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BEHAVIORAL PHENOTYPING MEAL PATTERNS IN *MUS MUSCULUS*: VALIDATION, TESTING AND APPLICATION

by

Christian Daniel Richard

A DISSERTATION

Presented to the Department of Behavioral Neuroscience
and the Oregon Health & Science University
School of Medicine
in partial fulfillment of
the requirements for the degree of
Master of Science

June 2008

School of Medicine
Oregon Health & Science University

CERTIFICATE OF APPROVAL

This is to certify that the M.S. dissertation of

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has been approved

Malcolm Low, Mentor

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Larry Trussell, Committee Member

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LIST OF ABBREVIATIONS

α-MSH	α-melanocyte stimulating hormone
A(y)	yellow agouti mouse strain
A-C	appetitive-consummatory
ACTH	adrenocorticotropic hormone
AgRP	agouti-related peptide
ARC	arcuate nucleus of the hypothalamus
BNST	bed nucleus of the stria terminalis
CCK	cholecystokinin
CeA	central amygdala
CNS	central nervous system
db	diabetes gene
EPSC	excitatory post-synaptic current
FR	fixed-ratio (reinforcement schedule)
MC	melanocortin
MC3RKO	melanocortin-3 receptor knockout mouse
MC4RKO	melanocortin-4 receptor knockout mouse
MD	meal duration
MM	minimum meal
MN	meal number
MS	meal size
nPOMCKO	neuron-specific POMC knockout mouse
nPOMCWT	neuron-specific POMC wild-type mouse
NTS	nucleus of the solitary tract
ob	obese gene
POMC	pro-opiomelanocortin
PRP	post-reinforcement pause
TMI	threshold meal interval
VBA	visual basic for applications
WB	work bout

ACKNOWLEDGEMENTS

"No man is an island, entire of itself; every man is a piece of the continent, a part of the main."
- John Donne, *Devotions Upon Emergent Occasions, Meditation XVII*

The training required to become a scientist and the concomitant production of scientific knowledge is best accomplished within a supportive community of peers, teachers and mentors. I am indebted to those who have embodied these social roles during the course of my graduate work here at OHSU.

I have been particularly lucky to have been surrounded by fellow lab members who have provided valuable advice, insights, and just as important, a friendly and accommodating social environment. Michael Haywood and Mandy Sharpe, as the resident behavioral experts, have been something akin to my older "siblings" in science, and I owe the better part of my molecular biological know-how to Veronica Otero-Corchon and Bryce Warren.

I would also like to express my gratitude to the members of my committee, and to faculty members who have consistently demonstrated genuine desire to act as teachers in the best sense of the word: (alphabetically) Drs. Greg Mark, Suzanne Mitchell, Jacob Raber, Andrey Ryabinin, and Larry Trussell. The support of the scientific community extends beyond the walls of this institution; my thanks to Dr. Eric Zorrilla of the Scripps Institute for clarifying some of the more difficult mathematical concepts central to the drinking-explicit method for meal definition, and to the venerable meal pattern researchers Gerry Smith and Henry Kissileff for their prompt responses to my emails.

Finally, I offer my sincerest thanks to my advisor, Dr. Malcolm Low, for giving me the opportunity to conduct my thesis work in his lab, for providing me with the freedom to be creative, and for being a scientific role model eminently worthy of emulation.

ABSTRACT

Significant advances have been made in our understanding of some of the neural circuits and molecular machinery underlying energy homeostasis, and the central melanocortin system has emerged as a particularly important player in this regard. Improvements in our behavioral measures, however, have not kept up with the molecular tools that have been so important to recent advances in the field. Measurements of cumulative food intake are simply insufficient to capture the complexity of ingestive behavior. This complexity must be properly accounted for if we hope to further illuminate the causal relationships between molecular and behavioral functioning. Meal measures are better suited to this task, and a well-validated method for analyzing meal patterns can make better use of molecular biological data.

An important part of this task has been to confront the historical challenges facing research into meal pattern. Perhaps the most serious has been the inability, or at best tremendous difficulty, of comparing results between labs. This is due in part to differences in experimental design. For example, it may be expected that meal patterns of animals given access to a liquid diet will differ from solid diet meals (Strohmayer and Smith, 1987; Ho and Chin, 1988). However, the lack of unanimity for how to define a meal has arguably been the greatest impediment to inter-laboratory comparisons (Geary, 2005). Meal definition determination is a prerequisite for calculating meal parameter values. Different meal definitions will produce different meal pattern results; moreover, different statistical methods have been used to identify the “correct” definition for those studies not resorting to the selection of a more or less arbitrary meal definition. A

comprehensive parametric study of meal patterns using mice offers the benefit of opening this field to the rapidly growing number of genetically engineered mouse strains. All of these issues will be the topic of chapter two.

Having developed and validated the tools for analyzing meal patterns in mice, I apply them to a concrete research problem in Chapter 3. While novel molecular biological details continue to emerge that illuminate the central melanocortin system's regulation of energy homeostasis, its effects on the initiation, maintenance and termination of meals remain largely unexplored. I report the results of two studies: first, I identify the meal pattern underlying the hyperphagia seen in mice deficient in neuronal POMC (nPOMCKO), and second, having characterized the aberrant meal pattern of nPOMCKO mice, I test whether the final adult meal pattern is present in 5 to 6 week-old nPOMCKO mice, and whether body weight covaries with meal pattern.

Finally, in chapter four I review the major findings, drawing conclusions and suggesting possible implications of the thesis and future directions. The remainder of this chapter, then, is devoted to discussion of the literature and issues underlying the controls of feeding behavior and energy homeostasis.

CHAPTER 1.0

GENERAL INTRODUCTION

1.1 *THERMODYNAMICS OF LIFE*

A fundamental feature of living organisms is their ability to maintain a steady state far from thermodynamic equilibrium by regulating the flow of energy and matter between themselves and their surroundings (Koshland, 2002; Ruiz-Mirazo *et al.*, 2004; Feinman and Fine, 2007). Organisms maintain energy homeostasis by coordinating the intake, expenditure and storage of energy. In many animals, including mammals, surplus carbohydrate and lipid are stored in distinct tissue compartments, the liver and adipose tissue respectively. Energy is continuously expended to drive metabolic processes throughout an organism, and intermittently during periods of physical activity. The energy demands of physical activity allow organisms to navigate through their environment, reproduce, and respond to predators. The search for sources of nutrition, their acquisition, and the mechanical processes of biting, chewing, swallowing, digesting and diet-induced thermogenesis all exact costs that must meet or exceed the energy derived from the effort. Energy intake is further complicated by uneven spatial and temporal distribution of nutrients. Organisms have evolved complex systems capable of maintaining energy homeostasis under conditions of duress and environmental uncertainty. Advancing our understanding of the regulatory controls over energy homeostasis is of key importance in the development of new treatments for obesity, diabetes, and cachexia.

1.2 *LEPTIN AND THE MOLECULAR BIOLOGY OF ENERGY HOMEOSTASIS*

The cloning of the obesity (*ob*) and diabetes (*db*) genes in mice over a decade ago brought the study of energy homeostasis into the molecular biology age, stimulating a

rapid acceleration in discovery rates (Zhang *et al.*, 1994; Gao and Horvath, 2008). The *ob*/*ob* and *db*/*db* mouse strains had been extensively studied as animal models of monogenic obesity (Johnson *et al.*, 1991; Hamann and Matthaei, 1996). The peptide product of the *ob* gene, leptin, was discovered to be a hormone secreted by adipose tissue to act as a humoral signal of energy stored as fat, and furthermore *ob*/*ob* mice fail to express functional leptin (Campfield *et al.*, 1995; Maffei *et al.*, 1995; Pelleymounter *et al.*, 1995). Leptin replacement restored normal body weight and food intake in *ob*/*ob* mice, but not in mice homozygous for the *db* mutation (Campfield *et al.*, 1995; Pelleymounter *et al.*, 1995). The *db* gene was found to code for the leptin receptor, and the failure of *db*/*db* mice to respond to leptin was shown to be the result of a loss of function mutation in the leptin receptor preventing detection of leptin signals from adipose tissue (Tartaglia *et al.*, 1995; Chen *et al.*, 1996; Lee *et al.*, 1996; Jequier, 2002). The discovery and characterization of the leptin receptor permitted investigation of the downstream targets of leptin signaling; neuroanatomical studies of leptin receptor message and immunoreactivity identified the central melanocortin system as a primary target (Hakansson *et al.*, 1996; Mercer *et al.*, 1996; Hakansson *et al.*, 1998; Shioda *et al.*, 1998).

1.3 *THE CENTRAL MELANOCORTIN SYSTEM*

An unusual feature of melanocortin signaling in the brain is that it is mediated by agonists derived from the propeptide pro-opiomelanocortin (POMC), and by the endogenous melanocortin receptor antagonist/inverse agonist AgRP, or agouti-related peptide (Ollmann *et al.*, 1997). Post-translational cleavage of POMC can generate several

melanocortin receptor agonists, including adrenocorticotropin (ACTH), α-, β-, and γ-melanocyte stimulating hormones (MSH), and the opioid β-endorphin (Tolle and Low, 2008a). The majority of neurons expressing POMC are found in the arcuate nucleus of the hypothalamus, with a much smaller population located in the dorsal vagal complex of the brainstem (Young *et al.*, 1998; Overstreet *et al.*, 2004). AgRP neurons are located exclusively in the arcuate nucleus, and coexpress neuropeptide Y (Broberger *et al.*, 1998). Although arcuate POMC neurons have a wider distribution of projections than AgRP neurons, there is substantial overlap in their respective terminal fields particularly in hypothalamic nuclei mediating neuroendocrine and autonomic functions, as well as in lateral septum and bed nucleus of stria terminalis (Haskell-Luevano *et al.*, 1999). Both AgRP and POMC-derived melanocortin agonists like α-MSH are high affinity ligands for melanocortin-3 (MC3) and melanocortin-4 (MC4) receptors (Fong *et al.*, 1997), the two melanocortin receptor subtypes expressed in the brain. Central MC3 receptor expression is localized primarily in the hypothalamus, while MC4 receptors have a much broader expression profile (Roselli-Rehfuss *et al.*, 1993; Mountjoy *et al.*, 1994; Fodor *et al.*, 1996). The neuroanatomical differences between MC3 and MC4 receptor distribution could be important in explaining their functionally disparate effects. Studies of MC3 receptor knockout (MC3RKO) mice reveal that these animals have increases body fat at the expense of lean mass, remarkably with little or no increase in overall body weight. Moreover, the daily food intake in these mutants is at or slightly below average; taken together these facts indicate that MC3 receptor activation appears to be important primarily in energy partitioning (Butler *et al.*, 2000; Chen *et al.*, 2000). Starkly contrasting to what has been found in MC3RKO mice, deletion of murine MC4 receptor

results in severe hyperphagia and significant increases in body weight and has led to the suggestion that MC4 receptor activation mediates changes in food intake. Consistent with this proposal, MC4 receptors are expressed in regions implicated in motivation and reward (Roselli-Rehfuss *et al.*, 1993; Mountjoy *et al.*, 1994; Alvaro *et al.*, 1996; Alvaro *et al.*, 1997; Alvaro *et al.*, 2003; Kishi *et al.*, 2003; Liu *et al.*, 2003; Hsu *et al.*, 2005).

1.4 *HOMEOSTATIC MODEL OF MELANOCORTIN FUNCTIONING*

Studies to date suggest a homeostatic regulatory model where changes in energy stores (body weight) initiate opposing compensatory alterations in food intake and energy expenditure to correct the change (Cone, 2005). Central melanocortin system alterations in food intake and energy expenditure are mediated predominantly through the MC4 receptor with POMC and AgRP neurons effecting changes by neuropeptide release. When there is a surfeit of energy, activation of MC4 receptors signal a reduction in food intake and an increase in energy expenditure; signaling conditions of energy deficit demand repletion and conservation of remaining stores, achieved through blockade or suppression of MC4 receptors (Hillebrand *et al.*, 2006).

Supportive evidence from studies within the past decade provide illustrative details. Consistent with the role of leptin as a signal of adipose energy stores, hypothalamic AgRP mRNA quantities in leptin-deficient (*ob*/*ob*) and leptin-insensitive (*db*/*db*) mice were an order of magnitude greater than wild-type mice (Shutter *et al.*, 1997). Daily leptin injections for five days significantly reduced hypothalamic AgRP mRNA in *ob/ob* mice and increased arcuate POMC mRNA in *ob/ob* and wild-type controls (Mizuno *et al.*, 1998; Mizuno and Mobbs, 1999). In contrast to these results, the

induction of negative energy balance (energy deficit) from a 48 hour fast increased wild-type AGRP mRNA, and decreased POMC mRNA in wild-type, *ob*/*ob* and *db*/*db* mice relative to fed controls (Schwartz *et al.*, 1997; Thornton *et al.*, 1997; Mizuno *et al.*, 1998; Mizuno and Mobbs, 1999).

The importance of central melanocortin receptor binding has been demonstrated in pharmacological and genetic studies. A variety of endogenous and exogenous melanocortin receptor ligands have been tested to determine their effect on food intake, and consistently melanocortin agonists and antagonists have decreased and increased food intake respectively (for review see (Irani and Haskell-Luevano, 2005)). Central administration of the potent melanocortin agonist MTII decreased food intake in fasted wild-type mice, and two obese mouse strains, *ob*/*ob*, and A(y) mice, a strain that ectopically expresses agouti, another endogenous melanocortin antagonist. The effects of MTII were blocked by an agouti-analogue, SHU9119 (Fan *et al.*, 1997). Genetic lesion of the murine MC4 receptor produces mice that are hyperphagic and develop early-onset obesity, leptin and insulin resistance (Huszar *et al.*, 1997). These traits also appear in mice that are POMC-deficient, and are reversed with replacement of α-MSH (Yaswen *et al.*, 1999). However, the absence of pituitary POMC precludes expression of the POMC-derived stress hormone ACTH and prevents appropriate development of adrenal glands in these mutants. Spontaneous loss-of-function mutations for both MC4 receptor and POMC in humans recapitulate the phenotypes described in mouse mutants (Krude *et al.*, 1998; Hinney *et al.*, 1999; Vaisse *et al.*, 2000; Krude *et al.*, 2003a; Krude *et al.*, 2003b). To address the confounding effects of adrenal insufficiency, a strain of POMC deficient mice with a transgene conveying pituitary POMC expression was generated (Smart *et al.*,

2006). The transgenic “rescue” of pituitary POMC in these mice results in obesity and hyperphagia that is more severe than seen in the global POMC knockout mice.

1.5 *LIMITATIONS OF CUMULATIVE FOOD INTAKE AS A DEPENDENT MEASURE*

Cumulative food intake is a measurement of the amount of food that has been consumed between two time points. Acquisition of this type of data is a simple matter of weighing food before and after it’s given to an animal, then calculating the difference. The ease of gathering this type of data likely contributes to the frequency of its use to represent feeding behavior. The simplicity comes at a price; cumulative food intake can say nothing about the temporal organization of the feeding behavior for which it is meant to account. The same amount of food consumed by an animal could be accomplished in a single enormous, hurried binge, or by frequent small snacks throughout the measurement period. This loss of information is not trivial. For most animals, rats and mice in particular, food intake occurs episodically, or colloquially put, in meals (Richter, 1927; Collier, 1980). Unless these patterns are measured, there are no data to improve our models. Eventually researchers will have to attend to the temporal aspects of food intake to move forward.

1.6 *DEFINING MEALS*

A primary difficulty of measuring meals comes from defining two boundary criteria, (1) quantity, and (2) time. The first criterion defines the minimum quantity of food that must be eaten to be considered a meal, commonly referred to as the minimum meal size (Castonguay *et al.*, 1986). The second criterion is used to cluster feeding events

together into meals; its value represents the amount of time that can pass between two consecutive feeding events and still belong to the same meal (Kissileff, 1970; Castonguay *et al.*, 1982; Castonguay *et al.*, 1986; Zorrilla *et al.*, 2005a). Researchers have used different terms for the same concept, commonly "meal criterion" (Tolkamp *et al.*, 1998; Clifton, 2000), but since it refers to a length of time that splits intra-meal and inter-meal intervals, and there is a precedent, I will use the term "threshold meal interval" (Zorrilla *et al.*, 2005a; Zorrilla *et al.*, 2005b; Tabarin *et al.*, 2007).

The difficulty lies in the method used to select defining values, especially for the threshold meal interval, and the consequences of the selection. Methodological studies of meal patterning in rats showed that different definitions could yield significant changes in meal values (Kissileff, 1970; Castonguay *et al.*, 1982). Despite this fact, some researchers have relied on arbitrarily chosen definitions to calculate their meal values (Ho and Chin, 1988; Azzara *et al.*, 2002; Zheng *et al.*, 2005; Berthoud *et al.*, 2006). Others have utilized empirical methods to define the threshold meal interval. Statistical methods take advantage of the bimodal distribution of inter-feeding intervals. Inter-feeding interval frequency histograms reveal distinct but overlapping distributions of the shorter intra-meal intervals and longer inter-meal intervals. Log-survivorship analysis has been employed by many to determine the boundary between intra-meal and inter-meal interval distributions, which should be the correct threshold meal interval (Fagen, 1978; Castonguay *et al.*, 1986; Glendinning and Smith, 1994; Clifton, 2000).

