COMPARISON OF LEVER PRESS AND NOSE POKE OPERANTS FOR AN ANALYSIS OF FOOD INTAKE AND MEAL PATTERNS IN MICE

By

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2008
To my mom, dad, and my advisor Dr. Neil E. Rowland
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Comparisons of Lever Press and Nose Poke Operants for an Analysis of Food Intake and Meal Patterns in Mice

By

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Chair: Neil E. Rowland
Major: Psychology

Much research in the field and in laboratory studies has focused on behavioral economics of food intake in several species. Operants such as lever press, nose poke, or key peck have been used to generate demand functions that express the relationship between the cost of food and the amount of food consumed. There have been very few such studies of motivated food seeking and demand in mice, and none has examined systematically consummatory cost or meal patterns.

Using albino (CD1) male mice, the present study compares food intake and meal patterns across a series of ratio consummatory schedules. Two operants, lever press and nose poke, were compared in a between groups design. A closed economy was used in which the mice were in the test chambers for 23 h/day and earned all of their food via the operant under four fixed (FR5, FR10, FR25, FR50), variable (VR10, VR20, VR50) and progressive (PR1.25, PR1.5, PR1.75) ratios. When averaged across all schedules, mice in the nose poke group consumed more food. Mice were run for 4 days at each ratio; there were no systematic differences between the first and last day indicating that behavioral adjustments to schedule changes occurred very rapidly. Meal number significantly differed when two criteria for the definition of `a meal’ (15 and 30 min) were used.
CHAPTER 1
INTRODUCTION

Obesity is a serious threat to human health and has a growing incidence in the recent decades. According to the most recent WHO report, at least 400 million adults globally are obese (World Health Organization, Obesity: 2005). Different approaches including use of animal models have been developed to study eating and yield insights into the etiology of obesity. From the early studies, it has been known that homeostasis has a crucial role in the regulation of food intake and energy balance (Levin, 2002; Teitelbaum, 1964; Berthoud, 2002; Woods, 1991, 1998). In addition, there have been advances in molecular biology and genetics that have identified a large number of genes responsible for satiety and hunger (Lubrano-Berthelier et al., 2003a, 2003b; Branson, 2003). It has been estimated that 40 to 70 percent of the variation in obesity-related phenotypes in humans is heritable (Comuzzie and Allison, 1998). However, it has a complex etiology and is a multifactorial phenomenon (Blundell and Cooling, 2000; Erlanson-Albertsson, 2005) that arises from the interactions of multiple genes and brain neural systems (Gelegen et al. 2006; King, 2006; Petrovich and Gallagher, 2007), external factors from environment (Rolls et al., 2002; French, 2003), and behavior (Stellar, 1954; Saelens and Epstein, 1996; Drewnowski, 1995; Lowe and Butryn, 2007).

Today it is highly recognized that there are many central and peripheral factors involved in energy homeostasis and regulation of food intake, and understanding these mechanisms should lead to effective treatments in the control of obesity. For example, some studies reported that obesity in United States is to a great extent an economical issue (Drewnowski, 2004). Specifically, the cost of energy-dense foods is often low, and it has been shown that dietary energy density influences the regulation of food intake and body weight (Drewnowski, 2004; 2003). Thus, feeding behavior is highly influenced by the economic structure of the current
environment in which the individual lives and economic analysis provides a tool for understanding this type of behavior. My goal in this study is to combine and examine the economic concepts within the context of nutritional homeostasis.

Earlier studies with physiological, genetic or homeostatic animal models have failed to provide a systematic protocol to examine whether food intake changes under controlled laboratory conditions. Recently, the principles of economics, which relate the commodity to its price, have been applied to the field of behavior. In the case of eating, it has been shown food intake does change under different conditions of food availability (Sumpter et al., 1999; Rovee-Collier et al., 1982; Hursh, 1980; Hursh et al., 1988).

Economics is considered a science of highly organized human behavior and is defined as the computational analysis of anticipated cost and benefits (Hursh, 1984). In most research protocols with animal models investigating eating in terms of economics of behavior, the price is defined as responses required per reinforcement or reward (e.g., food, water). These designs employ various experimental protocols that require some effort (cost) to obtain the reward. For example, on a ratio schedule, a specified number of responses are required to obtain a particular commodity or reinforcement. The schedules used in the present study are:

- **FIXED RATIO (FR).** The ratio in which each unit of the item costs a fixed amount (e.g., number of responses),

- **VARIABLE RATIO (VR).** The ratio in which each food item costs a mean amount but the actual cost of each item varies around that mean, and

- **PROGRESSIVE RATIO (PR).** The ratio in which successive food items within an episode of feeding become progressively more costly.

FR schedules are believed to be the most direct and are the most commonly used method to set the price of a commodity (Bauman et al., 1996; Hursh, 1984). By using different schedules, researchers aim to understand how animal’s preference for food is shaped by the
different economic environments and by extrapolation to the human condition, how an individual’s food preferences may be affected in these economies where different types of food are usually available at all times.

