Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain

L. E. SCHEVING, W. H. HARRISON, P. GORDON, AND J. E. PAULY

Departments of Anatomy and Pharmacology, Chicago Medical School, and Departments of Neurology and Biochemistry, Presbyterian-St. Luke's Hospital, University of Illinois College of Medicine, Chicago, and Department of Anatomy, Tulane University, New Orleans, Louisiana

SCHEVING, L. E., W. H. HARRISON, P. GORDON, AND J. E. PAULY. Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain. Am. J. Physiol. 214(1): 166-173. 1968.—Fluorometric measurements were made of serotonin, dopamine, and norepinephrine extracted from brains which were obtained from different subgroups of adult male rats killed at hourly intervals over three separate 24-hr periods. With a method involving sampling at hourly intervals, a synchronized circadian rhythm in serotonin with a crest extending from 1130 to 1730, followed by a trough between 2030 and 2130 was demonstrated in rats maintained on a 12-hr photoperiod (0600 to 1800 CST) followed by 12 hr of darkness. Although a circadian rhythm was not detected for dopamine and norepinephrine in the whole brain, the data did suggest that these two catecholamines are characterized by reproducible higher frequency, ultradian rhythms. The importance of these studies involving the biogenic amines, their specific relationship to the physiology of sleep, and their pertinence to neurochemical studies are discussed.

serotonin; 5-hydroxytryptamine; daily periodicity; diurnal rhythmicity; 24 hr rhythm; norepinephrine; adrenaline; epinephrine; noradrenaline; catecholamine

Many physiological variables in the rat demonstrate a circadian rhythm when measured or determined at frequent intervals over the 24-hr time scale. This especially is well documented in functions such as motor activity and body temperature because of their ease of assay; but many other rhythms in physiological variables have been demonstrated in the blood, liver, and other tissues. The designation of a function as circadian implies or means that a major crest of activity followed by a trough can be demonstrated during a period of approximately 12 hr of light followed by 12 hr of darkness. Consequently, one can predict approximately the local clock time at which the crest or trough or any other phase of the rhythm will occur under identical standardized conditions. If the light-dark cycle is discontinued and the animal is subjected to continuous light or darkness (or blinded), the rhythm of the physiological function measured now may have a frequency close to but different from 24 hr; consequently the peak and trough will gradually drift in relation to local clock time.

At present the mechanisms which underlie circadian rhythms are unknown; in fact, at the outset of this investigation, it had not been established, especially in brain, whether all physiological variables fluctuate with a typical circadian pattern or whether different components fluctuate with different frequencies depending upon their function. For example, the brain, which plays a unique role in the control of body processes, has not been explored with sufficient detail to justify a generalization concerning the daily rhythms of its variables. Except for studies of circadian rhythms in the rat pineal gland (17, 18, 24) and the report of a circadian rhythm in the human electroencephalogram (7, 8), there is only one report of a biochemically determined rhythm, that of 5-hydroxytryptamine (serotonin) concentration in the whole brain of mice (1).

In order to better characterize the rhythm phenomena of the brain and the relationship of the rhythms to the functional processes of the brain as well as to provide information essential to neurochemical studies involving the time variables, the daily rhythms of the important biogenic amines of the brain (serotonin, dopamine, and norepinephrine) were determined. The data which are presented in this report establish the circadian nature of the serotonin rhythm and the ultradian nature of the dopamine and norepinephrine rhythms. A discussion is included which relates these findings to the phenomenon of sleep and also evaluates their pertinence to neurochemical studies on biogenic amines.
were picked up by the microphone, amplified, and fed to a recorder which operated 24 hr/day.

Mean of 24-hr period......

TABLE 1. Biogenic amines measured in whole rat brain at hour intervals over a 24-hr period

