Relations Between Muricide, Circadian Rhythm and Consummatory Behavior

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RUSSELL, J. W. AND G. SINGER. *Relations between muricide, circadian rhythm and consummatory behavior.* PHYSIOL BEHAV 30(1)23–27, 1983.—Three forms of behavior—muricide, eating, and drinking—have been studied at six photic periods during a 12/12 hr light/dark circadian cycle to which the subjects have been habituated. One hundred and eight rats served as subjects, 18 per photic period. The frequency of muricide was recorded for each period and subsequent food and water intakes were measured during a 1 hr test period. Results show a significantly higher frequency of muricide during the dark than during periods of light. Food intake covaried significantly with the incidence of muricide $r_s=0.89$, $p<0.05$), while no such relationship was found between muricide and water intake ($r_s=0.17$, $p<0.05$). The findings are consistent with reports of circadian changes in other rodent behaviors, including rhythmicity in home-cage and in shock-induced aggression. Covariation of muricide and eating does not establish a causal relation between the two. Three models of physiological mechanisms which might provide substrates for the covariance are discussed.

Muricide   Eating   Drinking   Circadian rhythm

MANY behavioral and physiological functions have been shown to vary synchronously with the natural alteration of night and day [4]. These circadian rhythms have ranged from the innate homeostatic processes of eating and drinking to the retention of some acquired behaviors [37]. Although there is experimental evidence that lighting conditions under which animals are reared and maintained can have significant effects on the amounts and pattern of intraspecific aggressive behavior [9], relatively little is known about how aggressive behaviors may covary with such well-documented rhythmicities as eating and drinking. Knowledge about such covariation may be useful in the search for biochemical mechanisms of action underlying aggressive behavior, as well as in an understanding of aggressive behavior *per se*.

Muricide, mouse killing by rats, has been viewed as a mammalian model of aggressive behavior [36]. During the past 15 years considerable speculation and some research has been directed toward understanding the physiological as well as the environmental determinants of this behavior. Observations of the phenomenon indicate that muricide is not an inevitable outcome of an encounter between a rat and a mouse. There is evidence that both genetic and environmental factors affect the frequency with which it occurs. Most experiments have shown that the mean muricidal behavior in male randomly bred rats is approximately 15%. Paul and colleagues [25] have reported that the spontaneous kill rate in their study of rats selectively bred from killer parents was significantly greater, i.e, 43%. On the environmental side, cyclic food deprivation has also been reported to be related to an increased incidence of muricide in randomly bred animals to 85% [24,28]. The combined prenatal and environ-

mental factors are associated with an incidence of killing which reaches 100% [25].

Such observations suggest that among the determinants of muricidal behavior some may be periodic, represented by regular cyclic changes in tissue conditions within the body. Tissue conditions accompanying deprivation of food is one obvious possibility. The fasting rat displays an increasing level of food-oriented activity as time of deprivation increases. Rats eat approximately twice the amount of food during the dark than during the light [4,12]. Underlying these changes in behavior are concomitant functionul changes in both peripheral and central tissues. The possibility that such periodic changes may also affect muricidal behavior has been stated in its extreme form as the hypothesis that muricide is, in fact, a form of food-getting behavior [23]. This hypothesis has led investigators to examine relationships between muricide and conditions underlying the consummatory response of eating.

Only a few studies of muricide have provided data in which circadian rhythms and consummatory behaviors have both been observed in the same experiments. Although there appeared some early anecdotal reports [6,35], one of the first systematic experiments [24] varied times of testing for muricidal behavior throughout the light/dark cycle. The incidence of muricide did not differ significantly when testing occurred five hours before or five hours after their regular feeding. Unfortunately these results may have been affected by a 23 hr food deprivation schedule which preceded the testing for 11 days and which may have altered the established feeding rhythm without influencing the circadian cycle. More recent attempts to examine relations between these rhythms have chosen too few time points for testing [33] or have found so

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low an incidence of muricide—4 of 55 rats [32]—as to make conclusions precarious.

The present study was designed to investigate possible covariations between muricide, circadian rhythm and consummatory behavior. It employed a correlational approach by which the incidences of muricide, eating and drinking were sampled systematically at six times during a 12/12 hr light/dark cycle. It was hypothesized that there would be a greater incidence of muricide and of eating during the dark phase and that measures of these two behaviors would be positively correlated. Because drinking is also greater during the dark than in light, the possibility that a similar relation might exist between this form of consummatory behavior and muricide was also tested.

