." We rather stressed the importance of vision and visual configurational qualities in the warblers' star orientation, their reliance on a grid of two celestial coordinates and on an internal chronometer. We pointed out the enormous variability, qualitatively and quantitatively, in stellar information that enabled the birds to take their bearings (*2*). We also stressed the limitations of our planetarium experiments and of our study, which dealt with only one of the factors that govern the nocturnal flights of these birds. That the inexperienced warblers responded spontaneously to the canopy of stars requires no genetic blueprint of a star map. With the exception of the warblers' probable configurational perceptive