

STUDY OF PHOTIC VERSUS NON-PHOTIC CUES AS ENTRAINERS OF CIRCADIAN RUNNING ACTIVITY IN RATS

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Summary : Though relative dominance of photic/non-photic cues on rodent circadian running activity is known, the exclusive role of non-photic entrainers in rats deprived of photic entrainers is not demonstrated and hence present work using retino-hypothalamic pathway blocked male rats (n=10) was initiated. Blocking is done by enucleation of eyeballs. Circadian running activity is studied before enucleation and after enucleation towards instinctual social cues, food, water and sexual cues provided in activity cage. Twenty four hour activity of rats was recorded kymographically for a fifteen day period before enucleation and for a similar period after enucleation. Analysis of the records revealed that eight animals had predominant diurnal rhythm whereas two animals had predominant nocturnal rhythm. Enucleation of diurnal rats neither altered total running activity pattern nor caused any significant change in the goal compartments thus showing absence of entrainment by photic cues. In contrast nocturnal rats showed gradual shift of activity towards day time on enucleation thus indicating that nocturnal animals were entrained by photic cues. The overriding influence of non-photic entrainers on photic entrainers is discussed.

Key words : non-photic entrainers
enucleation

diurnal rhythm

circadian running activity
nocturnal rhythm

INTRODUCTION

It is well known that animals such as rats move from place to place in search of means to satisfy at least four instinctual drives viz. hunger, thirst, sexual and social urge. For this purpose the animal used various sensory inputs such as vision, hearing, sense of vibration, smell, stereognosis, etc. as well as the experience previously acquired to locate

food, water and species of his colony for the purpose of cohabitation and fulfilment of sexual drive.

It is well known (5) that light is major synchronizer of circadian rhythms of animal's activity to satisfy its instinctual drives (hunger, thirst, sexual and social and social urge). A few other non-photic cues as social cues (1), electrostatic field (4), sound (7), periodic presentation of food (11) have also been shown to be capable of entraining circadian rhythms in absence of light dark cycles. Recently Dhume and Gogate (3) showed that periodic water presentation is as significant as periodic food presentation in entraining the circadian running activity of the rat.

As earlier investigations showing relative dominance of photic/non-photic cues on the circadian running activity (5,11) were conducted under bright and dim (twilight) luminosities simulating day-night environmental conditions, even during night environment photic inputs were still operating. Hence the present work is designed to record the circadian running activity in vision deprived rats towards instinctual cues provided in maze-cum-activity cage. In these rats not only retino-suprachiasmatic pathway is blocked but also the animal is deprived of important visual input as well; and therefore this is a better preparation to elucidate the role of non-photic cues in the control of circadian instinctual drives in rats.

MATERIAL AND METHODS

Adult albino male rats ($n=10$) housed in individual cages were used. Water and powdered rat food (Hind Lever) supplemented with vitamins and minerals were provided *ad lib*. A strict protocol of daily body weight, water intake and food intake was observed.

An indigenously prepared (2) maze-cum-activity cage with automatic recording system is used for evaluation of circadian incentive drives in rats. It was designed to record circadian time-shift patterns of activity towards four environmental cues viz. food, water, sex (animal of opposite sex) and social behavior (animal of same sex) at selected period of solar day. The recording system consisted of slow moving smoked paper mounted on *Inco Kymograph with extension arm*. *Four signal markers were arranged in such a way that the signals were recorded one below the other when the respective switches from the chambers were operated by the weight of the animal on the tilting plate of the goal compartment.*

The animals were adapted to maze-cum-activity cage environment for a period of 10 days when the activity was not recorded. After adaptation, the activity in the cage was recorded from all four goal compartments for a continuous period of 15 days or till the pattern was stabilized. The cage was opened once daily to clean the chambers, to measure daily water and food intake, both provided *ad libitum*. The cage was opened at different times between 9 A.M. to 12 noon to prevent conditioning of the animal to a fixed period of the day.

