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Strain-Dependency in Motor Activity and in Concentration and Turnover of Catecholamines in Synchronized Rats

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LEMMER, B., G. CASPARI-IRVING AND R. WEIMER. *Strain-dependency in motor activity and in concentration and turnover of catecholamines in synchronized rats.* PHARMAC. BIOCHEM. BEHAV. 15(2) 173–178, 1981.—Circadian rhythm in motor activity was studied with an Animex motimeter in six strains of rats (ACI, BH, BS, DA, LEW, TNO) synchronized by a 12 hr light: 12 hr dark cycle. ANOVA revealed significant interstrain differences in motor activity as well as in the concentration and turnover of central noradrenaline and dopamine. Strain-dependent differences were also found with regard to tyrosine hydroxylase inhibition on motor activity. However, no significant interstrain correlations were found between endogenous concentration and/or turnover rates of the catecholamines and motor activity in normal and drug-treated rats.

Circadian rhythm Motor activity Rat strains Catecholamines Turnover Animex

THERE is an increasing body of evidence demonstrating strain-dependent variations of various drug effects in rodents [1, 2, 7, 11, 16]. These findings are of special interest in the study of the effects of centrally acting drugs on the motility of mice and rats. Strained-dependent differences were reported after application of amphetamine, scopolamine, clonidine as well as after inhibitors of catecholamine biosynthesis [1, 2, 7, 11]. Furthermore, circadian phase-dependent variations in the effects of centrally acting drugs on the motility of rodents were described [8, 12, 14]. In an earlier investigation in synchronized male rats of one strain (TNO W.74) significant daily variations in the turnover of central dopamine—but not of noradrenaline—were described, whereas the concentration of both dopamine and noradrenaline exhibited significant daily variations [13]. However, in this particular rat strain there was no obvious circadian phase-dependent correspondence between the biochemical data and the level of motor activity [12,13]. On the other hand, pharmacological studies with antiadrenergic drugs have clearly shown that central catecholamines are involved in the regulation of motor activity ([2, 11, 12, 14] e.g.). Experiments with male rats of six different strains were performed under identical environmental and experimental conditions in order to evaluate whether interstrain differences can be observed in the circadian rhythm of motor activity as well as in the concentration and turnover of noradrenaline and dopamine in brain and whether there was a correspondence between these parameters. Since the turnover of the catecholamines was determined after inhibition of tyrosine hydroxylase, we were also able to study the effect of catecholamine depletion on the motor activity of rats of the different strains.

METHOD

Animals and Environmental Conditions

Male rats of the following strains were used: TNO W.74 (Wistar, Albino), ACI/Ztm (Agouti, white belt), BH Ztm (Black Hooded), BS/Ztm (Black), DA/Ztm (Dark Agouti), LEW/Ztm (Lewis, Albino). The strain TNO W.74 was supplied from Winkelmann Versuchstierzucht GmbH & Co. KG (Borchen-Kirchborchen, FRG), all other strains were obtained from Zentrales Tierlaboratorium der Medizinischen Hochschule Hannover (Hannover, FRG). Their approximate age at sacrifice was 6–8 weeks. The animals, which had already been synchronized by a light-dark cycle since birth by the respective suppliers, were maintained in our laboratory under controlled environmental conditions (L: 0700–1900 hr, 200 lux; D: 1900–0700 hr, <0.1 lux; temperature 23±1°C, humidity 43%) for at least 7 days before use.

The animals were randomly housed in groups of 5 rats [10] in plastic cages (Makrolon®, 500×350×200 mm) and had free access to food and tap water during the experiments. In order to minimize registration of gross exploratory and social behavior in a new environment the animals were housed with the same rats with which they were tested in the experiments on motor activity as well as in the biochemical studies [12–14]. The experiments were performed during April and May.