<table>
<thead>
<tr>
<th>Hour</th>
<th>Exp 1—9/13/65</th>
<th>Exp 2—5/5/66</th>
<th>Exp 3—9/12/66</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dopamine</td>
<td>Norepinephrine</td>
<td>Serotonin</td>
</tr>
<tr>
<td>0630</td>
<td>0.04±0.09</td>
<td>0.23±0.02</td>
<td>0.67±0.02</td>
</tr>
<tr>
<td>0730</td>
<td>1.09±0.10</td>
<td>0.24±0.02</td>
<td>0.67±0.02</td>
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<tr>
<td>0830</td>
<td>1.12±0.06</td>
<td>0.24±0.02</td>
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<tr>
<td>0930</td>
<td>1.11±0.04</td>
<td>0.24±0.02</td>
<td>0.70±0.02</td>
</tr>
<tr>
<td>1030</td>
<td>1.00±0.06</td>
<td>0.23±0.01</td>
<td>0.71±0.03</td>
</tr>
<tr>
<td>1130</td>
<td>0.96±0.05</td>
<td>0.24±0.01</td>
<td>0.73±0.03</td>
</tr>
<tr>
<td>1230</td>
<td>0.99±0.05</td>
<td>0.23±0.02</td>
<td>0.71±0.02</td>
</tr>
<tr>
<td>1330</td>
<td>1.00±0.05</td>
<td>0.21±0.02</td>
<td>0.69±0.02</td>
</tr>
<tr>
<td>1430</td>
<td>1.01±0.05</td>
<td>0.21±0.01</td>
<td>0.71±0.02</td>
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<tr>
<td>1530</td>
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<td>0.71±0.03</td>
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<td>1630</td>
<td>0.93±0.05</td>
<td>0.22±0.01</td>
<td>0.72±0.02</td>
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<tr>
<td>1730</td>
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<td>0.70±0.02</td>
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<tr>
<td>1830</td>
<td>1.06±0.07</td>
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</tr>
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<tr>
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<td>0.62±0.01</td>
</tr>
<tr>
<td>2230</td>
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<td>0.66±0.02</td>
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</tr>
<tr>
<td>0530</td>
<td>0.97±0.06</td>
<td>0.23±0.02</td>
<td>0.67±0.07</td>
</tr>
</tbody>
</table>

Mean of 24-hr period plus SE

<table>
<thead>
<tr>
<th></th>
<th>Exp 1—9/13/65</th>
<th>Exp 2—5/5/66</th>
<th>Exp 3—9/12/66</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.02±0.06</td>
<td>0.24±0.02</td>
<td>0.68±0.02</td>
</tr>
</tbody>
</table>

Values are means ± se of the mean expressed as μg/g of brain and calculated as 2-hr moving averages.

MATERIALS AND METHODS

Male Sprague-Dawley rats averaging 280 g were used. Four weeks prior to each study, the animals were housed, two to a cage, in a light tight room maintained at 23 ± 2°C. The room was illuminated artificially from 0600 to 1800 hr (cst) and completely darkened from 1800 to 0600. Rockland rat chow and water were available ad lib. The room was entered only three times per week, Monday, Wednesday, and Friday at exactly 1400 for cage cleaning and replenishing of food and water.

A record of the daily motor activity of a typical colony maintained under the above mentioned conditions was obtained by placing a sensitive microphone in the room. Noises emanating from the colony through feeding, scratching on wire cages, running, vocalizing, or fighting were picked up by the microphone, amplified, and fed into a capacitor which, in turn, discharged every 2 sec. This discharge drove a galvanometer in a strip chart recorder which operated 24 hr/day.

On each of 3 experimental days (9/13/65, 5/5/66, and 9/19/66), subgroups of eight animals on the first experimental day and subgroups of six on the other 2 days were brought into a room located across the hall from their quarters for sacrifice every hour on the hour through each of the three 24-hr periods. Great care was taken to minimize the disturbance to the animals during the transfer. If the transfer occurred during the dark period, animals were shielded from light by covering the cages with a lightproof cloth. Each of the eight or six animals were decapitated in succession, and care was taken to ensure that the rats following in line did not view the decapitation of the preceding animals. We have observed that merely by viewing the procedure of decapitation, rats will become excited. Brains were collected along with several other tissues, including blood. The entire procedure took from 40 to 50 min, depending on whether eight or six animals were used. No more than 7 min elapsed from the time of decapitation to the removal of the brain and its preparation for storage in the deep freezer. The individual brains were wrapped in aluminum foil and stored at −12°C until analysis. Other tissues were stored similarly.

Chemical assay. The contents of norepinephrine and dopamine in the whole-brain tissue of the animals in the experiments performed on 9/13/65 were analyzed by fluorometric measurement of their trihydroxyindole derivatives after their partial purification by adsorption. The fluorescent derivative of dopamine was formed by an iodine oxidation, followed by alkalinization and subsequent acidification according to the method of Drujan et al. (6), and that of norepinephrine was formed by ferricyanide oxidation followed by an alkalinization by the method of Crout (5).