The light in the room was subject to natural day-night environmental luminosity changes. After confirming the stabilization of running activity pattern at the end of scheduled period of 15 days, the animal was bilaterally enucleated. The operative procedure consisted in lifting the eyeball with fine curved mosquito forceps at the root of the eyeball with which the optic nerve and ophthalmic vessels were compressed. Ligature of linen thread was passed beneath the mosquito forcep and tied. With the help of scalpel, cornea was incised and intraocular material was drained. Subsequently the eyeball layers were cut, removed and the cut stump of the membrane was permanently ligated. Eye sockets were subsequently cleaned and local antibiotic ointment was applied for prevention of infection. Excepting for the operative day the activity of enucleated rats was recorded for further period of 15 days and thus each animal served as its own control.

The records from goal compartment taken after enucleation were compared with the records from same goal compartments taken before enucleation to identify any shift in circadian activity due to deprivation of photic cues as well as to assess the relative strength of each one of these non-photic entrainers on running activity.

The comparison of summated running activities in all four compartments in 24 hours period before and after enucleation may indicate the effects of visual cue deprivation on global day-night (24 hrs) circadian rhythm of the animals.

RESULTS

The daily movement of each rat towards individual four chambers viz water, food, male rat and female rat are plotted separately and the global (24 hrs) circadian running activity of the animal is plotted in the central channel. The representative activity records of diurnal animals and nocturnal animals are depicted respectively in Figs.1 and 2.

The data related to control period revealed that eight animals had predominant diurnal rhythm with the total activity extending from 8 a.m. to 12 p.m. (midnight) while

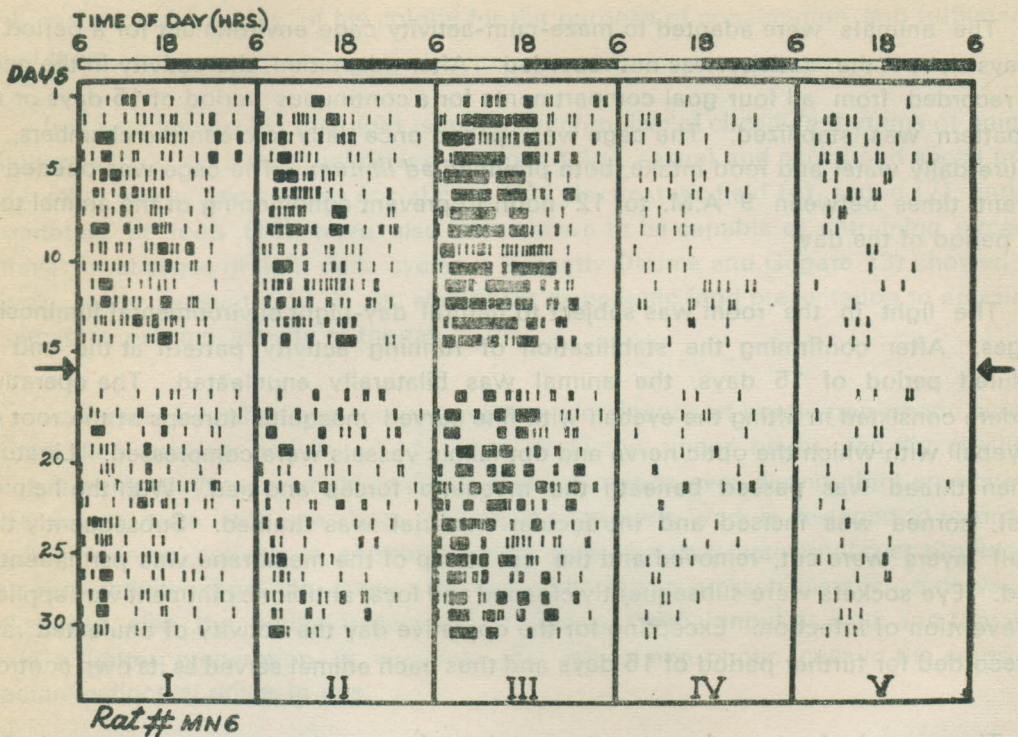


Fig. 1 : (Facsimile) Record of circadian running activity of the diurnal animal.

