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[COMMUNICATION]

Free-Running Locomotor Rhythms of Feeding-Entrained Goldfish

Richard E. Spieler and John J. Clougherty

Milwaukee Public Museum, 800 West Wells St., Milwaukee, WI 53233, USA

ABSTRACT—Groups of goldfish were held on a 12L:12D photoperiod and fed at one of four different circadian times (light onset at 6, 12, or 18 hr after light onset) for 46 days. The animals were then allowed to free-run on constant light or dark without food or disturbance for five days, and activity rhythms were remote monitored. Most groups maintained a circadian locomotor rhythm entrained to the approximate time of feeding. No consistent differences among fish on the different feeding regimes or between those on constant light or dark were noted.

INTRODUCTION

Meal-feeding a single daily meal can synchronize a host of daily rhythms in vertebrates including fishes. In many cases feeding is a more potent entrainer of the expressed rhythms (i.e., locomotor, circulating cortisol, agonistic behavior, etc.) than the light-dark cycle [1–3]. Whether meal-feeding is entraining an endogenous oscillator responsible for the expression of the rhythms is, however, not clear and may be species dependent. Previous research on the interrelationship of meal-feeding entrainment and the circadian locomotor rhythms of fishes have examined the feeding entrained rhythm in starved fish on a light-dark cycle [2] or examined the locomotor rhythm under conditions of constant light or dark while the animals continued to be fed [4, 5]. In either case, the fishes continued to receive a daily entraining stimulus and thus were not truly free-running. This study examined free-running locomotor rhythms of feeding-entrained, starved goldfish on constant light or dark.

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MATERIALS AND METHODS

Juvenile goldfish (body weight approximately 6 gm) of mixed sexes were obtained from a commercial supplier (Ozark Fisheries, Stoutland, MO) and placed in 18, 60-liter aquaria (12 fish/aquarium). Each aquarium received a continuous supply of filtered and aerated water ($13 \pm 2^{\circ}$C) and each had individual photoperiod control and was individually wired, via externally mounted ultrasound transducers, to an event recorder (Esterline Angus 2100). During periods of activity monitoring, disruption (activity) of a standing wave of ultrasound within an aquarium caused a change in voltage within a transceiver which was in turn recorded as an event. The event recorder totaled events per aquarium in 15 min blocks and these data were used in plotting rhythms and in statistical analysis. A more detailed description of the holding and recording equipment is given elsewhere [2].

The fish were held on a 12L:12D photoperiod and fed once daily (2.5% body weight - Biodiet, Bioproducts Inc. OR). All fish were fed at 1600 CST, however, the onset of light was staggered amongst the aquaria so that the fish received the food at one of four circadian times of day: light onset (0 hr), 6 hr after onset, 12 hr after onset, or 18 hr after onset. After 46 days on the feeding regime the fish were allowed to free-run under constant light (LL) or constant darkness (DD) (2 or 3 groups /each feeding regime) for 5 days. During this period the animals were not fed or disturbed (the aquarium room was not entered) and daily activity patterns were remote monitored.

The activity for the five day period was plotted for each tank and examined statistically by a Fourier time series analysis [6] and cosinor analysis [7]. One transceiver failed during the monitoring period and that tank was eliminated from the study.

RESULTS AND DISCUSSION

Total daily activity differed among days 1 through 5 in all tanks ($P<0.01$, ANOVA-Day 1> Day 4, 2, 3, 5 Tukey's Studentized range). This day-to-day difference in total activity produced some artifactual rhythms through the five day period in preliminary time series analysis. Therefore, mean daily activity for each tank was subtracted from the individual 15 min totals to yield a deviation about the daily mean per 15 min interval. These data were used, in turn, in subsequent analyses.