Each tissue sample was weighed and then homogenized with a Duall homogenizer in 10 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 2,000...
The individual plotted values, calculated as 2-hr moving averages, are the means (with standard errors) which indicate the pattern of brain serotonin over a single 24-hr period. Also, the activity pattern of a colony of rats over a 24-hr period based on noise emitted by the colony. Animals were maintained under a light-dark cycle (light from 0600 to 1800).

$X \cdot g$ and a measured amount (8–9 ml) of supernatant withdrawn and mixed with 10 ml of 0.2 M sodium acetate, 0.5 ml of 0.2 M disodium ethylenediaminetetraacetate and 500 mg alumina (Woelm, chromatographic grade). Then the pH was adjusted with stirring to 8.4 with 3 M NH$_4$OH and allowed to stir for 5 min to ensure absorption of the catecholamine onto the alumina. The suspension was then passed through a glass chromatography tube (standard 8-inch, Vitamin-B assay tube) plugged with glass wool; the resultant alumina column was washed with two 5-ml portions of H$_2$O and the catecholamine then eluted with 5 ml of 0.2 M acetic acid. In the analysis of norepinephrine, a 0.5-ml aliquot of the “acetic acid eluate” was mixed with 2 ml of 1 M acetate buffer (pH 6.5) and 0.1 ml of a 0.25% potassium ferricyanide solution was added to effect oxidation; after exactly 3 min, the oxidation reaction was stopped by addition of 0.5 ml of a mixture containing 9.0 ml 5 N sodium hydroxide and 1.0 ml 2.5% sodium sulfite. After exactly 3 min, 1.0 ml of a 5 N HCl solution containing 2.0 mg ascorbic acid/1.0 ml was added. The fluorescence of the trihydroxyindole derivative of dopamine was measured at 335 nm excitation and 385 nm emission. The recovery factor in this procedure was approximately 80%.

The analyses in the experiments performed on 9/12/66 and 5/5/66 were performed in a different laboratory than the 9/13/65 experiment. These analyses differed in that the partial purification was accomplished by an extraction procedure involving butanol (15). In this procedure, each tissue sample was homogenized in two volumes of 0.01 N HCl. Aliquots (6 ml) of this homogenate were mixed with 8 g NaCl and 60 ml n-butanol and shaken for 1 hr and then centrifuged to separate the layers. The butanol phase (40 ml) was added to 70 ml heptane and 5.0 ml 0.01 N HCl, shaken for 5 min, and then centrifuged to separate the layers. The aqueous phase was withdrawn and analyzed for catecholamines. In the analysis of dopamine, a 2-ml aliquot of the aqueous phase was mixed with 1 ml of 2 M acetate buffer (pH 6) and then 0.05 ml of 0.1 N iodine solution was added to effect the oxidation which was stopped in exactly 3 min.
by addition of 0.5 ml of an alkaline sulfite solution (504 mg Na₂SO₃, 11 ml H₂O, and 9 ml 10 N NaOH); after exactly 3 min, 0.5 ml 10 N HCl was added. After 90 min, the fluorescence of the trihydroxindole of dopamine was measured at 330 nm excitation and 380 nm emission. In the analysis of norepinephrine, 1 ml of the aqueous phase was mixed with 1 ml 2 mM acetic buffer, pH 5, and 0.05 ml 0.1 N iodine solution was added to effect oxidation which was stopped by addition of 0.10 ml 0.05 N sodium thiosulfate solution, after which 0.50 ml of an alkaline ascorbate solution (150 mg ascorbic acid, 15 ml H₂O, and 30 ml 10 N NaOH) was added. After 45 min, the fluorescence of the trihydroxindole derivative of norepinephrine was measured at 400 nm excitation and 510 nm emission. Also, routinely, oxidations at pH 3, at which norepinephrine is not oxidized, were carried out to obtain correction factors for any trace quantities of epinephrine which would be oxidized along with nor epinephrine at pH 5; this factor was negligible usually, but in a few samples traces of what appeared to be epinephrine were detected and corrected for. The recovery factor in this procedure was approximately 70%.

Serotonin was assayed in concentrated hydrochloric acid (Table 1, Fig. 1). The mean values (expressed as pg/g of brain) indicate that a major sustained crest occurred between 1130 and 1730, with minimum values between 2030 and 2130 hr (Table 1, Fig. 1). Comparison of the highest mean during the 24-hr period (0.73 μg/g at 1130) with the lowest mean (0.62 μg/g between 2030 and 2130) indicated an 18% flux in serotonin levels. Comparison of the phasing of the serotonin rhythm with the typical motor activity rhythm of the colony indicated a correlation of maximal serotonin levels with rest and minimal levels with activity; however, because of the oscillatory nature of serotonin, there were periods during the major activity and rest phases at which the serotonin levels were approximately similar (Fig. 1). Comparison of the highest and lowest levels of serotonin with the over-all 24-hr mean (0.60 μg/g) indicated statistically significant differences, P being <.02 and <.001 for the highest and lowest levels, respectively.