In order to survive animals must learn how to search for food. They often perform a series of behaviors traveling, catching and consuming this food. Earlier studies on behavioral economics found that feeding patterns vary according to the cost of access to the food (Collier, 1985; Collier et al., 1986, Hursh, 1980). There are at least three definable costs related to eating behavior (Morato et al., 1995):

- Cost of procuring access to food -procurement cost (travel effort and/or time)
- Cost of consuming food –consummatory cost (the cost within the patch such as digging or climbing for food items, sucking, catching, holding etc., which are the equivalent of operant schedules in an experimental setting in most studies)
- Cost of processing food (physiologic consequences/digestion)

Thus from the point of view of economics of behavior, the apparent question is how the animals will change the rate or amount of responding as the required effort to access to the commodity, the food is increased (Hursh, 1980). It is recognized that answer is a product of a complex interaction between the availability and/or cost of the food and hunger state of the organism, however it can be defined and summarized in a demand function which relates the price and food intake (Figure 1-1). The slope of the demand function is determined by the amount of the effort that an animal will work to obtain a commodity as the price for that commodity increases. The theory of economics claims that the consumption of most goods will decrease as price increases (Hursh et al., 1988).

Morato et al. (1995) compared the food intake in rats under low vs. medium vs. high procurement cost that were varied across days, and showed that when the price of procurement was stable for more than about three days, rats ate less frequent and relatively larger meals when
the price was high than the lower prices (Figure 1-2). Many behavioral economists working with animal models claim that animals optimize their food intake in accordance with 3 goals. First, animals adjust their eating behavior to maintain the daily food intake. Second, by eating less frequent, animals limit the time and energy spent for foraging. Third by adjusting the meal size, animals limit the physiological cost of processing ingested food. Thus, an optimal meal pattern represents a compromise between eating as infrequently as possible so as to minimize foraging cost, and eating meals as small as possible so as to minimize processing cost (Collier et al., 1986; Morato et al., 1995; Stephens and Krebs, 1995).

Morato et al. (1995) showed that for high cost requirement (FR400) condition rats did save foraging cost by reducing their food intake. Some rats did not consume anything at all on those `expensive` days, and thus did avoid paying the high procurement price. Some rats however, continued to eat on high-price days but their meals were not large enough to compensate for their reduced meal frequency, so their total food intake fluctuated. A new meal frequency was established on the first day that the new price was set but meal size seemed to change more slowly. A similar result was also found when change in caloric density was used rather than change in procurement cost change (Le Magnen, 1992). Rats can use this strategy to increase their efficiency with which they exploit resources. For example, if the procurement/consummatory cost is lower during the day than at night, rats will switch from a nocturnal to diurnal feeding (Jensen et al., 1983). As mentioned above, when a day of high cost is followed by a day of low cost rather than by another high cost day, intake is reduced or even eliminated on high cost day and the deficit is made up entirely on the low cost day (Morato et al., 1995). Since the predictability of the environment affected the meal patterns in rats, the present
study also tested this in mice by using random vs. ascending order reinforcement schedule ratios
(variable vs. progressive ratio schedules).

**Open and closed economies.** The literature of behavioral economics has offered two
different settings for analysis of eating behavior -open and closed economies- and these lead to
sometimes disparate results (Timberlake and Peden, 1987; Bauman, 1991; Killeen, 1995). In a
closed economy all of the commodity is earned in the experimental session which often is in
force all of the time. In an open economy protocol, a commodity may be earned during the
experimental session, which is time-limited, but in the case of food, additional food is usually
offered outside of the economy. For example, an animal may receive supplemental free rations
after a session to ensure that it maintains a specific body weight. Further, an open economy
usually requires food deprivation prior to the experimental session. Hursh (1980) demonstrated
that in an open economy as the rate of reinforcement schedule decreased, response rate slightly
decreased too. In contrast, in a closed economy, the response rate increased remarkably (Killeen,
1995). Hursh (1980) explains this dilemma by suggesting that in a closed economy, the
commodity and costs are present at all times and animals can only obtain food as a consequence
of the scheduled ratio of reinforcements. On the other hand, Killeen (1995) proposes that the
trade between the animal and the environment is guided to a great extent by the deprivation level
of the organism which he argues that in a closed economy setting it is not taken into much
consideration. Open economy experiments effectively study single meals, thus to acquire an
eating episode in a given experimental session, food deprivation prior to the session is essential.
In a closed economy protocol, no food restriction is imposed by the experimenter; any change in
meal taking is thus a direct consequence of the economic structure. However, it is known that
food restriction increases food consumption and also reinforcing value of food (Raynor and
Epstein, 2003). Collier (1985) argues that humans live in a closed economy as the food is almost always available, thus closed economy protocols may provide more realistic models for human eating behavior.

It has been shown that the meal size and subsequent interval to the next initiation of eating were positively correlated (LeMagnen, 1992); such a correlation which cannot be studied in a single meal session of the open economy environment. On the other hand, closed economy settings are concerned with sequence of meals or meal patterns. The present experiments thus employ a closed economy setting for studying meal patterning in mice.

Determining the relationship between food consumption and the price for food (demand function) is the one of the main foci of the economics of ingestive behavior. To acquire such demand functions, systematic analyses on meal patterns have been done with various animal models, with various schedules of reinforcement. To reach a consistency between days for a day-to-day meal pattern for each animal, researchers use time frames ranging from several days to weeks for each schedule of reinforcement (Morato et al., 1995, Collier et al., 1986). To obtain a demand curve for the rats, Hursh (1980) reported that it required 5 to 6 months. They adapted a rapid method for determining demand curves but it still required 40 days. Of great significance to this project, it has been reported in a study using rats with a lever press operant that a demand function can be generated in a much shorter period (Raslear et al. 1988). They suggested that in an environment where the cost for food is changing and unpredictable, rats are able to adjust their food intakes very rapidly probably less than one day.

