Physiology & Behavior 181 (2017) 10–15

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Circadian and economic factors affect food acquisition in rats restricted to discrete feeding opportunities

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A R T I C L E I N F O

Keywords:
Closed economy
Unit price
Operant behavior
Zeitgeber
Satiation

A B S T R A C T

The purpose of this study is to examine aspects of operant behavior-modeled economic choice for food in rats in closed economy protocols in which food is available for only a few discrete times per daily 23-h session, designed to emulate clustering of human food intake into meals. In the first experiment, rats performed lever press responses for food pellets in an ascending series of ratios or fixed unit prices (FUP) when food was available for four 40-min food opportunities (FO) per day. Daily intake at low FUP was comparable to ad libitum intakes. Intake declined as FUP increased and was not distributed equally among the four FOs. In particular, the last FO of a session (occurring at about lights on in a 12:12 cycle) was the smallest, even when total intake was low due to the response requirement at high FUP. Within FOs, satiation was evident at low FUPs by a decrease in rate of intake across a 40 min FO; at high FUPs responding was evenly distributed. In the second experiment, rats had a choice of responding on two levers for either intermittent inexpensive (II; low FUP according to a four FO schedule) or costly continuous (CC; 20-fold higher FUP but available throughout 23-h sessions) food. Most (73%) of the rats consistently chose almost all of their food from the II source. Further, as the timing of the four II FOs were changed relative to the light: dark Zeitgeber, the time of the smallest meal changed such that the smallest meal (s) were during the light period regardless of ordinal position within a session. These data are discussed in terms of economic and Zeitgeber effects on consumption when food is available intermittently, and are contrasted with results from comparable protocols in mice.

1. Introduction

Starting with the work of Curt Richter [1], rats (*Rattus norvegicus*) have been the subjects of an overwhelming majority of scientific studies on mechanisms of food intake. Early studies showed that, with free access to food, rats eat in ∼10 well-defined episodes or meals per day separated by substantial periods of no eating (intermeal interval, IMI). Further, food intake is higher at night, with shorter IMIs, than during the daytime [1–3]. These studies led to the belief that the meal is the fundamental neurobiological unit of eating [4] and numerous studies have investigated possible physiological mechanisms. This meal pattern is conserved when rats have to perform an operant task for small food pellets in a closed economy using ratio or fixed unit price (FUP) schedules [5–7] although, consistent with economic demand theory, total intakes decline at the highest FUPs [5–10]. In contrast, when the operant cost is instead imposed at the point of obtaining access to food, rats adopt a cost-minimizing or global economic strategy of fewer but larger meals as access cost increases [5,11]. The mechanism behind this change in meal strategy is unknown.

Several reports from our laboratory have described the behavior of mice (*Mus musculus*) in operant protocols for food acquisition. As in rats, intake of mice declines as FUP increases, often more sharply because mice seem to emit or tolerate a lower maximum number of operant responses per day than rats [12]. In contrast to rats, mice spend at least the first half of the night engaged in almost continuous locomotor activity during which many small but frequent eating episodes or grazing occur [6,14]. Meals, as such, are poorly defined during this period but tend to be better defined in the late night and daytime [6]. These observations led us to investigate what would happen to regulation of intake if mice were forced to take discrete meals, in the limit emulating the typical pattern of humans [15], by enabling them to work for food only during discrete intervals or food opportunities (FOs). Two important findings emerged from that work. First, when food was available in four 40-min FOs spaced 4-h apart, intake was not the same at each FO but showed a characteristic variation that appeared in part to be due to the light-dark cycle and/or an endogenous circadian oscillator [13,16,17]. Second, within an FO, the rate of intake declined monotonically and most individuals stopped responding and eating

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http://dx.doi.org/10.1016/j.physbeh.2017.09.003
Received 3 July 2017; Received in revised form 22 August 2017; Accepted 3 September 2017
Available online 05 September 2017

before the end of the 40 min [13]. This within-FO behavioral satiation occurred at all FUPs, including at higher costs at which total intake was substantially reduced and weight loss was rapid and would have resulted in death if the animals had not been removed from the experiment. In contrast, when a fixed-interval schedule for earning food was imposed to slow (at higher values) the rate at which food could be procured, pellets were earned evenly across a FO with no evidence of satiation [13]. Thus, a characteristic of higher ratio (FUP) schedules in mice is to produce a maladaptive pattern of early satiation despite inadequate intake.

The purpose of the first experiment in this paper is to examine whether rats will exhibit within-FO satiation similar to those shown by mice using ratio schedules. This will address the question of whether there is or is not a significant difference in mechanism or behavioral strategy of satiation between rats and mice in this situation and whether rats, like mice, show circadian variation in FO size. The second experiment examines choice strategy and meal structure of rats as a function of economic factors. To approach this question, rats were allowed continuous access to costly food (i.e., high FUP) intercalated with intermittent short periods of inexpensive food (i.e., low FUP; effectively, cheap FOs). Further, to investigate the role of timing relative to the light: dark (Zeitgeber) cycle on size of successive FOs, this experiment was performed in three segments using standard, phase advanced, or phase delayed timing of the II episodes. In a prior study, we showed that in mice exposed to this experimental protocol, light exerted a suppressive effect on food intake and had a greater modulatory effect than economic choice [17]. We have been surprised by both early satiation and circadian sensitivity findings in mice, given the context of a large and diverse literature in rats. It is thus important to validate this potential species difference using exactly analogous protocols. If the species difference holds, then rats should show less early satiation (exp. 1) and greater economic sensitivity (exp. 2) than mice.

