Visiting Fellow of the Schweizerische Stiftung für biologisch-medizinische Stipendien; present address: Psychiatrische Universitätsklinik, CH-4025 Basel, Switzerland.

1 Richter, C.P., Biological Clocks in Medicine and Psychiatry. Charles C. Thomas, Springfield, Ill., 1965.
2 Wehr, T.A., Wirz-Justice, A., Goodwin, F.K., Duncan, W., and Gillin, J.C., Phase advance of the sleep-wake cycle as an antidepressant. Science *206* (1979) 710-716.
3 Wehr, T.A., Goodwin, F.K., Wirz-Justice, A., Craig, C., and Breitmaier, J., 48-Hour sleep-wake cycles in manic-depressive illness: naturalistic observations and sleep deprivation experiments. Archs gen. Psychiat. *39* (1982) 559-565.
4 Wallen, E.P., Hess, M., Johnson, L., and Stone, W., Rhythm monitoring using a microcomputer system. Physiol. Behav. *23* (1979) 611-614.
5 Nakatsu, K., and Owen, J.A., A microprocessor-based animal monitoring system. J. Pharmac. Meth. *3* (1980) 71-82.
6 Sokolove, P.G., and Bushnell, W.N., The Chi-square periodogram: its utility for analysis of circadian rhythms. J. theor. Biol. *72* (1978) 131-160.
7 Wirz-Justice, A., and Campbell, I.C., Antidepressant drugs can slow or dissociate circadian rhythms. Experientia *38* (1982) 1301-1309.
8 Theeuwes, F., and Yum, S.I., Principles of the design and operation of generic osmotic pumps for the delivery of semisolid or liquid drug formulations. Ann. biomed. Engng *4* (1976) 343-353.

Antidepressant drugs can slow or dissociate circadian rhythms[1]

Anna Wirz-Justice[2] and Iain C. Campbell[3]

Clinical Psychobiology Branch, National Institute of Mental Health, Bethesda (Maryland 20205, USA), 15 June 1982

Summary. The circadian rest-activity cycle of female hamsters was lengthened by chronic administration of the monoamineoxidase inhibitor antidepressant drug clorgyline. Chronic treatment with clorgyline or the tricyclic antidepressant drug imipramine also induced dissociation of circadian activity rhythm components. Thus these drugs may be added to the very small group of substances (including the prophylactic antidepressant and antimanic drug lithium) that modify circadian frequency and/or coupling between circadian rhythms.

Introduction

The temporal stability of circadian oscillations is extremely important for their time-keeping and time-measuring functions[4,5]. Homeostasis of circadian oscillations is reflected in their resistance to chemical fluctuations in the internal milieu. However some endocrinological manipulations have been found to change the free-running circadian period (τ = the duration of 1 complete cycle in an environment free of time cues): ovariectomy, and replacement oestrogen and progesterone in hamsters[6,7]; castration and replacement testosterone in mice[8]; pinealectomy and replacement melatonin in birds[9]; and perhaps also thyroidectomy and replacement thyroid in hamsters[10] and man[11].

Although the number of drugs known to modify circadian rhythms in mammals is small, it is expanding[12]. The first substance to be extensively investigated was deuterium oxide[13]. Carbachol, a cholinergic agonist, and α-bungarotoxin, a nicotinic cholinergic receptor blocker, have been shown to exhibit phase-shifting properties mimicking and blocking, respectively, the effects of light[14,15]. Lithium, used as a prophylactic antidepressant and antimanic drug, slows circadian rhythms and delays phase-position in many species from hamsters to man (reviewed in Wirz-Justice[16]).

There are reasons to suspect that not only lithium, but that antidepressants as a class may alter circadian time-keeping. The timing of many circadian rhythms in physiological, behavioral, and endocrinological parameters appears to be abnormal in depression and mania (for reviews, see Wehr and Goodwin[17] and Wehr and Wirz-Justice[18]). It is therefore of interest for circadian hypotheses of the pathophysiology of affective disorders to investigate whether drugs used in the treatment of this illness act on the circadian system.

The free-running period and the phase-response curve for some standard perturbation, are the only parameters of an overt rhythm that can be measured and said to reflect the corresponding parameter of the underlying oscillator(s)[19]. Studies in both rodents and man on the properties of circadian pacemakers have measured the period (τ) of the rest-activity (sleep-wake) cycle under constant conditions of isolation from external time cues. We report here that drugs selected from the 2 major classes of antidepressants, a monoamine-oxidase inhibitor (MAOI) - clorgyline - and a tricyclic - imipramine - can slow or dissociate circadian rest-activity rhythms; we propose that these effects may be an important mechanism in their antidepressant and mania-inducing actions.

Methods

Data collection and reduction. Hamsters were housed in steel cages attached to running wheels (⌀ 36 cm), with free access to food and water, in an airconditioned room maintained at 22 °C. The room was

Table 1. Effect of chronic antidepressant drugs on the period ($\bar{\tau}$) of the circadian rest-activity cycle

Treatment	N	Period ($\bar{\tau}$; h; mean ± SEM)[a]		t-Value[b]	$\Delta\bar{\tau}$
		Pretreatment	During treatment		
Clorgyline	12[d]	24.20±0.08	24.53±0.08	6.03 $p<0.0001$	0.33±0.05[c]
Empty implant	13	24.28±0.07	24.31±0.09	0.43 n.s.	0.03±0.06
Imipramine 10 mg/kg/day	9	24.31±0.10	24.25±0.07	0.69 n.s.	−0.06±0.08
Empty implant	6	24.33±0.12	24.25±0.17	1.58 n.s.	−0.08±0.05
Imipramine 20 mg/kg/day	13	24.25±0.06	24.21±0.06	0.78 n.s.	−0.04±0.05
Empty implant	5	24.35±0.10	24.32±0.21	0.27 n.s.	−0.03±0.11

[a] The period was estimated by the periodogram method ($p<0.01$ for a significant peak) using days − 10–0 for the pretreatment baseline, and days 4–14 for the treatment period. [b] Paired t-test. [c] $p<0.05$, Student's t-test clorgyline vs empty implant. Comparison of baseline $\bar{\tau}$ of controls and drug-treated animals was in no instance significantly different. [d] Excluding 1 hamster with an extreme lengthening of τ by 3.5 h.

entered at irregular intervals to replenish food and water, clean cages, and service equipment. Revolutions of each activity wheel activated an IR photocell detector wired to one channel of a specially-designed recording system[20]; total revolutions/15 min interval, date and time, were displayed on a CRT terminal and recorded on magnetic cartridge tape. The data were transferred weekly to continuous files on a cartridge disk in a PDP-11/40 computer, and visualized as double plots of 48-h running wheel activity with a Versatec plotter. As there was no manual back-up recording system, occasional recording failures resulted in loss of data.