We do not know whether all these findings on rats reflect the facts about mice there has not been much consistency on the field about meal patterns in mice. Studies suggested that the average number of meals per day shows substantial variation for different animal species
(Berthoud, 2002). When several factors are controlled, such as stress, existence of predators or social competitors, light-dark cycle and given adequate food, species-specific meal patterns seem to become apparent (Madden, 2005).

The initiations and terminations and of meal to meal intervals (MMI) are called “the meal pattern” of rodents. However, defining a meal within each session, and differentiating a pause within a meal (intra-meal intervals) from an MMI require a suitable time resolution (typically <5 min). 2 min after animals stopping eating, the probability of animals resume eating within the next 10 min, was found to be maximal. That probability decreases from 10 min to 40 min, and is minimal at 40 min and increases again for longer (because the animal is probably hungry again). Thus a meal has been defined as an eating episode initiated after at least 40 min of non-eating period (Kissileff, 1970; LeMagnen, 1992; Clifton, 2000). In mice, spontaneous food intake occurs in number of eating bouts separated by periods of non-eating or inter-meal or meal-to-meal intervals that typically are at least 30 min in length (Clifton, 2000). However, there is a drastic discrepancy between different laboratories for the number of meals taken per day in experiments with mice (Petersen and McCarthy, 1981; Gannon et al., 1992; Strohmayer and Smith, 1987; Vaughan and Rowland, 2003; Fox and Byerly, 2004; Richard and Low, 2007). Table 1-1 summarizes these results for operant and non-operant conditions.

Although rats typically do not show so much between-laboratory variability in results, some studies with rats have suggested differences with use of a nose poke compared with a more traditional lever press operant (Ettenberg et al., 1981). Also it has been claimed that changes in the meal patterns in different studies with rats might arise from the effects of the experimental manipulations; such as single vs. multiple presentation of food, method of choosing between two options, water availability, ratio or interval schedules etc. (Clifton et al., 1984). In their study,
dose-dependent reduction in response rates was not found when nose poke operant was used. According to their report even at the highest doses, rats continued to respond. Thus, they concluded the effect of the drugs on responding rate was partially a result of the instrumental paradigm that has been employed in the experimental design.

Roche and Timberlake (1998) emphasized the importance of designing protocols that examine the natural behavioral traits and behavioral hierarchies that may be species rather than responses that are completely arbitrary. It has been demonstrated that there is a species-typical perceptual/motor organization in rats related to common maze equipment, such as straight alleys, and radial arm mazes and in many studies it is suggested that the operant has a great deal of importance (Roche and Timberlake, 1998; Schindler et al., 1993; Marusich and Branch, 2006). It is has been suggested that the baseline level of nose poke operant response was high and acquisition with food reinforcement occurred rapidly, particularly when compared with a lever press operant response. Therefore, the nose-poke response appeared to be particularly useful for the study of the acquisition of operant responses (Schindler et al., 1993).

Thus, we compared two different operants, nose poke which has not been much used and lever press which has been most commonly used in these types of protocols. For analytic simplification in defining the parameters of feeding behavior in this particular species, we designed a parametric study with an operant (nose poke) that to our knowledge has not been used previously in an eating task in mice and using various environmental conditions of food availability.
<table>
<thead>
<tr>
<th>MOUSE STRAIN</th>
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<th>DIET</th>
<th>#OF MEALS/DAY</th>
<th>REFERENCE</th>
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<tr>
<td>Small 'S' and large 'L' inbred SWR/J</td>
<td>Overhead door panel</td>
<td>Powdered rodent chow</td>
<td>12 (5min)</td>
<td>Petersen and McCarthy (1981)</td>
</tr>
<tr>
<td>C57BL/6J (lean and ob/ob)</td>
<td>Recess at the floor level</td>
<td>Powdered rodent chow</td>
<td>36 (5min)</td>
<td>Gannon et al (1992)</td>
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<tr>
<td>C57BL/6J</td>
<td>Sipper spout in cage</td>
<td>Liquid diet EC116</td>
<td>50(male)</td>
<td>Strohmayer and Smith (1987)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>30(female)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1 and 5min)</td>
<td></td>
</tr>
<tr>
<td>C57BL/6J (lean and ob/ob)</td>
<td>Lever press</td>
<td>Noyes 20mg pellets</td>
<td>~8 (24 h food access through FR schedules)</td>
<td>Vaughan and Rowland (2001)</td>
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<td>C57BL/6J</td>
<td>Lever press and food receptacle</td>
<td>Noyes 20mg pellets</td>
<td>2-10, function of access cost (10min)</td>
<td>Vaughan and Rowland (2003)</td>
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<td>129/B6 (wild type for BNDF +/-)</td>
<td>Pellet removal from trough</td>
<td>BioServ 20mg pellets</td>
<td>~12 (18 hr food access)</td>
<td>Fox and Byerly (2004)</td>
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<tr>
<td>129/B6 (wild type for BNDF +/-)</td>
<td>Liquid diet from 0.02ml dipper</td>
<td>Isocal-High fat liquid</td>
<td>~15 (18 hr food access)</td>
<td>Fox and Byerly (2004)</td>
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<td>129/B6 (wild type for MC4R +/-)</td>
<td>Lever press and food receptacle (procurement cost)</td>
<td>Noyes 20mg pellets</td>
<td>2-7, function of procurement cost (10min)</td>
<td>Vaughan et al (2005)</td>
</tr>
<tr>
<td>C57BL/6J (wild type)</td>
<td>Lever press (procurement cost-FRs)</td>
<td>BioServ 20mg pellets</td>
<td>(4g per day) (735sec)</td>
<td>Richard and Low (2007)</td>
</tr>
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Figure 1-1. The demand function. The figure summarizes the relation between the cost and demand. The slope of the function is determined by the amount of the effort that a person or an animal will work to obtain a commodity as the price for that commodity increases (the numbers do not indicate any real data; the graph was drawn for conceptual purposes).
Figure 1-2. The demand function. The slope of the demand function is determined by the amount of the effort that an animal will work to obtain a commodity as the price for that commodity increases. The theory of economics claims that the consumption of most goods will decrease as price increases (the numbers do not indicate any real data; the graph was drawn for conceptual purposes).
CHAPTER 2
MATERIALS AND METHODS

