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Effect of day-night cycle on distribution of food intake and economic choice among imposed food opportunities in mice

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HIGHLIGHTS

- Mice fed at four 40-min opportunities per day show a sequencing of meal size.
- That sequencing is changed by phase advance or delay of light:dark Zeitgeber.
- Mice with intermittent inexpensive food eat ~50% from a constant costly source.
- Intake from the intermittent inexpensive food occurs only nocturnally.
- Food intake and choice in mice is highly sensitive to the light:dark Zeitgeber.

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ABSTRACT

We have shown previously that mice given access to four discrete feeding opportunities (FOs) per day show a characteristic sequence of sizes across ordinal FOs. The purpose of the present experiments was to determine the relative contributions of external and internal factors on the sequencing of FO size. The external factors were the light:dark Zeitgeber and the cost of food, imposed *via* different fixed unit prices (FUP) in a closed operant economy, and the internal factors were signals relating to energy status including time since last food and weight loss. In the first experiment, mice were given 4 FOs spaced 4-h apart, but with the timing of the FOs relative to the Zeitgeber altered by a 4-h Zeitgeber advance or delay of the cycle. Food intake, and associated body weight, declined as price increased, but the temporal order of FO size was invariant within a Zeitgeber condition. The Zeitgeber advanced group showed clear evidence of a shift in meal sequence relating to the light:dark cycle. Thus, external factors seem to be a more important determinant of total intake and sequencing than internal factors. In the second experiment, mice were given the choice between continuous costly (CC) and intermittent inexpensive (II) food. II food was available for four-15 min intervals every 4-h, and the timing of the 15 min intervals was varied relative to the Zeitgeber cycle. In spite of a 20-fold difference in price between CC and II food, mice took approximately equal amounts from each, and all food intake took place during the dark phase. Mice consumed II food only if it was available during the dark phase. Food intake was strongly linked to the light:dark cycle, largely independent of food cost.

1. Introduction

Pioneers in the study of free feeding behavior in rats described a pattern of episodic high rates of eating (meals) flanked by longer periods of not eating (inter-meal interval, IMI) modulated by a circadian pattern in which feeding occurred primarily at night [1–3]. The meal, and determinants of IMI, thus became important foci of neurobiological study of eating. Starting about 20 years ago, house mice (*Mus musculus*) became a species used extensively in study of eating; the meal and IMI criteria that had been successfully used in rats were applied with little critical evaluation [4,5]. It is now clear that these criteria do not adequately describe feeding in mice [6,7]. In particular, we and others have shown that mice engage in several hours of slow, continuous feeding (grazing) at the start of the night [7,8]. We turned this question around by asking how mice would eat when food was available only in episodic feeding opportunities (FOs) that would force meal structure [9,10]. The present experiments will use a temporal arrangement of FOs that to some extent emulate human eating patterns [11], namely four FOs distributed evenly over a 12-h part of each 24-h cycle. In our previous work, each 40-min FO has been during or immediately contiguous

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with the dark phase of a 12:12 cycle [9,10,12], to maintain similarity to the spontaneous nocturnal pattern of free-feeding mice.

Additionally, our studies have manipulated consummatory cost of food, imposed *via* a specified number of behavioral responses required to receive each food pellet; this cost is the fixed unit price (FUP) for food. As FUP increases, daily food intake settles at progressively lower levels (*i.e.*, food demand is elastic) despite progressive weight loss [5,7]. Compared with continuous access, mice in the four FO protocol show greater elasticity of demand, yet they do not show high or maximal rates of responding through the available time [9,10]. This suggests that neither metabolic (*i.e.*, weight loss) nor behavioral ceiling effects can explain the high elasticity of food demand in meal-eating (four FO) mice.

In our previous four-FO work, we find that the first and last meals (corresponding roughly to dusk and dawn, respectively) are the smallest, while meals in the middle of the night are the largest [5,7,12]. This suggests that circadian oscillator(s) may modulate intake in these FOs, and eventually impact the effects of consummatory costs. The present experiments directly test the hypothesis that the light:dark Zeitgeber will affect distribution of food intake in FO-constrained mice. In the first experiment, the effect of relative timing of four FOs with respect to the Zeitgeber is examined. In the second experiment, employing an economic choice situation in which mice with continuous access to relatively costly food additionally have four short daily periods of cheap food availability, we examine whether the timing of the cheap food relative to the Zeitgeber affects choice.

2. Methods

2.1. Animals and maintenance

Experiment 1 used 23 male ICR:CD1 mice (Harlan, Indianapolis IN), approximately 5 mo of age. They had served previously in an operant experiment, using what we will later refer to as a standard schedule of four FOs, to investigate the effect and time-course of an anorectic agent [12]. For the present experiment, groups were formed that were matched for prior experience. Mice were housed individually in conventional polycarbonate shoebox cages for 2 mo between the previous experiment and the present with free access to Harlan #7912 pelleted chow and autoclaved water. Contact bedding (Sani-Chips, Harlan) was changed weekly. Fluorescent vivarium lights were on 0700–1900-h (equivalent to Zeitgeber time ZT 0–12), ambient temperature was 23–24 °C, and relative humidity was 40–70%. The University of Florida Animal Care and Use Committee approved all procedures in this protocol with the stipulation that mice were removed from study on the day that body weight loss first exceeded 15% from that at the start of the experiment.