Animals and experimental conditions. Adult female hamsters (7–8 weeks old, 100 g at the beginning of each experiment) were obtained from Charles River, Lakeview, N.J., and kept initially under the same 14:10 light:dark (LD) schedule used by the breeder and supplier for 2 weeks. Constant conditions were established using a continuously lit photographic red bulb (designated RR, to distinguish from complete darkness, DD).

Three separate experiments were carried out between June 1979 and February 1980. Clorgyline (2 mg/kg/day) or imipramine (10 or 20 mg/kg/day) in distilled deionized water were placed in Alzet® osmotic minipumps[21] designed to deliver drug at a constant rate of 0.5 µl/h for a minimum of 2 weeks. Baseline activity records in RR were inspected after 2–3 weeks to estimate expected time of activity onset (=circadian time CT 12) for each hamster. Short-lasting halothane anesthesia was used to implant the pumps s.c. (in dim red light) at CT 7–9, where it was hoped that least perturbation of the circadian system would occur (the 'dead zone' of the phase-response curve towards light)[19]. The pumps were not removed.

Biochemical pharmacology. Prior to the wheel-running experiments, preliminary biochemical studies were carried out to ascertain that chronic drug application was adequate. Hamsters implanted with minipumps containing clorgyline were killed at various times thereafter to determine the time course of MAO inhibition throughout and after the drug regime.

Table 2. Effect of chronic antidepressant drugs on the incidence of dissociation of circadian activity components

Treatment	Number of hamsters showing dissociation*	Stable free-running period	χ^2	p
Clorgyline 2 mg/kg/day	13	6	7.13	<0.01
Empty implant	3	11		
Imipramine 10 mg/kg/day	2	7	0.23	n.s.
Empty implant	2	4		
Imipramine 20 mg/kg/day	9	8	4.28	<0.05
Empty implant	0	5		

*Dissociation defined as more than one significant period in the circadian range in the periodograms of days 4–28 after drug implant. There was some overlap between animals showing a stable free-running period during days 4–14, with dissociation during days 15–28 (included here) and those whose data were used to calculate the results in table 1 (where only days 4–14 were used).

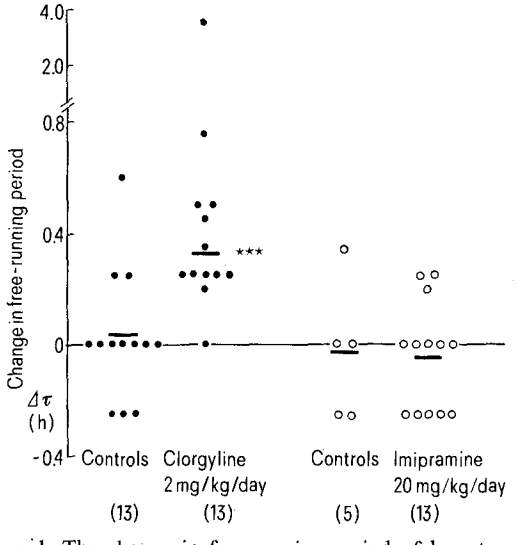


Figure 1. The change in free-running period of hamsters in RR after 2 weeks clorgyline, imipramine, or empty implants, is shown as $\Delta\tau$ (post-implantation τ minus pre-implantation τ in hours: a negative sign indicates shortening of τ, a positive sign lengthening of τ). Only those hamsters with a single peak in the circadian range in the periodograms during this time were used for the calculation of τ. A statistically significant lengthening of τ with clorgyline was found: paired t-test = 6.03, *** $p<0.001$ (the outlying value of $\Delta\tau=3.5$ h was not included in the calculation of either significance or mean $\Delta\tau$).

MAO activity was measured in brain homogenates using phenylethylamine and serotonin as substrates, by methods described previously[22].

Data analysis. The free-running period, τ, was measured by eye-fitting lines to activity onset by 2 independent observers, and later, by periodogram analysis[20,23]. Only the latter 'objective' data are presented here, even though comparisons of both methods yielded similar values for τ. Baseline τ in RR was estimated using the last 10 days free run before implantation: post-implantation τ from days 4–14 for both controls and drug-treated animals. Statistical analysis was by means of paired t-tests for pre- vs post-implantation τ for each hamster. Only those hamsters whose periodograms showed a single peak in the circadian range in the pre- and 2-week-post-implantation phase were used for τ calculations. Those hamsters whose periodograms showed 2 or more significant peaks in either the 2-week-post-implantation and/or the following 2 weeks, were considered for estimation of the incidence of dissociation of circadian activity components.

The data analysis programmes[20] also computed total wheel revolutions/24 h as well as estimating activity onset and offset times. These were used to measure the effect of drugs on motor activity per se (A), and on total activity time (α).

Results

1. The circadian rest-activity cycle

Effects of antidepressants on the circadian period. Chronic administration of clorgyline lengthened the circadian period: imipramine had no significant effect on τ (table 1, fig. 1). Examples of lengthening of τ by clorgyline, together with the corresponding periodograms (portions b. cf. a.) are shown in figures 2A and 2B. Lengthening often persisted after drug cessation (portion c.). Controls showed slight changes in both directions (figs 2C and 3C).

Effects of antidepressants on circadian activity components. During the baseline free-run in constant conditions the active phase of the rest-activity cycle frequently showed 2 distinct components, a long 'evening' activity bout and a shorter 'morning' bout[24]. In many imipramine-treated (20 mg/kg/day) animals, τ of the 'morning' activity component appeared to be preferentially lengthened such that it eventually broke away from the 'evening' activity component and traversed the rest phase. This was accompanied by a phase-advance of the 'evening' activity component. A similar phenomenon was observed in clorgyline-treated animals with or without an initial lengthening of τ of the undissociated rhythm. Examples of dissociation of circadian activity components after imipramine (fig. 3) and clorgyline (fig. 2) are shown with their corresponding periodograms.

Effects of antidepressants on total motor activity and activity time. Total wheel revolutions/24 h (A) were calculated for each day and each animal. The range of values between animals and between experiments over the last 10 days in RR before implantation was very small, indicating a very stable mode of running in hamsters (calculated to be about 1.6 km an hour). Halothane anesthesia and implants of empty capsules had no effect on A (see controls, arrow in fig. 4). However a gradual decline in total running activity of about 100 wheel revolutions/24 h occurred in RR during the course of each experiment.

Clorgyline treatment immediately decreased the amount of motor activity, even though behaviorally the animals did not appear sedated. During the 1st week of clorgyline treatment a significant decrease to 40%, during the 2nd week to 73% of control values occurred (fig. 4). Imipramine had no effect on A (fig. 4).