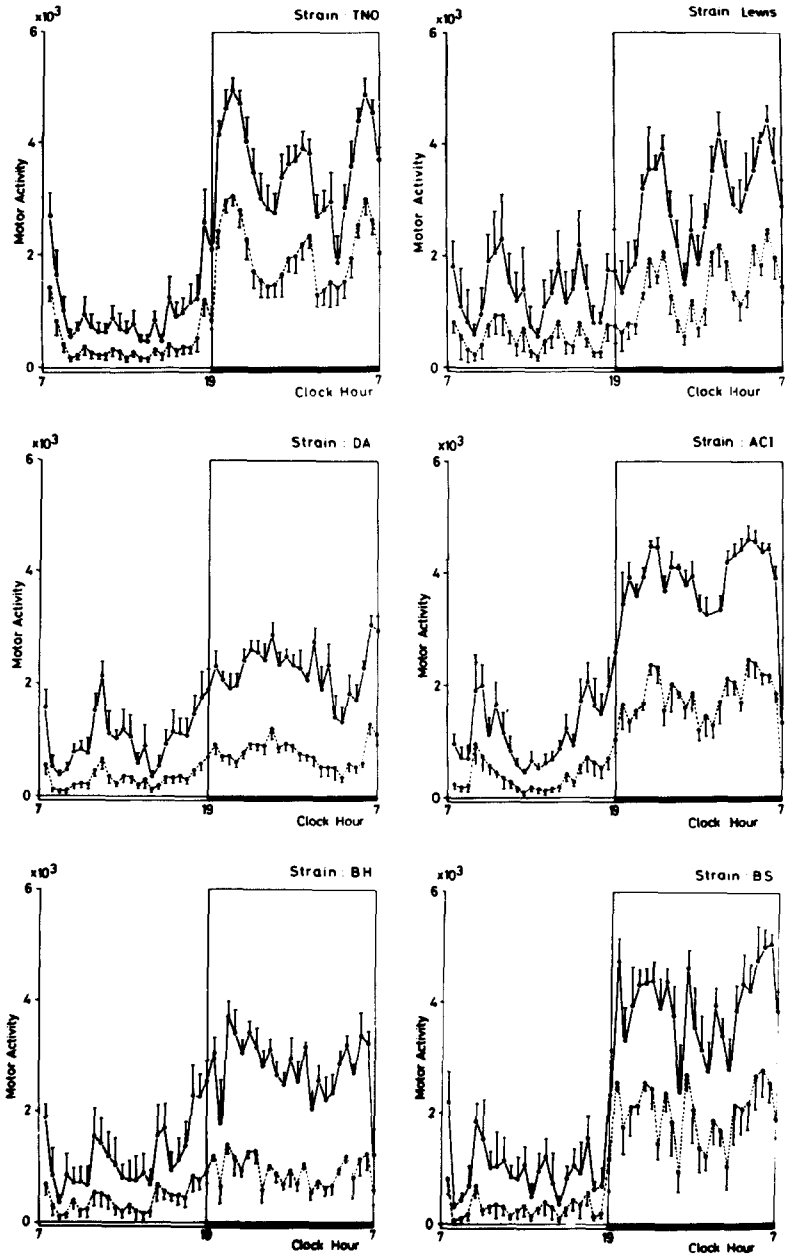

FIG. 1. Circadian variations in the motor activity in six strains of male rats. Motor activity was registered in groups of 5 rats with a two-channel Animex motimeter, thereby differentiating between total motor activity (47 μA, solid line) and running activity (10 μA, dashed line). Shown are the mean activity counts±SEM of 4–8 groups of rats, registered every 30 min.

Studies on Motor Activity

Motor activity of groups of 5 rats per cage for all strains was studied with a two-channel Animex motimeter (AB Farad, Stockholm). Based on earlier experiments total motor activity was registered on channel A at a sensitivity setting of 47 μA and running activity was registered at a sensitivity setting of 10 μA on channel B. The counting interval was 30 min (for details see [12,14]). In order to exclude the possibility of one extreme value unduly influencing a group value, motor activity was recorded in two groups of rats per strain each for 2–4 days. Since the 12-hr and the 24-hr mean values as well as the circadian pattern in motor activity did not differ significantly between the two groups of rats of each strain, the experimental data of both groups were combined for further statistical analysis.