The most accurate method of determining when rodents have finished a meal is the behavioral satiety sequence assay. The behavioral satiety sequence refers to a set of post-meal behaviors reliably expressed by rodents that can be used to determine when

meals are complete (Halford *et al.*, 1998). As rats and mice become satiated, feeding behavior is replaced by grooming and finally a sustained period of rest. Two important preconditions for expression of the behavioral satiety sequence are the presence of a caloric load in the stomach, and release of gut peptides associated with satiation. Rats fed a glucose solution but not a saccharin one will express the satiety sequence (Kushner and Mook, 1984). Furthermore, the behavioral satiety sequence is not expressed in rats with gastric fistulas preventing the accumulation of food in the stomach unless they are also injected with the satiety factor cholecystokinin (Antin *et al.*, 1975). The accuracy of this method also makes it the most time-consuming, prohibitively so as the number of subjects and the session durations increase.

The behavioral satiety sequence assay is ideally suited to test the validity of less time-consuming methods for defining meals, and has recently been used for this purpose. A novel method has been described recently for rats that compares multiple meal definitions to find the one where meal values change the least, and test the hypothesis that the inclusion of drinking events along with feeding events make meal definitions more accurate (Zorrilla *et al.*, 2005a). The authors confirm the predictive validity of this "drinking-explicit" model for expression of the behavioral satiety sequence. Using this meal definition method, the probability of new meals being initiated is low immediately following the end of the old meal and increases with time as expected by the concept of satiety (Tolkamp *et al.*, 2000).

1.7 *LEPTIN AND THE SIGNIFICANCE OF MEAL DEFINITION VALIDITY*

Zorrilla and colleagues complemented their methodological study of drinking-explicit meals (Zorrilla *et al.*, 2005a) with an investigation of the effects of leptin on the meal patterns of rats (Zorrilla *et al.*, 2005b). Previous attempts to determine the role that leptin plays in the control of meal pattern had led to divergent findings: a reduction in meal frequency (Blevins *et al.*, 1996), reduced meal size and eating rate (Hulsey *et al.*, 1998), and reductions in meal frequency, size, duration and eating rate (Flynn *et al.*, 1998). With the exception of one study which did not report their meal definition (Blevins *et al.*, 1996), each of the earlier studies used arbitrary threshold meal intervals that were longer than the one arrived at with the empirically-determined, validated method. Zorrilla and colleagues' findings agreed with Blevins et al. (1996) where rats responded to central leptin with longer inter-meal interval durations that reduced meal frequency, and not with decreased meal sizes. Insofar as these results are correct, leptin exerts its effects on meal pattern by enhancing post-meal satiety responsible for suppressing the initiation of new meals, rather than through within-meal satiety mechanisms that determine how much food is eaten before the meal is terminated (Blundell, 1991; Blundell, 2006). These comparisons further highlight the importance of getting the meal definition right to accurately distinguish between different mechanisms controlling meal pattern.

CHAPTER 2.0

METHODOLOGICAL STUDY OF MEAL PATTERNS IN C57BL/6 MICE

2.1 *INTRODUCTION*

Remarkable advances in the molecular genetics and neurobiology of energy homeostasis spanning the past two decades have largely relied on dependent measures of total food intake over predetermined periods of time. The primary disadvantage of such measures is that the temporal organization of feeding behavior is unrepresented, and may confound interpretations of the data or render undetected potentially interesting results. Total food intake is determined by two variables, meal size (MS) and meal number (MN) (Meguid *et al.*, 1998), both reflected in the temporal organization of an organism's feeding behavior. Compared to measurements of total food intake, meal pattern analysis allows one to ascertain when feeding behavior is taking place across time, the quantities ingested, and rate of eating during each feeding episode. Since energy intake is not an abstract concept but rather a function of actual ingestive events, a complete account of energy homeostasis will ultimately need to elucidate the causal relationships linking molecular, cellular and electrophysiological activity with the initiation, maintenance and termination of meals across short and long term time scales (Smith, 2000; Geary, 2005).

Investigations into meal pattern have suffered from lack of unanimity over the best method for defining meals. A recent study was conducted to exhaustively compare the most frequently used meal definition methods with a "drinking-explicit" model (Zorrilla *et al.*, 2005a). Unlike previous models, which only include feeding events in the definition of meal boundaries, the drinking-explicit model includes both feeding and drinking events. This model satisfies two predictions of meal pattern that previous meal definitions do not; first, the probability of initiating a new meal increases with time as expected by the concept of satiety. Second, rats are much more likely to exhibit a

behavioral sequence associated with meal termination, the so-called behavioral satiety sequence (Halford *et al.*, 1998). Thus, this model more accurately characterizes meal taking in rats.

The purpose of the current study was to determine whether the drinking-explicit model is appropriate for meal analysis in mice. We measured self-initiated responding for food and water in wild-type male C57BL/6 mice living in operant conditioning chambers in a series of experiments to establish the validity of our measurements before testing three different meal definition methods. Custom software was developed to accomplish these tasks. A meal definition that includes both eating and drinking events appears to provide more accurate meal measurements in mice similar to what has been shown in rats.

2.2 *MATERIALS AND METHODS*

Subjects

Male wild-type C57BL/6J mice were born and reared in our vivarium using breeder pairs originally obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were kept on a 12:12 hr light-dark cycle (lights on at 07:00) and provided ad libitum access to chow containing 28.0 kcal% protein, 12.1 kcal% fat, and 59.8 kcal% carbohydrate (Rodent chow diet no. 5001; PMI Feeds Inc., St. Louis, MO) and water. Mice were group housed after weaning and then individually housed in home cages for at least one week prior to the start of meal pattern experiments. All procedures were approved by the Institutional Animal Care and Use Committee and followed the Public Health Service guidelines for the humane care and use of experimental animals.

Equipment

Four 16×14×13 cm and four 22×18×13 cm instrumental conditioning chambers with acrylic walls and floors consisting of 2.5 mm stainless steel rods spaced 10 mm on center were used in meal pattern studies. All chambers were outfitted with a food lever to the right of the food magazine. Water was supplied from a sipper tube located on the wall opposite to the food magazine. Lick-o-meters were installed on each sipper tube assembly to record drinking events. Sipper tubes were recessed 0.5 cm from the chamber wall to prevent false licks but still allow drinking access. A house light (100mA) was situated above the access opening for the sipper tube, and a 7.9 mm LED stimulus light above the food magazine. A 4.4 cm^2 acrylic platform was secured to the grid floor of each chamber to provide mice with a solid surface to rest. Each chamber was enclosed in a light- and sound-attenuating, ventilated cabinet. Control of the apparatus and records of lever presses, pellet deliveries and lick events were made through a computer interface and MedPC for Windows software (Med-Associates, St. Albans, VT). Numerical data records for individual mice were graphically rendered into cumulative records for visual inspection using Med-Associates SoftCR 4.0 for Windows.

General Procedures and Experimental Design

Mice lived in operant conditioning chambers continuously while their spontaneous responses to obtain food and water were recorded. Sessions started between 16:00 and 17:00 hours. At the beginning of each session, a response lever in each chamber was extended. Fixed-ratio (FR) reinforcement schedules were employed to set

the “cost” per food pellet; performance of a fixed number of lever presses resulted in the delivery of a single nutritionally complete 20 mg food pellet (FO163; Bio-Serve, Frenchtown, NJ). The macronutrient composition of the food pellets was similar to standard chow with 23.9 kcal% protein, 10.3 kcal% fat, and 65.7 kcal% carbohydrate. House lights within each chamber were programmed to turn off at 19:00 and turn back on at 07:00 in synchrony with the room lighting. When the session was over, levers were retracted but house lights remained on to maintain diurnal illumination conditions. Mice were gently coaxed out of their chambers into a plastic beaker, weighed and placed in their homecages without food or water for approximately 45 minutes. During this time, sipper tubes were weighed to measure water consumption, and bedding trays were removed to count uneaten pellets, washed thoroughly with water and dried before replacement in their respective chambers.

Pilot Studies

We first conducted a series of pilot studies to establish several critical features of the final operant behavioral paradigm to analyze meal patterns in mice. Instrumental training is typically performed with food-restricted mice to increase their motivation to perform and learn the contingent behavior, but we wanted to avoid a deprivation state that might confound the subsequent meal patterns. Therefore, the initial training was conducted in free-feeding mice simply placed overnight in the apparatus on an FR1 reinforcement schedule. This strategy immediately proved to be inadequate because the mice obtained so many pellets (> 1000) that the food magazines jammed. Similarly, an FR5 schedule was too easy and associated with excessive food wastage, leading us to

settle on an FR10 schedule as optimal for the training day with wild-type C57BL/6 mice. The noise or vibration produced by retraction of the levers appeared to be detrimental to training in some mice on the first night, therefore we standardized the procedure so that starting on day two of experiments a 10 sec lever retraction time-out period would occur each time the mouse satisfied the schedule requirements. Activation of the stimulus light simultaneously with pellet delivery was eliminated after day 1 of training because it was unnecessary to maintain instrumental responding. Finally, we determined the minimal number of days that mice required to stabilize their instrumental responding at a given schedule before escalation. Three-day intervals were too rapid for many mice, leading to a progressive drop-off in lever pressing, food intake, and body weight. However, all mice readily tolerated five-day intervals between schedule changes, so this timeframe was adopted for the detailed experimental analysis of drinking-explicit meal pattern.

Study 1

Mice (n=8) were 6-7 weeks old when they began living in operant conditioning chambers with access to food on a FR10 reinforcement schedule. On day 1, delivery of each food pellet was accompanied by a brief (1 sec) illumination of the stimulus light above the food magazine. On all subsequent days, levers were programmed to retract for 10 sec immediately following completion of the FR schedule criterion. This was done to prevent perseverative lever pressing identified in pilot studies, and to encourage pellet consumption immediately after delivery. Mice worked for food under the FR10 schedule from day 1 to day 5. On day 6, the reinforcement schedule was increased to FR20, and was doubled every five days thereafter up to FR80 (days 16-20) before returning to FR10

for the last five days of the experiment. The MedPC code for these operant schedules is contained in the Appendix.

Study 2

To determine whether 24-hour body weight, food and water intake were affected by the escalating schedules and increased effort imposed on the mice to acquire food, we used additional cohorts of animals to record these variables before, during and after living in the operant conditioning chambers on a prolonged FR40 schedule. Male mice (n = 16), 9-10 wk old, were individually housed in home cages outfitted with wire top racks and 500 ml water bottles with 1 mm weep-hole openings. Remaining food and water were weighed daily for 2 weeks (cohort 1) or 4 weeks (cohort 2) before testing in operant conditioning chambers. Within each cohort, mice were subdivided into an operant feeding group (n=4) whose food was contingent on lever presses, and a non-operant feeding group (n=4) whose members were given *ad libitum* access to the same food type. Every 24 hours the food remaining in each container was weighed and replenished with another 8-10 grams of food pellets, a quantity well in excess of normal daily food intake. On day 1 in the chambers, food delivery for the operant feeding group was contingent on 10 responses on lever (FR10); the stimulus light was illuminated after every 10^{th} lever response as was done in study 1 for both operant and non-operant groups. The schedule increased to FR40 on day 2, with a 10 sec lever retraction after pellet delivery; both conditions persisted unchanged for the remainder of time in chambers regardless of group. After gathering data for the first five nocturnal periods, sessions were lengthened to 47-hours in order to acquire data for uninterrupted 12 hour diurnal periods as well as

additional nocturnal periods. Video recordings under red light illumination were captured for each mouse in the operant feeding groups during the first 4 hours of the third nocturnal period under FR40 and time-stamped for later comparison to data obtained from the operant chambers. 24-hr body weight, food and water intake were recorded for 1 additional week after the return to the free-feeding conditions of their home cages.

Data exclusion

Exclusion criteria for data from meal pattern measurement periods were as follows: data from the training day (day 1), data from the first day at each new schedule, data from individual mice on days where equipment failure was noted post-session, e.g. clogged pellet delivery tube, lickometer not counting licks, food hopper not dispensing pellets, and food lever not extending properly. Only 1.1% of sessions satisfied one of these individual-specific exclusion criteria in study 1, and 2.9% of sessions in study 2.

Meal pattern analysis

Custom software, written in Visual Basic for Applications (VBA) to run on Excel (Microsoft Corp., Redwood, WA) was developed to automate each processing step in meal pattern analysis. Raw data were structured as temporally consecutive intervals, in seconds, between lever press, pellet delivery and/or lick events representing the ingestive activity of an individual mouse while living in an operant conditioning chamber. Data were transferred from MED-PC files into Excel spreadsheets using MED2XL (Med-Associates). The algorithm we created for meal definitions was based on the recently developed “drinking-explicit” model (Zorrilla *et al.*, 2005a). Meal parameter values were

calculated using our software from the identified threshold meal interval (TMI) and minimal meal (MM) criterion. The annotated VBA code for our meal pattern analysis software is included in the Appendix [Supplemental Files 1-3] and is freely available electronically on request from the authors.

Determination of the optimal TMI relied on an iterative process where an average meal duration was calculated for each possible TMI ranging from 11 to 40,134 sec (~12 hr). Possible TMI values were drawn from a natural logarithmic scale (e^n) in decimal increments starting with n = 2.4, e.g. $e^{2.4}$ = 11 sec, $e^{2.5}$, $e^{2.6}$… $e^{10.6}$ = 40,134 sec because logarithmic scales have been shown previously to be efficacious in separating intra-meal and inter-meal intervals (Tolkamp and Kyriazakis, 1999). For each possible TMI, consecutive intervals between pellet delivery and/or lick events that were less than or equal to that TMI were summed while pellet deliveries within the possible meal were counted. When an interval was reached that was greater than the TMI, the number of pellet deliveries within that possible meal was compared to the MM criterion, initially set to a default value of one food pellet so that all possible meals would be included. If pellet deliveries taking place within a possible meal were equal to or greater than the MM criterion, then it was included in the average meal duration for that possible TMI. At the completion of this process, a zero-order curve was generated by plotting the average meal duration for each possible TMI. Similar zero-order curves were generated based on either meal size or meal number. First-order derivations from the zero-order curves were used to identify the most likely TMI. The first-order curves were generated by subtracting adjacent average MDs (or MSs) from each other and normalizing for the increasing lengths of possible TMIs by dividing by $e^{(n-2.4)}$. The optimal TMI was located in the nadir

between the two peaks of the first-order curves at which the least rate of change in average meal duration and meal size occurred.

The TMI was subsequently used to generate a meal size frequency histogram to determine the correct MM criterion. The MM criterion distinguishes incidental pellet deliveries from small meals. In the event of a bimodal meal size distribution the MM was taken as the meal size falling at the threshold between the two distributions. TMIs were established for each schedule period, and across all schedules and together with MM criterion values arrived at in this analysis were used to calculate meal parameter values.

Putative TMIs were calculated using a drinking-explicit first-order derivative method, log-survivorship functions and log-frequency histograms of inter-feeding intervals (Fagen, 1978; Tolkamp and Kyriazakis, 1999; Zorrilla *et al.*, 2005a). These three methods for defining meals were compared on the basis of their ability to accurately predict characteristic post-prandial behaviors of the behavioral satiety sequence. The four hours of video data for each mouse from study 2 were broken into 1 minute sample intervals using AVS Video ReMaker 2.4 (Online Media Technologies Ltd, London, UK), and each of the 240 sample points was scanned for 5 seconds to assess which of five mutually exclusive behaviors was being exhibited: 1) eating; 2) drinking; 3) resting; 4) grooming; or 5) activity, including non-food directed lever pressing or locomotion. If the mouse was engaged in lever pressing at a sample point, then the observer determined whether food delivery and consumption immediately followed, in which case the behavior was scored as eating and the time taken to eat the food pellet was recorded. Otherwise, the behavioral event was scored as activity.

Statistics

Initial statistical analyses indicated that none of the measured dependent variables were affected by the differences in chamber area between the two sets of apparatus, therefore all further analyses combined the data collected from both chamber sizes. Daily measures of body weight, lever presses, total pellets delivered, uneaten pellets (food wastage), percentages of nocturnal and diurnal pellet deliveries, and water drunk for individual mice were averaged within each schedule. Daily food intake was determined by subtracting the number of uneaten pellets from total pellets delivered for that day. These averages were analyzed using one-factor repeated measures ANOVA by schedule with Tukey's Multiple Comparisons post hoc test, except for uneaten pellets which required Friedman's test because of a violation of normality (individual x schedule). One-factor repeated measures ANOVA by day was used to detect differences in the daily food intake of individual mice across all days in the initial and final FR10 schedule periods (days 2-5 and 22-25) of study 1.