Neither clorgyline nor imipramine modified activity time (α). If we use only the time spent running (excluding resting bouts scattered throughout the active phase), as a modified measure of α to allow for the complex patterns seen with drugs, there was no significant effect on running time with drugs: imipramine, 7.8±0.3 h/day compared with baseline RR 9.2±0.3 h; controls, 7.7±0.8 h compared with 9.1±0.7 h; clorgyline, 10.6±0.5 h compared with baseline RR 10.5±0.3 h; controls, 8.7±0.5 h compared with 9.7±0.5 h.

2. Biochemical pharmacology

Figure 5 shows that the dose of clorgyline used in these studies, 2 mg/kg/day, caused rapid and substantial inhibition of hamster brain MAO-A activity (the MAO-A form of the enzyme is preferentially inhibited by clorgyline[25], and hamster brain contains predominantly MAO-A[26] – our studies have found a ratio of MAO-A:B of 90:1). Since the minipumps were not removed after 2 weeks, the exact time of cessation of drug administration in each animal was not known. However it can be seen that only little recovery of MAO activity had occurred even many weeks later.

Imipramine plasma levels were not measured. This lack of information as to adequate imipramine levels was compounded by a problem related to the local anesthetic properties of the tricyclic drug itself: after both modes of application, necrosis of tissue around the implants was observed, that may have impaired delivery of drug, and have been a source of variance in the individual plasma levels attained. This variability and possible low drug concentration may have been one reason for the negative effects of imipramine on τ. Indeed, a recent study[27] where plasma levels of imipramine were measured, showed negligible steady state concentrations and no effect on

τ. The problem of drug application in circadian rhythm experiments is discussed in detail elsewhere[16].

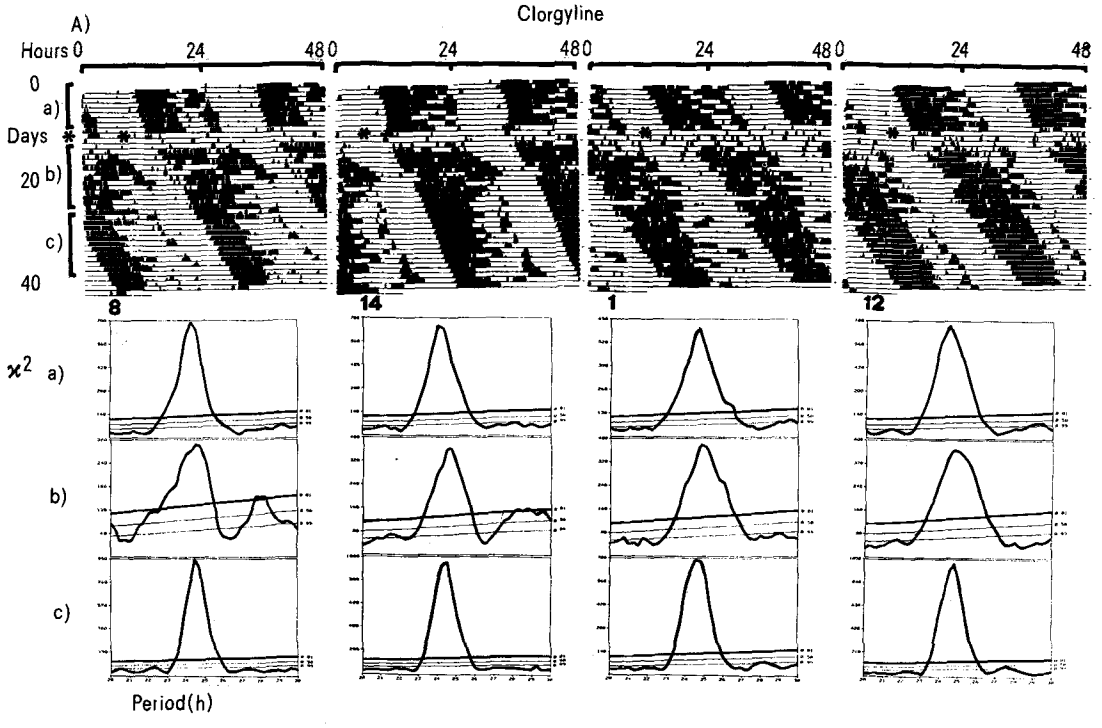
Discussion

These studies extend previous findings that lithium can lengthen τ to another class of antidepressant drugs. Although our first analysis of the data using visual estimation of τ, indicated that imipramine, as well as clorgyline, could lengthen τ[28], this was not substantiated for imipramine using the more rigorous criteria of a) periodogram analysis and b) excluding all animals showing dissociation of activity components. The magnitude of the change in τ after clorgyline is not trivial: a difference of 0.2-0.3 h has been documented for lithium and for steroid hormones (the latter changes being associated with important behavioural events)[6].

Slowing the circadian pacemaker driving the rest-activity cycle would be predicted to delay the phase-position (ψ) of entrained rhythms[29]. This occurs with lithium[16], clorgyline, and pargyline (another MAOI) in rats[30] and hamsters[31]. A recent investigation of imipramine in rats (administered by stomach intubation to ensure adequate dosage) has also noted a phase-delay of activity onset in an LD cycle[32]. Thus even though only clorgyline was found to lengthen τ, both clorgyline and imipramine have been found to delay ψ, even in neurochemical rhythms[18,33-35]. It is important that lengthening of τ is reflected in a delay of ψ, since these drugs are used to treat depressive patients who are living under entrained conditions. The abnormal circadian rhythms often observed in depressive patients have been interpreted as a phase-advance with respect to the sleep-wake or the day-night cycle[17,18,36]: if this advance in ψ is correlated with pathophysiology, then a delay in ψ induced by antidepressants may, correspondingly, be correlated with their clinical efficacy.

Clorgyline and imipramine also promote dissociation and relative coordination of activity components. Although this sometimes occurred spontaneously in dim red light, the changes in controls occurred significantly less often than was found with drug-treated animals (see table 2)[37]. Thus dissociation between evening and morning activity bouts did not arise de novo as a response to drugs, but, under our particular conditions was a normal, though infrequent response[37] that was facilitated by drug treatment.