In additional experiments motor activity was studied after injection of the tyrosine hydroxylase inhibitor H 44/68 (200 mg/kg, IP), which was used in order to determine catechol-

TABLE 1

24-HOUR MEANS IN THE MOTOR ACTIVITY OF SIX STRAINS OF MALE RATS

Animex Setting	24-Hour Mean Activity Counts (Counts/30 min, Mean ± SD) in the Strains						
	BS	TNO	ACI	LEW	BH	DA	ANOVA
Channel A	2586 ± 145	2365 ± 83	2527 ± 167	2128 ± 102	2018 ± 172	1643 ± 97	F(5,27)=44.057 $p<0.001$
Channel B	1288 ± 175	1231 ± 85	1092 ± 134	977 ± 102	672 ± 70	549 ± 76	F(5,27)=41.527 $p<0.001$
	(4)	(8)	(6)	(5)	(4)	(6)	
B/A (%)	49.8	52.1	43.2	45.9	33.3	33.4	

Motor activity was studied with a two-channel Animex motimeter (channel A: sensitivity 47 μA, total motor activity; channel B: sensitivity 10 μA, running activity) in (n) groups of 5 rats which were synchronized by light (0700 hr–1900 hr) and darkness (1900 hr–0700 hr). ANOVA revealed significant interstrain differences in 24-hr means in channel A and channel B; F-values, degrees of freedom and probabilities are shown.

TABLE 2

MOTOR ACTIVITY IN THE LIGHT PERIOD AND DARK PERIOD IN SIX STRAINS OF RATS

Photoperiod/ Animex Setting	12-Hour Mean Activity Counts (Counts/30 min, Mean ± SD) in the Strains						
	BS	TNO	ACI	LEW	BH	DA	ANOVA
Light Period (L)							
Channel A	1071 ± 194	1038 ± 149	1200 ± 162	1262 ± 60	1204 ± 157	1041 ± 115	F(5,27)=2.319 $p>0.05$
Channel B	378 ± 121	419 ± 82	410 ± 86	558 ± 118	412 ± 48	292 ± 89	F(5,27)=4.606 $p<0.01$
Dark Period (D)							
Channel A	4102 ± 254	3694 ± 188	3856 ± 224	2993 ± 207	2832 ± 302	2244 ± 106	F(5,27)=61.997 $p<0.001$
Channel B	2197 ± 334	2043 ± 216	1775 ± 206	1396 ± 104	933 ± 133	806 ± 80	F(5,27)=47.790 $p<0.001$
Ratio of 12-Hour Mean D/L							
Channel A	3.83	3.56	3.21	2.37	2.35	2.16	
Channel B	5.81	4.88	4.33	2.50	2.26	2.76	

Motor activity was studied with a two-channel Animex motimeter (channel A: 47 μA; channel B: 10 μA) in 4–8 groups of 5 synchronized rats; same experiments as in Table 1. ANOVA was used to test for interstrain differences in the 12-hr means in activity counts in either photoperiod and at either channel sensitivity; F-values, degrees of freedom and probabilities are shown.

*Correlation coefficient r(4)=0.941, $p<0.01$; †correlation coefficient r(4)=0.920, $p<0.01$.

amine turnover (see below). Motor activity for the H 44/68 treated animals was tested for 3.5 hr in the light phase (0730–1100 hr) and in the dark phase (1930–2300 hr) of the LD photoperiod. In these studies the same groups of animals were used as those for which motor activity had been registered in the control period.