Meal parameter values of meal duration, meal size, meal number, within-meal eating rate and intermeal interval durations were calculated for individual mice by day using a MM criterion of 2 food pellets with two different TMIs (665 sec and 735 sec) and averaged by schedule for each mouse. Two-factor (schedule x TMI) repeated measures ANOVA was employed in order to identify any significant effects of TMI on meal parameter values that would preclude the use of a single TMI for all meal calculations. Meal parameter values for each TMI were also analyzed with repeated measures ANOVA using schedule as the single factor. Differences between schedules were resolved with Tukey's Multiple Comparisons post hoc test. The non-parametric

Friedman's test was used to analyze meal size (individual x schedule). Individual successive meal parameter values for the nocturnal and diurnal periods were calculated using a TMI of 665 sec and averaged for each mouse. Statistical analyses were generated using Graphpad PRISM v.4.03 for Windows and *P* values < 0.05 were considered significant.

2.3 *RESULTS*

Study 1: Effects of reinforcement schedule on body weight, pellet delivery, and food wastage

Average daily body weight of the mice in study 1 across all schedules is shown in Figure 2.1A. Body weight steadily increased over the five days of the initial FR10 schedule, but overall was significantly lower than for all subsequent schedule periods [$F(4,28) = 43.59$, $P < 0.0001$; FR20, $P < 0.05$; FR40, $P < 0.05$; FR80, $P < 0.001$; final FR10, $P < 0.001$]. Weight then remained stable during the FR20, FR40, and FR80 periods despite the increased work requirements for pellet acquisition. In the final FR10 period mice weighed more than during all previous schedule periods [FR20, $P < 0.001$; FR40, $P < 0.001$; FR80, $P < 0.001$].

Figure 2.1B shows that the average number of lever presses emitted per session increased commensurately with increases in the FR schedule [$F(4,28) = 197.4$, $P<0.0001$]. Only the initial and final FR10 periods were not statistically different from each other.

Average food intake decreased as the schedule increased [$F(4,28) = 6.87$, $P < 0.001$] (Figure 2.1C). Mice ate the most food during the initial and final FR10 periods.

Food consumption during the initial FR10 period was greater than the food intake when mice were under the FR40 (P <0.05) and FR80 (P <0.01) schedules. Similarly, more food was consumed during the final FR10 than both the FR40 (P <0.05) and FR80 periods (P <0.01). No difference was found in total daily food intake among the last four days of the initial and final FR10 schedule periods [$F(7,49) = 0.11$, P >0.05]. Daily water consumption by schedule mirrored the changes seen in food intake at different reinforcement schedules [$F(4,28) = 6.2$, P<0.01] with mice drinking the most water during the initial FR10 period (4.2 ± 0.2 mL), significantly more than at the FR40 (3.7 ± 0.2 mL, p<0.01) and FR80 period (3.6 ± 0.2 mL, p<0.001), when it was the least. The schedule also had a marked effect on the number of uneaten pellets [$\chi^2 = 19.9$, df = 4, P <0.001]. Uneaten pellets were greatest during the initial FR10 and FR20 schedule periods, and were significantly greater than uneaten pellet counts at FR80 [FR10-1 vs. FR80, $P < 0.01$; FR20 vs. FR80, $P < 0.01$], with most of the variation due to 2 out of the 8 mice studied. Counts of uneaten pellets fell during the FR40 schedule period to approximately 5% of the total pellets delivered and then to < 1% in the FR80 schedule period. Interestingly, the number of uneaten pellets remained low (5%) when the schedule returned to FR10 in the last five days of the experiment.

Total daily pellet deliveries were divided into those occurring during the nocturnal and diurnal periods. Averages included both eaten and uneaten pellets as it was not possible to distinguish a priori which pellet deliveries were followed by immediate consumption. Schedule had a significant effect on the percentage of both nocturnal and diurnal pellet deliveries [$F(4,12) = 12.86$, P <0.001]. Nocturnal percentages were statistically indistinguishable at all schedules (FR20, 73.4 ± 0.9%; FR40, 73.4 ± 0.7%;

Figure 2.1

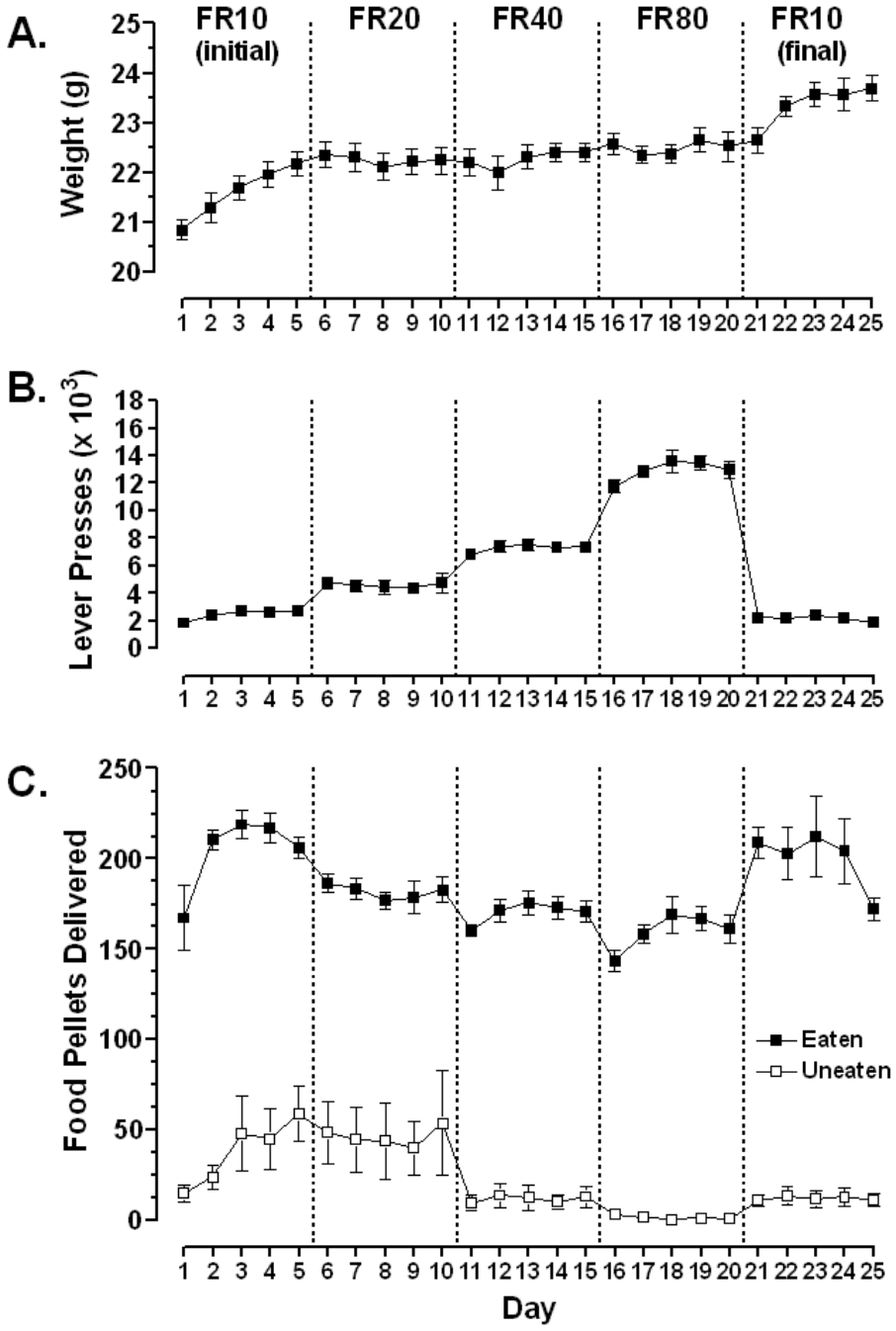

Figure 2.1. Daily measures of body weight, lever presses, eaten and uneaten food pellets during the entire 25-day course of study 1. Pellet reinforcement schedules were changed every five days. (A) Body weights increased during the initial and final FR10 periods and remained stable during the intervening 15 days despite increases in FR schedule. (B) Mice modified their total daily lever press events in proportion to the demands of varying reinforcement schedules. (C) Daily food pellet consumption (black squares) was calculated by subtracting uneaten food pellets (white squares) from total daily food pellet deliveries. All data are means ± SEM, n = 8 mice.

FR80, 73.7 ± 0.6%; final FR10, 68.8 ± 1.5%) as were diurnal percentages (FR20, 26.6 ± 0.9%; FR40, 26.6 ± 0.7%; FR80, 26.3 ± 0.6%; final FR10, 31.2 ± 1.5%) except during the first FR10 period, which was significantly different than the rest (P <0.01; nocturnal, 57.0 ± 4.4%; diurnal, 43.0 ± 4.4%). Since there were no wasted pellets during the FR80 period, the circadian percentages of pellet deliveries under this schedule almost certainly reflect actual nocturnal and diurnal pellet ingestion. Preservation of a circadian division in pellet deliveries under FR20, FR40, FR80, and the final FR10 schedules suggest that the instrumental paradigm did not disrupt normal nocturnal-diurnal feeding patterns.

Study 1: Descriptions of appetitive and consummatory measures

A meal is a unit of food intake defined as a cluster of temporally proximal feeding events bounded on either side by longer periods of non-feeding events. Identification of these two boundaries, the points of meal initiation and termination, relies on two variables: the TMI and the MM criterion (Castonguay *et al.*, 1986; Zorrilla *et al.*, 2005a). The TMI defines the cut-off between intra-meal and inter-meal intervals, that is, it represents the maximum amount of time that can pass between two ingestive events such that they can still be considered part of the same meal. Meal definitions have traditionally used intervals between feeding events as the exclusive basis of meal pattern analyses (Gannon *et al.*, 1992), however, for reasons enumerated subsequently in study 2 we adapted a drinking-explicit model based on the report of Zorrilla et al. (Zorrilla *et al.*, 2005a) in rats to define the temporal boundaries for each meal in mice, and hence the TMI. The MM criterion sets a lower limit to the amount of food eaten that defines a meal. A frequency histogram of all nocturnal feeding events across all schedules revealed a

clear bimodal distribution with a marked drop in the frequency of meal sizes between 1 and 3 food pellets (data not shown). Therefore, the MM criterion used in all subsequent meal pattern calculations was set to 2 food pellets.

Drinking-explicit meals are defined as clusters of temporally proximal feeding and drinking bouts separated by IMIs where episodes of non-ingestive behavioral bouts, e.g. locomotion, resting and grooming bouts occur. As shown in the representative cumulative record of a mouse working for food pellets at FR40 (Figure 2.2), the majority of meals defined by a TMI of 665 sec were concluded by a drinking bout.

Feeding and drinking behaviors, like most motivated behaviors, consist of an appetitive phase when an organism exerts effort required to procure access to food or water followed by a consummatory phase during which the acquired food or water is ingested (Craig, 1917). A fundamental assumption of the meal pattern paradigm outlined in this study is that mice consume virtually every earned food pellet immediately after delivery into the magazine. If this assumption holds, then the appetitive-consummatory (A-C) behavioral sequence representing ingestion of individual food pellets will be reflected in the event record by a work bout (WB), defined by the amount of time passing between the first and last lever press required to trigger a pellet delivery, followed by a post-reinforcement pause (PRP) during which the mouse retrieves and consumes the earned pellet (Figure 2.2, inset). PRPs represent the duration between pellet delivery and the first lever press or lick event to follow. Consecutive series of WB→PRP sequences while mice are actively engaged in meal-taking constitute feeding bouts. A bout is a repetitive occurrence of some behavioral sequence that ends with the initiation of a competing behavioral sequence (Lehner, 1996). The A-C behavioral sequence for a drinking bout,

Figure 2.2

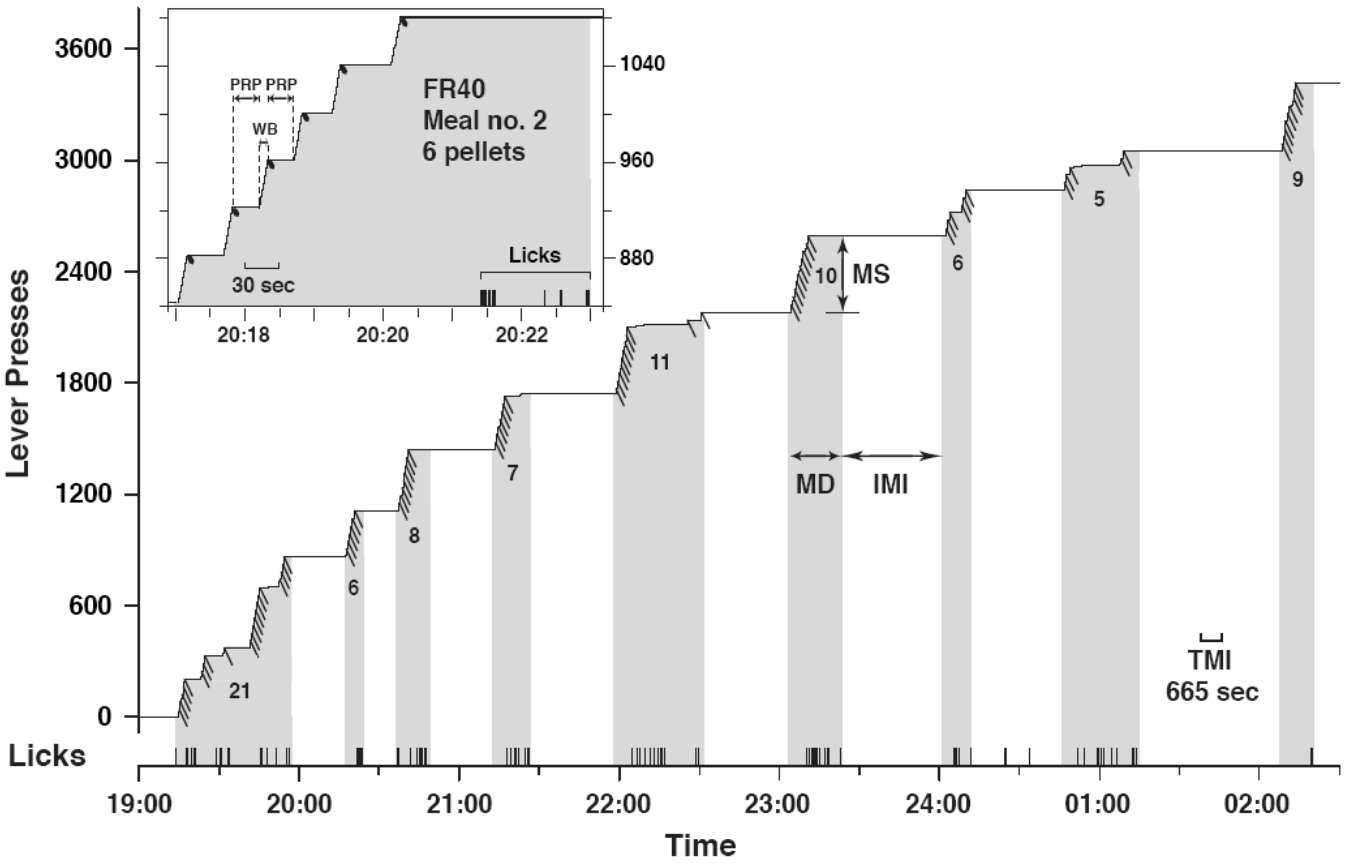

Figure 2.2. Representative nocturnal meal pattern of a single mouse over 7.5 hours under the FR40 schedule in study 1. The cumulative record of feeding and drinking events performed by one mouse after the start of the dark cycle (19:00 hours) is depicted in the main diagram. Each diagonal tick mark represents the delivery of a single 20 mg food pellet triggered after the mouse completed 40 consecutive lever presses. Lever presses during the period are summed across the *y*-axis. Vertical ticks situated immediately above the *x*-axis represent times when the mouse was drinking water. Each vertical tick may contain multiple lick events (recorded at 10 msec resolution) occurring in close temporal proximity, depending on its thickness. Vertical gray stripes are superimposed over the sections of the cumulative record determined to be individual meals. Stripe width reflects meal duration (MD), and the integer within each stripe denotes the meal size in food pellets (MS). Width of the unshaded stripes, interdigitated between meals, represents intermeal interval durations (IMI). Meal value calculations were based on a threshold meal interval (TMI) of 665 seconds (11 min 5 sec) and a minimum meal criterion of two pellets. The inset depicts the second nocturnal meal in greater detail. A stair-stepping pattern reflects the alternating work bouts (WB) of 40 lever presses each, and post-reinforcement pauses (PRP) during which the mouse retrieved and consumed the acquired food pellet. As in the main diagram, pellet delivery events are recorded as diagonal tick marks and vertical ticks running along the *x*-axis are drinking events.

while not explicitly analyzed here, presupposes water spout approach immediately preceding a series of lick events.