Dissociation could be considered as a drug effect modifying coupling between oscillators driving these activity components. To our knowledge, this is the first description of such an effect promoted by pharmacological agents. Deuterium oxide and lithium, which also lengthen τ, do not induce dissociation or 'splitting'[13,16]. The only 'splitting' (defined as the persistence of 2 separated activity components in a stable phase relationship and with the same periodicity)[24] consistently observed in hamsters occurs after lengthening of τ in bright white light[24]. However there are important differences between splitting in LL and the dissociation found with antidepressants: in splitting in LL the 2 activity components rapidly find a new stable phase-relationship; in the case of antidepressant drug-induced dissociation in RR, the 2 components rarely maintain a stable phase-relationship. Examples in figure 3B (hamsters 18, 23) do show



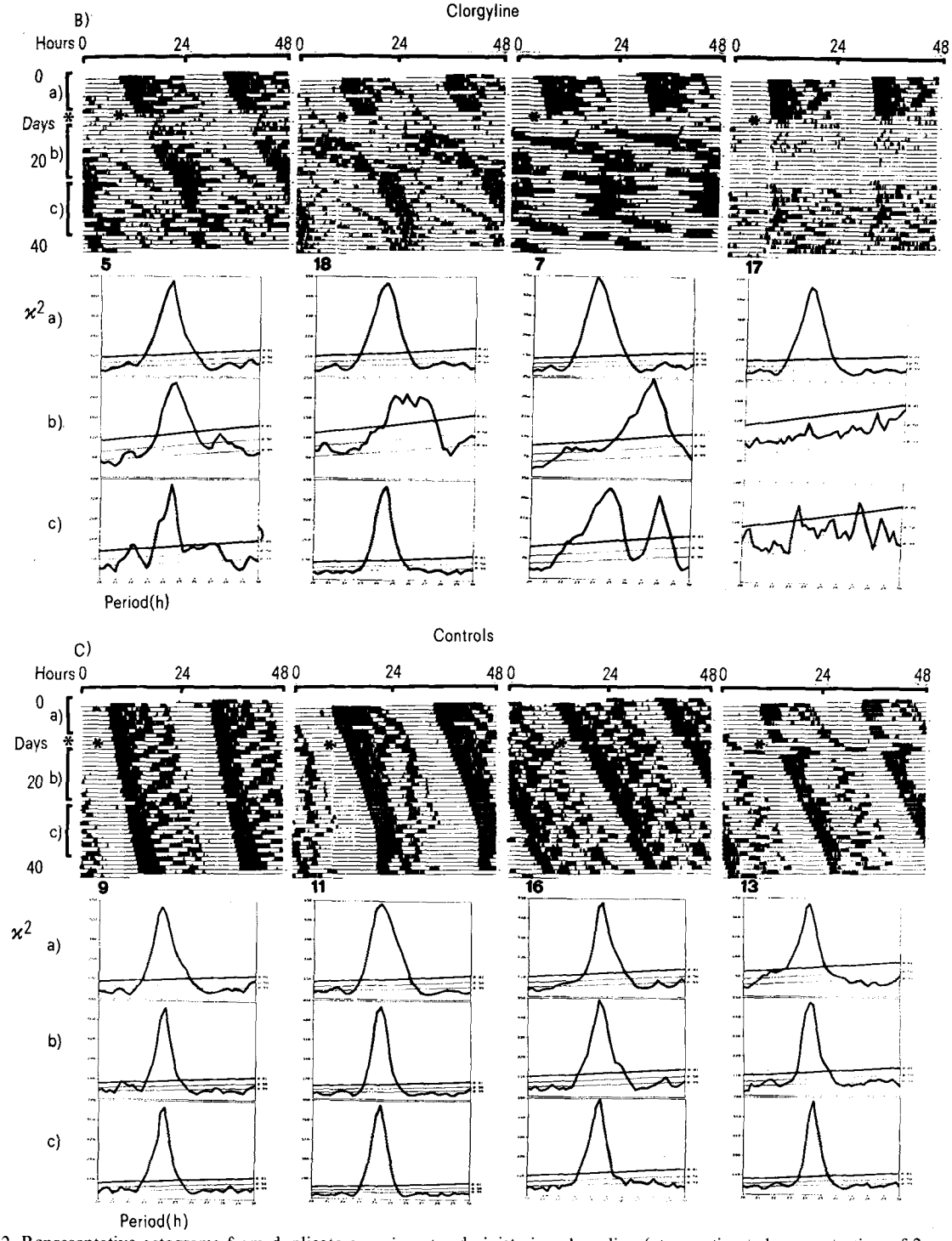
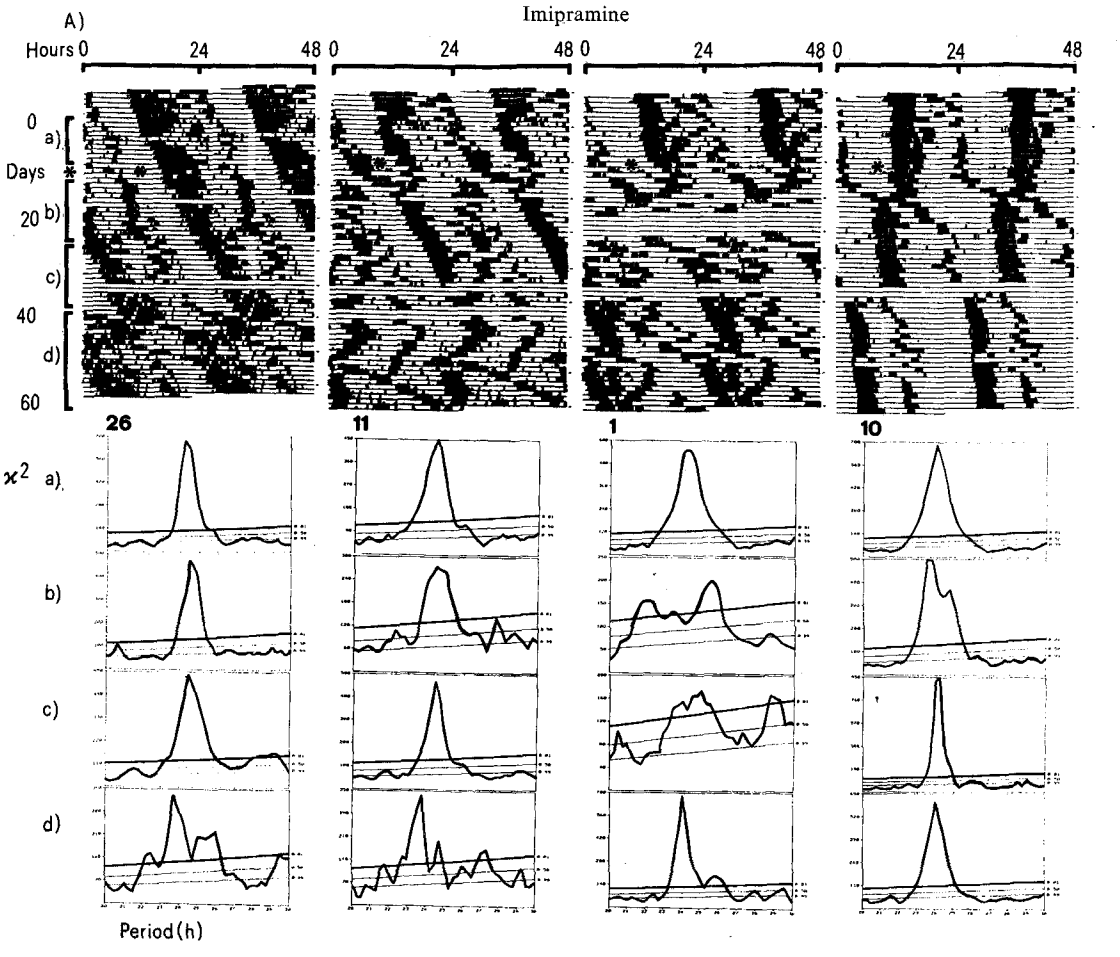
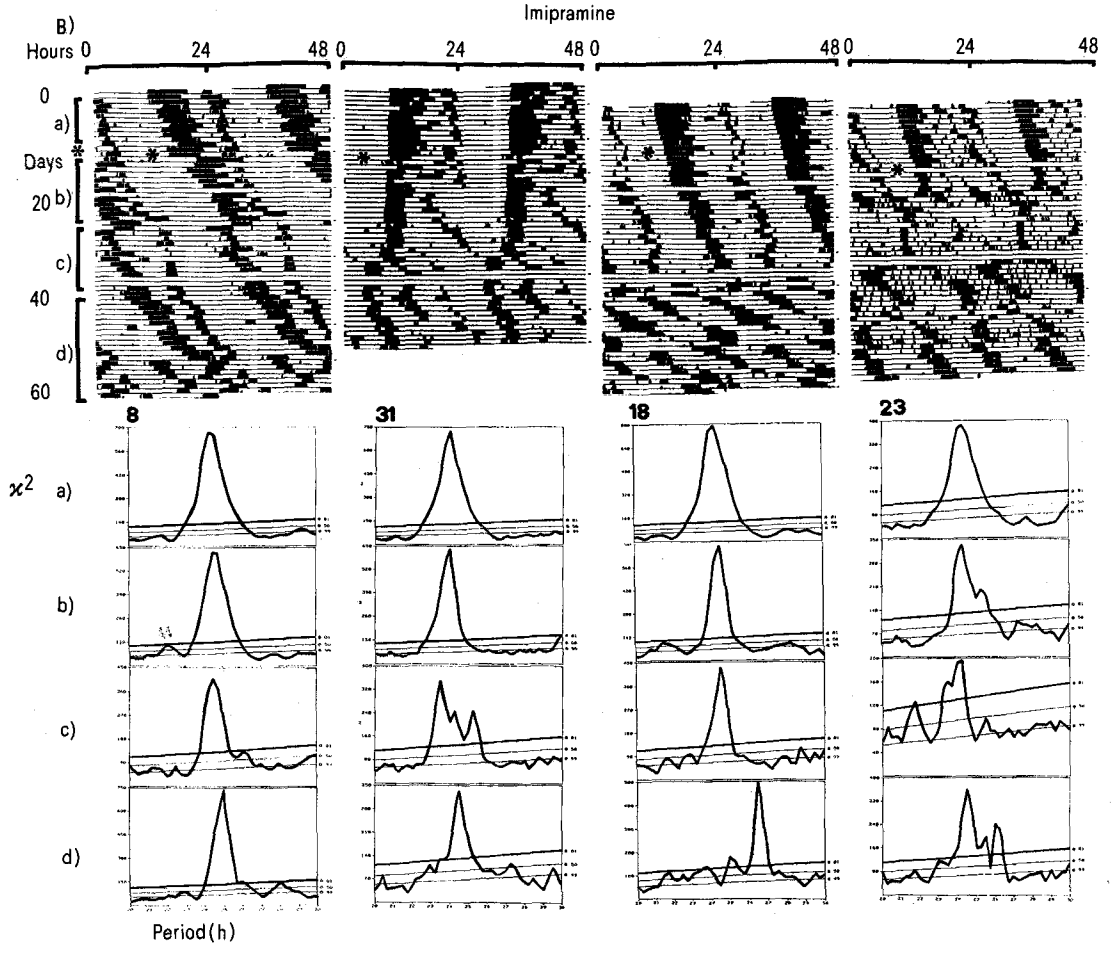