Biochemical Studies

Four groups of five rats per strain were used in these experiments. In either photoperiod the catecholamine turnover was determined from the logarithmic decline of the endogenous amine concentration after inhibition of tyrosine-hydroxylase with H 44/68 (D, L-α-methyl-p-tyrosine-methylester·HCl, Labkemi AB Stockholm) as described in detail [13]. H 44/68 was dissolved in 0.9% saline solution and a 200 mg/kg dose was injected (1 ml/100 g body weight). The drug was injected either at 0730 hr or at 1930 hr. Groups of five rats were sacrificed 0 and 4 hours after injection of the synthesis inhibitor. The concentration of noradrenaline and dopamine was determined spectrofluorometricaly by modifications of the procedures described by Schlumpf *et al.* [17] and by Chang [4]. The hypothalamus/midbrain and striatum were dissected according to [9]. The parameters of the turnover ($t_{1/2}$=half-life, turnover rate) were calculated from the respective regression lines according to Costa [6].

TABLE 3

CONCENTRATION AND TURNOVER OF NORADRENALINE IN HYPOTHALAMUS-MIDBRAIN REGION OF 5 RAT STRAINS

	Light Period (L)			Dark Period (D)			
Strain	NA (μg/g)	$t_{1/2}$ (hr)	Turnover Rate (μg/g/hr)	NA (μg/g)	$t_{1/2}$ (hr)	Turnover Rate (μg/g/hr)	Turnover Rate D/L
TNO	0.81 ± 0.05	6.3	0.039	0.92 ± 0.07	4.7	0.059	1.51
ACI	1.05 ± 0.17	13.1	0.024	1.03 ± 0.10	17.6	0.018	0.75
LEW	0.97 ± 0.06	4.1	0.072	1.17 ± 0.08	2.7	0.130	1.81
BH	1.91 ± 0.08	7.2	0.050	1.07 ± 0.13	7.0	0.046	0.92
DA	1.24 ± 0.06	7.3	0.051	1.14 ± 0.13	7.6	0.046	0.90

The concentration of noradrenaline (mean ± SEM, n=5) in hypothalamus/midbrain region was determined in synchronized rats (L: 0700–1900 hr, D: 1900–0700 hr) of the strains indicated either at 0730 hr or at 1930 hr. The parameters of turnover (half-life=$t_{1/2}$, turnover rate) were calculated from the logarithmic decline of the endogenous noradrenaline concentration after inhibition of tyrosine hydroxylase with H 44/68 (200 mg/kg, IP), for details see text. ANOVA revealed strain dependent variations in noradrenaline concentrations at 0730 hr, $F(4,17)=3.600$, $p<0.05$, but not at 1930 hr, $F(4,18)=0.888$, $p>0.05$.

TABLE 4

CONCENTRATION AND TURNOVER OF DOPAMINE IN STRIATUM OF 5 RAT STRAINS

	Light Period (L)			Dark Period (D)			
Strain	DA (μg/g)	$t_{1/2}$ (hr)	Turnover Rate (μg/g/hr)	DA (μg/g)	$t_{1/2}$ (hr)	Turnover Rate (μg/g/hr)	Turnover Rate D/L
TNO	4.32 ± 0.35	4.7	0.276	6.41 ± 0.57	3.6	0.538	1.95
ACI	7.67 ± 0.30	3.0	0.762	7.27 ± 0.53	3.5	0.618	0.81
LEW	5.22 ± 0.42	5.6	0.282	8.26 ± 0.10	4.5	0.553	1.96
BH	9.19 ± 0.53	2.7	1.020	8.74 ± 0.92	2.1	1.253	1.23
DA	7.67 ± 0.90	3.5	0.654	6.10 ± 0.64	4.1	0.445	0.68

The concentration of dopamine (mean ± SEM, n=5) in striatum was determined in synchronized rats (L: 0700–1900 hr, D: 1900–0700 hr) of the strains indicated either at 0730 hr or at 1930 hr. The parameters of the turnover (half-life=$t_{1/2}$; turnover rate) were calculated from the logarithmic decline of endogenous dopamine concentration after inhibition of tyrosine hydroxylase with H 44/68 (200 mg/kg, IP), for details see text. ANOVA revealed strain-dependent variations in dopamine concentrations at 0730 hr, $F(4,16)=18.421$, $p<0.001$, and at 1930 hr, $F(4,17)=3.362$, $p<0.05$, respectively.