Subjects

A total of sixteen male albino (CD1) mice, initially about 3 months of age were used. We did not include female mice in our design to avoid the possibility that estrous cyclicity would interact with our analysis of meal patterns. The average weight of the animals was 39.7±2.7g at the beginning and 45.8±4g at the end of the experiments (Mean ± s.d.).

During the experimental periods, mice lived in the operant chambers for 23 hr per day. The mice were weighed daily during a 1 hr cleaning period and were kept in empty holding cages. The operant chambers were wiped with 70% ethanol solution and distilled water each time before the mice were placed. When not in experiments, mice were housed in a standard, polycarbonate cages (separate vivaria) with Purina Chow pellets and tap water available ad libitum and a 12:12 light cycle in place (lights on 0700). During the experiments, mice obtained 20 mg; complete nutritional mouse pellets (Research Diets Inc) when they completed an imposed cost determined by the reinforcement schedule. Our preliminary studies demonstrated that the spillage with this type of pellets was typically very little. Tap water was available freely from a sipper tube.

The Psychology department vivaria are part of the centralized University of Florida Animal Care program with full AAALAC accreditation. Animal use is approved by a campus-wide IACUC and is compliant with the recommendation of the Guide for the Care and Use of Laboratory Animals (1996).

Apparatus

Sixteen operant chambers (Med Associates: 13x13x12 cm with Plexiglas and metal walls and stainless steel grill floor plus solid nesting platform) enclosed in ventilated, noise attenuating
cabinets with the same 12:12 light cycle as the vivarium (a 15 watt bulb in a standard light fixture run from a 24 hr timer) were used in the experimental procedures in the present study. All chambers were equipped with one lever press and one nose poke operant device, located 2cm above the floor, situated on one wall on either side of a food aperture. Water was supplied from sipper tubes mounted on the wall opposite side to the food magazine and the two operant devices. In each chamber and for a given mouse only one manipulandum was active during the whole experimental protocol.

A record of the total pellets obtained by mice and number of responses (nosepoking and leverpressing) were acquired by the MEP-PC IV computer software (MED Associates, St. Albans, VT). The computer recordings allowed an accurate analysis of the number of meals and the amount eaten at each meal. Data were accumulated in each 15 min (for FR and VR) and 5 min (for PR) time bins for each 23 hr period.

**Procedure**

To investigate whether the form of the operant (nosepoking vs. leverpressing) influences the economic analysis of meal patterning, mice were divided randomly in two groups of 8, with one group obtaining food pellets by pressing the lever and the other group by nosepoking.

Prior to the study, to habituate mice to the operant chambers and to the novel pellets, a 1 hr training period was applied with free food was available in the food magazine of the operant chambers without any cost. Later, a fixed ratio-1 (FR1) where a pellet was delivered as a consequence of one response on the active manipulandum, was used as a magazine training for a day or two to acclimate mice to the operant conditioning protocol. For the training, a mouse was considered to have learned the conditioning paradigm if they earned enough pellets to maintain their body weight. No food deprivation protocol was used prior to the experimental
sessions. After they successfully learned to press the lever or nose poke, animals were exposed to several reinforcement schedules as the experimental design.