Figure 2. Representative actograms from duplicate experiments administering clorgyline (at an estimated concentration of 2 mg/kg/day for 2 weeks) (A and B) or empty implants (C), together with their corresponding periodograms. The time scale runs from left to right, and the activity record has been doubled to permit visulization of the continuity of free-running rhythms (x-axis = 48 h). Successive days' activity are shown beneath each other (y-axis = circa 40 days). After 40 days a breakdown in the data-collecting system occurred, lasting about 10 days: the last 4 weeks are not shown because of lack of visual continuity, but were used to calculate total activity (A, fig. 4). Periodogram analysis of baseline (= portion labelled a) used the last 10 days in RR before implantation (shown by * in the actogram at CT 7–9), the following days 4–14 (= b) for analysis of the drug effect, and days 15–28 (= c) for post-drug effect. Where no dissociation into 2 or more significant activity components (the upper horizontal line in the periodogram indicates the $p < 0.01$ level) occurred, $\Delta\tau$ averaged 0.33 h. Of interest in the clorgyline-treated hamsters is that the periodogram shows periodicities that cannot be estimated visually: e.g. in A), portion (b), hamsters 8 and 14 show that clorgyline lengthens activity onset τ by 0.5 h, but also lengthens activity offset τ to a greater extent (2nd peak circa 3.75 h and 4.5 h); in B), portion (c), hamsters 5, 7, 17, show a shorter activity component ($\Delta\tau$ of -2.25, $+0.75$, -0.5 h respectively) as well as a long activity component ($\Delta\tau$ of 3.0, 3.5, 3.25 h as compared with baseline τ). In B), hamster 17 is shown as an example of apparent arrhythmicity (b) and extreme sedation induced by clorgyline (no significant peak in periodogram), followed by (c) which does however yield significant periods. This extreme drug effect was rare, but has been seen when clorgyline is administered directly into the SCN.