All tanks had highly significant rhythmic activity during the free-running period ($p<0.01$, Fisher's Kappa and Bartlett's Kolmogorov-Smirnov tests). In the majority of cases a 24 hr rhythm was the predominant (12 groups) or secondary (3 groups) peak in the periodogram; only 2 groups lacked 24 hr intervals (Fourier time series analysis, unweighted). The mean acrophase of the five day period for the 17 groups ranged from 13:00$\pm$1.4 (s.e.) to 22:54$\pm$1.0 CST with an overall mean of 17:00$\pm$0.82 (cosinor analysis); shortly after the acclimation feeding time (16:00). Likewise, a visual examination of the data reveals a peak of activity about the time of feeding, especially for the first two days of free-run, for most groups (Fig. 1). There were no consistent differences apparent, either visually or statistically, in rhythm phasing among the four feeding/light-dark regimes or the two free-running regimes (L:L or D:D).

Thus, meal-feeding does entrain the locomotor rhythm in goldfish and this circadian rhythm will free-run in the apparent absence of other obvious zeitgebers (i.e., light-dark cycles, feeding, disturbance). Kavaliers [8], working with groups of light-dark entrained goldfish on LL, has reported a free-running rhythm of about 24.5. The exact 24 hr rhythms recorded in our study are most likely due to the 15 min activity intervals used in the recording apparatus rather than the different entraining stimulus. As has been previously demonstrated in fishes [2, 4] and mammals [see 9] the onset of activity in this study anticipated the scheduled feeding (Fig. 1). In free-running rats it is mainly this anticipatory phase of the circadian locomotor rhythm that is entrained by meal-feeding; the remaining portion of the rhythm remains locked to the light-dark entrained oscillator [9, 10]. In contrast, with goldfish there is not an apparent splitting of the locomotor rhythm (this study) and it appears that the bulk of the activity is entrained to the time-of-feeding. We caution, however, that another study has demonstrated that the daily activity rhythm of a fish (Medaka) can be composed of component rhythms and these individual rhythms can be entrained to different environmental stimuli [3].

The mechanism whereby feeding entrains rhythms in fishes as well as mammals remains unresolved. At this time it does not appear to be a nutritional component of the diet. In previous research from this laboratory the absence of dietary tryptophan or tyrosine and phenylalanine did not affect the ability of meal-feeding to phase-shift locomotor activity in goldfish [11]. Although some other dietary constituent may play a role in meal-feeding entrainment, work with primates, fed intergastrically, has led to the conclusion that the diet per se is at best a weak synchronizer of circadian rhythms and that some aspect(s) of the pre-gastric feeding process is the major entraining stimulus [12]. The disturbance associated with feeding may play a role in the entrainment mechanism. A hypothesis supported in part by a study with fishes wherein feeding entrainment of anticipatory behavior could be altered by disturbance (feeding adjacent aquaria) [4]. Another aspect of the entraining mechanism is the social interaction induced in a feeding group. Social interaction does facilitate circadian activity and feeding in goldfish [8, 13]; and although preliminary research at this laboratory indicates single fish can be entrained to meal-feeding there