Experimental sessions lasted 23 hr. A short protocol (4 days with each ratio) was used because previous studies in our laboratory (Vaughan et al., 2006) have indicated that mice adjust to changes in ratios within a day or so. Mice were exposed to an incrementing series of fixed ratios (FR1, FR5, FR10, FR25, FR50) and then, variable ratios (VR10, VR20, VR50), and finally progressive ratios (PR1.25, PR1.5, PR1.75). In the VR, the actual ratios selected randomly by the program were VR10; 1, 5, 10, 15, 19, for VR20; 2, 10, 20, 30, 38, for VR50; 5, 25, 50, 75, 95. In the PR, the number of responses required for the next (n+1)th pellet in a series, \( R_{n+1} = R_n \times 1.25 \) and \( R_{n+1} = R_n \times 1.5 \) and \( R_{n+1} = R_n \times 1.75 \) (\( R_n \) = nth response requirement). The resulting number was rounded to the nearest integer, giving the following sequences: for PR1.25; 1, 2, 2, 2, 2, 4, 4, 5, 6, 8, 10, 12, 15, for PR1.5; 1, 2, 2, 3, 5, 8, 11, 17, 26, 38, 58, 86, 130 and PR1.75; 2, 3, 5, 9, 16, 29, 50, 88, 154, 269, 471, 825. Further, in the PR series, whenever 15 min elapsed without a response the ratio was reset to the initial value of the particular schedule. This reset allows the animals to effectively quit eating when the unit cost of a pellet becomes too high and shift to another “patch” (in this case with a 15 min temporal boundary). For comparison, we additionally ran a PR 1.5 reinforcement schedule using a 30 min reset criterion.

In the final phase of the experiment, after all the above schedules were completed, to determine whether mice can follow schedule changes even more rapidly as well as to determine whether differences that we observed across schedules were not merely due to experience or age, each reinforcement schedule was employed for one day consecutively.

**Analyses and Data Acquisition**

In the present experiment, two different meal-to-meal interval (MMI) criteria (15 and 30 min) were applied. The raw data from the computer recordings, showed how many responses
were made and the number of pellets earned at each 15 min (for FRs and VRs) and 5 min (for PRs) throughout the whole 23 hr period (1380 min) each day. Non-responding (non-eating) episodes were showed as zero for each 15min time bin in the computer software and a minimum of 15 or 30 min was used to distinguish separate meal events. After the numbers of meals for each mouse were counted by the experimenter for each day of each schedule from the raw data, the mean meal size was derived by dividing the number of total pellets by the number of meals for the particular day. With one exception, no systematic difference was noted across the four days for pellet intake per day. Thus, the mean for number of meals, total pellets earned and meal sizes were computed for each mouse and for each reinforcement schedule by averaging them over for four days. Parameters were analyzed for significance with SPSS computer software by using repeated measures analysis of variance (ANOVA), with the operant (nosepokers vs. leverpressers) as between-subject variable and schedules as within-subject variable. Analyses within each ratio schedule type (FR, VR and PR), One-way ANOVAs were used to measure the significance of each variable. Independent t-tests were used where necessary. In all cases, \( p < 0.05 \) was considered significant. Graphs are drawn using Sigma-plot computer software.
CHAPTER 3
RESULTS

When averaged across all of the ratio schedules, mice consumed (Mean ± S.D.) 259.5 ± 56.7 pellets per day (Figure 3-1) distributed as either 20 ± 7.5 or 11 ± 3.2 meals at the 15 and 30 min MMI, respectively. The corresponding meal sizes were 15 ± 6 and 28 ± 17.8 pellets. Since each pellet is 20mg, this corresponds to mean meal sizes approximately of 0.2 and 0.4 g.

With the exception of FR10 \([F(3,60)=8,193; \ p < 0.01]\), under each schedule, meal numbers, meal sizes and the number of pellets did not differ significantly across the four days of each schedule. Thus, data were averaged across 4 days to give a single datum for each mouse.

When averaged across schedules, nosepokers (NP) consumed significantly more pellets than leverpressers (LP) (Mean ± S.D.; NP: 274.5 ± 51.7, LP: 244.6 ± 58). ANOVA analyses resulted in a group effect for the operant \([F(1,174) = 13.086; \ p<0.01]\).

FR Schedules

The number of pellets taken in the FR phases is shown in Figure 3-2, the meal numbers in Figure 3-3a, b and the meal sizes in Figure 3-5, 3-6. Total pellets earned per day differed significantly across the four FR (FR5, FR10, FR25, FR50) schedules \([F(3,60)=5.763; \ p < 0.05]\). Post-hoc Bonferroni test showed that mice at FR50 mice consumed fewer pellets compared to their intake on the other three FR schedules (Figure 3-2).

Across FR schedules, there were significant differences between LP and NP for total pellets \([F(1,62)=7.818; \ p < 0.01]\) (Figure 3-2), meal numbers at 30min MMI \([F(1,62)=4.802; \ p < 0.05]\) (Figure 3-4), and meal size at 15min MMI \([F(1,62)=10.763; \ p < 0.01]\) (Figure 3-5).

Post-hoc \(t\)-tests showed that, NP consumed more number of pellets daily \([t (62) = -2.796; \ p < 0.01]\), had more meals with the 30 min MMI definition criterion \([t (62) = -2.191; \ p < 0.05,]\] and larger meals with the 15 min MMI criterion \([t (62) = -3.281; \ p < 0.01]\).
**VR Schedules**

The number of pellets taken in the VR phases is shown in Figure 3-7, the meal numbers in Figure 3-8, 3-9 and the meal sizes in Figure 3-10, 3-11. Total pellets earned per day differed significantly across the three VR (VR10, V20, VR50) schedules \( F(2,45)=15.728; p < 0.01 \). Post-hoc Bonferroni test showed that mice at VR50 mice consumed fewer pellets compared to their intake on the other two VR schedules (Figure 3-7).

