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Effect of imipramine and amitriptyline on circadian locomotor activity rhythm in the field mouse *Mus booduga*

P SUBRAMANIAN and R SUBBARAJ*
Department of Animal Behaviour, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. We have performed experiments on the influence of imipramine and amitriptyline (antidepressant drugs) offered through drinking water on the period length (τ) of the circadian locomotor activity rhythm in the field mouse *Mus booduga* under continuous darkness (DD). Ingestion of imipramine to mice under freerunning conditions caused lengthening of the activity rhythm whereas amitriptyline caused dissociation of activity components. Furthermore, the amount of activity (α) is significantly reduced ($P < 0{\cdot}005$) during imipramine as well as amitriptyline treatment. The action of these antidepressants may be explained by their action on serotonergic terminals in the suprachiasmatic nucleus, the putative circadian pacemaker in mammals.

Keywords. *Mus booduga*; period lengthening; imipramine; amitriptyline; amount of activity.

1. Introduction

Drugs have proven to be valuable in probing the mechanism(s) underlying mammalian circadian system (Wehr and Wirz-Justice 1982). Several investigators have described the period modifying effects of antidepressant drugs on circadian rhythms (Wirz-Justice and Campbell 1982). Lithium ions were first reported to cause period lengthening in the circadian rhythm of petal movement of a crassulacean flower and in the locomotor activity of a desert mouse (Engelmann 1973) and has since been known to have significant effects in several other systems (Subbaraj 1981). Imipramine, one of the tricyclic antidepressant drugs was reported to cause period lengthening and dissociation of activity components in the locomotor activity of freerunning hamsters kept under constant darkness (DD) (Wirz-Justice and Campbell 1982). Clorgyline, another type of antidepressant drug, was also reported to promote dissociation of activity components (Duncan *et al* 1988). Furthermore, clorgyline and imipramine were found to delay the phase position of many neurotransmitter receptor rhythms (Wirz-Justice *et al* 1982).

We have studied the influence of imipramine and amitriptyline (tricyclic antidepressant drugs) on the locomotor activity rhythm of the field mouse *Mus booduga* offered through drinking water. Imipramine was found to cause period lengthening of the activity rhythm under freerunning conditions in DD whereas amitriptyline dispersed the activity components of the freerunning rhythm. There was a significant reduction of amount of activity (α) during the treatment of both these drugs.

*To whom all the correspondence should be addressed.

2. Materials and methods

The mice were captured from fields surrounding the University campus. They were kept in light-tight, temperature controlled experimental cubicle maintained at $31 \pm 1°C$. All experiments were performed in constant darkness (DD). Food comprising of millets, maize, grains and water were available *ad libitum*. Dim red light of 610–700 nm was used while feeding the animals (Viswanathan and Chandrashekaran 1985) and the hours of routine care were varied. An eccentrically placed magnet on the wheel temporarily made and broke contacts in an electrical circuit with every turn of the wheel. The revolutions were picked up by channels of an A 620X Esterline Angus Event Recorder. Actograms were constructed and double plotted in a manner which is now routine in chronobiological research (Pittendrigh and Daan 1976).

Imipramine (2 mg/ml) and amitriptyline (2 mg/ml) were administered through drinking water for its wide acceptance (Subbaraj 1981; Aschoff 1989) and as a reliable and convenient mode of drug administration (Melżacka *et al* 1985). (LD_{50} for imipramine = 3·5 mg/ml; LD_{50} for amitriptyline = 3 mg/ml for *M. booduga*). Stable values of period length (τ) were established while mice were in DD and drinking tap water. After 14 days of freerun, the water bottles were replaced with particular concentration of the drug. Drug administration was continued for the consecutive days of the experiment. For pharmacological studies on circadian rhythms, each animal acts as its own control (Kayser and Hildwin 1977). Final steady values of period length (τ) obtained while mice were ingesting the drug were calculated and compared to τ prior to drug administration.