A)
Imipramine
Hours 0 24 48 0 24 48 0 24 48 0 24 48
0
a)
Days
20 b)
c)
40
d)
60
26
11
1
10
χ² a)
b)
c)
d)
Period (h)


B)
Imipramine
Hours 0 24 48 0 24 48 0 24 48 0 24 48
0
a)
Days
20 b)
c)
40
d)
60
8
31
18
23
χ² a)
b)
c)
d)
Period (h)

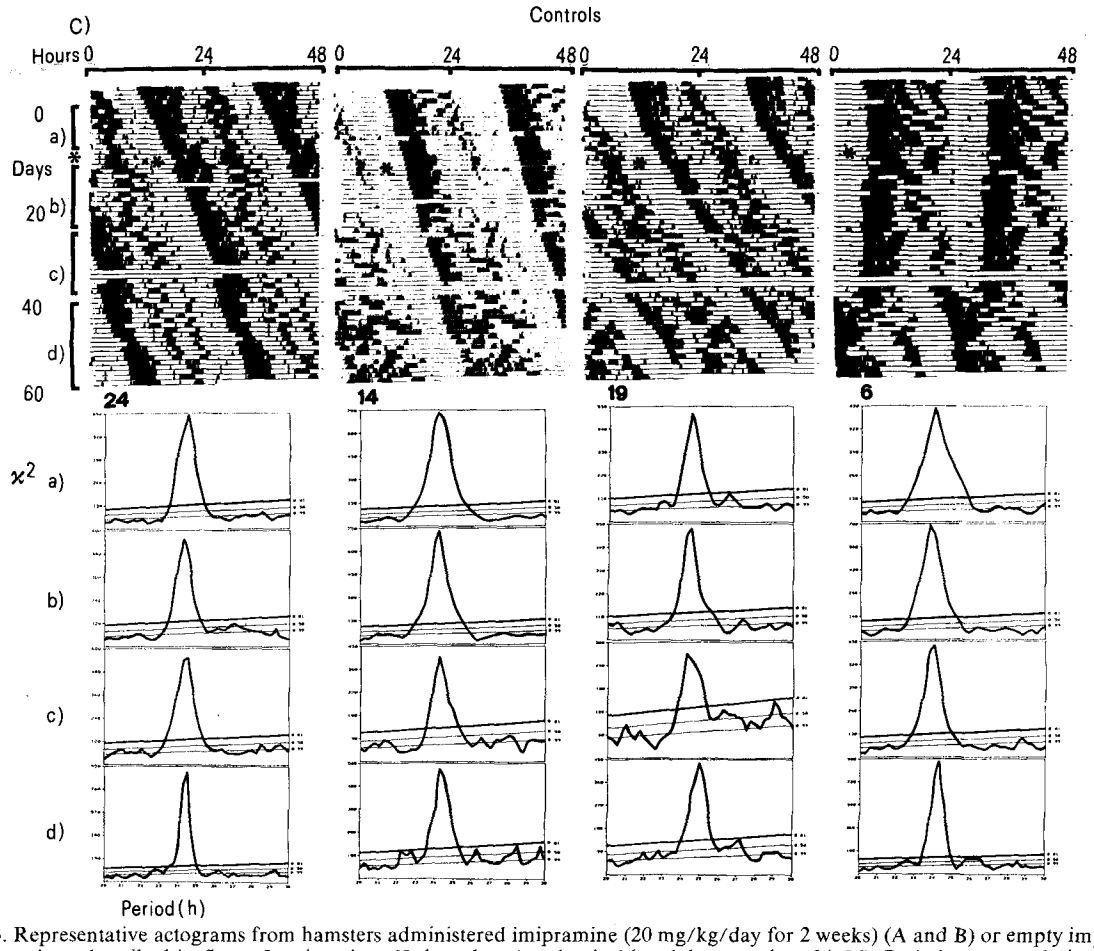


Figure 3. Representative actograms from hamsters administered imipramine (20 mg/kg/day for 2 weeks) (A and B) or empty implants (C). The format is as described in figure 2, using circa 60 days data (mechanical breakdown at days 34-36). Periodogram analysis of baseline (a), and post implantation (* at CT 7-9) days 4-14 (b), 15-28 (c), and 29-50 (d) are shown. There were few effects on τ = (A), hamsters 26, 11 and B), hamsters 8, 18, show initial lengthening of τ. Various patterns of dissociation of activity components after imipramine are seen; the selective lengthening of τ of the morning activity bout is particularly clear in A), hamster 10. As with clorgyline, there are some examples of dissociation into an activity component with shorter period underlying the activity bouts with longer period (A), all actograms at some stage; B), hamsters 31, 18, 23). In B), hamster 23 is shown as an example of dissociation and decreased rhythmicity immeditely after implantation: in this hamster 2 Alzet minipumps were implanted at a total dose of 50 mg/kg/day, indicating some dose dependence of effect (the other hamster given this dose died). In this animal too, 'splitting' is clearly seen at the end of the experiment. Control hamster 6 showed a phase jump; otherwise changes in controls were small and in both directions.

stable split activity components at the end of the experiment. However a further occasional finding was that the rest-activity cycle as a whole could be markedly lengthened and apparently break away from a shorter, underlying rhythmic component (e.g. fig. 3A, hamsters 26, 11; fig. 3B, hamsters 18, 23; fig. 2B, hamsters 5, 7). The latter in particular resembles actograms in humans under free-running conditions when the rest-activity cycle breaks away from the temperature rhythm[37]. A somewhat similar pattern also occurs in conditions of entrainment in certain manic-depressive patients when they undergo spontaneous, or tricyclic- or MAOI-induced switches out of depression into mania[18,38]. Imipramine- or clorgyline-induced switches into mania may be related to the capacity of these drugs to dissociate circadian rhythms.

One caveat needs to be added: in these hamsters (as a group, both male and female) we have noted an instability over many years, of baseline free-running activity rhythms that is unusual for the species. No reason has yet been found for why animals from the same dealer should be more labile in our laboratory than in others. This baseline susceptibility may be one explanation for the variability of effects seen with antidepressant drugs; it does not however undermine the validity of the observations.

Until now, very few drugs have been found to modify the frequency of the circadian pacemaker[4,5,12]. Our studies raise the question of whether other clinically effective antidepressants can slow or dissociate circadian rhythms, and whether these effects are specific for antidepressants alone. Under entrained conditions another MAOI, pargyline, has been observed to delay ψ of activity onset[30,40] and we have recently found that methamphetamine, a psychoactive drug sometimes used as an antidepressant, can delay ψ of activity, temperature, and corticosterone rhythms[41].

That this occurs through lengthening of τ has been indicated in an early study of amphetamine in wood-mice[42].

Another question concerns the primary site of action of these drugs: do they act on the circadian pacemaker itself? In preliminary studies[12,43], administration of clorgyline and imipramine directly to the suprachiasmatic nuclei of the anterior hypothalamus (the putative mammalian circadian pacemaker) disrupted or slowed circadian rhythmicity, whereas drugs administered in nearby brain structures had no effect.