An assessment of appetitive and consummatory behaviors by the analysis of WB and PRP durations revealed schedule-dependent changes. WB durations increased in proportion to the difficulty of the schedule as reflected in the orderly rightward shifts of the WB distributions at FR20, FR40 and FR80 compared to the initial FR10 (Figure 2.3A). Peak WB duration doubled from FR20 (6.0 sec) to FR40 (11.0 sec), and again to FR80 (22.2 sec) before falling below the highest frequency seen during the initial FR10 (4.5 sec) to 2.0 seconds during the final FR10 period. Lever press rates at these peak frequencies also increased with schedule from 2.2 lever presses/sec during the initial FR10 period to 3.3 lever presses /sec at FR20, and 3.6 lever presses /sec for both FR40 and FR80. The WB duration distribution for the final FR10 period was shifted further to the left than all schedules, including the initial FR10 period, having the highest peak lever pressing rate at 5.0 lever presses /sec. The uppermost limit for the rate at which mice were capable of sustained lever pressing calculated from the leftmost non-zero frequency for each distribution appeared to be between approximately 6 to 8 lever presses /sec with the highest rate found during the final FR10 period (initial FR10, 7.4 lever presses /sec; FR20, 6.7 lever presses /sec; FR40, 6.0 lever presses /sec; FR80, 5.9 lever presses /sec; final FR10, 8.2 lever presses/sec).

PRP durations distributed bimodally with peaks at 10-14 sec and 22-45 sec (Figure 2.3B). Differences in PRP frequency by schedule were found at both the 10-14 sec peak [$F(4,28) = 5.22$, $P < 0.01$] and at the 22-45 sec peak [$F(4,28) = 8.49$, $P < 0.001$]. Within the 10-14 sec peak the initial FR10 and FR20 were significantly larger than FR80

(initial FR10, $P < 0.01$; FR20, $P < 0.01$). PRP frequencies at the 22-45 sec peak showed FR80 having the highest frequency of PRPs, more than at both initial FR10 ($P < 0.001$) and FR20 ($P < 0.01$). The frequency of 22-45 sec PRPs at FR40 was also significantly greater than at the initial FR10 ($P < 0.01$).

Study 1: Comparison of nocturnal meal parameter values at varying reinforcement schedules

The combined measurements of meal duration, meal size, meal number, within-meal eating rate, and inter-meal interval characterize meal patterns. We initially calculated these values using two alternative TMIs (TMI 665 and TMI 735) found at the schedules when uneaten food was minimal. The FR20 period schedule-specific TMI was not included in this analysis due to evidence of degradation of meal pattern from both the unacceptably large number of uneaten food pellets and the results from the PRP frequency analysis. This decision was further reinforced by the finding that apart from the FR20 schedule period, the TMI for all other schedules was either 665 sec or 735 sec. Two-factor repeated measures ANOVA by schedule and TMI revealed no interactions between TMI and schedule [$F(1,56)$ for meal duration = 0.09, meal size = 0.02, meal number = 0.09, eating rate = 0.01, inter-meal interval = 0.03], nor any effect of TMI on meal parameter values [$F(4,56)$for meal duration = 0.35, meal size = 0.11, meal number = 0.75, eating rate = 0.18, inter-meal interval = 0.05]. Meal parameter values for subsequent results were therefore calculated using a TMI of 665 sec and a MM2 criterion to comport with the TMI found at the optimal schedule, i.e. FR40.

Figure 2.3

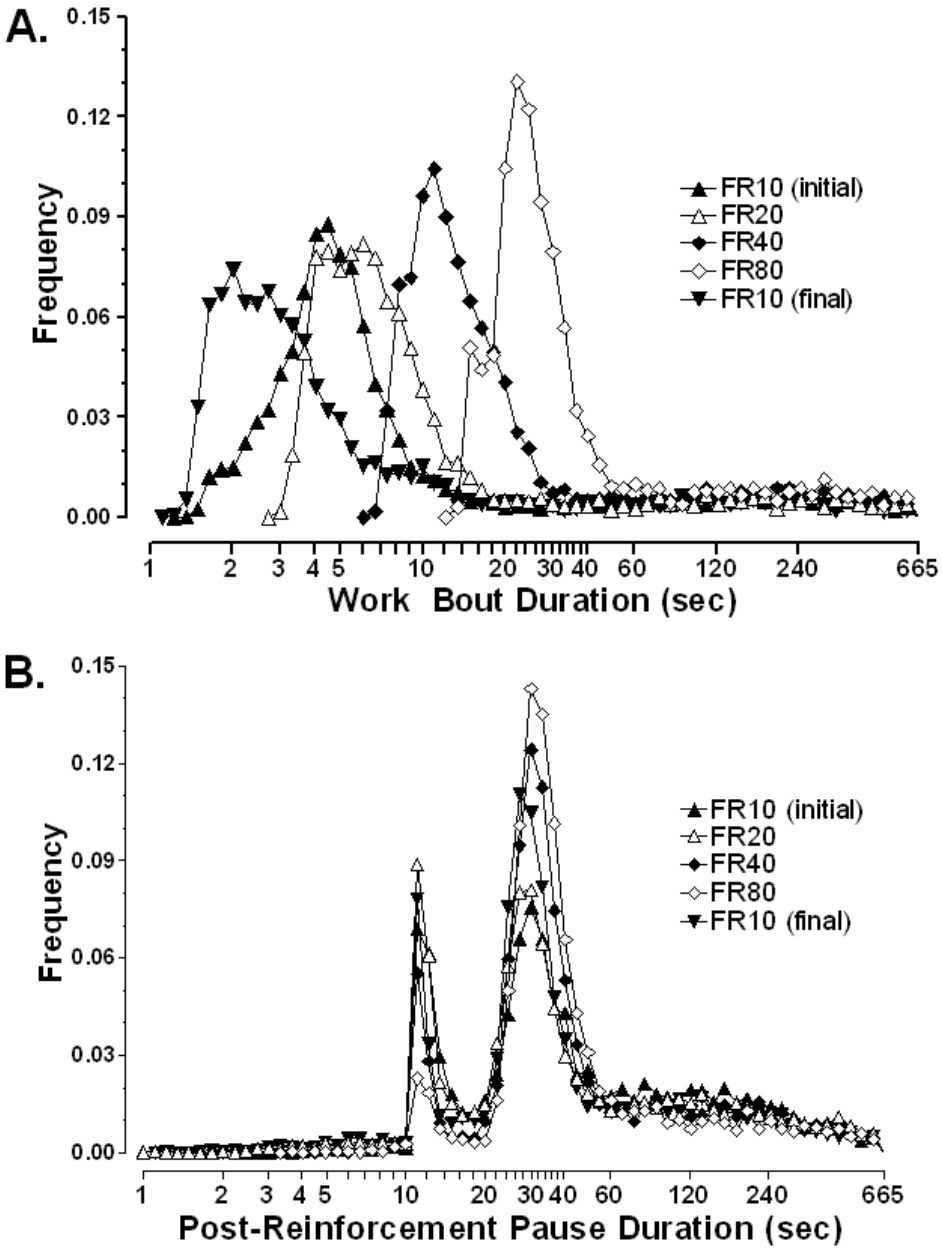

Figure 2.3. Frequency histograms for durations of all nocturnal work bouts (WB) and post-reinforcement pauses (PRP) split by pellet reinforcement schedule in study 1. (A) The frequency distribution of WB durations, defined by the time from first to last lever press required to satisfy a reinforcement schedule for pellet delivery, is schedule-specific. (B) The frequency distribution of PRP durations, defined as the length of time between a pellet delivery and the next recorded event (either lever press or lick event), is bimodal. Although the two peak durations are superimposeable regardless of schedule, the proportion of PRPs represented in each peak varies by schedule. Time bins for both the WB and PRP histograms were derived using a natural logarithmic scale ranging from e^0 (1 second), with the exponent increasing in 0.1 increments, to $e^{6.5}$ (665 seconds) to include all possible within-meal intervals for a TMI 665.

Results for average nocturnal meal parameter values are shown in Table 1. There were no differences in average meal number by schedule [$F(4,28) = 2.03$, $P > 0.05$]. meal size was also unaffected by schedule [$\chi^2 = 3.37$, df 4, $P > 0.05$]. Meal duration under the FR20 schedule was longer than during the final FR10 period [$F(4,28) = 3.61$, $P < 0.05$]. A significant effect of schedule was found on eating rate [$F(4,28) = 7.96$, $P < 0.001$]. The eating rate at FR80 was lower than at both FR10 periods (initial FR10, $P < 0.05$; final FR10, $P < 0.001$), and at FR20 ($P < 0.05$). eating rate under FR40 was less than at the final FR10 ($P < 0.05$) when mice ate at the greatest rate. The only differences found in the inter-meal intervals were between the initial FR10 and the FR20 periods [$F(4,28) = 2.81$, $P < 0.05$].

Study 2: Maintenance of energy balance in mice during prolonged FR40 operant feeding

Although body weights and food intake of mice remained relatively stable during the course of escalating FR schedules in study 1, there was a significant increase in both measures in the last 5 days when mice returned to the lower FR10 schedule. To determine whether these increases were simply overcompensation as the result of a sharp contrast in work requirements from 80 down to 10-lever presses/pellet or an indication of a chronic caloric deficit, we performed a follow-up study in a second cohort of male C57BL/6J mice. These mice were fully mature adults, on average 3 weeks older, 4 g heavier, and at a near plateau in body weight gain at the start of experiments compared to study 1. Instead of an escalating schedule in 5-day steps, mice in study 2 transitioned directly from 1 day of training at FR10 to 14 days of operant responding for all their food requirements at a constant FR40 schedule. Overall, there were no significant differences

Table 2.1

	Study 1 (nocturnal periods only)					Study 2 (both periods)	
	Initial FR10	FR20	FR40	FR80	Final FR10	Nocturnal FR40	Diurnal FR40
Meal number	11.4 ± 0.4	11.8 ± 0.8	12.1 ± 0.6	11.5 ± 0.4	13.1 ± 0.6	10.1 ± 0.8	7.1 ± 0.7 [a]
Meal duration (min)	26.3 ± 2.4	30.5 ± 3.6	26.6 ± 1.9	26.7 ± 2.0	20.7 ± 1.3	29.3 ± 2.4	16.7 ± 1.8 [b]
Meal size (20 mg pellets)	13.4 ± 1.3	15.1 ± 2.5	11.6 ± 1.1	10.8 ± 0.7	10.9 ± 0.4	13.7 ± 1.7	9.1 ± 1.6 [a]
Eating rate (pellets/min)	0.84 ± 0.08	0.86 ± 0.07	0.73 ± 0.04	0.64 ± 0.05	0.95 ± 0.09	0.68 ± 0.03	0.70 ± 0.03
Intermeal interval (min)	36.5 ± 1.5	31.8 ± 2.3	33.4 ± 2.0	35.7 ± 1.1	33.9 ± 1.9	48.3 ± 8.0	93.4 ± 15.2 [a]

Table 2.1. Average meal parameter values by operant schedule and lighting period

[a]$P < 0.05$, [b]$P < 0.001$ compared to nocturnal FR40 expt. 2 (two-tailed paired Student's t-test) All meal pattern values were calculated based on the drinking-explicit meal definition and a minimum meal size of two food pellets. A threshold meal interval of 665 sec (TMI 665, ~11 min) was used for the nocturnal periods in Studies 1 and 2. A longer TMI of 898 sec (TMI 898, ~15 min) was used for the diurnal periods in Study 2. Values represent means ± SEM, n = 8 mice.

in body weight of the mice comparing the initial 1-3 wk of individual housing in home cages, 2 wk in the behavioral chambers (either free-feeding or under operant schedules), and the 1-3 wk of follow-up in home cages under free-feeding conditions (data not shown).

In order to obtain uninterrupted records of feeding and drinking data during the light period of the light-dark cycle, mice in study 2 underwent several, sequential 47 hr sessions in the operant chambers in addition to the standard 23 hr sessions used in study 1. The overall pattern of feeding behavior exhibited by the mice as a group is illustrated by histograms of total food pellet deliveries (Figure 2.4). Analysis of the videotaped records showed that 100% of the nocturnal WB (129 observations) were followed immediately by pellet retrieval and consumption in an average of 21.7 ± 1.9 seconds followed by a 5-10 second latency before reinitiating lever pressing. This time is almost identical to the duration of the second PRP peak (Figure 2.3B), validating our assumption in other data analyses that virtually every pellet delivery recorded by the equipment during FR40 sessions is equivalent to a pellet eaten. There is a clear diurnal rhythm of feeding during both the 23 hr (Figure 2.4A) and 47 hr sessions (Figure 2.4B) with maximum activity near the light-dark transition through the first half of the dark period followed by a nadir in the first half of the light period. The peak of behavior that occurs 1-2 hr prior to onset of darkness is moderately accentuated in the 23 hr records and the first light-dark transition of the 47 hr records compared to the second, probably due to the 1 hr immediately prior when the mice are removed from the operant chambers and housed in their home cages without food. Clearly, however, there is a significant increase

Figure 2.4

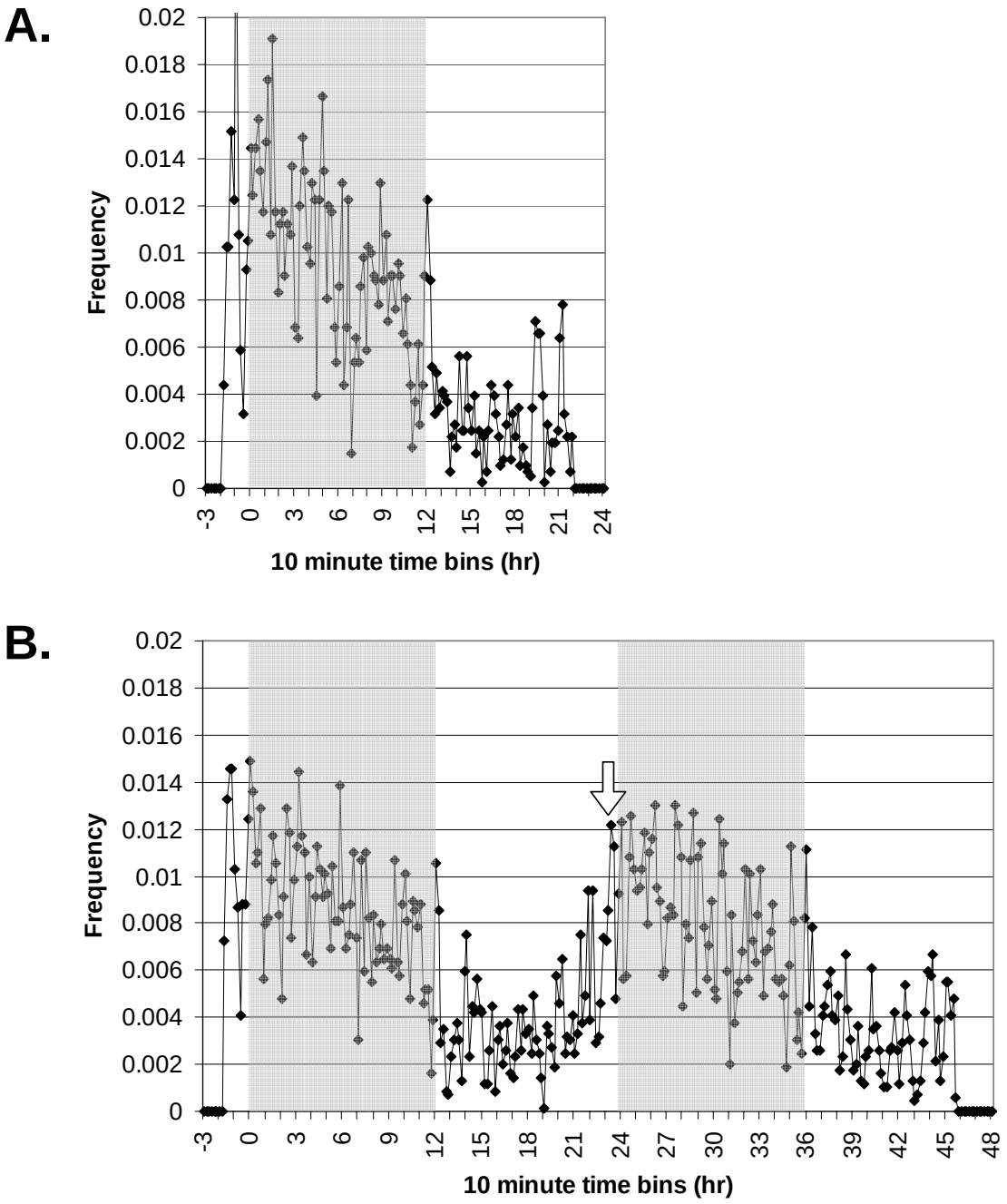

Figure 2.4. Circadian rhythm of food pellet deliveries across all consecutive 23 hr (A) or 47 hr (B) operant sessions at FR40 in study 2. Pellet deliveries were combined for all 8 mice across the 2-3 initial 23 hr sessions and the 4-5 subsequent 47 hr sessions. The data were plotted as histograms with the frequency of pellet deliveries normalized to 23 hr periods and separated into 10 min bins. The shaded areas represent the 12 hr nocturnal period from 19:00 to 07:00 and the unshaded areas represent the 12 hr diurnal period from 07:00 to 19:00. The arrow indicates a significant increase in feeding behavior that anticipates the onset of darkness.

in feeding behavior that anticipates the onset of darkness at the end of the uninterrupted diurnal sessions (Figure 2.4B, arrow).