METHOD

Animals

The subjects consisted of 108 naive male Wistar derived rats ranging from 90–120 days old. All were housed individually and maintained on ad lib food and water.

One hundred and eight naive female Swiss mice, weighing approximately 30 grams, were used as prey.

Apparatus

Rats were tested individually in wire mesh cages measuring 40×20×20 cm. The experimental room in which the animals were housed was maintained on a 12 hour on and 12 hour off light/dark cycle. Three 40 watt red lights remained on at all times to facilitate observations during the dark phase. Room temperature was kept at 22°C±1°.

Procedure

Rats were habituated to their home cages for at least one week prior to testing. Following this period, they were randomly assigned to one of six experimental groups. Each group contained 18 subjects. Three groups were tested at different times during the light phase: first hour of light (L+1), sixth hour of light (L+6), and last hour of light (L+11). The remaining three groups were tested during the dark phase: first hour of dark (D+1), sixth hour of dark (D+6), and eleventh hour of dark (D+11). Apart from the time at which testing took place, all subjects were treated the same, i.e., food was available ad lib and all were handled the same number of times.

Following adaptation to the home cage, rats were tested for muricide at times in accordance with their group assignment. The one hour test consisted of removing both food and water and then placing a mouse into the front of the cage. The animals were characterized as either mouse-killers or non-killers. For those that killed, the carcass was removed for the remainder of the hour in order to prevent ingestion of the prey and the kill latency was recorded. For those that failed to kill, the trial was terminated at the end of one hour by removing the mouse.

Following the one hour muricide test, pre-measured food and water were placed in the cage for one hour, after which intakes of both were recorded.

Statistical analysis

For frequency data like number of killers, chi square analysis was used, for continuous data such as food and water intake, analysis of variance was used, and data on the
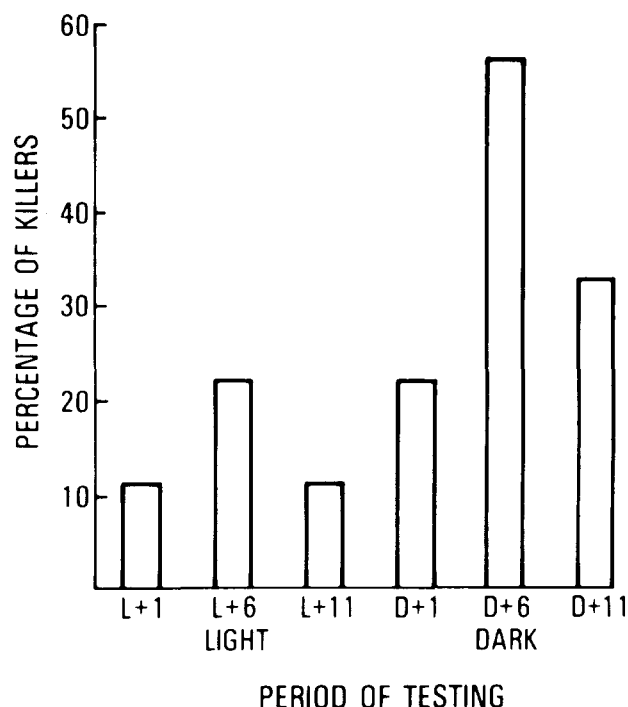

FIG. 1. Frequencies of muricide at various periods during the 12/12 hr circadian cycle. Frequencies are expressed as percents of animals in each group (n=18) that engaged in muricidal behavior.

relationship between muricide and consummatory behavior was analyzed by using the Spearman Rank Correlation Coefficient and the Mann-Whitney U-test.

RESULTS

Muricide

Figure 1 presents the percentage of killers at each period of testing. This figure appears to support the hypothesis under test, i.e., that muricidal behavior is more prevalent during the dark than the light. Comparing the number of killers in the light conditions (L+1, L+6, L+11) with the number of killers in the dark (D+1, D+6, D+11) confirmed the hypothesis ($\chi_1^2=5.83$, corrected for continuity, $p<0.02$).

Comparisons between the three periods during the light phase showed no significant differences, while similar comparisons during the dark indicated a significant difference between the (D+1) and the (D+6) groups ($\chi_1^2=2.92$, corrected for continuity, $p<0.10$).

Analysis of median kill latencies for animals tested in the dark (mean=55 seconds) compared to those tested in the light (mean=105 seconds) showed no significant difference (U=57.5, $p>0.05$). Further comparisons also failed to show any significant differences.