Thus there is some evidence for a selective action of antidepressants on circadian pacemaker frequency. Nevertheless, other effects may be as important: for example, clorgyline interferes with the effect of light on the circadian rhythm of pineal melatonin[31,40], and may also affect visual imput to the SCN at the level of the retina itself[39].

Finally, we need to relate the effects of antidepressant drugs on circadian timekeeping to their known neuropharmacology. In this respect, our findings that both clorgyline and imipramine delay the phase-position of many neurotransmitter receptor rhythms are of particular interest, since they form a link between research fields that are usually separate. In a recent review[12], we have considered which neurotransmitters or neuropeptides have been unequivocally identified in the SCN, which pharmacological manipulations of the SCN or its afferents modify circadian phase or period, and which of the multiplicity of effects of antidepressants on neurotransmission are relevant to the effects of these drugs on the circadian system. Our present state of knowledge implicates the serotonergic and cholinergic systems as the most likely substrates: iontophoretic application of clorgyline and imipramine in the SCN potentiate both the effect of iontophoresized and endogenously released serotonin[43]. Antidepressant drugs thus provide powerful new tools to investigate circadian physiology and its neurochemical basis. Conversely, modulation of circadian frequency may be an important mode of action of the widely disparate group of antidepressant drugs.

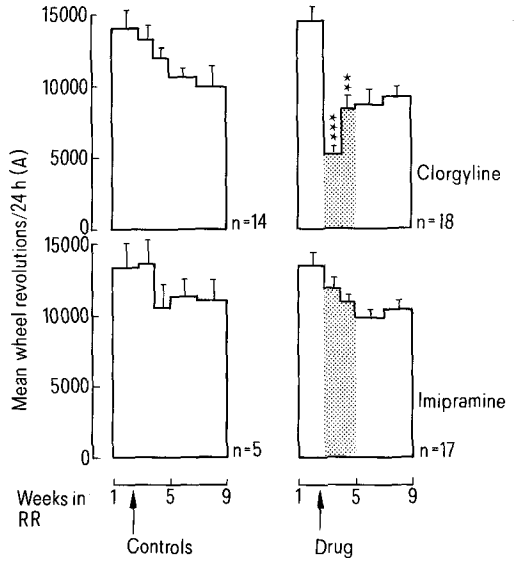


Figure 4. Total wheel revolutions/24 h (A) were averaged for each hamster over time and for all hamsters in a group. The arrow after 10 days baseline indicates halothane anesthesia and implantation. The striped area indicates the estimated time of drug release. The mean±SEM of (A) is shown for 10 days baseline in RR, week 1 of drug/empty implant, week 2, weeks 3+4, weeks 5+6. Although there was a decrease in motor activity over the course of the experiment in RR in controls, only clorgyline administration was found to cause a significant reduction during drug release. t-test drug vs controls during the same time-span $^{***}p < 0.001$; $^{**}p < 0.01$.

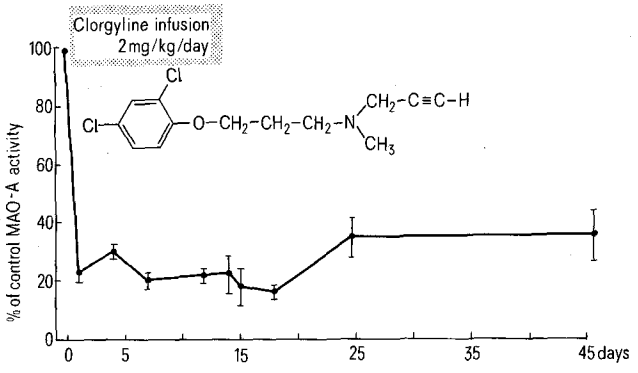


Figure 5. Hamster brain MAO-A activity using ^{3}H-serotonin as substrate, expressed as percentage of control animals. Clorgyline was administered in minipumps, and hamsters (N=3-6/time point) killed at various times after. MAO-A was inhibited 80% by the 1st day, and had not increased substantially (64% inhibition) even up to 30 days after presumed cessation of drug infusion. On the basis of the dose used we would have predicted total inhibition of MAO-A. However, the actual level of inhibition may indeed have been greater than 80%, since the level of MAO activity relative to blank values was extremely low.

Acknowledgments. The authors thank Carolyn Craig for careful assistance with collection of the data; Newlin Morgan for maintenance of the hardware in spite of the vagaries of escaped hamsters; Bill Vaughn and Wayne Rasband for development of the programmes for analysing and displaying the data; and Gerard Groos for exigent criticism of earlier versions of the manuscript.

1 A preliminary analysis of these results was presented at the 18th Annual Meeting of the American College of Neuropsychopharmacology, Puerto Rico 1979[28].
2 Visiting Fellow of the Schweizerische Stiftung für biologisch-medizinische Stipendien; present address: Psychiatrische Universitätsklinik Basel, CH-4025 Basel, Switzerland.
3 Neuropharmacology Branch, National Institute of Mental Health; present address: Institute of Psychiatry, London, England.

4 Pittendrigh, C.S., and Caldarola, P.C., General homeostasis of the frequency of circadian oscillations. Proc. natl Acad. Sci. USA *70* (1973) 2697-2701.
5 Richter, C.P., Biological clocks in medicine and psychiatry. Charles C. Thomas, Springfield, Ill. 1965.
6 Morin, L.P., Fitzgerald, K.M., Rusak, B., and Zucker, I., Circadian and neural organization of hamster reproductive rhythms. Psychoneuroendocrinology *2* (1977) 73-98.

7 Takahashi, J.S., and Menaker, M., Interaction of estradiol and progesterone: effects on circadian locomotor rhythm of female golden hamsters. Am. J. Physiol. *239* (Regulat. integr. comp. Physiol. *8*) (1980) R497–R504.

8 Daan, S., Damassa, D., Pittendrigh, C.S., and Smith, E.R., An effect of castration and testosterone replacement on a circadian pacemaker in mice *(Mus musculus)*. Proc. natl Acad. Sci. USA *72* (1975) 3744–3747.

9 Turek, F.W., McMillan, J.P., and Menaker, M., Melatonin: effects on the circadian locomotor rhythm of sparrows. Science *194* (1976) 1441–1443.

10 Beasley, L., and Nelson, R., Thyroid gland influences the period of hamster circadian oscillations. Experientia *38* (1982) 870–871.

11 Kamgar-Parsi, B., Wehr, T.A., and Gillin, J.C., Successful treatment of human non-24-hour sleep-wake syndrome. Submitted for publication.

12 Wirz-Justice, A., Groos, G.A., and Wehr, T.A., The neuropharmacology of circadian timekeeping in mammals, in: Vertebrate Circadian Systems: Structure and Physiology. Eds J. Aschoff, S. Daan, and G.A. Groos. Springer, Berlin, Heidelberg 1982, in press.