Statistical Analysis

ANOVA (analysis of variance) was used to test for interstrain differences in motor activity and endogenous catecholamine concentrations, respectively.

RESULTS AND DISCUSSION

Figure 1 shows pronounced interstrain differences in circadian phase-dependent motor activity expressed as activity counts per 30 min measured with an Animex motimeter. These strain-dependent differences could be observed in total motor activity (channel A, 47 μA) as well as in running activity (channel B, 10 μA). A detailed analysis of the experimental data is compiled in Tables 1 and 2: ANOVA revealed significant ($p<0.001$) interstrain differences in 24-hr means in total motor activity and in running activity, respectively (Table 1). Also, the relative amount of running activity expressed as percent of total motor activity (activity counts channel B/activity counts channel A, Table 1) varied between 33.3% (strain BH) and 52.1% (strain TNO) as demonstrated in Figure 1.

In Table 2 the 12-hour mean activity counts obtained for six rat strains in the light period and the dark period are summarized. It can be seen that interstrain differences in mean activity counts were most prominent in the dark period, in which ANOVA revealed highly significant interstrain variations at either motimeter channel sensitivity setting (Table 2). In the light period significant strain-dependent variations in motor activity were observed only with the motimeter channel B, representing mainly the running activity. Table 2 further shows that the relative increase in mean motor activity in the dark period as compared to the light period (D/L ratios of the 12-hr means) ranged from 2.16 (strain DA) to 3.83 (strain BS) in channel A and from 2.26 (strain BH) to 5.81 (strain BS) in channel B. These ratios represent strain-dependent variations in circadian amplitude of motor activity, which is evident already from Figure 1.

Furthermore, within the six rat strains there was a significant positive correlation between the 12-hr means of the dark period (but not of the light period) and the respective D/L ratios, the correlation coefficients for channel A and B being 0.941 and 0.920 ($p<0.01$), respectively (Table 2). Thus, the

TABLE 5

CORRELATION BETWEEN MOTOR ACTIVITY AND BIOCHEMICAL PARAMETERS

Interaction		Degree of Freedom	Correlation Coefficient (r) Light Period	Dark Period
MA_A	vs [NA]	3	0.0671	−0.7279
"	vs TR_{NA}	3	0.3329	−0.2183
"	vs [DA]	3	0.2179	−0.0064
"	vs TR_{DA}	3	0.1935	−0.0818
MA_B	vs [NA]	3	0.5550	−0.7335
"	vs TR_{NA}	3	0.5041	−0.2183
"	vs [DA]	3	0.4719	−0.2248
"	vs TR_{DA}	3	0.4583	−0.3506
$MA_{H44/68}$	vs TR_{NA}	2	0.8748	0.3289
"	vs TR_{DA}	2	0.6861	0.2824

The strain-dependent variations in the 12-hr-means in motor activity in channel A and B (Table 2, MA_A, MA_B) or in the activity counts after H 44/68 (Table 5, $MA_{H44/68}$) were correlated with the strain-dependent variations in concentrations ([NA], [DA]) or in turnover rates (TR_{NA}, TR_{DA}) of noradrenaline and dopamine, respectively (Tables 3, 4). No correlation coefficient differed significantly from zero ($p>0.05$).

experiments with the Animex motimeter clearly demonstrate significant interstrain variations in motor activity of grouped male rats of six different strains.

Various behavioral studies [3, 15, 18] and pharmacological experiments with adrenergic [1, 2, 7–9, 20] or antiadrenergic [2, 12–14, 19] drugs indicate that central catecholamines play an important role in the regulation of motor activity. Therefore, in five of the six rat strains the concentration and turnover of dopamine in the corpus striatum and of noradrenaline in hypothalamus/midbrain was measured. The results of the biochemical studies are summarized in Tables 3 and 4. Significant interstrain differences in endogenous noradrenaline concentration in the hypothalamus/midbrain region was observed at 0730 hr but not at 1930 hr (Table 3), whereas endogenous dopamine concentrations in striatum showed significant strain-dependent differences both at 0730 hr and at 1930 hr, respectively (Table 4). Interstrain differences could also be observed in terms of half-lives and turnover rates of noradrenaline and dopamine (Tables 3 and 4).