**Dopamine (9/13/65).** In this, the first experiment, three peaks and troughs in dopamine levels occurred during the 24-hr period. The three peaks occurred at 0030, between 1930 and 2030 and at 0030; whereas the troughs preceding these peaks occurred at 0330, 1530, and 2230 (Table 1, Fig. 2). The three peaks were approximately equal in amplitude and were statistically significant when compared to the preceding troughs, with P always between <.05 and <.01. The over-all 24-hr mean was 1.02 μg/g of brain.

**Dopamine (9/12/66).** In the second experiment there also were three statistically significant peaks of dopamine activity along the 24-hr time scale (Table 1, Fig. 2). There was a remarkable degree of synchrony in the phasing of the three peaks observed in the first and second experiments; however, a significant difference in the over-all level of dopamine in the two experiments was observed, the over-all 24-hr mean being 0.60 μg/g for the second as compared to 1.02 μg/g for the first. We noted that these two experiments were done with two different methods for separation of amines. The second method employed tissue homogenate-aqueous-butanol distribution while the first involved absorption onto an alumina slurry of a TCA supernatant following tissue homogenization, protein precipitation, and centrifugation in TCA. The exact reason for the dopamine discrepancy is not presently known; the two methods differed in respect to the nature of the initial purification step, the use of disodium ethylenediaminetetraacetate and ascorbic acid, the time of development of fluorescence and in other aspects of reaction conditions, all of which have been described. Attempts are being made to determine whether any of these variables are responsible for the differences. A survey of the literature indicates that although any given method gives consistent results the normal ranges reported for different methods vary from 0.00 μg/g (3) to 1.0 μg/g (22).

**Norepinephrine (9/13/65).** The data of experiment 1 (Table 1) indicated two peaks equal in magnitude over the 24-hr period in the levels of norepinephrine in whole brain. The first peak occurred between 2130 and 2230, the second at 0330. There was one rather sustained trough between 1330 and 1530, each peak representing
2. The individual plotted values, calculated as 2-hr moving averages, are the means expressed as percent changes from the 24-hr mean, which indicate the pattern of brain dopamine over two 24-hr periods. Also, activity pattern of a colony of rats over a 24-hr period based on noise emitted by the colony. Animals were maintained under a light-dark cycle (light from 0600 to 1800).

19% increase over the low period. Both peaks in norepinephrine levels were statistically significant when compared to the levels at the above mentioned trough hours (P<.02). When the daily low was compared with the over-all 24-hr mean (0.24 µg/g), the differences were statistically significant (P < .05).

Norepinephrine (5/3/66). In experiment 2 the lowest levels of norepinephrine occurred at 1330 (Table 1, Fig. 3) at which time the lower levels in experiment 1 occurred. Comparison of the 1330 low level with the over-all 24-hr mean (0.27 µg/g) indicates a statistically significant difference (P < .02). There were two peaks in norepinephrine levels, one occurring at 1930, the other at 0330; these peaks were statistically significant when compared to the lowest level at 1330 (P < .01). A smaller but nonsignificant peak occurred at 2230. The maximum variation between the lowest and highest recorded values was 55%.

Norepinephrine (9/12/66). In experiment 3 the same trough of activity which characterized experiments 1 and 2 in phasing of norepinephrine levels occurred in the early afternoon hours and was sustained for approximately 4 hr. Figure 3 demonstrates the repetition of the saw-tooth pattern in fluctuation in norepinephrine levels described for experiments 1 and 2. The statistical significance of the data of experiment 3 was comparable to those of experiments 1 and 2. The maximum variation between the lowest and highest recorded values was 41%. The over-all 24-hr mean value was 0.26 µg/g of brain.

DISCUSSION

Serotonin. The results for the one 24-hr experiment carried out indicate a circadian rhythm in the levels of serotonin in the whole brain. The fluctuation is of relatively small amplitude, the daily change being only 18%, however, the peak and trough values are noted to be different from the over-all mean at highly significant values of P. Before the peak and trough times are established as predictable it will certainly be important to have them confirmed by other studies.