13 Daan, S., and Pittendrigh, C.S., A functional analysis of circadian pacemakers in nocturnal rodents. III. Heavy water and constant light: homeostasis of frequency? J. comp. Physiol. *106* (1976) 267–290.

14 Zatz, M., and Herkenham, M.A., Intraventricular carbachol mimics the phase-shifting effect of light on the circadian rhythm of wheel-running activity. Brain Res. *212* (1981) 234–238.

15 Zatz, M., and Brownstein, M.J., Injection of α-bungarotoxin near the suprachiasmatic nucleus blocks the effects of light on nocturnal pineal enzyme activity. Brain Res. *213* (1981) 438–442.

16 Wirz-Justice, A., The effects of lithium on the circadian system, in: Basic Mechanisms in the Action of Lithium, pp.249–258. Eds H.D. Lux, J.B. Aldenhoff, H.M. Emrich. Excerpta Medica, Amsterdam 1982.

17 Wehr, T.A., and Goodwin, F.K., Biological rhythms and psychiatry, in: American Handbook of Psychiatry, Vol.7, 2nd edn, pp.46–74. Eds S. Arieti and H.K.H. Brodie. Basic Books, New York 1981.

18 Wehr, T.A., and Wirz-Justice, A., Circadian rhythm mechanisms in affective illness and in antidepressant drug action. Pharmacopsychiatry *15* (1982) 31–39.

19 Daan, S., and Pittendrigh, C.S., A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. J. comp. Physiol. *106* (1976) 253–266.

20 Morgan, N., Vaughn, W., Rasband, W., Wehr, T.A., and Wirz-Justice, A., A computer-based system for collection and analysis of circadian rest-activity data. Experientia *38* (1982) 1296–1301.

21 Theeuwes, F., and Yum, S.I., Principles of the design and operation of generic osmotic pumps for the delivery of semisolid or liquid drug formulations. Ann. Biomed. Engng *4* (1976) 343–353.

22 Campbell, I.C., Robinson, D.S., Lovenberg, W., and Murphy, D.L., The effects of chronic regimens of clorgyline and pargyline on monoamine metabolism in the rat brain. J. Neurochem. *32* (1979) 49–55.

23 Sokolove, P.G., and Bushnell, W.N., The chi-square periodogram: its utility for analysis of circadian rhythms. J. theor. Biol. *72* (1978) 131–160.

24 Pittendrigh, C.S., and Daan, S., A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: a clock for all seasons. J. comp. Physiol. *106* (1976) 333–355.

25 Johnstone, J.P., Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmac. *17* (1968) 1285–1297.

26 Edwards, D.J., and Malsbury, C.W., Characteristics of monoamine oxidases in rat brain and other organs of the golden hamster. Biochem. Pharmac. *27* (1978) 959–963.

27 Aschoff, J., personal communication.

28 Wirz-Justice, A., Wehr, T.A., Goodwin, F.K., Kafka, M.S., Naber, D., Marangos, P.J., and Cam bell, I.C., Antidepressant drugs slow circadian rhythms i ı behaviour and brain neurotransmitter receptors. Psychoph ırmac. Bull. *16* (1980) 45–47.

29 Aschoff, J., The phase-angle differenc in circadian periodicity, in: Circadian Clocks, pp.263–276. Ed. J. Aschoff. North-Holland, Amsterdam 1965.

30 Rommelspacher, H. von, Bade, P., Blu lau, J., and Strauss, S., Hemmung der Monoamineoxidase und Tag-Nacht-Rhythmus: Korrelation zwischen physiologischen und biochemischen Parametern. Arzneimittel-Forsch. *26* (1976) 1078–1080.

31 Craig, C., Tamarkin, L., Garrick, N., and Wehr, T.A., Long-term and short-term effects of clorgyline (a monoamineoxidase type A inhibitor) on locomotor activity and on pineal melatonin in the hamster, in: Abstract No. 229.14, Society for Neuroscience 11th Annual Meeting, 1981.

32 Mason, R., personal communication.

33 Wirz-Justice, A., Kafka, M.S., Naber, D., and Wehr, T.A., Circadian rhythms in rat brain α- and β-adrenergic receptors are modified by chronic imipramine. Life Sci. *27* (1980) 341–347.

34 Wirz-Justice, A., Kafka, M.S., Naber, D., Campbell, I.C., Marangos, P.J., Tamarkin, L., and Wehr, T.A., Clorgyline delays the phase-position of circadian neurotransmitter receptor rhythms. Brain Res. *241* (1982) 115–122.

35 Wirz-Justice, A., Antidepressant drugs: effects on the circadian system, in: Circadian Rhythms in Psychiatry. Eds T.A. Wehr, and F.K. Goodwin. Boxwood Press, Los Angeles 1982, in press.

36 Wehr, T.A., Wirz-Justice, A., Goodwin, F.K., Duncan, W., and Gillin, J.C., Phase advance of the sleep-wake cycle as an antidepressant. Science *206* (1979) 710–713.

37 Wirz-Justice, A., and Wehr, T.A., Uncoupling of circadian rhythms in hamsters and man, in: Sleep 1980, pp.64–72. Ed. W.P. Koella. Karger, Basel 1980.

38 Wehr, T.A., Goodwin, F.K., Wirz-Justice, A., Craig, C., and Breitmaier, J., 48-Hour sleep-wake cycles in manic-depressive illness: naturalistic observations and sleep deprivation experiments. Archs gen. Psychiat. *39* (1982) 559–565.

39 Remé, C., and Wirz-Justice, A., Disk shedding and autophagy in rat retina: antidepressant drug modifies retinal circadian rhythms. Suppl. Invest. Ophthalmol. *22* (1982) 140.

40 Wehr, T.A., Lewy, A.J., Wirz-Justice, A., Craig, C., and Tamarkin, L., Antidepressants and a circadian rhythm phase-advance hypothesis of depression, in: Brain Neurotransmitters and Hormones, pp.263–276. Eds R. Collu, J.R. Ducharme, A. Barbeau and G. Tolis. Raven Press, New York 1982.

41 Morimasa, T., Wirz-Justice, A., Kräuchi, K., and Feer, H., Chronic methamphetamine phase delays rat circadian rhythms. XIII International congress of the International Society of Psychoneuroendocrinology, Tübingen 1982; abstract.

42 Bodman, K. von, Die Wirkung von Pharmaka auf Kurzzeitschätzung und circadiane Periodik von Säugetieren. Z. vergl. Physiol. *68* (1970) 276–292.

43 Groos, G.A., and Mason, R., An electrophysiological study of the rat's suprachiasmatic nucleus: a locus for the action of antidepressants. J. Physiol., Lond. *330* (1982) 40 P.