Study 2: Direct comparison of drinking-explicit and drinking-naïve meal pattern analysis

The large data set, including 47-hr operant sessions, obtained in study 2 allowed us to formally compare the drinking-explicit meal analysis paradigm with more commonly used drinking-naïve analyses of both nocturnal and diurnal feeding behavior in mice (Figure 2.5). Zero-order curves for average predicted meal number, meal size, and meal duration at all possible TMIs across nocturnal sessions were generated as described in the Materials and Methods (Figure 2.5A). These curves show the reciprocal relationships between meal number and meal size or meal duration at different intervals. First order curves plotting the instantaneous rate of change in meal size and meal duration at all possible intervals (Figure 2.5B) exhibit a consistent inter-peak minimum of 665 sec, designated as the TMI. The TMI is shifted to the right (898 sec) or to the left (221 sec) when a drinking-explicit meal definition is applied to diurnal sessions or when a drinking-naïve meal definition is applied to the nocturnal sessions, respectively (Figure 2.5 C, D). Two other drinking-naïve analyses by log-normal frequency histogram or log-survivorship function from inter-feeding intervals also predict significantly lower TMIs of approximately 300 sec and 90 sec for both nocturnal and diurnal sessions (not shown) compared to the drinking-explicit analysis (Figure 2.5 E, F).

Figure 2.5

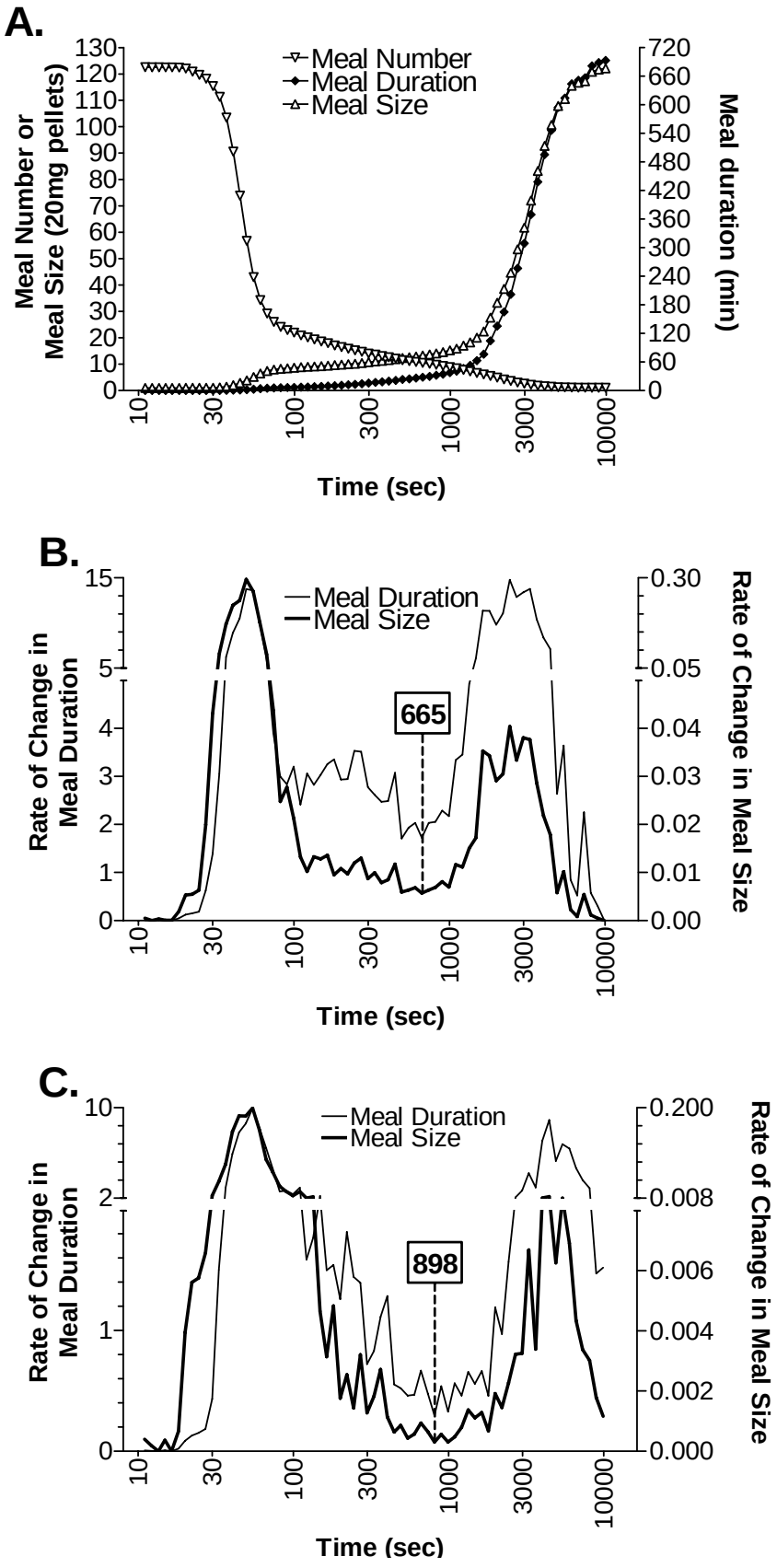
A.
Meal Number
Meal Duration
Meal Size
Meal Number or
Meal Size (20mg pellets)
Meal duration (min)
Time (sec)
B.
Meal Duration
Meal Size
Rate of Change in
Meal Duration
Rate of Change in Meal Size
665
Time (sec)
C.
Meal Duration
Meal Size
Rate of Change in
Meal Duration
Rate of Change in Meal Size
898
Time (sec)

Figure 2.5

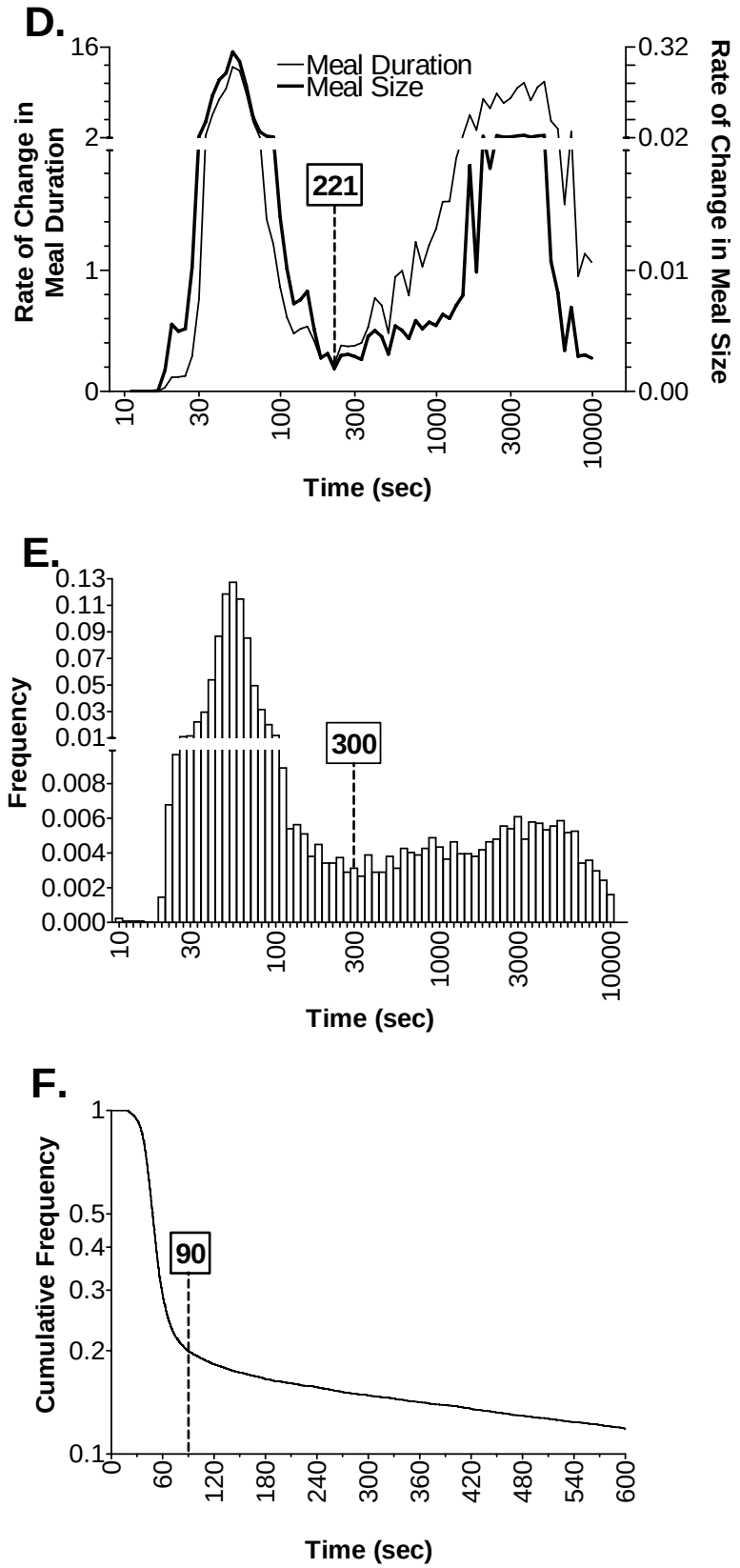
D.
Meal Duration
Meal Size
Rate of Change in Meal Duration
Rate of Change in Meal Size
221
Time (sec)
E.
Frequency
300
Time (sec)
F.
Cumulative Frequency
90
Time (sec)

Figure 2.5. Comparison of analytical methods to determine the optimal threshold meal interval (TMI) for meal value calculations based on a common data set obtained in study 2. (A) Zero-order curves illustrating average meal values for all possible time intervals within a 12-hour nocturnal period based on a drinking-explicit meal definition. Time interval data corresponding to consecutive lick, lever press, and/or pellet delivery events from all mice during the nocturnal periods are plotted on a natural logarithmic scale progressing from $e^{2.4}$ (11 seconds), with 0.1 increments of the exponent, to $e^{10.6}$ (40,134 seconds). (B) First order curves to estimate the optimal nocturnal TMI. Average meal durations (MD) and meal sizes (MS) at each possible time interval on the corresponding zero order curves (panel A) were used to calculate local rates of change in the respective meal values between adjacent time intervals. The TMI of 665 sec corresponding to a minimum in the rate of change for both curves occurs at the boundary between distributions for within-meal and between-meal intervals. (C) First order curves to estimate the optimal diurnal TMI. Data were collected from the 12-hour diurnal periods and analyzed identically to the previous data. The TMI of 898 sec is shifted slightly to the right compared to nocturnal values. (D) First order curves to estimate the nocturnal TMI using a drinking-naïve meal definition. Only time interval data corresponding to consecutive lever press and/or pellet delivery events from all mice during the nocturnal periods were used to calculate the corresponding zero order curves (not shown). The TMI of 221 is shifted considerably to the left compared to the drinking-explicit nocturnal TMI. Values shown for panels A-D are within-subject means, n = 8 mice. (E) Nocturnal inter-feeding interval frequency histogram. The proportion of time intervals corresponding only to consecutive pellet delivery events from all 8 mice over all nocturnal periods is plotted against bin sizes on a natural log scale (e^n) with increments of 0.1 for n. The TMI of 300 was estimated to be near the threshold between the distribution of short, intra-meal intervals to the left and the distribution of long, inter-meal intervals. (F) Log-survival curve based on nocturnal inter-feeding intervals. The TMI of 90 is estimated from the point of intersection of tangents corresponding to the double exponential curves.

Behavioral scores of the mice obtained from analyses of videotaped records during the operant sessions were aligned to the termination point of meals calculated according to the different meal-definition paradigms and resulting TMIs. Using a TMI 665 derived from the drinking-explicit analysis of nocturnal sessions, mice exhibited principally grooming behavior in the first 5 min following meals, gradually replaced by resting behavior over the subsequent 10 min (Figure 2.6A). There were occasional locomotor or eating and no drinking events. This behavioral constellation is consistent with the satiety sequence reported to coincide with true meal completion (Antin *et al.*, 1975). In contrast, an identical behavioral analysis performed with drinking-naïve based TMIs of 300 (Figure 2.6B) or 90 (data not shown) showed relatively stable levels of locomotor activity > grooming > resting > drinking > eating over the entire 15 min window. To disambiguate the effects of longer TMI from the inclusion of drinking events in meal definitions, we determined meal termination time points when a TMI of 665 seconds was applied to the drinking-naïve data (Figure 2.6C). Use of this longer TMI on interval data that included only inter-feeding intervals produced no appreciable differences in the behavioral patterns that were found when using the shorter TMIs.

The validity of the different TMIs to predict true meal termination was further examined by calculating the instantaneous probability of new meal initiation from the nocturnal data set at FR40. The probability should be zero immediately after a meal and then gradually increase as time elapses. Figure 2.7 shows that a TMI of 665 sec best accords with this postulate. Both TMIs of 300 and 90 sec produce non-zero probabilities in the first 7 min after putative meal termination and then exhibit more shallow slopes over the next 35 min compared to TMI 665. These features, together with the post-meal

Figure 2.6

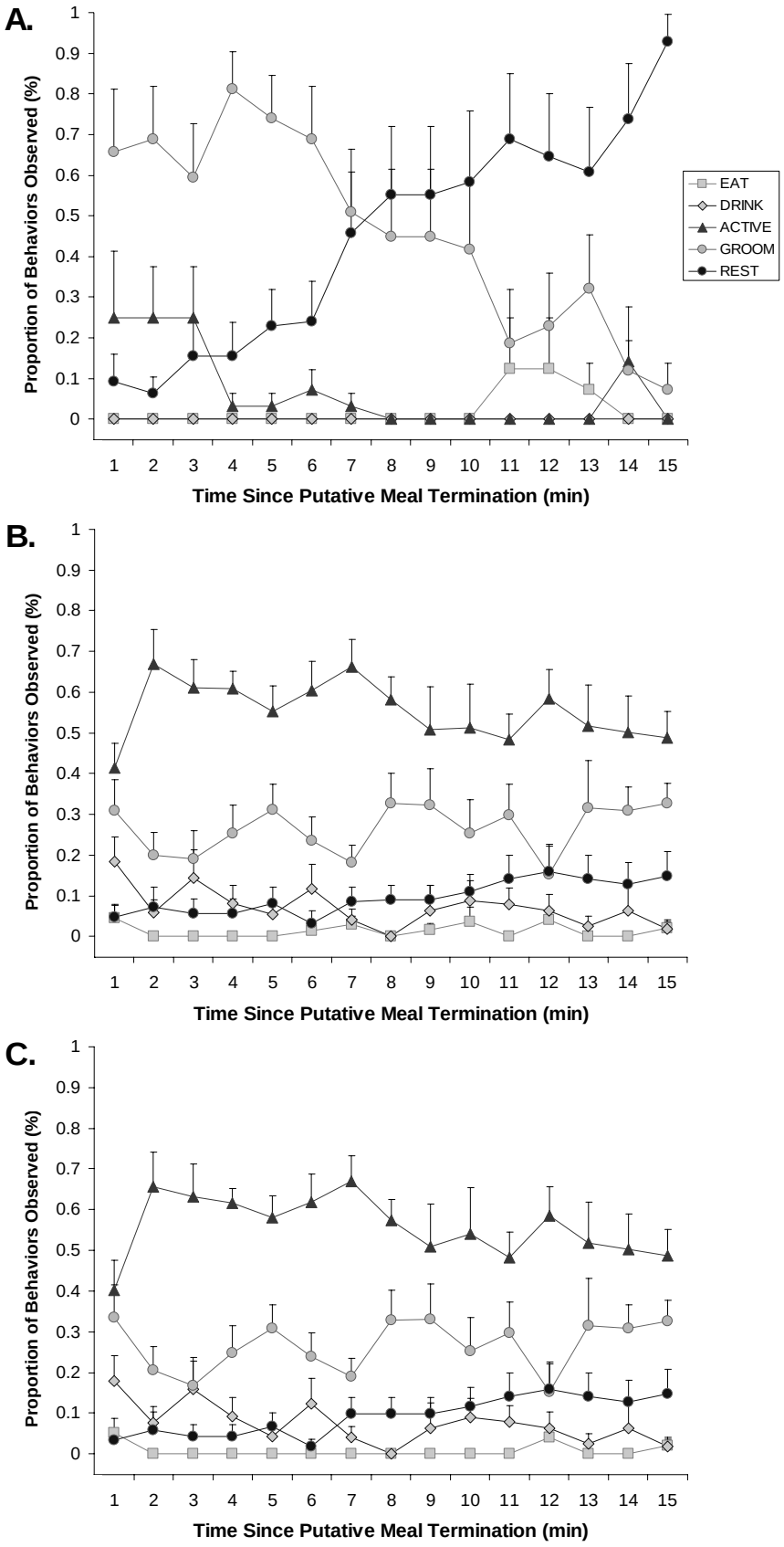
A.
B.
C.
Proportion of Behaviors Observed (%)
Time Since Putative Meal Termination (min)
EAT
DRINK
ACTIVE
GROOM
REST

Figure 2.6. Comparison of the temporal organization of postprandial behaviors following meal termination in study 2 based on drinking-explicit and –naïve meal definitions. Mutually exclusive behavioral counts for eating (squares), drinking (diamonds), activity (triangles), grooming (open circles), and resting (shaded circles) were scored at 1-min intervals from videotaped records of the first 4 hr of nocturnal operant sessions of 8 individual mice. The same time-stamped data arrays were then aligned with each mouse's respective meal pattern calculated on the basis of either a nocturnal TMI of 665 sec, derived from drinking-explicit first order curves (A), a drinking-naïve nocturnal TMI of 300 sec (B) or 665 sec (C). Meal definitions included both feeding and drinking events (A) or only feeding events (B, C). Alignments of the 15-min periods immediately following meal termination as defined by the different TMIs and meal definitions are shown. Values are the mean ± SEM, n = 8 mice.