3. Results

The activity recordings of imipramine treated animals are shown in figure 1. Imipramine had period lengthening effect in all the cases studied ($n = 7$). Period change was observed immediately after the administration of the drug. The values of τ (mean ± SD) were $23{\cdot}50 \pm 0{\cdot}25$ h before treatment and $23{\cdot}62 \pm 0{\cdot}25$ h after imipramine treatment ($P < 0{\cdot}05$; Student's *t*-test). Dissociation of activity components of the freerunning rhythm was observed during the treatment of amitriptyline (figure 2) ($n = 6$). The amount of activity (α) became lesser during the treatment of both these drugs. The reduction of α during drug treatment is statistically significant ($P < 0{\cdot}005$, Student's *t*-test; table 1). After the termination of drug treatment, an increase in the amount of activity was observed.

4. Discussion

The mice *M. booduga* exhibit very precise onset on nocturnal motor activity which permits an accurate measurement of τ. Furthermore, the mice exhibit very stable period length (τ) and the exogenous influences have very little effect. Thus, this resistance to manipulation lowers the probability of spurious results (Viswanathan and Chandrashekaran 1985).

Antidepressant drugs are reported to lengthen τ, dissociate activity components and to reduce amount of activity (Wirz-Justice and Campbell 1982; Aschoff 1989) in

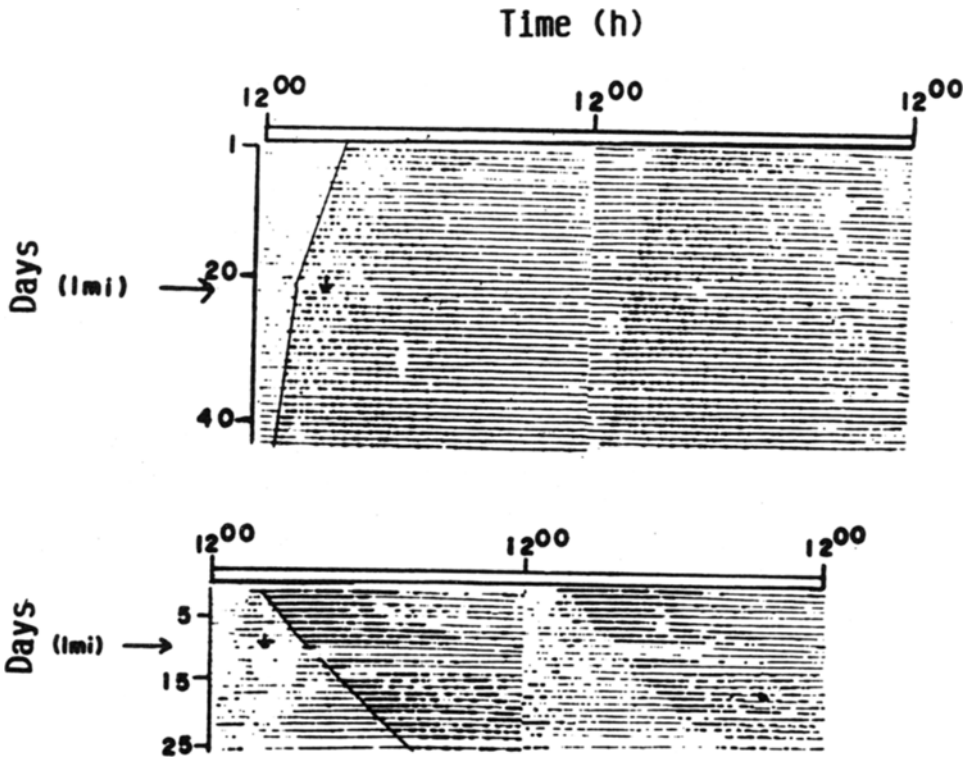

Figure 1. Representative actograms showing the effect of imipramine (2 mg/ml) on freerunning rhythm of *M. booduga*. A period lengthening occurred after the drug treatment.

hamsters and our studies substantiate these findings. Moreover, the magnitude of change in τ during drug treatment is small and a difference of only 0·2–0·3 h has been documented (Wirz-Justice and Campbell 1982). In our studies also, similar results were obtained. Antidepressant drugs are known to exert their mechanism by increasing serotonin levels in suprachiasmatic nucleus (SCN), the putative circadian hypothalamic pacemaker (Greco *et al* 1988). The SCN is particularly rich in serotonergic terminals (Groos *et al* 1983) and it was proved that imipramine binding in the SCN was highest than in any other brain regions (Wirz-Justice *et al* 1983). Such pharmacological manipulation of serotonin might modify circadian phase or period in mice. For instance, Wirz-Justice *et al* (1982) reported that clorgyline delayed the phase position of several neurotransmitter receptor rhythms in rats under entrained conditions and suggested that these delayed phase position of receptors may be reflected in the frequency of the central circadian pacemaker.