Published information on periodicity in brain relating to serotonin is apparently limited to that of Albrecht et al. (1) who found time dependent fluctuation in the levels of 5-hydroxytryptamine (serotonin) in mouse brain. When one carefully compares the results of this present study on the rat with that of Albrecht et al., a similarity is detected in phasing of the two species. Differences seen probably are due to variation in the intervals between sampling employed by the two investigators. A second
striking similarity in the two studies is the close approximation in the absolute values of serotonin obtained for mice and rats. In Table 1 of Albrecht’s data, the absolute values for his series 2 mice indicate that at 1230 the serotonin level of the mouse brain was 72 μg/g; for our rats the level was 73 μg/g at 1130 and 71 μg/g at 1230. At 2030 in the same series 2 data, the serotonin level in the mouse brain was 61 μg/g at 2030; whereas in the rats we found serotonin to be 62 μg/g at this same time point. Although these similarities may be coincidental, there is a strong suggestion that mouse and rat are essentially similar in both phasing and amplitude. The similarity in phasing is not surprising when we consider that the animals of both studies were subjected to identical light-dark cycles with a photoperiod from 0600 to 1800.

As mentioned above, the amplitude of the serotonin rhythm in brain is small; it is only a minor fraction of that of the pineal gland where serotonin demonstrates a 900% flux (17). The rhythms in serotonin, of whole brain tissue as demonstrated by our data and of the pineal as demonstrated by Quay (17), are identical in respect to both phasing and profile. The above similarities in phasing suggest a strong synchronization in phasing of the serotonin rhythm to the light-dark cycle even though serotonin presumably has a melatonin precursor function in the pineal and a neuronal function in the brain. There was precisely the same phase relationship in time of occurrence of the onset of darkness to the over-all daily low and subsequent upswing which occurred at 0400; whereas in the present experiment the same stimulus occurred at 0600. After considerable experimentation, Quay (17) concluded that the phasing of the pineal rhythm in rats was more strongly dependent on the stimulus of “lights off” than on “lights on.” Our data would support this conclusion, and it would appear that the rhythm in serotonin in both the brain and pineal is somehow synchronized in a similar manner to the light-dark cycle. This seems contrary to a statement made by Quay (17) after he had analyzed the similarities and differences between the mouse data of Albrecht et al. and his own data on pineals in the rat. Quay’s (17) statement was, “It appears unlikely at this time that the rhythm in pineal serotonin is directly referable to a general rhythm in brain serotonin.” Further study is needed to resolve this point.

Dopamine. Unlike serotonin and numerous other sub-

Since this investigation was completed and written, a preliminary communication of a circadian rhythm for serotonin in rat brain with an 11% variation over the 24-hr period has been reported (Life Sci. 6: 755, 1967). The interval of sampling in this study was 6 hr as compared to our hourly intervals over the 24-hr period. Another difference making it difficult to make meaningful comparisons between the two studies is the fact that rats in the recent report were adapted to a reversed light-dark cycle. In spite of methodological differences, comparison of both studies do suggest similar phasing of the rhythms with peak serotonin occurring during the middle of the light period when animals normally would be sleeping.
stances in other organs such as liver or in blood, the daily pattern of fluctuation does not resemble what is normally considered to be a characteristic circadian rhythm. Instead there is a trimodal pattern along the 24-hr time scale. Each of the three peaks in the oscillating pattern represents approximately a 21–35% increase over the troughs which preceded them. The reproducibility of the phasing in the two separate studies indicates that the pattern is “ultradian” rather than circadian. The adjective ultradian is defined by Halberg et al. (10) as a rhythm having “1 cycle/1 hr to 1 cycle in 19.9 hr.” In the case of the dopamine rhythm there would be 1 cycle in approximately 5–8 hr. The fluctuation pattern for dopamine is the pattern that can be predicted for similar rats maintained under identical standardized conditions and employing identical experimental procedures.

Norepinephrine. Like dopamine the daily pattern in the fluctuation of norepinephrine was not a circadian one. Furthermore, the pattern is considerably different from that of dopamine, but the striking similarity in the phasing of norepinephrine found in the three different experiments indicates that the observed saw-tooth fluctuations are reproducible if animals are maintained under identical standardized conditions. One conspicuous resemblance in the three experiments was the statistically significant trough which occurred just before or about the same time the animals show the first signs of arousal, and it is precisely at this time that the serotonin levels were shown to be maximal. Wurtman and Axelrod (24) reported a daily rhythm with a fourfold fluctuation for norepinephrine in the pineal gland of the rat with a typical major crest and trough over the 24-hr period. The profile they observed for the pineal norepinephrine rhythm did not resemble in any manner the profile observed in the study for total brain norepinephrine; this is in contrast to serotonin which appears to fluctuate similarly in the pineal and total brain.