- Channel I — Food
- Channel II — Water
- Channel III — Temporally summated circadian activity from all four goal compartments.
- Channel IV — Female rat
- Channel V — Male rat

The activity is indicated by a vertical line, the thickness of which indicates the period of stay. Each day's activity corresponds to one horizontal strip. The activity of subsequent days are arranged one beneath the other strip. Arrow indicates the day of enucleation. The portion of the record above arrow represents activity during control period, the portion of the record below arrow represents activity after enucleation.

two rats had predominant nocturnal rhythm having total activity extending from 5 p.m. to about 3 a.m. In all these animals, diurnal as well as nocturnal, the period from 3 a.m. to 8 a.m. was a period of relative inactivity.

As far as the individual goal compartment's activity is concerned, it is seen that, at the outset the movements of all animals are made to satisfy consummatory drives, that is towards food and water chamber. The animals' activity shifts towards non-consumma-

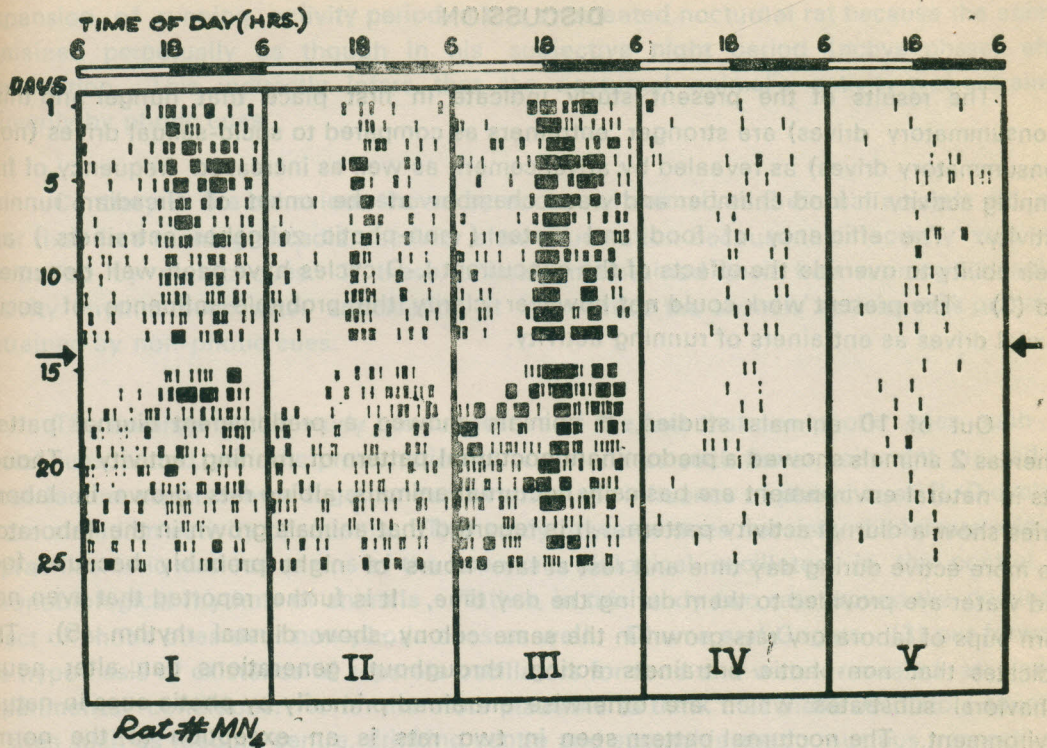


Fig. 2 : (Facsimile) Record of circadian running activity of the nocturnal animal. Explanation is the same as for Fig. 1.

tory drives, were seen only after fulfilling consummatory drives. It was further seen that the activity of the animals towards food and water chamber was more frequent as compared to the activity recorded in other two compartments which presented non-consummatory, socio-sexual drives. No significant preferential difference was shown in movements during control period towards sex and social chambers.

