depth of modulation was increased until a threshold criterion was reached (*12*). At the best rate of AM, threshold was reached at the smallest depth of modulation; at lower or higher rates, the depth of modulation had to be increased in order to maintain a threshold level of response (*13*). In some cases, units sharply tuned to a particular rate of AM were entirely unresponsive to pure tones. (Rise and fall times were varied from 10 to 50 msec.)

Since the r.m.s. sound pressure level and spectral content of amplitude-modulated white noise remained constant as the rate of modulation was varied, the differential selectivity of auditory units in the torus is a function only of the temporal information in the stimulus (the rate at which the amplitude is modulated over time). This selectivity was not related in any direct way to a unit's best excitatory frequency to tones. Our finding that some AM tuned units failed to respond to pure tones supports the notion that the response of higher order auditory neurons to complex stimuli often cannot be predicted on the basis of their responses to pure tones alone (*14*).

Our results indicate that a transformation occurs in the manner in which the rate of AM is coded in the anuran's peripheral and central nervous systems. Unlike auditory nerve fibers, many central auditory neurons respond selectively to particular rates of AM. Further studies should reveal at what level of the auditory system this selectivity first develops. Similar transformations may underlie the encoding of other forms of temporal information in acoustic signals and in other sensory systems.

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1. A sound may vary in frequency or amplitude over time. That is, the sound may contain frequency modulation or amplitude modulation (AM), respectively.
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6. Analogous to frequency selectivity, where a neuron responds best to a particular tonal frequency or frequency band, a temporally selective unit responds best when the rate of AM is of a certain value or values.
7. G. A. Miller and W. G. Taylor *J. Acoust. Soc. Am.* **20**, 171 (1948). However, in a short-term spectral analysis of AM white noise, an energy peak can occur at the frequency (rate) of AM. To control for this deviation from the theoretical long-term analysis, the AM stimulus was filtered through a high-pass filter so that any energy at the modulating frequency was at least 20 dB below the spectrum level of the signal (this procedure did not alter the gross temporal structure of the AM signal).
8. The stimulus was delivered through an earphone (Permoflux PDR-10) housed in a T-shaped brass coupler. A condenser microphone (Bruel and Kjaer 4134) was inserted in one end of the T. A tapered rubber fitting, having a tip diameter slightly larger than the outer margin of the tympanic ring, was located on the other end of the coupler. The end of this fitting was placed close to but not touching the tympanum, and then sealed around the tympanic ring with silicone grease. In this manner a closed acoustic system (± 5 dB over the range 50 to 4000 Hz) was achieved without any direct mechanical connection to the eardrum. All distortion products were at least 46 dB below the level of the primary tone.
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10. Since most toral units had little, if any, spontaneous activity, threshold was defined as the sound pressure level required to elicit a response on at least 80 percent of the trials. For units having spontaneous activity greater than 2 spikes per second, threshold was defined as the sound pressure level at which the activity of the unit was 50 percent above that of spontaneous activity.
11. A midbrain auditory unit was classified as being tuned to a particular rate of AM only if the following criteria were met: (i) The response at the preferred rate of AM had to be at least twice as great as that to very low or very high rates of AM. (ii) The temporal selectivity of the unit could not be altered by high-pass filtering the AM signal at 200 Hz.
12. Our criterion for threshold of each unit (in spikes per second) was based on its firing rate to modulated white noise compared with that to unmodulated white noise:

$$\text{Threshold} = \frac{R_m - R_u}{4} + R_u$$

where R_m represents the response to white noise 100 percent amplitude modulated at the best rate of AM and R_u represents the response to unmodulated white noise.
13. The temporal selectivity of these units was invariant over at least a 30-dB range in sound pressure level.
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Free-Running Activity Rhythms in the Rat: Entrainment by Melatonin

Abstract. *The pineal gland hormone melatonin may play a role in synchronization of rat circadian rhythms. Free-running activity rhythms of the rat were entrained by a daily melatonin injection, with entrainment occurring when the onset of activity coincided with the time of daily injections. When injections were stopped, activity rhythms became free-running again. Thus in pharmacological experiments, the time of day of melatonin administration is crucial.*

For more than a decade, it has been suggested that the rodent's pineal gland is involved in entrainment of daily activity rhythms to abrupt shifts in the light-dark cycle. Pinealectomized young rats, hamsters, and feral mice entrain faster to reversals in the lighting schedule than rats subjected to a sham operation, whereas pinealectomized old rats are slower to entrain than controls (*1*). A complete inversion of lighting (180° shift) is not necessarily the optimum strategy for testing the rate of entrainment. When an 8-hour advance of the dark period is applied, the entrainment rate of adult rats, pinealectomized when young, is greatly enhanced (*2*). Because of methodological problems in many studies, alternative interpretations of findings have been suggested (*3*). We found that entrainment is much faster for pinealectomized adult rats than for controls after a 5-hour advance of the dark period (*4*). The mechanism by which the pineal facilitates entrainment is not known.