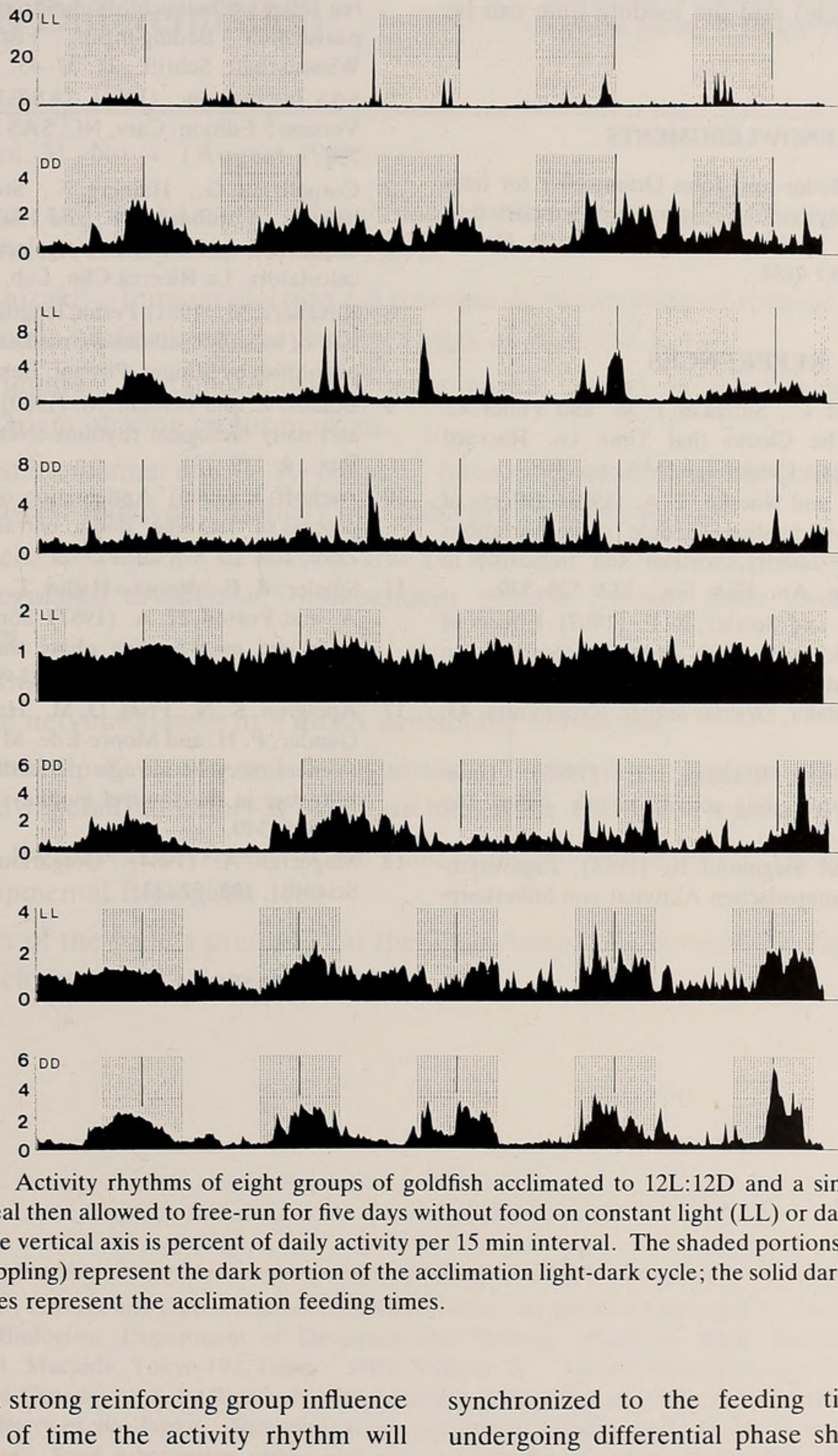

FIG. 1. Activity rhythms of eight groups of goldfish acclimated to 12L:12D and a single daily meal then allowed to free-run for five days without food on constant light (LL) or dark (DD). The vertical axis is percent of daily activity per 15 min interval. The shaded portions (vertical stippling) represent the dark portion of the acclimation light-dark cycle; the solid dark vertical lines represent the acclimation feeding times.

appears to be a strong reinforcing group influence on the length of time the activity rhythm will remain entrained to the feeding time in starved fish (Spieler *et al.*, unpubl.).

Also unresolved is the question of whether or not meal-feeding is entraining an endogenous oscillator in fishes. Previous studies [2, 3] as well as the present study support an entraining hypothesis; in the present study all groups remained synchronized to the feeding time rather than undergoing differential phase shifts as would be anticipated if groups were using some aspect of the light-dark cycle to cue on and/or feeding induced rhythms were masking an endogenous locomotory rhythm. Further research is required, however, before the possibility that the animals are remembering the interval between a point on the endogenous oscillator(s) (presumably entrained by the light-dark cycle) and the feeding time can be discounted.

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