Figure 2.7

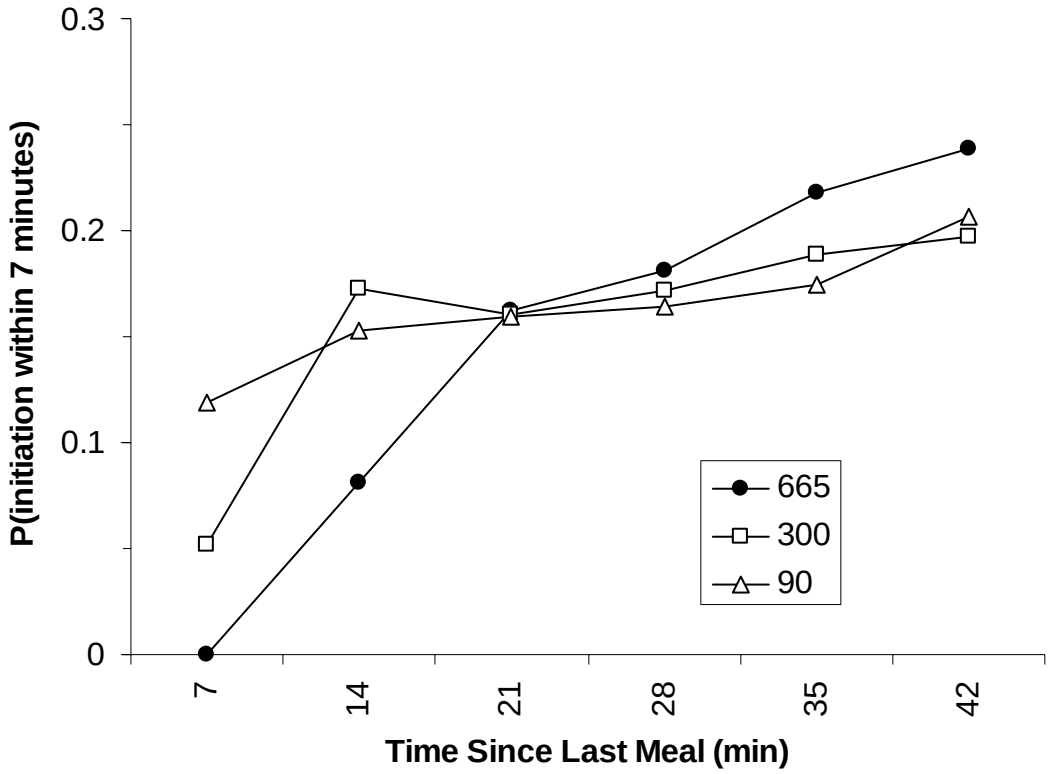

Figure 2.7. Probability of new meal initiation following meal termination based on different TMIs. The instantaneous probability of meal initiation within the next 7 min is plotted against the time elapsed from the last meal. Calculations were performed on the same data set, including all nocturnal periods from 8 mice in study 2, with meal definitions based on TMIs of 665 (circles), 300 (squares), or 90 (triangles).

behavioral analysis. suggest that the shorter TMI values derived from drinking-naïve meal definitions inappropriately parse “true” meals into smaller feeding bouts or snacks.

Study 2: Dynamic features of meal pattern across the nocturnal and diurnal periods

All average meal values were calculated separately from the nocturnal or diurnal sessions using the drinking-explicit and light-cycle-specific TMI values of 665 and 898, respectively (Table 2.1). Meal number, meal duration, and meal size for the 12 hr diurnal periods were significantly less than the 12 hr nocturnal periods. Consequently, average diurnal intermeal intervals were markedly longer, consistent with the longer TMI. The nocturnal values at FR40 in study 2 are similar to those obtained in study 1, however unlike all the schedules in study 1, food consumption was split 67% nocturnal/33% diurnal instead of 75%/25%. This may explain the relatively subtle differences between the studies for meal number, meal size, and inter-meal interval.

Because the pellet delivery histograms in Figure 2.4 clearly showed a diurnal rhythm for feeding behavior, we formally analyzed changes in serial meal values over the course of the nocturnal and diurnal periods (Figure 2.8). Repeated measures ANOVAs were performed on data from the first three meals in each period because all mice consumed at least 3 meals for every session. Meal duration and meal size were both greatest for the first nocturnal meal and then decreased. Diurnal period values were similar to the final nocturnal values for the first 3 meals, but then increased by the last meal in the light period. The same analyses were performed across all FR schedules for mice in study 1 and the results and conclusions were similar (see appendix), indicating that the

Figure 2.8

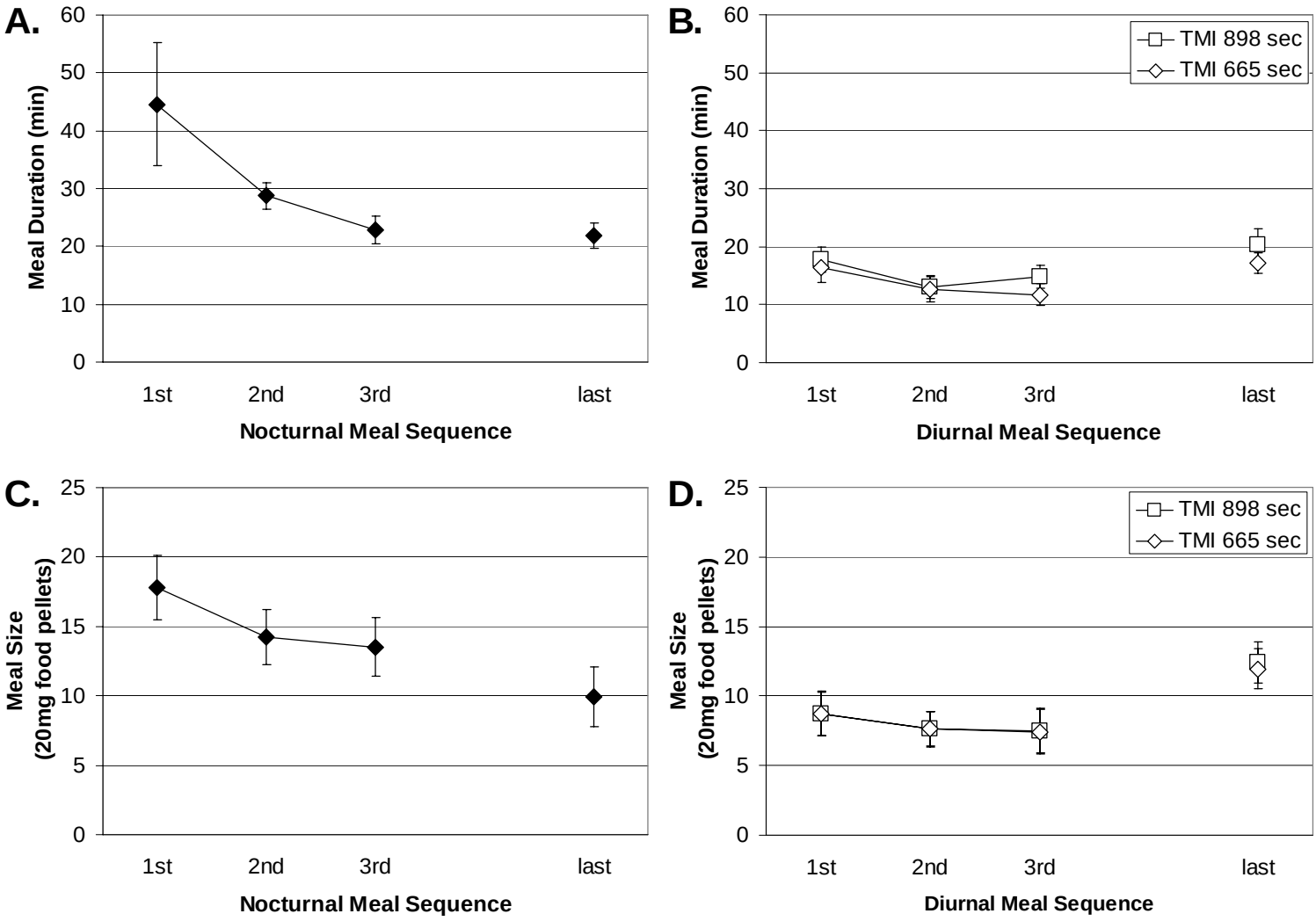

Figure 2.8. Sequential meal parameter values for the first three and last meals during nocturnal (A, C) and diurnal (B, D) periods from study 2. Individual meal durations (A, B) and meal sizes (C, D) were calculated using the drinking-explicit meal definition and TMIs of 665 (diamonds) or 898 (squares, diurnal only). Values are within-subject means ± SEM during 12 consecutive nocturnal and 5 uninterrupted diurnal periods at FR40, n = 8 mice.

that the essential diurnal rhythm of feeding behavior remained intact despite large differences in the difficulty of pellet acquisition.

2.4 *DISCUSSION*

Over the past four decades there have been several studies in rats conducted to address methodological issues of meal pattern investigation (Kissileff, 1970; Collier *et al.*, 1972; Panksepp, 1973; De Castro, 1975; Castonguay *et al.*, 1982; Castonguay *et al.*, 1986; Glendinning and Smith, 1994; Zorrilla *et al.*, 2005a). Recent technological developments have made the mouse an attractive subject for meal pattern analyses, however a comparable corpus of methodological investigations have yet to be developed for the mouse. This study was undertaken to address those issues that we encountered in the process of measuring meal patterns of mutant mice generated in our lab using an operant procedure.

The prerequisite for all subsequent analyses was to maximize the fidelity of our data upon which meal pattern calculations are based; to this end we demonstrated unequivocally that food pellet delivery events represent immediate eating events. Food wastage decreases when operant costs increase, but an FR40 schedule of reinforcement for 20mg food pellets is demanding enough to discourage uneaten pellets without putting mice into a negative energy balance. Our results further demonstrate that mice can learn the operant task within a single 23 hour session, and without using food deprivation.

Most mouse meal pattern studies have not treated drinking as an integral component of a meal. We show that for mice, like rats, drinking is an essential component of meals. Drinking-explicit meals have better predictive validity for the

behavioral satiety sequence than if using drinking-naïve data, and best fit expectations of the satiety concept. We present analyses of meal pattern at three different time-scales: averaged across days, consecutive nocturnal and diurnal meals, and within-meal work bouts and post-reinforcement pauses.

We were able to accomplish these tasks by developing freeware that provide users with all the tools necessary for meal pattern analyses from start to finish: automated extraction of inter-event interval information from raw data in proprietary Med-Associates format, calculation of zero and first-order curves for meal definition analyses, and generation of multiple meal pattern values for final output. The improvements in meal pattern analysis reported here follow in the footsteps of earlier studies making the best use of limited technology available to them at the time.

Validation of operant meal patterns of wild-type C57BL/6 mice

The first goal of this study was to confirm the validity of the measurements used to calculate meal patterns for mice. We demonstrated that mice were able to learn the contingency between lever pressing and food acquisition within 24 hours of the day 1 session. In study 1, there were no differences in total daily food intake among any of the days within the initial and final FR10 schedule periods, and average food intake during these two periods was consistent with measures of food intake recorded in our lab under free feeding conditions. Mice defended their daily food intake by appropriately increasing the number of lever presses they performed as the schedule became more difficult and then decreasing lever presses during the final FR10 period to levels indistinguishable from those found during the initial FR10 period. Investigation of the cumulative records

from day 2 onward revealed PRP durations appropriate for food retrieval and consumption that were sandwiched between uninterrupted bouts of lever pressing. Finally, all of the mice in study 2 were able to perform at FR40 after the first 23 hours in the operant chambers earning food under an FR10 schedule. Collectively, these findings support the contention that all mice learned the contingency in the 23 hr before the end of day 1.

Determination of the TMI and subsequent calculation of meal parameter values rely on the assumption that all food pellet delivery events in the data record correspond to actual pellet consumption events. The session data records do not explicitly distinguish between eaten and uneaten pellets, nor when a dispensed pellet is consumed. Uneaten pellet counts are a direct measure of the number of pellet delivery events that violate the assumption upon which meal definition is based, and therefore minimizing uneaten pellets was one way to reduce the number of unrepresentative pellet delivery events. Pilot experiments showed that if the cost was too low, mice accumulated uneaten pellets suggesting the alternative possibilities of food-hoarding or non-food directed lever pressing. If the cost was too high, mice had progressive decreases in total daily food intake and body weight from their free feeding baselines. The objective was then to employ an escalating reinforcement schedule to identify a pellet cost in lever presses high enough to discourage non-food directed lever pressing and low enough so that daily food intake and body weight were not significantly affected. The results of study 1 showed that uneaten pellets were greatest during the initial FR10 and FR20 schedules, and fell at FR40 and FR80 proportionally to the difficulty of the schedule, then remained low during

the final FR10 period. Furthermore, the minimal number of uneaten pellets found at FR40 in the first study was replicated in study 2.

The positive effect of higher schedules on the fidelity of meal pattern measurements was moderated by concurrent negative effects on body weight and daily food intake. Daily food intake was significantly decreased at FR80 from consumption levels at the lowest schedules when food intake was closest to that found under free feeding conditions (~ 4 g per day). Despite the schedule-associated decreases in food intake, consumption levels at FR40 were comparable to free feeding measurements obtained in our own lab as well those reported in other studies (Kurokawa *et al.*, 2000; Bachmanov *et al.*, 2002). There was no evidence of body weight loss as the schedule escalated from FR20 to FR80, however at 8 to 10 weeks of age, mice are still in the dynamic stage of weight gain. It is likely that body weight gains were mildly restrained during the course of the experiment as evidenced by the significant rebound in food intake and upsurge in body weight when the schedule returned to the final FR10 from FR80. This issue was clarified in study 2 by measuring body weights of adult mice before, during and after testing in the operant chambers at FR40; we found no changes in daily food intake or body weight indicating that the FR40 schedule was not putting these mice into a state of negative energy balance.

Minimizing uneaten pellets by itself did not guarantee that all pellets were immediately consumed. Eaten pellets should be followed by a PRP long enough to represent the time it takes for a mouse to complete the consummatory sequence of retrieving and consuming the earned pellet before initiating another bout of lever presses. Frequency histograms of PRP durations in study 1 revealed a bimodal distribution with

well-defined peaks centered between 10 to 14 seconds, and 22 to 45 seconds, regardless of schedule. The second PRP peak conformed to our observations of PRP duration, strongly indicating the instances when mice were consuming food pellets just after their delivery. This interpretation was supported by the fact that the range for the PRP peak did not vary by reinforcement schedule, which should have no effect on how long it takes mice to complete the consummatory sequence. PRPs of 10 to 14 sec most likely represented occasions leading to the accumulation of uneaten pellets. The frequencies of 10 to 14 sec PRPs were proportional to the number of uneaten pellets and varied by schedule. Frequencies of the short PRPs were highest during the initial FR10 and FR20 schedules, the same schedules with the greatest uneaten pellet counts, and lowest at FR80 when uneaten pellets were minimized. Both uneaten pellet counts and frequencies of 10 to 14 sec PRPs were intermediary in value at the FR40 and final FR10 schedules. The virtual absence of PRPs shorter than 10 sec was attributable to the 10 sec time-out period when the food lever was retracted concurrent with pellet delivery. This time-out was imposed to encourage mice to reorient to the food magazine and consume the dispensed pellet. The moderate frequency of PRPs lasting 10 to 14 sec suggest that mice were waiting for the lever to re-extend rather than consuming the pellet acquired in the previous completed WB. Insofar as this interpretation is correct, the first PRP peak reflects lever pressing that was not food-directed. Reinforcement schedules under which mice generate high frequencies of PRPs lasting 22 to 45 sec, and low frequencies of 10 to 14 sec PRPs should increase the likelihood that pellet deliveries and their ingestion are temporally contiguous. Videotaped observations of mice engaged in unambiguous food-

directed lever pressing followed by immediate pellet consumption indicated that the PRP was roughly 27 to 32 seconds.