Across VR schedules, there was no significant difference in the number of pellets taken by NP vs. LP (Figure 3-7). However, the type of operant showed a significant between-subjects effect on meal number \( F(1,46)=5.213; p < 0.05 \) (Figure 3-8) and meal size under with 15min MMI definition criterion \( F(1,46)=7.123; p < 0.01 \) (Figure 3-10). The t-test revealed that nosepokers took fewer but larger meals than leverpressers [for meal numbers; \( t(46) = 2.283; p < 0.05 \), for meal size; \( t(46) = -2.669; p < 0.01 \)].

**PR Schedules**

The number of pellets taken in the PR phases is shown in Figure 3-12, the meal numbers in Figure 3-13, 3-14 and the meal sizes in Figure 3-15, 3-16. Total pellets earned per day differed significantly across the four PR (PR1.25, PR1.5, PR1.75, PR1.5/30min) schedules \( F(3,60)=8.500; p < 0.01 \). Post-hoc Bonferroni test showed that at PR1.25 mice consumed more pellets compared to their intake on the other three PR schedules (Figure 3-12).

In PR schedules, operant type displayed a significant between-subjects effect for total pellets earned per day \( F(1,62)=6.640; p < 0.05 \) (Figure 3-12), and meal number with 30min MMI \( F(1,62)=5.676; p < 0.05 \) (Figure 3-9b). Meal sizes did not differ significantly (Figure 3-15, 3-16).

The comparison between 15 min and 30 min reset criteria applied in PR1.5 schedules did not seem to make any difference for total pellets (Figure 3-17), number of meals (Figure 3-18) or
meal size (Figure 3-19) per day except when 30min MMI used \[ F(1,30)=15.896; \ p < 0.01 \]. A follow-up independent t-test showed when 30min MMI used, mice had more meals per day with the 30 than the 15 min reset criterion \[ t(30)=-3.987; \ p < 0.01 \].

**Comparison Between Schedule Types**

Daily pellet intake showed significant variation between three types of ration \[ F(2,173)=17.846; \ p < 0.01 \]. A follow up post-hoc analysis showed that mice took more pellets per day under PR schedules compared to FR and VR schedules. PR schedules also resulted in more number of meal intakes but only when 15min MMI was used \[ F(2,173)=233.987; \ p < 0.01 \].

**Last Phase**

The last phase of the experiment that each schedule were employed for one day in the exact same order with the whole procedure, showed no significant difference on the total number of pellets earned daily when averaged over the schedules. However, when one to one comparisons between the number of pellets computed by the average of previous four days for a schedule and the number of pellets per day as the last phase for the same schedule, some reached the significance \[ \text{for FR5: } t(15)=-3.629; \ p < 0.01, \text{ for VR50: } t(15)=5.385; \ p < 0.01, \text{ for PR1.25: } t(15)=2.474; \ p < 0.05, \text{ and for PR1.75: } t(15)=3.142; \ p < 0.01 \]. Table 3 summarizes the result.

In addition, mice obtained more number of pellets \[ F(2,173)=5.667; \ p < 0.01 \] and more number of meals per day \[ \text{for 15min MMI } F(2,173)=79.679; \ p < 0.01 \text{ and for 30min MMI } F(2,173)=4.356; \ p < 0.05 \] in PR schedules compared to FR and VR schedules in this last phase of consecutive one-day employment of the each schedule.

When 30min MMI was used to define ‘a meal’, there was a significant between-subjects effect of operants \[ F(1,174)=8.758; \ p < 0.05 \]. Independent t-test conducted as a follow-up showed that nose pokers had more meals per day \[ t (174) = -2.959; \ p < 0.05 \]. However, when
15min MMI criteria was used to define a meal, the group effect of operants did not reach significance. The difference between the meal sizes nose pokers and lever pressers on the other hand, reached statistical significance for 15min MMI but not for 30min MMI definition criteria \[ F(1,174)=13.143; p < 0.01 \]. The \( t \)-test analysis concluded that nosepokers took larger meals when 15min MMI definition criteria was used \( t (174) = -3.625; p < 0.01 \).

Regardless of the operants, defining a meal by 15min MMI vs. 30min MMI resulted in different number of meals and the meal size per meal. Statistical significance were at \( F(1,350)=210.049; p < 0.01 \) for number of meals and \( F(1,350)=86.933; p < 0.01 \) for meal size. Mice ate significantly fewer and larger meals when 30min MMI used compared to 15min MMI \( t (350) = 14.493; p < 0.01 \) and \( t (350) = -9.324; p < 0.01 \) respectively.
Figure 3-1. Total pellets per day by operant with FR schedules.
Figure 3-2. Total pellets per day by operant across FR schedules.
Figure 3-3. Daily number of meals with 15min MMI by operants across FR schedules.
Figure 3-4. Daily number of meals with 30min MMI by operants across FR schedules.
Figure 3-5. Meal size with 15min MMI by operant across FR schedules.
Figure 3-6. Meal size with 30min MMI by operant across FR schedules.
Figure 3-7. Total pellets per day by operant across VR schedules.
Figure 3-8. Daily numbers of meals with 15min MMI by operants across VR schedules.
Figure 3-9. Daily number of meals with 30min MMI by operants across VR schedules.
Figure 3-10. Meal size with 15min MMI by operant across VR schedules.
Figure 3-11. Meal size with 30min MMI by operant across VR schedules.
Figure 3-12. Total pellets per day by operant across PR schedules.
Figure 3-13. Daily number of meals with 15min MMI by operants across PR schedules.
Figure 3-14. Daily number of meals with 30min MMI by operants across PR schedules.
Figure 3-15. Meal size with 15min MMI by operant across PR schedules.
Figure 3-16. Meal size with 30min MMI by operant across PR schedules.
Figure 3-17. Comparison of PR1.5 and PR1.5 schedules with 30min Program resetting criteria (daily number of pellets).
Figure 3-18. Comparison of PR1.5 and PR1.5 schedules with 30min program resetting criteria (meal size).
Figure 3-19. Comparison of PR1.5 and PR1.5 schedules with 30min program resetting criteria (daily meal numbers).
Table 3.1  The average for total pellets per day for main procedure and last phase of scheduling (mean±s.d.)