The second experiment used 12 experimentally naive, male C57Bl/6J mice. Animals were obtained from Harlan laboratories at ~8 weeks of age and allowed to acclimate to our animal facility for one week before starting experiments. Other maintenance details were similar to Experiment 1.

2.2. Behavior test chambers

Experiment 1 used 23 individual mouse operant conditioning chambers (Med Associates, St. Albans, VT) enclosed in ventilated, sound and light-attenuating cubicles (relative humidity ~45%; ambient temperature ~23 °C). A 7-w light in each cubicle provided illumination according to the schedule specified below. Chambers measured 14 × 14 × 12-cm inside and were made of Plexiglas, with aluminum front and rear panels and steel rod floor (0.5-cm spacing). A paper-lined pan was placed 4.5 cm below the floor. A nose poke recess measuring 1-cm in diameter was located on the front panel, 3-cm from the right wall and 2-cm above the floor. A 0.75-cm diameter cue light 5-cm above the nose-poke recess was illuminated whenever food was available. A food trough was horizontally centered on the front panel, 1.5-cm to the left of the nose-poke recess and 1-cm above the floor. Food (5TUM, Test Diet, Richmond VA; 20-mg pellets) was dropped into the trough from a dispenser outside the cage. Water was available freely from a sipper spout. A computer running Med-PC IV software recorded responses and controlled pellet deliveries in daily 23-h sessions.

In Experiment 2, the chambers were larger (20 × 24 × 21 cm) with a mouse-appropriate (0.5 cm spacing) stainless-steel rod floor. The front wall of each chamber was equipped with two low force, mouse-appropriate levers (Med Associates ENV-310M) each with a cue light above and located on either side of a food trough. A 7-w light in each cubicle provided illumination on a 12:12-h light:dark cycle, with lights on at 0600-h. Other details were as in Experiment 1.

2.3. Procedure experiment 1

Groups of 7–8 mice, matched for prior experience, were assigned to three groups, as shown in Fig. 1. For the standard group, lights in their experimental chambers were the same as the vivarium (on 0700–1900-h). For the Zeitgeber advanced group, the lights in the chambers were on 0300–1500-h and for the Zeitgeber delayed group, lights in the chambers were on 1100–2300-h. The cubicles were located inside the vivarium with the standard light cycle and, while they were nominally lightproof, some stray ambient light may have entered through cable conduits and exhaust fans. Mice were removed from the test condition each day between 1400 and 1500-h, coinciding with a period of chamber lights on in all groups, in order to weigh them and service the chambers. During this hiatus, mice were placed in individual holding cages without food.

Mice were exposed to their new light cycle at the start of retraining in the operant chambers at unit price 2 and with progressive tapering of food access to four FOs (over 11 days total). FOs were delivered simultaneously to all experimental groups at 1500, 1900, 2300, and 0300-h, but these represent different ZTs for each group. After retraining, mice weighed a mean of 34.8 g and then were tested in contiguous 3-day segments at unit prices of 2, 10, and 25.

2.4. Procedure experiment 2

For initial training, mice were exposed to a unit price of two lever presses per 45-mg pellet of food (5TUM). Mice were kept at this price for four consecutive days followed by an incrementing series (5, 10, 25, 50) each for 3 days. Both levers were operational and delivered pellets at the same unit price during this training phase.

At the beginning of the experiment, mice weighed a mean of 25.1 ± 0.29 g. At this time, the batch of 5TUM diet was prone to crumbling in the dispenser, and we switched all mice to a semisynthetic approximately isoenergetic pellet (AIN93-G; Test Diet). Mice were tested in

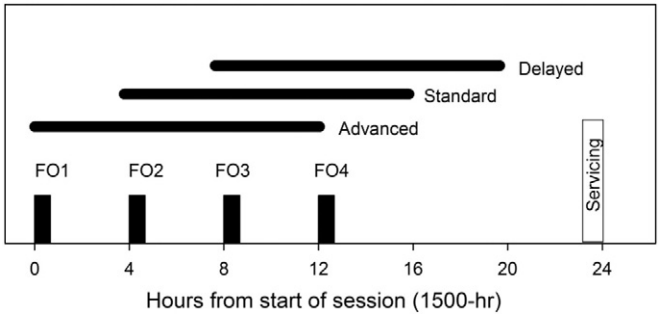

Fig. 1. Schematic of temporal organization of Experiment 1. Mice received four 40-min feeding opportunities (FOs, ordinally labeled). FO1 started at 1500-h, immediately the daily session began following the servicing period. The 12-h night periods of the three groups (4-h Zeitgeber advanced, standard, or delayed) are indicated as black horizontal bars.