The frequency distribution of WB durations shifted to the right in proportion to the number of lever presses needed to earn food pellets. Concomitantly, the lever press rate increased, judging from the ratio of the WB duration at peak frequency within a schedule to the number of lever presses fulfilling schedule requirements. Similar to reports of increased rates of responding by rats earning food in closed economies, the lever press rate increased between FR40 and FR80 as predicted by economic models of meal pattern (Collier and Johnson, 2004). This rate increase is interpreted as an animal's attempts to maximize caloric gains but minimize time costs. Interestingly, the lever press rate appeared to be highest once the schedule was returned to FR10 and not during the FR80 period.

Drinking-explicit meal pattern analysis of mice

The earliest incidence, to our knowledge, of drinking-explicit meal pattern analyses in mice may be credited to a study comparing "complete meals" of male C57BL/6J *ob*/*ob* mice with lean littermates under free feeding conditions (Ho and Chin, 1988). The authors provided no justification for their choice of a 12 minute TMI, very close to the one we identified, but it was likely the result of measurement limitations. The limits of the temporal resolution for feeding (food hopper vibrations) and drinking (lick counts) were measured in 6 minute epochs. Despite these limitations, meal number was somewhat comparable to those reported in this study, with lean control mice having 7.3 ± 0.8 nocturnal meals, fewer than what we calculated, and 6.9 ± 0.6 diurnal meal to the 7.1

± 0.6 meals reported here. However, the average nocturnal and diurnal meal sizes were reportedly larger than ours. There was no description of how meal sizes were calculated, nor were there any reported attempts to test the important assumption that food hopper vibrations were always synonymous with eating activity. There is no way to tell whether the differences are the result of free feeding versus operant feeding, or from measurement inaccuracies.

The first application of the drinking-explicit method outlined by Zorrilla and colleagues to mouse meal patterns was conducted with CRF2 receptor knockout mice (Tabarin *et al.*, 2007). Mice were initially required to nosepoke under an FR1 schedule to earn 20mg food pellets, however the authors increased the schedule to FR2 to increase the likelihood that nosepokes were being performed for food. Under these conditions the authors found, as we did, that the probability of meal initiation was very low immediately after the end of a meal. However, they reported drinking-explicit meal definitions of 360 seconds for the nocturnal period, almost half the TMI arrived at in our study. TMIs for the diurnal period, while closer, were still divergent with Tabarin and colleagues reporting 720 seconds compared to 898 second TMI identified in our study. The discrepancy could be attributed to mouse strain, although in meal pattern analyses of another mouse strain in our lab we have identified a similar TMI to the one reported here.

There are two possibilities which, if true, have larger implications for the determination of meal definitions. Differences in the operant method could have an effect on the TMI at which meal duration and size are most stable. The intervals between consecutive food pellet deliveries under an FR2 schedule of nosepokes we would expect to be much shorter than ours. It is possible that this could reduce the length of the

"correct" TMI. The broader implications being that TMI, itself, may differ under different experimental conditions, precluding the universal adoption of a "real" TMI. We expect, however, that the discrepancy in TMI between the two studies is likely attributable to the number of TMIs tested. The zero-order curve and first-order derivation calculations in the current study used 40 possible TMIs between 60 and 3000 seconds ($e^{6.1}$ to $e^{8.0}$ seconds) compared to the 23 data points represented in the authors' zero and first-order curves. If too few possible TMIs are tested then one may appear to be correct that would be rejected with zero and first-order curves of higher resolution. The meal pattern analysis software we developed gives users control over the range, number and identity of possible TMIs to be tested. The decision to make the meal pattern analysis software that we've developed freely available, we hope, will help to resolve these kinds of issues and facilitate future studies of meal pattern to advance our understanding of the physiological controls initiating, maintaining, termination of meals.

CHAPTER 3.0

MEAL PATTERNS OF MICE SELECTIVELY DEFICIENT IN NEURONAL PRO-OPIOMELANOCORTIN

3.1 *INTRODUCTION*

For over a decade the central melanocortin (MC) system has been an important focus of investigation for the role it plays in regulating food intake and energy homeostasis (Cone, 2005; Ellacott and Cone, 2006; Tolle and Low, 2008a). Melanocortin agonists are derived by post-translational cleavage of pro-opiomelanocortin (POMC), a propeptide expressed in the arcuate nucleus of the hypothalamus (ARC) and in the nucleus tractus solitarius (NTS) of the brainstem. The melanocortin agonist *alpha*-melanocyte stimulating hormone (α-MSH) inhibits feeding by acting on central MC3 and MC4 receptors (Cone, 2005). The opposite effect is produced by agouti-related peptide (AgRP), an endogenous MC receptor antagonist and putative inverse agonist expressed in the hypothalamus (Tolle and Low, 2008b). Antagonism of central MC receptors with AgRP potently stimulates feeding (Haskell-Luevano *et al.*, 1999). Disruption of central melanocortin signaling by genetic lesions of the MC4 receptor or POMC both result in hyperphagia and early development of obesity (Huszar *et al.*, 1997; Yaswen *et al.*, 1999).

Despite these advances, little is known about how the central melanocortin system regulates the temporal expression of feeding episodes that underlie increases or decreases in food intake. Yet most if not all mammals, including humans, eat in discrete episodes, or meals (Collier, 1980). We employed meal pattern analysis to investigate the role that central melanocortin signaling plays in the initiation, maintenance and termination of meals in a strain of neuron-specific POMC knockout (nPOMCKO) mice generated in our lab (Smart *et al.*, 2006). Mice with global POMC deficiency exhibit a profound loss of stress hormones and adrenal atrophy that confounds the effects of POMC and glucocorticoids on the hyperphagia and obesity seen in these mice (Yaswen *et al.*, 1999).

nPOMCKO mice possess a transgene that ‘rescues’ production of the POMC-derived peptide ACTH in the pituitary thereby restoring adrenal development and function while preserving POMC deficiency in the CNS. nPOMCKO mice are hyperphagic, have dysfunction of energy balance, and develop a profoundly obese phenotype more severe than global POMC deficient or MC4RKO mice.

The first objective of this study was to determine the underlying meal pattern responsible for the hyperphagia of adult nPOMCKO mice. There is more than one way that hyperphagia may be expressed: with larger meal sizes, more frequent meals, or a combination of both. This hyperphagic phenotype is evident in nPOMCKO mice as early as 6 weeks old who, remarkably, consume the same amount of food per day as the adult mutants (Smart *et al.*, 2006). It is not known whether this increased daily food intake is mediated by identical meal patterns regardless of age. It is possible that the meal pattern of young nPOMCKO mice is still undergoing developmental changes, or that the final meal pattern phenotype does not appear until after 6 weeks when the nPOMCKO mice begin dramatic weight gains. The second objective was to test these hypotheses by measuring meal patterns of young nPOMCKO mice. We recorded the temporal structure of feeding and drinking events occurring across multiple sequential 23-hour sessions in adult nPOMCWT and nPOMCKO mice in the first study, then 5 week old nPOMCKO mice before the onset of obesity, with body weights comparable to adult wild-type mice.

the right of the food magazine and a retracted dummy lever opposite of the food lever. Water was supplied from sipper tubes mounted on an extension-retraction device opposite of the food magazine. Lick-o-meters were installed on sipper tube assembly to record drinking events. Each chamber was enclosed in a ventilated cabinet that was light and sound attenuating. Control of apparatus and record of lever press, pellet delivery and lick events were made through computer interface and MedPC for Windows software (all equipment - Med-Associates, St. Albans, VT).

Procedure

Prior to the start of experiments, groups were counterbalanced in order to control for effects of chamber size. Once mice were moved to the operant conditioning chambers they lived in them continuously for 14 days except for ~ 1 hour each day when they were weighed and moved to a homecage without food or water while data were gathered and preparations were made for the next session. All sessions ran for 23 hours and started between 16:00 and 17:00 hours. At the beginning of each session, the response lever and sipper tube in each chamber were extended to allow mouse access, and the house light (100 mA) was illuminated. Extended sipper tubes were set 0.5 cm back from the chamber wall to prevent false licks while still allowing drinking access. House lights turned off at 19:00 and turned back on at 07:00. When mice completed the required number of lever presses a single nutritionally complete food pellet was delivered (20 mg; PJAI-0020; Noyes Precision Pellets, Research Diets Inc., New Brunswick, NJ). Except for training day 1, food levers were programmed to retract for 10 seconds following satisfaction of FR criterion to prevent perseverative lever pressing identified in pilot studies, and to

encourage pellet consumption immediately after delivery. At session end, food lever and sipper tube were retracted, and house light was kept on to maintain diurnal illumination conditions. Mice whose body weights fell below 80% of starting weight during the initial training sessions were removed from the experiment and excluded from subsequent data analyses (nPOMCKO, n=2). Chambers and cabinets were thoroughly cleaned after each cohort.

Experimental design

During the first five days the fixed-ratio schedule for food pellets was increased at the beginning of each new session, from FR1, to FR5, FR10, and FR20 until the nPOMCKO mice were working for food under a FR30 schedule. This protocol was adopted following pilot studies showing that adult nPOMCKO mice were not lever pressing for food if the reinforcement increased too rapidly. FR30 was chosen for the meal pattern measurement period from pilot studies because it simultaneously minimized pellet waste/loss while still reproducing *ad lib* daily food intake previously reported for both genotypes. The nPOMCWT mice started at FR10, and reached FR30 by day 2. Once FR30 was achieved the schedule did not change for the remainder of the experiment. Only feeding and drinking event data from day 6 to day 14 were used to calculate meal patterns. Data gathered during days where equipment malfunction prevented mice from acquiring food or failed to record drinking events were not used.

Two studies were conducted. The first study compared meal patterns of adult nPOMCKO mice (n=10) and nPOMCWT littermates (n=11). Three genotype-matched cohorts were run over the course of four months. The second study, conducted several

months later, was conducted to determine whether the hyperphagia exhibited by adult nPOMCKO mice is a cause or effect of their obesity, and to test whether the same aberrant meal pattern phenotype of the adult mutants is present in 5-7 week old nPOMCKO mice. The body weights of nPOMCKO mice this early in development are indistinguishable from those seen in adult wild-type mice. Male nPOMCKO mice (n=6) were acclimated for 1 week to individual housing before moving them into the operant chambers. All young nPOMCKO mice were 37-39 days old at the start of experiments. The same protocol used for adult nPOMCKO mice was used for the young nPOMCKO mice.

Meal pattern analysis

A meal is the basic unit of food intake and is typically defined as a cluster of temporally proximal feeding events bounded on either side by longer periods of ingestive inactivity. Meal definitions rely on two variables: the threshold meal interval (TMI) and the minimum meal (MM) criterion (Castonguay *et al.*, 1986). The TMI defines the cut-off between intra-meal and inter-meal intervals, that is, it represents the maximum amount of time that can pass between two ingestive events such that they can still be considered part of the same meal. The MM criterion sets the lower limit to the amount of food that must be eaten to be considered a meal. Meal definitions have traditionally used intervals between feeding events as the basis of meal pattern analyses (Gannon *et al.*, 1992), however, Zorrilla et al. (Zorrilla *et al.*, 2005a) have shown that the inclusion of drinking events along with feeding events in the meal definition results in meal values that more accurately predict the likelihood of meal initiation based on time since last meal, and

post-prandial satiety sequence of behaviors when compared to drinking-naïve meal definitions. We have used this drinking-explicit model on our data to define meals, i.e. to calculate TMI and MM variables.

Data gathered for meal pattern analysis were structured as consecutive intervals, in seconds, between sequential lever press, pellet delivery and lick events representing the activity of an individual mouse across a 23 hour session. Algorithms written in Microsoft proprietary language Visual Basic for Applications were used to separate nocturnal period intervals from session data sets and to perform calculations for the drinking-explicit model of meal definition on these data as described in Zorrilla et al. (Zorrilla *et al.*, 2005a).

The MM criterion was dependent on the frequency distribution of meal sizes and was meant to distinguish "snacks" from small meals. In the event of a bimodal distribution distinguishing bona fide meals from "snacks", the MM was taken as the threshold value between the two distributions, otherwise MM was set to 1 pellet (20mg). In order to determine the correct TMI, average meal durations were calculated using all possible TMIs ranging from 11 seconds to 40,134 seconds (~12 hours). Possible TMI values were drawn from natural log scale in decimal increments, e.g. $e^{2.4}$ = 11 seconds, $e^{2.5}$, $e^{2.6}$… $e^{10.6}$ = 40,134 seconds as logarithmic scales have been shown to be efficacious in separating intra-meal and inter-meal intervals (Tolkamp and Kyriazakis, 1999). For each possible TMI, consecutive intervals between lever press and lick events that were less than or equal to that TMI were summed while pellet deliveries within the possible meal were counted. When an interval was reached that was greater than the TMI, the number of pellet deliveries within that possible meal were compared to the MM criterion.

If pellet deliveries taking place within a possible meal were equal to or greater than the MM criterion, then it was considered a bona fide meal. Meal duration and meal size for that meal was included in average meal duration and meal size calculations at that possible TMI, and meal number was incremented up by 1.

The average meal duration calculated from each possible TMI when plotted depict a zero-order curve. First-order derivations from the zero-order curve were used to identify the most likely TMI. The first-order curve was generated by subtracting adjacent average meal parameter values from each other and normalizing for interval length by dividing by e^{n}, where n starts at 0.0 for the first interval, $e^{2.4}$, and increments up in 0.1 units for each subsequent interval, e.g. $e^{0.1}$ for $e^{2.5}$, $e^{0.2}$ for $e^{2.6}$, etc. The resulting first-order curve reflects the bimodal distribution of intra- and inter-meal intervals where the inflection point as least rate of change in meal parameter values represents the interval right at the threshold between the two distributions. The TMI was identified by finding the interval at which the least rate of change took place along the first-order curve for each genotype. The average meal parameter values calculated at this TMI, e.g. meal duration, meal size and meal number, were used for comparisons between experimental and control groups of mice to determine meal pattern. In the event that the TMI was different between the groups, an average of the TMI for both groups was used to make sure that differences in TMI were not the cause of differences in meal pattern.

Statistics

Graphs of daily body weight, food and water intake were replaced with averages of day 6 to day 14 on days when failure of equipment allowing mice to acquire food

resulted in unrepresentative decreases in body weight, food or water intake. Only 2 of 140 days of the adult nPOMCKO mouse data (1.4%), and 7 of 154 days of adult nPOMCWT data were affected (4.6%).

Meal definitions varied by genotype, and group comparisons required using a single definition. Feeding and drinking interval data for each group were used to calculate two sets of meal values from different meal definitions, either 545 or 898 second TMIs. Meal values sets for each group were compared with 2-way ANOVA (TMI x day) to test the inter-compatibility of each meal definition by determining whether or not meal values were unchanged when using either of the two meal definition.

Average nocturnal meal values for each group were calculated from individual mouse meal averages from day 6 to day 14. Comparisons between the adult nPOMCWT, nPOMCKO and young nPOMCKO mice were made using 1-way ANOVA with Bonferroni posttest.

Meal patterns at the level of individual meals were investigated by averaging the first five nocturnal meal sizes, durations and inter-meal intervals across the meal measurement period from day 6 to day 14 for each mouse. Sequential meal patterns for adult nPOMCWT, nPOMCKO, and young nPOMCKO mice were then compared with 2-way repeated measures ANOVA (group x sequence) with Bonferroni posttests. Significance was set at $p<0.05$.

3.3 *RESULTS*

Body weights of adult mice from both genotypes were stable throughout the study following an initial drop when moved into operant conditioning chambers (Figure 3.1).

Body weights of nPOMCKO mice that were 37-39 day old at the start of the study were comparable to adult wild-type mice, and continued to rise without interruption. Food intake was most variable during the first five days when the reinforcement schedule was being increased each day (Figure 3.2A). Average intake fell for all groups after the first schedule increase from FR1 to FR5 on day 2, gradually recovering even with daily increases in the reinforcement schedule. Once mice were acquiring food under a FR30 schedule from day 5 on daily food intake stabilized with nPOMCKO mice consuming over 6 grams of food per day regardless of age and control mice eating ~4 grams. Daily water intake followed similar trends as those seen in food (Figure 3.2B). Overall nPOMCKO mice drank more water than controls, although adult nPOMCKO mice consumed intermediate quantities of water during the first five days when their daily food intake was lower.