General considerations. Any interpretation made concerning the amine patterns observed requires first a recognition that assay of amine levels per se in brain does not identify relationship of changes seen to any of the components: amine synthesis, storage, release, and catabolism.

We have found serotonin to be highest in rat brain during the normal sleep time of the colony. In harmony with this, serotonin release in the median raphe system has been found by others to be associated with induction of sleep and its maintenance (12–14). It would appear then that peak serotonin physiological activity in the normal brain may be associated with the highest measured brain levels of this amine. In contrast, lowest norepinephrine levels are probably associated with maximum norepinephrine activity. This latter contention is supported by a recent study carried out in our laboratory (to be published elsewhere) identifying a revealing similar pattern of flux in brain and adrenal catecholamine content, between 1000 and 2000 hr. When the major norepinephrine trough developed in brain at approximately 1200 hr, a similar trough developed in adrenal catecholamine content; at the same time norepinephrine in plasma manifests a threefold increment. Taken collectively these findings identify a generalized increase in sympathetic activity. Further support for this is gained from the fact that peak plasma levels of corticosterone in the rat standardized under identical conditions occur during this period (19). We note that covariance is commonly found between secretion of adrenocorticoids and secretion of catecholamines under conditions of stress or other types of heightened central sympathetic activity.

As stated, evidence has been offered by others (12–14) that serotonin secretion in critical areas of the brain stem induces light sleep in the awake animal. Also, it has been found that brain serotonin levels in the normal animal correlate inversely with spontaneous activity in the awake state (20). Norepinephrine secretion in brain, on the other hand, has been proposed as a contributor to the initiation of deep (paradoxical or activated) sleep; the latter is associated with eye movement, muscle atony, and EEG patterns of arousal, and in the human with dreaming (12). The crest serotonin levels found at the sleep time of the colony could reflect therefore an altered amine metabolism related to the modulation by this amine of sleep induction and maintenance. In turn, development of trough norepinephrine levels at 1200 hr could reflect an increased and imperfectly compensated release from stores of norepinephrine. The observation of Michel et al. (16), that paradoxical sleep intervals tend to aggregate, leads us to conjecture that the norepinephrine trough in question reflects a time during which the colony is maximally devoted to paradoxical sleep. Alternatively, the enhanced sympathetic activity occurring at this time may be related to restorative metabolic mechanisms which prepare for the period of sustained motor activity to follow. Again it is not unreasonable to speculate that both sleep and operation of the restorative metabolic machinery reflect similar functions at different levels of organization and thus are interrelated.

It may be argued that the multiple peaks in norepinephrine simply represent the summation of multiple circadian rhythms with different phasing in different parts of the brain which only in aggregate appear ultradian. However, dopamine also manifests a high frequency rhythm and is far more localized in distribution, the major portion of the brain dopamine being found in the corpus striatum (2, 3). The rhythms for dopamine then probably represent a genuine ultradian pattern. Although dopamine is a precursor of norepinephrine, a number of studies recently reviewed by Hornykiewicz (11), as well as a recent finding of Gordon et al. (9), indicate that a major portion of brain dopamine may not participate in this synthesis. It is therefore not surprising that the profiles of the rhythms for the two amines are considerably different from one another.

Since increasing the availability of the amino acid precursors (tryptophan, tyrosine, or phenylalanine) apparently either does not increase brain amine levels at all or does not increase these levels prominently (21, 23),

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amine flux, in biochemical terms, would appear to represent largely a changing brain metabolism reflected in an interplay of the activities of transport functions, and synthesizing and catabolizing enzymes. Clearly then, flux in activities of the pertinent systems over the 24-hr time span deserves careful study so that the action mechanisms involved ultimately may be defined. Lastly, we would point out that since some of the differences generated by daily flux in brain amine values are as great as 55%, the fact of their occurrence would appear to be of importance to the neurochemist interested in reproducible analytical values. At least some of the variations that in the past may have been uncritically ascribed to random variation now can be explained.

We are grateful to Mr. Stanley Tsai, Miss B. J. Hill, and Mr. J. Krause for their technical assistance.

REFERENCES