Dissociation of activity components was observed during the treatment of amitriptyline in mice similar to the treatment of clorgyline in hamsters (Duncan *et al* 1988). The period lengthening effect of imipramine and dissociation of activity components of amitriptyline may also be explained by the predictions based on the Pittendrigh-Daan model of the complex circadian pacemaker (Pittendrigh and Daan 1976). According to this model, the phase relationship between two coupled

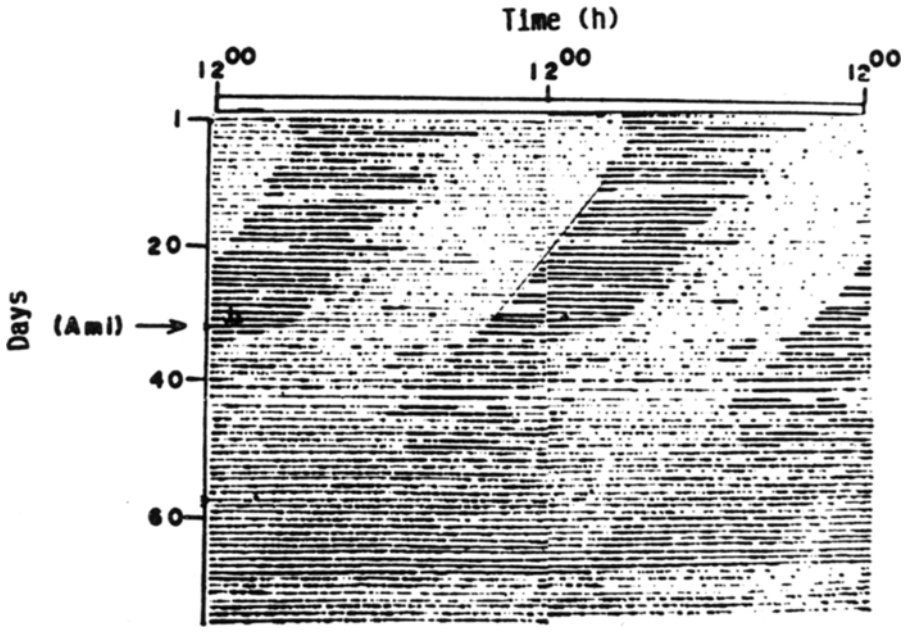

Figure 2. Effect of amitriptyline (2 mg/ml) on the freerunning rhythm of *M. booduga*. Dispersed activity components can be seen after the drug treatment.

Table 1. Effect of imipramine and amitriptyline on the amount of activity.

Amount of activity (α)					
Before drug treatment (h)		During drug treatment (h)		After drug treatment (h)	
Imipramine (2 mg/ml)	Amitriptyline	Imipramine (2 mg/ml)	Amitriptyline	Imipramine (2 mg/ml)	Amitriptyline
6·91	9·06	4·46	6·96	6·7	10·1
6·7	12·23	4·14	2·13	6·97	4·34
6·97	9·4	6·24	7·48	7·47	7·56
9·66	6·52	6·43	1·28	6·6	3·2
6·6	7·14	6·58	6·54	6·95	6·91
6·9	4·73	3·14	1·47	4·93	1·6

oscillators determines the period of freerunning activity as well as the ratio of activity to rest. Our drug induced changes in the overt locomotor rhythm can be interpreted as resulting from a change in this phase relationship (Duncan *et al* 1988). There was a reduction of α during methamphetamine treatment in hamsters (Aschoff 1989) similar to our results obtained in *M. booduga*. Antidepressant drugs thus provide powerful tools to investigate circadian physiology and its neurochemical basis. Conversely, modulation of circadian frequency may be an important mode of action of widely disparate group of antidepressant drugs.

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