In our laboratory the effect on free-running activity rhythms of substances that are produced by or act upon the pineal have been investigated. Daily injections of arginine vasotocin, melanocyte-stimulating hormone, and the β-adrenergic blocker, pindolol, did not entrain free-running activity rhythms. Some interference with the expression of the free-running rhythm was evident in the rats treated with arginine vasotocin. However, of particular interest was the finding that in three out of four rats the free-running activity rhythm was entrained by daily melatonin injections (*5*). We now report that daily administration of melatonin can entrain free-running circadian activity rhythms in the rat. This demonstration of entrainment of mammalian circadian rhythms by chemical means may provide a methodology for examining the process by which neural signals triggered by light are transduced into chemical messages that synchronize internal biological rhythms.

Twenty Long-Evans male rats (280 to 340 g) were housed individually in a five-

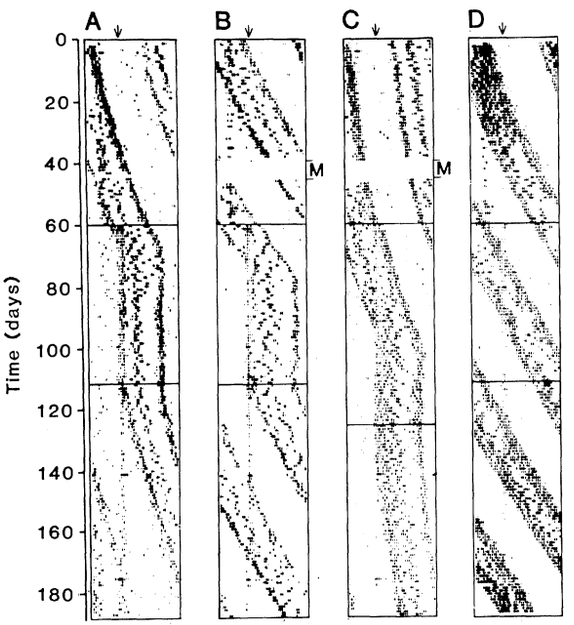

Fig. 1. The 24-hour activity plots of three rats receiving daily melatonin injections (A, B, and C) and one rat receiving daily injections of a control solution (D). Solid horizontal lines divide the records into preinjection (stage 1), injection (stage 2) and postinjection (stage 3) periods. Arrows indicate the time of injections in stage 2. *M*, missing data.

tiered rack in constant, dim red light. Powdered food and water were freely available (*6*). Rats were maintained under these conditions for 6 weeks until free-running activity rhythms were well established. Running-wheel activity was then monitored every 15 minutes by a minicomputer situated in an adjacent room, and a 60-day baseline period was established for each rat (stage 1). From day 61 ten rats were given daily subcutaneous injections of melatonin (1 mg per kilogram of body weight) (*7*), and control rats also received injections without melatonin (stage 2). Rats were injected in random order starting at 1530 hours, and the total time spent by the experimenter in the laboratory was approximately 20 minutes. Daily injections were continued for at least 52 days. Injections were discontinued once the entraining effect of melatonin was well established and it was evident that results were not due to the phenomenon of masking—that is, it was necessary to establish that the pacemaker itself has been entrained and not just the overt behavior (*8*). Therefore, in a number of cases the phase of the free-running rhythm before entrainment was extrapolated and care taken that melatonin injections were not terminated at a point at which the free-running rhythm would naturally reemerge. Injections of a similar number of control rats was discontinued at the same time. After daily injections were stopped, activity was monitored for at least 30 days (stage 3).