Post-Test Food Intake

As Fig. 2 suggests, animals tested during the dark phase consumed more food than those tested during the light. There was a significant overall effect, $F(5,102)=4.51$, $p<0.05$. Tukey post-hoc tests revealed that the major differences were between (D+7) and all the other photic periods
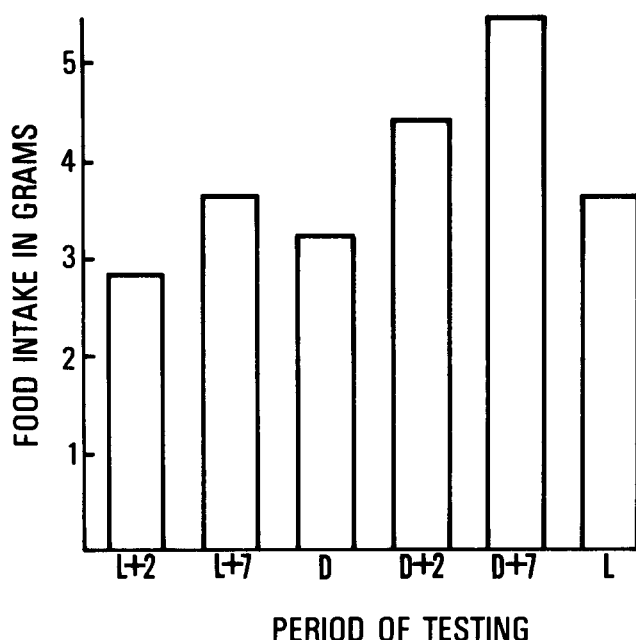
FIG. 2. Food intake (g) at various periods during the 12/12 hr circadian cycle. Intake was measured during a one hour free feeding period immediately following the test for muricide.
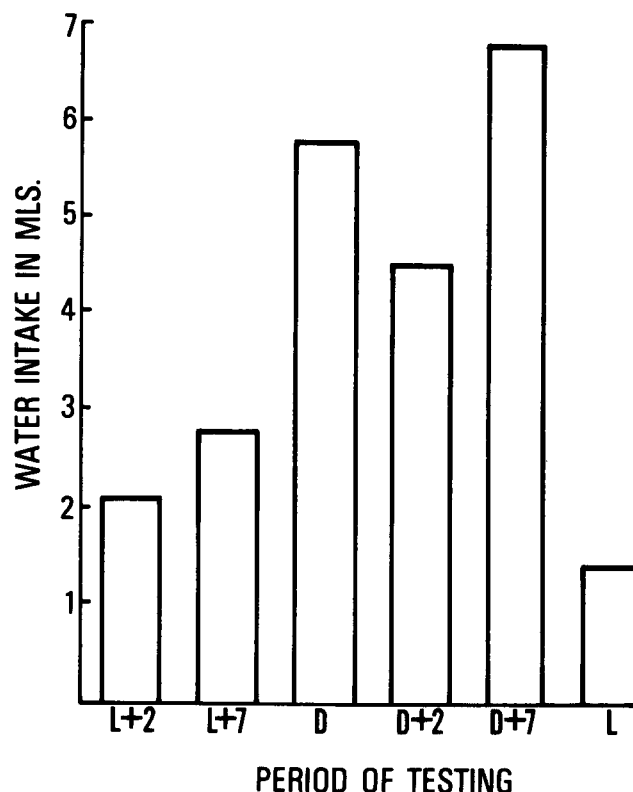

FIG. 3. Water intake (ml) at various periods during the 12/12 hr circadian cycle. Intake was measured during a one hour free feeding period immediately following the test for muricide.

with the exception of (D+2). There were no significant differences between other groups.

Post-Test Water Intake

Figure 3 presents the mean water intakes for each of the photic periods. Although analysis of variance indicated a significant overall effect, $F(5,102)=14.86$, $p<0.05$, this was not attributable to differences betwen intakes during the light and dark phases. As Fig. 3 suggests, the largest intake occurred at the beginning of dark (D), (D+2), and (D+7). Tukey tests showed that water intakes at these three points were significantly different from intakes at the other three, i.e., (L+2), (L+7), and the beginning of light (L). In addition, (D+7) was significantly different from (D+2). The last period of testing (D) exhibited a lower mean intake when compared to all the other periods, with the exception of (L+2).