<table>
<thead>
<tr>
<th>schedules</th>
<th>Main procedure</th>
<th>Last phase of schedules</th>
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<tr>
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<td>261±30</td>
<td>270±40</td>
</tr>
<tr>
<td>FR25</td>
<td>227±68</td>
<td>226±50</td>
</tr>
<tr>
<td>FR50</td>
<td>190±63</td>
<td>188±61</td>
</tr>
<tr>
<td>VR10</td>
<td>293±45</td>
<td>297±38</td>
</tr>
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<td>VR20</td>
<td>268±34</td>
<td>254±56</td>
</tr>
<tr>
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<td>215±40</td>
<td>160±56</td>
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<td>PR1.5-30MIN</td>
<td>275±42</td>
<td>256±57</td>
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</table>
CHAPTER 4
DISCUSSION

The main focus of the present study was to design an instrumental conditioning paradigm that would allow us to conduct a systematic analysis for meal patterns in mice as a function of effort and with an explicit comparison of two different operants used.

Mice consumed ~250 pellets (~5g/day) but the meal distribution was critically dependent on the MMI criterion: at the 15min MMI, the overall mean was ~28 meals with a meal size of ~10 p-ellets (~0.2g), whereas at the 30 min MMI the overall mean was ~15 meals with a meal size of ~18 pellets (~0.4g) a day, regardless of the operant. The number of meals and average total intake consumed (grams) per day did show consistency with most of the previous research in the literature (Table 1). This might suggest that using different operants and MMI criteria for defining a meal have reasonable effects on the results for eating behavior analysis in mice (Kissileff, 1970).

One of the findings of the present study was that for each reinforcement schedule, the meal pattern of mice did not significantly differ across four days of each schedule with the exception of FR10. However, the statistical significance for the FR10 condition disappeared when the data from first day was excluded from the four days of FR10 schedule and analysis was conducted across the second, third and fourth days. It might be possible that mice had difficulty adjusting to the novel eating condition as the first day of FR10 schedule was the first day that they encountered a cost that required a relatively more effort compared to free access or FR1 or FR5 schedules. This particular effect for the initial FR10 schedule shown in this study was also found in other studies examining meal patterns in mice (Richard and Low, 2007).

The rapid adaptation of mice to the changing schedules, usually 1 but at most 2 days shown here agree with a study by Raslear et al. (1988) with rats in an operant task that showed a
stable relationship between food consumption and price for food throughout seven consecutive
days after schedule change. Thus, our findings in mice, along with Raslear’s results in rats
indicate that for consummatory costs, rodents have a substantial capability to adapt rapidly the
changes in the schedules of reinforcement. Future use of short-term protocols like the procedure
in this study should allow further research using anti-obesity drugs that have short half-lives
and/or to shorten the time period that is needed to complete an economic profile.

We have presented a parametric study with an animal model to examine the role that
operant plays on meal pattern analysis in mice. Hence, nosepokers obtained considerably more
pellets per day when averaged across all schedules. Also, when analyzed separately, in each
group of ratio schedule (FR, VR and PR) operant type appeared to be an important factor
affecting the total number of pellets eaten. This showed that in reinforcement schedule
paradigms, using different operants has a crucial effect on the results and the nose poke operant
is particularly useful in this type of research. These findings agree with some of the studies with
rats comparing nose poke and lever press operants (Schindler et al., 1993; Ettenberg et al., 1981;
David et al., 2001). Schindler et al. (1993) suggested that acquisition of the nose poke response
in rats occurred much more rapidly than of other operants. In addition they reported that if there
is no experimenter intervention such as shaping, acquisition of lever pressing response occurs
rather slowly. Thus, a nose poke operant might be useful whenever short-term procedures are to
be used (Schindler et al., 1993). On the other hand some studies with rats also indicated that the
type of operant (lever press vs. nose poke) did not have a significant effect on the acquisition of
intravenous heroin/cocaine self-administration or dose-related responding (David et al., 2001).
However, in that particular study, only two types and low ratio schedules (FR1 and FR3) were
used. In order to make an accurate comparison, more variety of reinforcement schedules is required.