Thus, these data give further evidence to strain-dependent differences in the adrenergic nervous system of rodents as reported for the activity of phenylethanolamine-N-methyl-transferase [5], tyrosine hydroxylase [5,18], noradrenaline sensitive adenylate cyclase in brain [18] and the cyclic-AMP-generating system in brain slices [19]. However, no significant correlations were found between the 12-hr means (channel A and B) in either photoperiod and the respective concentrations and turnover rates of either noradrenaline or dopamine in the attempt to correlate strain-dependent differences in motor activity with the biochemical data (Table 5). Furthermore, strain-dependent differences in D/L ratios in motor activity (Table 2) showed no significant correlation ($p>0.05$) with strain-dependent variations in the D/L ratios of the turnover rates of both noradrenaline (Table 3) and dopamine (Table 4). Though the tyrosine hydroxylase inhibitor H 44/68 decreased both central noradrenaline and dopamine concentration in each rat strain (Tables 3 and 4), this inhibitor affected the motor activity quite differently in the different rat strains. Table 6 shows that motor activity was reduced by H 44/68 in strains BS, TNO and ACI with a more pronounced effect in the dark period. An increase in motor activity was observed, however, with strains BH and DA during the light period, with no effect during the dark period. Strain-dependent differences in the effect of H 44/68 on motor activity were not significantly correlated with neither noradrenaline nor dopamine turnover (Table 6). These data support findings of Anisman and Kokkinidis [2] who reported strain-dependent differences in the effects of inhibitors of tyrosine hydroxylase and

TABLE 6

EFFECTS OF TYROSINE HYDROXYLASE INHIBITOR ON MOTOR ACTIVITY OF FIVE STRAINS OF RATS

Strain	Activity Counts/30 min: During 0730–1100 hr (Light Period) Control	Drug	% Change	During 1930–2300 hr (Dark Period) Control	Drug	% Change
BS	987	885	−10	4171	2939	−30
TNO	882	475	−46	3946	2381	−40
ACI	1314	948	−28	4008	2233	−44
BH	808	1350	+67	3052	2920	− 4
DA	760	1232	+62	2312	2421	+ 5

Groups of 5 synchronized (L: 0700 hr–1900 hr, D: 1900 hr–0700 hr) male rats of the strains noted were injected either at 0730 hr or at 1930 hr with the inhibitor of tyrosine hydroxylase H 44/68 (200 mg/kg, IP) and motor activity (Animex motimeter, channel sensitivity 47 μA) was registered for up to 3.5 hr after drug injection. Motor activity is expressed as activity counts per 30 min; activity counts are mean values of 4–8 (control) or 1–2 (drug) groups of rats. For details see Method.

of dopamine-β hydroxylase on spontaneous as well as amphetamine-induced motility in three strains of mice.

In conclusion, behavioral and biochemical results obtained under identical environmental and experimental conditions in different strains of male rats clearly demonstrate significant interstrain differences with regard to the level and circadian pattern of motor activity in normal and drug-treated (H 44/68) animals. Further interstrain differences were observed with regard to concentration and turnover of the central catecholamines. There was, however, no simple interstrain relationship between motor activity and central noradrenaline or dopamine. The results of our experiments show that beside circadian-phase-dependent variations interstrain differences also have to be taken into account when studying the effects of centrally acting drugs on the motility of rats.