2. Methods

2.1. Subjects

The same 6 male and 6 female Sprague-Dawley rats (Harlan/Envigo Labs, Indianapolis IN), initially 4 months old were used in both experiments. They had previously served in an operant study that examined the pattern of food pellet acquisition in daily sessions as a function of price, using an increasing fixed unit price schedule of reinforcement [6]. Between studies, rats were housed individually in polycarbonate cages with free access to standard pellets of Purina 5001 and autoclaved water. Fluorescent vivarium lights were on 06–18 h, ambient temperature was ~24 °C, and relative humidity was ~50%. We did not assess the estrous cycle of females. All procedures were approved by the Institutional Animal Care and Use Committee of University of Florida with the stipulation that rats were removed from the study as soon as body weight loss exceeded 15% of initial.

2.2. Behavior test chambers

During experiments, rats were housed individually in operant conditioning chambers (Med Associates, St. Albans, VT) enclosed in ventilated, sound-attenuating cubicles (relative humidity ~45%; ambient temperature ~ 23 °C). Indirect light from a 7-W bulb inside the cubicle provided the same 12:12-h light-dark cycle as the vivarium with lights on 0600–1800 h local time, equivalent to Zeitgeber time (ZT) 00–12. Chambers measured 30 × 24 × 21 (L, W, H) cm and were made of Plexiglas with aluminum front and rear panels, and standard rat stainless steel rod floor (~1 cm between rods). A paper-lined stainless steel pan was placed below the floor. In experiment 1, the front wall of each chamber was equipped with a single fixed lever located on the left side of a food trough. A cue light was illuminated above the lever when food was available. Grain-based 45-mg food pellets (Purina Test Diet 5TUM) were dropped into the trough from a dispenser outside the cage. Water was available ad libitum from a sipper spout. A computer running custom programs in Med-PC IV software recorded responses and controlled pellet deliveries in daily 23-h sessions.

In experiment 2, the front wall of each chamber was equipped with two levers, each with a cue light above that was illuminated when the lever became operational, located on either side of a the food trough. Other details were as in experiment 1.

2.3. Experimental procedures

2.3.1. Experiment 1

These animals had previously served in a continuous access operant food study [6] and re-adaptation to the chambers was rapid, achieved in one day during which one food pellet was delivered for every two lever presses (FUP2). At the start of the experimental phase, males and females weighed means of 402 g and 264 g, respectively. Food access was then tapered to 16-h/day for three days, then to four 40-min feeding opportunities (FOs) each 23-h session for six days. FOs started at 1800, 2200, 0200, and 0600-h (FOs #1–4, respectively and ZTs 12, 16, 20, and 00), during which time food pellets were delivered according to the prevailing FUP schedule. The animals were removed from the testing chambers for one hour during the middle of each day (11:30–12:30-h) during which they were weighed and the chambers serviced. During the formal experimental phase, rats were tested for four contiguous daily sessions at a given FUP after which the number of responses required to deliver a food pellet was increased sequentially to 2, 5, 10, 25, 50, 100, and 200. Some animals did not complete the higher FUPs due to weight loss > 15%.

2.3.2. Experiment 2

The goal of this experiment was to examine whether the timing of cheap food relative to the ambient light Zeitgeber (lights out ZT 12-00) affects economic choice. Following a 30-day rest period in their home cage, rats were allowed to re-adapt to the test chambers and the presence of two levers. For initial training, animals were exposed to FUP2 per 45-mg food pellet for two consecutive days followed by an incrementing FUP series (5, 10, 25, and 50 for 1–2 days each) to again adapt the animals to higher food costs. The animals were then tested in three successive segments that differed in the timing of four inexpensive FOs, a protocol previously described by Minaya et al. [17]. Briefly, in each segment, one lever (continuous costly, CC) was operational throughout each 23-h session and delivered one 45-mg food pellet upon completion of 100 responses. The other lever (intermittent inexpensive, II) was operational only during four 15-min FOs and delivered a food pellet upon completion of 5 responses; the shorter inexpensive FOs in this experiment were determined on the basis of results at FUP5 in experiment 1. The relative position of the levers was switched every 2 days to account for and avoid side preference. In segment 1, the inexpensive 15-min FOs (#1–4, respectively) were available on the II lever starting at ZT 13, 17, 21, and 01. In segment 2, the inexpensive FOs were 4-h earlier relative to segment 1 with the II lever operational starting at ZT 09, 13, 17, and 21. In segment 3, the inexpensive FOs were delayed 4-h relative to segment 1, with the II lever operational starting at ZT 17, 21, 01, and 05. Data were collected for eight consecutive days in each segment.

2.4. Statistical analysis

Data are presented as group means with statistical comparison using two-way RM ANOVA and Holm-Sidak post hoc contrast (Sigma Plot); $P < 0.05$ was used as the criterion for statistical significance. Intakes were not adjusted for spillage from the pan because this was minimal and, in any event, we could not determine when any food spillage occurred within a session. In the first experiment, to ensure analysis of the most stable behavior, data from the last two days at each FUP were