Activity rhythms of all ten rats treated with melatonin became entrained by the daily melatonin injections. In all cases entrainment did not occur until the onset of the active period of the free-running rhythm (established in stage 1) coincided with the time of injection (Fig. 1, A through C). Three rats entrained as soon as the injection was introduced, three rats took 7, 11, and 18 days, respectively, and four rats took much longer (29, 38, 39, and 44 days). Melatonin did not influence the activity rhythm until the timing of the injection closely approached onset of motor activity. This effect was established by periodogram analysis (*9*) in 14-day blocks of data from the four rats that took the longest time to entrain. For example, in the 14 days before melatonin was administered, data for the rat illustrated in Fig. 1C showed a statistically significant free-running period of 24.25 hours. This period continued for the first 28 days of stage 2. For the 14 days during which activity onset and injection time coincided, periodogram analysis revealed no single significant period. After this portion of the data until the end of stage 3, the period was 24.0 hours. When injections ceased the activity rhythms of eight rats became free-running almost immediately. The free-running period of the two other melatonin-treated rats took much longer to become established (Fig. 1C). In four rats it appeared that the duration of activity (α) increased once rats were entrained (Fig. 1B). However, close scrutiny of nonreduced plots reveals that this was not the case. Anticipatory activity, or activity induced by the experimenter entering the laboratory at the same time every day, gives an apparent extended duration of α. This induced activity could be seen to persist in stage 3 (Fig. 1, A and B).

Activity patterns of eight control rats were not affected by the injection treatment. Their activity rhythms were free-running throughout all three stages of the experiment (Fig. 1D). One control rat became entrained by the daily injection schedule, and a second showed signs of synchronization for a number of days before the activity rhythm became free-running again. It is not clear whether the one control rat that was entrained to the daily injection schedule became entrained to daily handling, the stress of daily injections, or the chemical content of the solution. This effect, however, emphasizes the need for a judicious use of controls.

Our results show that melatonin injections can influence the daily activity cycle of rats and that daily injections are followed by onset of activity. In contrast, injections of melatonin to starlings entrain them to the end of activity (*10*). Endogenous concentrations of pineal melatonin peak in the dark in both diurnally active birds and nocturnally active rodents (*11*). Thus it seems that melatonin injections mimic the effect of the onset of darkness in both species and that melatonin exerts its action on a timing mechanism controlling the switch from behavioral quiescence to activity or vice versa. It is feasible that the circadian variation in endogenous melatonin concentrations serves a similar function.

It appears that exogenous melatonin's entraining action is limited to a critical phase in the circadian cycle, an observation consistent with other research findings. These, taken together, strongly suggest that target organs are receptive to melatonin only at certain times of the day. Injected melatonin is effective in regressing hamster gonads only when administered late in the light period (*12*). In rats with constant estrous anovulatory syndrome induced by continuous light, late afternoon melatonin injections reestablish normal cyclicity. Melatonin injected daily from the commencement of the continuous light schedule prevents the development of this syndrome. Injected melatonin may act as a lights-off signal in maintaining ovarian cyclicity (*13*). Growth of tumors is stimulated by morning injections of melatonin but inhibited by late afternoon injections (*14*). Although the function of nocturnal release of melatonin in humans is not known (*15*), our results suggest strongly that effects of any pharmacological administration of melatonin in clinical trials will be dependent on the time of day.

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7. Melatonin (Sigma) was dissolved in 2 percent alcohol and 0.01*M* acetic acid, then brought to isotonicity by sodium chloride. The dosage (1 mg/kg), although high compared to endogenous levels in the pineal gland, was based on previous behavioral, biochemical, and entrainment studies employing similar dose levels [A. J. Kastin, M. C. Miller, L. Ferrell, A. V. Schally, *Physiol. Behav.* **10**, 399 (1972); P. C. Datta and M. G. King, *Pharmacol. Biochem. Behav.* **11**, 172 (1979); F. Anton-Tay, C. Chou, S. Anton-Tay, R. J. Wurtman, *Science* **162**, 277 (1968); Gwinner and Benzinger (*10*)]. Circulating melatonin is rapidly metabolized by the liver [I. J. Kopin, C. M. B. Pare, J. Axelrod, H. Weissbach, *Biochim. Biophys. Acta* **40**, 377 (1960)]. Because the effects of melatonin appear to depend upon its ability to reach certain brain areas, the systemic dose of melatonin required to produce a given effect may be much greater than the level of endogenous melatonin that normally produces this effect [F. Anton-Tay, *The Pineal Gland* (Churchill Livingstone, Edinburgh, 1971), pp. 213–227].
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Coping and the Stress-Induced Potentiation of Stimulant Stereotypy in the Rat