Eating behavior in animals occurs in episodes (Collier and Johnson, 2004). The size of each bout depends heavily on the eating environment of the animal such as the availability of food resources and effort that requires consuming the particular resource. Adjusting the meal size for required costs for food is part of the economizing strategy of the animal in eating behavior. In the present design, meal size was affected by the operant type, as nose pokers ate bigger meals regardless of the different schedule requirements. It has been shown that rats increased their meal size as they decreased the frequency of meals (decreasing meal numbers) in a compensatory fashion as the required cost to access to food increased (Mathis et al., 1995; Collier et al., 1998; Collier et al., 2002). However, total number of pellets earned per day showed significant changes under each schedule which indicated that for our results daily intake was not unaffected by the changes in the schedules. In other words, daily intake was not maintained as it was suggested in the literature (Morato et al., 1995; Collier et al., 1998). Nevertheless, it was also claimed that at the highest costs, animals made a sacrifice by decreasing their food intake, thereby avoid paying the expensive price (Morato et al., 1995). Thus, at the highest costs for each ratio in our experiment, it could be argued that mice avoided excessive consummatory cost by sacrificing some of their intakes and reduced the number of pellets earned a day. This is, of course, the defining feature of a demand function. The schedule-associated decrease in food intake was apparent in FR and VR schedules as at FR50 and VR50 mice consumed less pellets. This was also in agreement with a recent mice meal pattern study suggesting that FR40 cost was not enough to alter the meal pattern of mice from the baseline (Johnson and Low, 2007). For PRs, significance was found at the lowest PRs as PR1.25 was resulted in more pellet intake per day.
PR schedules were found to result in higher number of pellets earned per day when compared to the FRs and PRs. However, since a considerable amount of time (~5 months) elapsed between the first day of FR5 and the first day of PR schedules, it is reasonable to argue that PR schedules did result in more number of pellets per day because of that amount of the time that has passed. In an effort to control this issue, after the last PR schedule, we conducted each schedule for only one day in the same order to see if the intake was comparable with the previous 4-day of intake. When averaged across all eleven reinforcement schedules of one day, the total pellets earned per day did not differ from the previous 4-day scheduling when they are averaged across schedules. On the other hand, comparing each schedule separately with the 4-day average of the same schedule resulted in a significant difference between the pellets obtained per day for some of the reinforcement schedules (FR5, VR50, PR1.25 and PR1.75). However, opposite to what we wanted to control as a potential confounding of increase in intake as the time passed, the number of pellets decreased-not increased when compared to the previous 4-days of scheduling. Thus, the argument for the possible confounding effect of the time passage on the higher number of pellets for the PR schedules was eliminated. It can be concluded that PR schedules resulted in significantly more number of pellets compared to the FR and VR schedules.

In addition, in this one day protocol of each reinforcement schedule, the comparison between the three reinforcement schedules (FR, VR, PR) in terms of total pellets and meal numbers per day, agree with the results from the same type of comparison of FR, VR and PR of the main 4-day procedure. Thus, mice took more pellets and meals (both with 15min MMI and 30min MMI) daily in PR schedules compared to FR and VR schedules also at the 1-day phase of the experiment.
Our data revealed that the criterion used for the definition of ‘a meal’ has an important effect on the results. Mice appeared to take considerably more meals when 15min was used as MMI compared to 30min MMI. Reciprocally, 30min MMI resulted in larger meal sizes when compared to 15min MMI. The group effect of the operants also differed when different MMIs were used. All these comparisons in the food intake parameters between the two MMIs indicated that defining ‘a meal’ has a crucial influence on the detail of the meal pattern of animal models. Although it would be hard to determine which of the MMIs is a better representation for ‘a meal’ for this strain of mice, 15min MMI seemed to concur with some of the earlier studies with mice (Petersen and McCarthy, 1981; Vaughan and Rowland, 2003; Fox and Byerly, 2004; Vaughan et al., 2005; Richard and Low, 2007) although strain differences cannot be excluded as a source of variance.

Comparison between 30min vs. 15min reset criteria applied separately on PR1.5 schedules indicated no different meal patterns. Since the first few pellets in each meal were the cheapest, mice ate many small meals by quitting eating as the cost increased and letting the program reset itself to the lower initial cost. However, mice maintained the total pellet intake same in both PR1.5 schedules.

**Future directions.** In our study we looked at the consummatory cost, which is the equivalent of the near-the-patch cost. We are currently conducting a foraging cost design study by applying both Collier’s two costs; procurement and consummatory costs with the same strain of mice. We may further look at the genetic models to see how these meal patterns change in genetically mutated obese mice as an implication for the human obese models.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Deniz Atalayer graduated from Bogazici University-Istanbul-Turkiye in 2004 with a Bachelor of Science degree in psychology. Her interest in neuroscience began in the last two years of undergraduate as she worked in a psychobiology lab specifically conducting research on circadian rhythmicity with rats. She also worked in a behavioral analysis/learning lab on sexual preference with quails. After completing her bachelor’s degree in psychology, she began to seek for a graduate degree combining the behavioral work and neuroscience research. In fall 2005, she was admitted to the behavioral neuroscience program in psychology department at the University of Florida, and started to work with Dr. Neil Rowland. Her field of study includes eating behavior and obesity research both from genetic, neurological, physiological, and neuroeconomical perspectives. She defended her master’s thesis in fall 2007 which concerns the effects of the use of different operants in the meal pattern analysis on mice, and she is currently seeking candidacy to pursue a Ph.D. in the same program.