It is further noted that after enucleation though all diurnal rats' total activity was relatively reduced, there was neither a change in the pattern of the activity nor was there a change in the pattern of the activity in individual goal compartment. Contrary to diurnal rats' behavior, the nocturnal animals showed a striking change of the pattern after enucleation. It was gradually shifted towards day time and at the end of the schedule period of two weeks the recording pattern was stabilised to that of the diurnal animals i.e. shift the activity towards the period of 8 a.m. to 12 p.m. (midnight). There were also parallel changes of running activity in the goal compartment.

DISCUSSION

The results of the present study indicate in first place that hunger and thirst (consummatory drives) are stronger entrainers as compared to socio-sexual drives (non-consummatory drives) as revealed by advancement as well as increased frequency of free running activity in food chamber and water chamber at the onset of circadian running activity. The efficiency of food and water (non-photic zeitgeber entrainers) and their ability to override the effects of the concurrent L-D cycles have been well documented (3). The present work could not however clarify the probable influence of socio-sexual drives as entrainers of running activity.

Out of 10 animals studied, 8 animals showed a predominant diurnal pattern whereas 2 animals showed a predominant nocturnal pattern of running activity. Though rats in natural environment are basically nocturnal animals, albino rats grown in laboratories show a diurnal activity pattern. It is reported that animals grown in the laboratory are more active during day time and rest at late hours of night probably because food and water are provided to them during the day time. It is further reported that even new born pups of laboratory rats grown in the same colony show diurnal rhythm (5). This indicates that non-photic entrainers acting throughout generations can alter neural behavioral substrates which are otherwise entrained primarily by photic cues in natural environment. The nocturnal pattern seen in two rats is an exception to the normal activity pattern of laboratory animals and probably these two rats belong to different strain of rats in which non-photic cues were not powerful enough as entrainers to override the innate influence of photic cues and hence they continue their natural nocturnal pattern.

The present study elucidates further the relative strength of photic cues and non-photic cues in the entraining of circadian running activity. Whereas diurnal animals (8 rats) did not show any substantial phase shift of circadian running activity after enucleation, the nocturnal animals (2 rats) showed significant phase advance towards day time with expansion of running activity period after enucleation. Rusak and Zucker (9, 10) reported failure of blind adult mammals to be entrained by L-D cycles. The SCN is claimed to be dominant light entrained oscillator in the rodent circadian system and therefore it is plausible to conceive that enucleated animals fail to be entrained by photic cues. In fact, the nocturnal animal is primarily dependent on L-D cycles; during the subjective day (inactive phase) as long as the light stimulates SCN, the animal is kept inactive; during the subjective night (active phase) the absence of SCN's stimulation by light, the animal's activity is increased. It is therefore easy to understand the phase advance as well as

expansion of running activity period in this enucleated nocturnal rat because the animal remained perpetually as though in his subjective night period (active phase) after enucleation. This indirectly infers that the nocturnal animal's activity was entrained primarily by photic cues.

Contrary to the behaviour shown by nocturnal animal, all 8 diurnal animals did not alter their free running circadian activity after enucleation because their activity was not controlled by photic cues. If these animals maintained their free running circadian activity rhythm even after enucleation it is because the animals' activity was primarily entrained by non-photic cues.

The findings of this study are suggestive of the fact that non-photic cues such as social cues, sound, periodic presentation of water and as such the entrainment created by these factors separately or together can override the influence of photic cues (L-D cycles) in the circadian activity of rodents. This study however does not deny the inmate role of suprachiasmatic nucleus as one of the important biological oscillators in the control of chronobiological rhythms of animals. Rather, in this study two rats proved the dominant effect of photic cues over non-photic cues as well. Dhume and Gogate (3) put forward the hypothesis of existence of separate oscillators for food and water revealed at similar experimental conditions. It is therefore plausible to think that these oscillators situated at sites such as feeding centre, drinking centre and suprachiasmatic nucleus respectively are coupled to each other so that when all the cues are presented in natural condition they work in congruence but one entrainer may override and thus dominate other entrainers.

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