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REFERENCES

1. Anisman, H. and D. Cygan. Central effects of scopolamine and (+)-amphetamine on locomotor activity: Interaction with strain and stress variables. *Neuropharmacology* **14:** 835–840, 1975.
2. Anisman, H. and L. Kokkinidis. Effects of scopolamine, d,amphetamine and other drugs affecting catecholamines on spontaneous alternation and locomotor activity in mice. *Psychopharmacologia* **45:** 55–63, 1975.
3. Büttner, D. Die Variabilität von Herzfrequenz und Sauerstoffverbrauch der gestressten und ungestressten Ratte. Lecture, Neuherberg, May 31-June 3, 1977.
4. Chang, C. C. A sensitive method for spectrofluorometric assay of catecholamines. *Int. J. Neuropharmac.* **3:** 643–649, 1964.
5. Ciaranello, R. D., R. Barchas, S. Kessler and J. D. Barchas. Catecholamines: Strain differences in biosynthetic enzyme activity in mice. *Life Sci.* **11:** 565–572, 1972.
6. Costa, E. Simple neuronal models to estimate turnover rate of noradrenergic transmission in vivo. In: Biochemistry of simple neuronal models. *Adv. Biochem. Pharmac.* **2:** 169–204, 1970.
7. Davis, W. M., M. Babbini, S. F. Pong, W. T. King and C. L. White. Motility of mice after amphetamine: Effects of strain, aggregation and illumination. *Pharmac. Biochem. Behav.* **2:** 803–809, 1974.
8. Evans, H. L., W. B. Ghiselli and R. A. Patton. Diurnal rhythm in behavioural effects of methamphetamine, p-chlormethamphetamine and scopolamine. *J. Pharmac. exp. Ther.* **186:** 10–17, 1973.
9. Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the rat brain—I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-dopa in various regions of the brain. *J. Neurochem.* **13:** 655–669, 1966.
10. Halberg, F. Physiological 24-hour periodicity. General and procedural considerations with references to the adrenal cycle. *Z. Vitam.-Horm.- u. Fermentforsch.* **10:** 225–296, 1959.
11. Hano, J., J. Vetulani, M. Sansone and A. Oliverio. Effect of clonidine, amphetamine, and their combinations on the locomotor activity of CD-1 and S57BL/6 mice. *Pharmac. Biochem. Behav.* **9:** 741–746, 1978.
12. Lemmer, B. and T. Berger. Diurnal variations in the motor activity of the rat: Effects of inhibitors of the catecholamine synthesis. *Naunyn-Schmiedeberg's Arch. Pharmac.* **303:** 251–256, 1978.
13. Lemmer, B. and T. Berger. Diurnal rhythm in the central dopamine turnover in the rat. *Naunyn-Schmiedeberg's Arch. Pharmac.* **303:** 257–261, 1978.
14. Lemmer, B. and K. Wenda. Diurnal rhythms in noradrenaline turnover and motility after reserpine and 6-hydroxydopamine. *Pharmac. Biochem. Behav.* **10:** 361–368, 1979.
15. Liang, B. and D. A. Blizard. Central and peripheral norepinephrine concentrations in rat strains selectively bred for differences in response to stress: Confirmation and extension. *Pharmac. Biochem. Behav.* **8:** 75–80, 1978.
16. v. Mayersbach, H. Time—A key in experimental and practical medicine. *Archs Toxic.* **36:** 185–215, 1976.
17. Schlumpf, M., W. Lichtensteiger, H. Langemann, P. G. Waser and F. Hefti. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. *Biochem. Pharmac.* **23:** 2437–2446, 1974.
18. Skolnick, P. and J. W. Daly. Norepinephrine-sensitive adenylate cyclase in rat brain: Relation to behaviour and tyrosine hydroxylase. *Science* **184:** 175–177, 1974.
19. Skolnick, P. and J. W. Daly. Strain differences in responsiveness of norepinephrine-sensitive adenosine 3′, 5′-monophosphate-generating systems in rat brain slices after intraventricular administration of 6-hydroxydopamine. *Eur. J. Pharmac.* **41:** 145–152, 1977.
20. Walker, C. A. Implications of biochemical rhythms in brain amine concentrations and drug toxicity. In: *Chronobiology*, edited by L. E. Scheving, F. Halberg and J. E. Pauly. Stuttgart: Thieme Publ., 1974, pp. 205–208.