Correlation Between Muricide and Consummatory Behaviors

In order to test the degree of concordance between muricide and post-test consummatory behaviors, the Spearman Rank Correlation Coefficient was used. Comparison between the rank orders for the incidence of muricide and food intake across the light-dark phases yielded a significant positive correlation ($r_s=0.89$, $p<0.05$). Changes in water intake and the incidence of muricide over the same phases were not significantly related ($r_s=0.17$, $p>0.05$). There was some correlation between food and water intakes ($r_s=0.50$, $p>0.05$) which, however, was not statistically significant.

DISCUSSION

Muricide and Light/Dark Cycle

One main objective of the present experiment was to test the hypothesis that the incidence of muricide varies systematically during an established 24 hour light/dark cycle, i.e., muricidal behavior is greater during the dark than during the light phase. The results are unequivocably in support of the hypothesis. As such, they clarify observations reported earlier [6, 24, 32, 33] which were confounded by features of the research designs. Within the two photic periods, rats tended to have a higher incidence of killing in the middle and towards the end of the dark than during the light and beginning of the dark. The present results are consistent with reports of diurnal variations of rhythmicity in home-cage and in shock-induced aggression [9,13]. This finding is also in general agreement with conclusions concerning circadian changes in other rodent behaviors [1, 12, 22, 32].

Covariations: Muricide and Consummatory Behavior

A second major goal of the present research design was to study covariations between the rhythmicity of muricidal behavior and the innate consummatory processes of eating and drinking. Diurnal rhythms in the latter are generally entrain with those just described for the former. However, correlations between empirical measures of actual performance showed a significant relation between muricide and eating,

but not between muricide and drinking. Although food deprivation is known to increase the probability of muricide [14, 25, 28], water deprivation has no such effect. That this should occur is not surprising, for several investigators [10, 19, 27] have recently reported research results which ". . . leave no doubt that drinking is characterized by a circadian rhythm which is independent of food intake ([3] p. 125)."

The fact that the two behaviors, muricide and eating, covary significantly during the dark/light cycle does not establish a causal relation between the two, as some investigators have proposed (e.g., [23]). To establish that one rhythm is driven by another requires not only that the two covary, but also that one is the antecedent and the other, the consequent in their temporal relations. The "systems approach" in the biobehavioral sciences [29] also expects to find that the two rhythms have biochemical and neural substrates which are either related or in common. Covariations of two or more behavioral periodicities may mean that all are manifestations of a common set of physiological conditions, that one is the antecedent of the others, or that each has its own individual basic condition(s) and that all of these are activated concomitantly. In the latter instance rhythmicities occur independently, even when their expressions are synchronized. There is ample evidence for such dissociation of circadian rhythms [3, 19, 27].

Implications of Covariations

Of these three models, the first, i.e., that muricidal and eating behaviors share a common physiological origin, appears unlikely. While there has been general agreement that

under conditions of food deprivation the incidence of muricide increases [5, 24, 25], there is evidence which strongly suggests that the killing response is mediated through brain mechanisms other than those associated with hunger [14, 20, 24]. The seocnd and third models need fuller testing in terms of the criteria discussed above.

Both of the latter models imply independent physiological mechanisms of action for various biological rhythms. The identities of neural circuits for eating and drinking have been known for some time to involve hypothalamic among other sites [21]. More recently evidence has come to light suggesting that sites within the hypothalamus itself, the suprachiasmatic nuclei, are broadly involved in circadian rhythms [18, 26, 34]. Neural sites provide places for the occurrence of dynamic biochemical events which underlie the biological rhythms of concern to the present study. The search for the nature of these events has already attracted considerable attention. One study has demonstrated in the hippocampus that ". . .daily change in synaptic responsiveness covaries with a number of reported behavioral fluctuations ([2] p. 91)." Diurnal oscillations have been reported in concentrations of neurotransmitter substances [11] and in hormones from such tissues as the adrenal cortex, the pituitary gland and the median eminence of the hypothalamus [7,16]. Specifically implicated in muricide are variations in the transmitter, serotonin [8, 17, 30, 39]. Neurotransmitter receptor populations have been observed to show unimodal circadian cycles with peaks occurring during dark periods [22]. Circadian rhythms in behavior and in brain neurotransmitter receptors have been altered experimentally by various pharmacological manipulations, e.g., slowed following administration of lithium and antidepressant drugs [15, 22, 38].

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