Abstract. *It has been shown that stressed rats display increased stereotypy in response to a subsequent amphetamine challenge. Evidence is presented showing that stress potentiates cocaine stereotypy as well. These effects of stress were found to be particular to stress that could not be controlled in that rats receiving an identical amount of stress from footshock, but allowed to control its duration, displayed no more stereotypy than did nonstressed rats. These findings have implications for the role of stress and coping in amphetamine and cocaine psychoses, endogenous psychoses, and some forms of schizophrenia.*

A psychosis brought on in humans by chronic consumption of large doses of amphetamine resembles paranoid schizophrenia (*1*), and both are blocked by neuroleptic treatment (*2*). Amphetamine and other stimulants elicit stereotyped behaviors in animals that increase gradually with chronic administration (*3*), are blocked by neuroleptic treatment (*4*), and may be analogous to behavior seen in amphetamine psychosis (*3*) and schizophrenia (*3, 5*). These findings indicated that these effects of amphetamine and other stimulants might serve as useful models of endogenous psychoses (*3, 5*), stimulant-induced psychoses (*3, 5, 6*), and some forms of schizophrenia (*5, 6*).

Stress can precipitate schizophrenic episodes (*7*) and reinstate amphetamine psychosis in abstinent individuals in remission (*8*), and it is capable of making rats more sensitive to the stereotype-inducing effects of amphetamine (*9*). Such sensitization to amphetamine seems to be general (footshock, food deprivation, and repeated sessions of mild tail pressure are all effective) (*9, 10, 11*) and may operate through the mesolimbic and mesocortical dopamine systems—systems implicated in schizophrenia (*12*) and amphetamine psychosis (*13*)—and not the nigrostriatal dopamine system (*10, 14*). Footshock has also been shown to alter dopamine turnover in the same two systems but not the nigrostriatal (*15*). Because of the similarity in the neurochemical effects of stress and stimulants, it was suggested that the psychotogenic effects of stimulants may be caused by their stress-mimicking effects (*16*). It has also been reported that repeated stress and a series of amphetamine injections cause the same degree of sensitization to amphetamine (*9*).

Although the sensitization to amphetamine caused by stress may be a useful animal model for the role of stress in stimulant-induced psychoses, endogenous psychoses, and some schizophrenias, not all high-risk patients develop schizophrenia when stressed (*17*). The ability to cope with stress may be a major factor mediating the interaction of stress and schizophrenia (*17–20*), a proposal supported by findings that schizophrenia patients in remission who use coping responses, such as social withdrawal, in stressful situations relapse less than those who do not (*21, 22*). We found that rats exposed to footshock become sensitized to both amphetamine and cocaine only if they cannot cope with or control the shock.

We studied the effects of stress on the class of amphetamine-induced stereotypy characterized by sniffing, rearing, and repetitive head movements for several reasons: (i) this class of stereotypy, unlike the biting, licking, and gnawing class, increases with repeated amphetamine administration (*23, 24*) and is thought to operate through the mesolimbic dopamine system (*25*); (ii) others studying the potentiation of amphetamine stereotypy induced by stress have measured this behavior (*9, 10, 11*); and (iii) scores assigned in a preliminary test by observers using a simple rating scale were correlated ($r = .736$, $P < .0001$) with the dose of amphetamine administered (*26*).

Pairs of rats received shocks on three consecutive days. Each session consisted of 180 2.5-mA scrambled grid shocks with 8 seconds between shocks. The rats of a pair received shock separately in identical shuttle boxes (34.5 by 20.5 by 19.5 cm). One rat received controllable shock (CS) and could terminate each shock, which was received by itself and its pairmate, by running through an archway cut in the barrier that divided the shuttle box in two. The rat receiving uncontrollable shock (UCS) had to depend on its CS partner to terminate the shocks. Shocks began at the same time for both rats and terminated for both either when the CS subject ran through the archway or automatically after 5 seconds of shock (< 1 percent of the trials). A third group of rats did not receive shocks.

Twenty-eight hours after the last treatment session the rats received injections intraperitoneally of 4.0 mg of *d*-amphetamine sulfate per kilogram of body weight, and stereotypic behavior was rated by an observer unaware of treatment group (*26*) (Fig. 1). Groups showed essentially no differences in weight at this time. Other rats, treated identically but injected with physiological saline, displayed no stereotypic behavior, as defined by either the amphetamine or the