The first-order curves shown in Figure 3.3 are derivatives of the average nocturnal meal durations calculated for each of 82 possible threshold meal intervals (TMI) ranging from 11 seconds to 12 hours. As the TMI increases in length temporally distant ingestive events determined to belong to separate meals at shorter TMI will be subsumed into a single, longer, meal. The relative rate of change between average meal durations calculated from two possible TMIs may differ depending on the TMIs used; rates of change are expected to be greatest when the TMI are inappropriately splitting or coalescing ‘real’ meals, and to drop to its lowest point or region of points as the correct TMI is approached. The first-order curve for nPOMCWT mice revealed a region of low rates of change with possible TMIs ranging from 493 to 898 seconds (Figure 3.3A). The absolute minimum rate of change at a TMI of 545 seconds was selected to define meals

3.2 *MATERIALS AND METHODS*

Subjects

Adult male mice (18 – 23 wk old) were used in the first set of meal pattern experiments comparing neuron-specific POMC knockout mice (nPOMCKO) with wild-type littermates (nPOMCWT) who, like their siblings, were hemizygous for the POMC 'rescue' transgene. Young male nPOMCKO mice (n=6) were used in the second meal pattern experiment. These mice were all 37-39 days old at the start of experiments. Transgenic mice were generated by crossing POMC(+/-) mice with mice possessing either one or two copies of a POMC 'rescue' transgene. The transgene construct, containing a functional POMC gene downstream of a pituitary-specific promoter, was designed to restore POMC expression, and thus ACTH production, in the pituitary glands of mice globally deficient in POMC. The hybrid genetic background of resulting mice was approximated at 80% C57BL/6, 10% DBA/2, and 10% 129X1;129S6. All mice were bred on-site and tested for absence of neuronal POMC expression and the presence of the transgene that rescued pituitary POMC expression (Smart *et al.*, 2006). Mice were kept on a 12:12 hour light-dark cycle (lights on at 07:00). Mice were individually housed at least a week prior to the commencement of experiments. All procedures were approved by the Institutional Animal Care and Use Committee and followed the Public Health Service guidelines for the humane care and use of experimental animals.

Apparatus

Four 16×14×13 cm and four 22×18×13 cm operant conditioning chambers were used in meal pattern studies. All chambers were outfitted with two levers, a food lever to

in nPOMCWT mice. A region of low rates of change was also seen in the first-order curve for adult nPOMCKO mice. In contrast to the nPOMCWT first-order curve, however, the rates of change are much lower in this region and they encompass a much broader range of possible TMIs (Figure 3.3B). These features are present in the first-order curve for the young nPOMCKO mice as well (Figure 3.3C). The TMIs at which average meal durations change the least in the young and adult nPOMCKO mice are at 812 and 898 seconds respectively, both showing virtually no change in meal duration unlike the nPOMCWT mice.

In the nPOMCWT mice, use of the two meal definitions (TMI 545 seconds vs. TMI 898 seconds) produced significantly different meal sizes ($F_{1,166}$=15.2, $p<0.0001$), meal number ($F_{1,166}$=22.1, $p<0.0001$) and meal durations ($F_{1,166}$=26.3, $p<0.0001$). In contrast, only meal durations were found to differ based on the two TMIs in both young ($F_{1,90}$=5.7,$p<0.05$) and adult nPOMCKO mice ($F_{1,154}$=16.5, $p<0.0001$). Therefore, all subsequent meal pattern analyses were based on the most parsimonious meal definition of 545 seconds and a minimum meal of two 20mg food pellets.

The hyperphagic phenotype of nPOMCKO mice appears to be primarily attributable to meal size (Figure 3.4A); nPOMCKO mice ate significantly larger meals than controls regardless of age ($F_{2,24}$=10.9, $p<0.001$), but no group differences in meal number were found (Figure 3.4B). Adult but not young nPOMCKO mice had significantly longer inter-meal interval durations when compared to nPOMCWT controls ($F_{2,24}$=5.4, $p<0.05$) although there was a trend for longer inter-meal intervals in young nPOMCKO mice (Figure 3.4C). Increased inter-meal intervals would be expected following large meals if the mechanisms regulating satiety, i.e. that suppress initiation of

new meals, were intact; this is in contrast to satiation, which is responsible for terminating a meal. The only significant difference found between young and adult nPOMCKO mice was in average nocturnal meal duration (Figure 3.4D). Adult nPOMCKO mice had shorter meals than both young nPOMCKO and the adult nPOMCWT mice ($F_{2,24}$=6.1, p<0.01).

Analysis of sequential meal durations revealed significant effects of group ($F_{2,96}$=5.6, p<0.05) and meal sequence ($F_{4,96}$=2.9, p<0.05) on meal durations (Figure 3.5A). A significant interaction effect was also discovered ($F_{8,96}$=2.5, p<0.05). The duration of the first meal for nPOMCWT mice was significantly longer than both adult nPOMCKO mice (t_{19}=4.6, p<0.001) and young nPOMCKO mice (t_{15}=3.2, p<0.05). When sequential meal sizes were analyzed only a significant interaction effect between meal sequence x group was found ($F_{8,96}$=2.8, p<0.01). During the fifth meal the nPOMCWT mice ate less than nPOMCKO adults (t_{19}=2.8, p<0.05), and young mice (t_{15}=3.1, p<0.05) (Figure 3.5B). The size of the fourth meal was significantly smaller for nPOMCWT mice than the young POMCKO mice (t_{15}=2.7, p<0.05). Sequential inter-meal intervals analysis showed main effects of group ($F_{2,96}$=8.4, p<0.01) and inter-meal interval sequence ($F_{4,96}$=25.7, p<0.0001) (Figure 3.5C). Adult nPOMCKO mice inter-meal interval durations were found to be significantly longer than all five of the nPOMCWT mice inter-meal intervals: 1st (t_{19}=2.9, p<0.05), 2nd (t_{19}=3.8, p<0.01), 3rd (t_{19}=4.1, p<0.001), 4th (t_{19}=4.2, p<0.001), 5th (t_{19}=3.4, p<0.01).

Figure 3.1

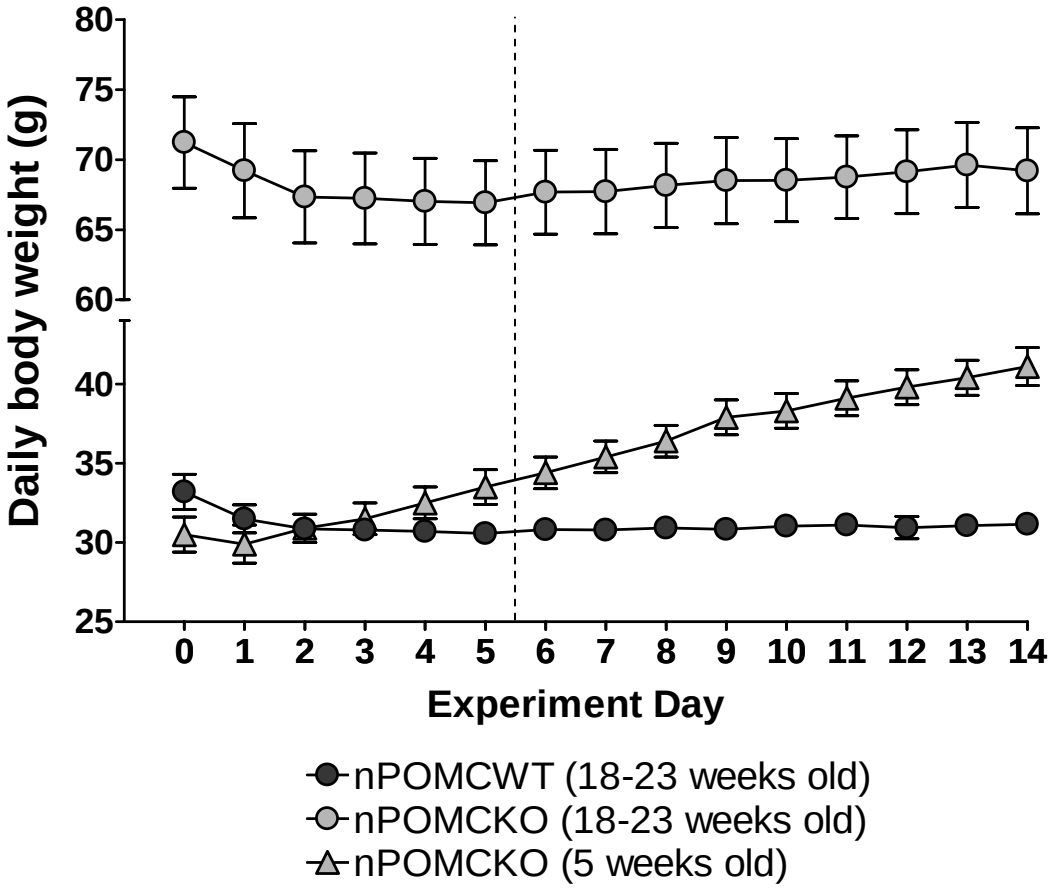

Figure 3.1 Daily body weight averages in young and adult groups of neuron-specific POMC knockout (nPOMCKO) mice and adult wild-type littermates (nPOMCWT). The dotted line indicates the end of the training period, after which all mice responded for food pellets on a FR30 schedule.

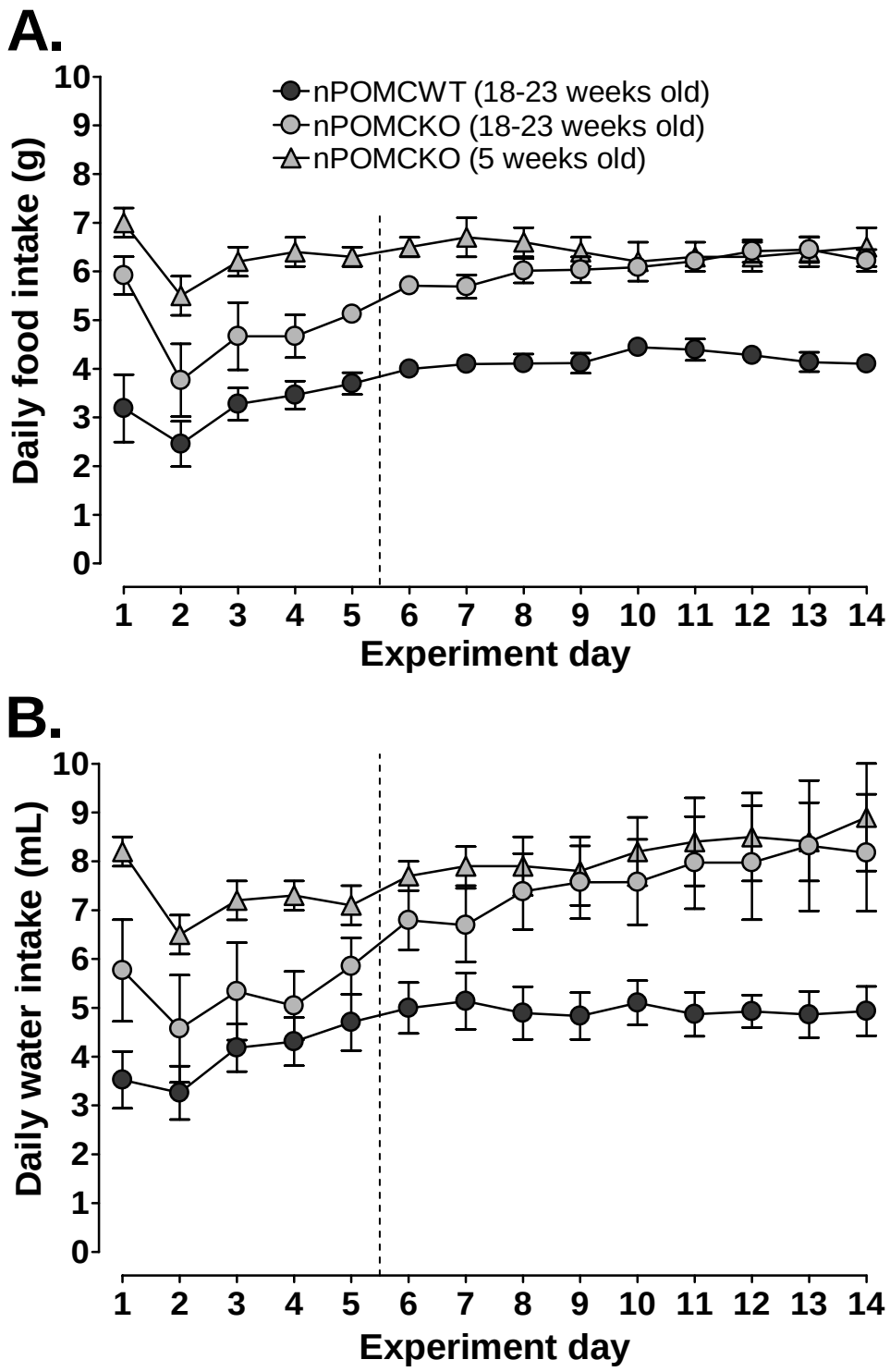

Figure 3.2 Daily food and water intake in young and adult nPOMCKO mice and adult wild-type littermates**.** The dotted line indicates the end of the training period, after which all mice responded for food pellets on a FR30 schedule.

Figure 3.3

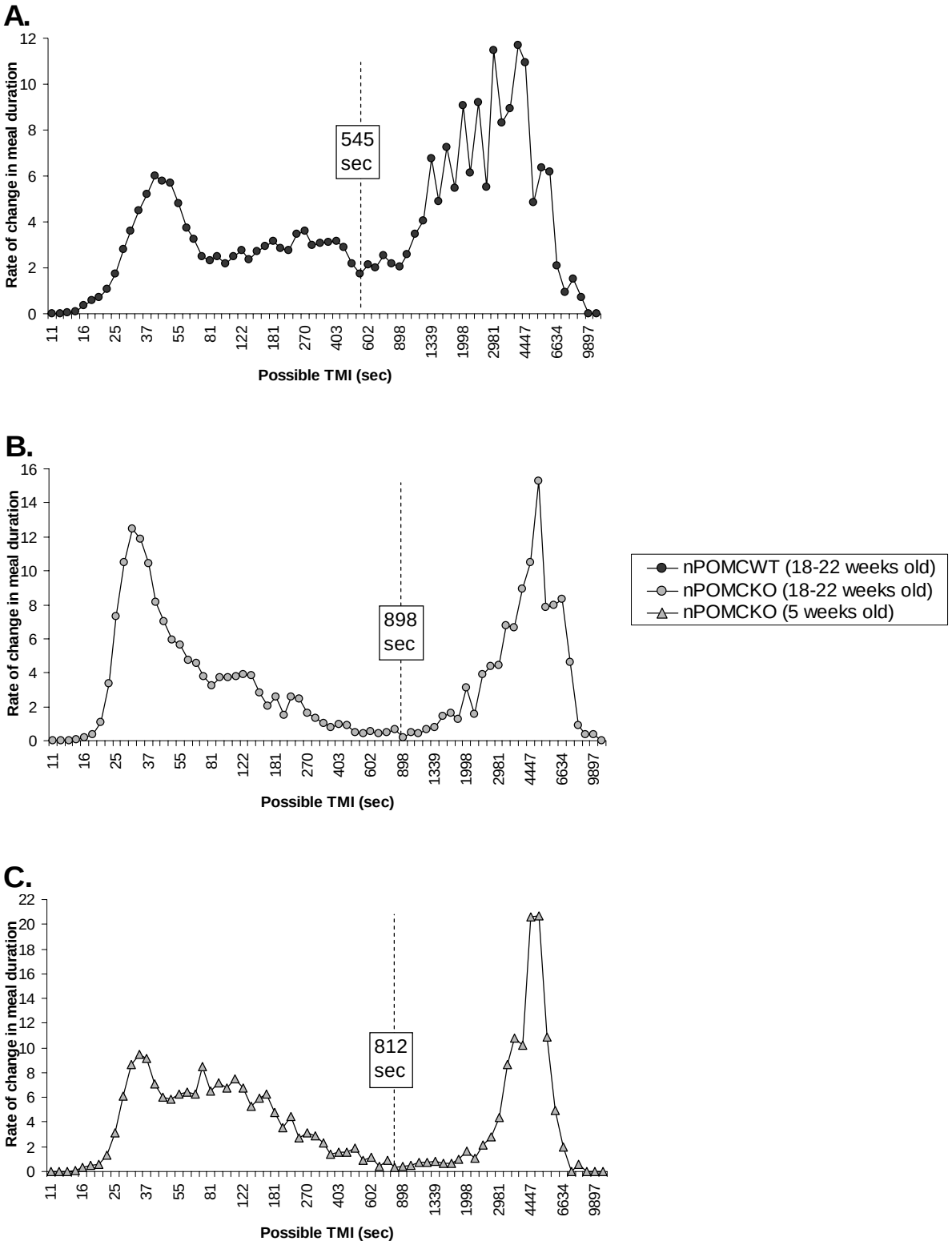

Figure 3.3 Drinking-explicit meal definitions for young and adult nPOMCKO mice and adult nPOMCWT littermates during the nocturnal period.

Figure 3.4

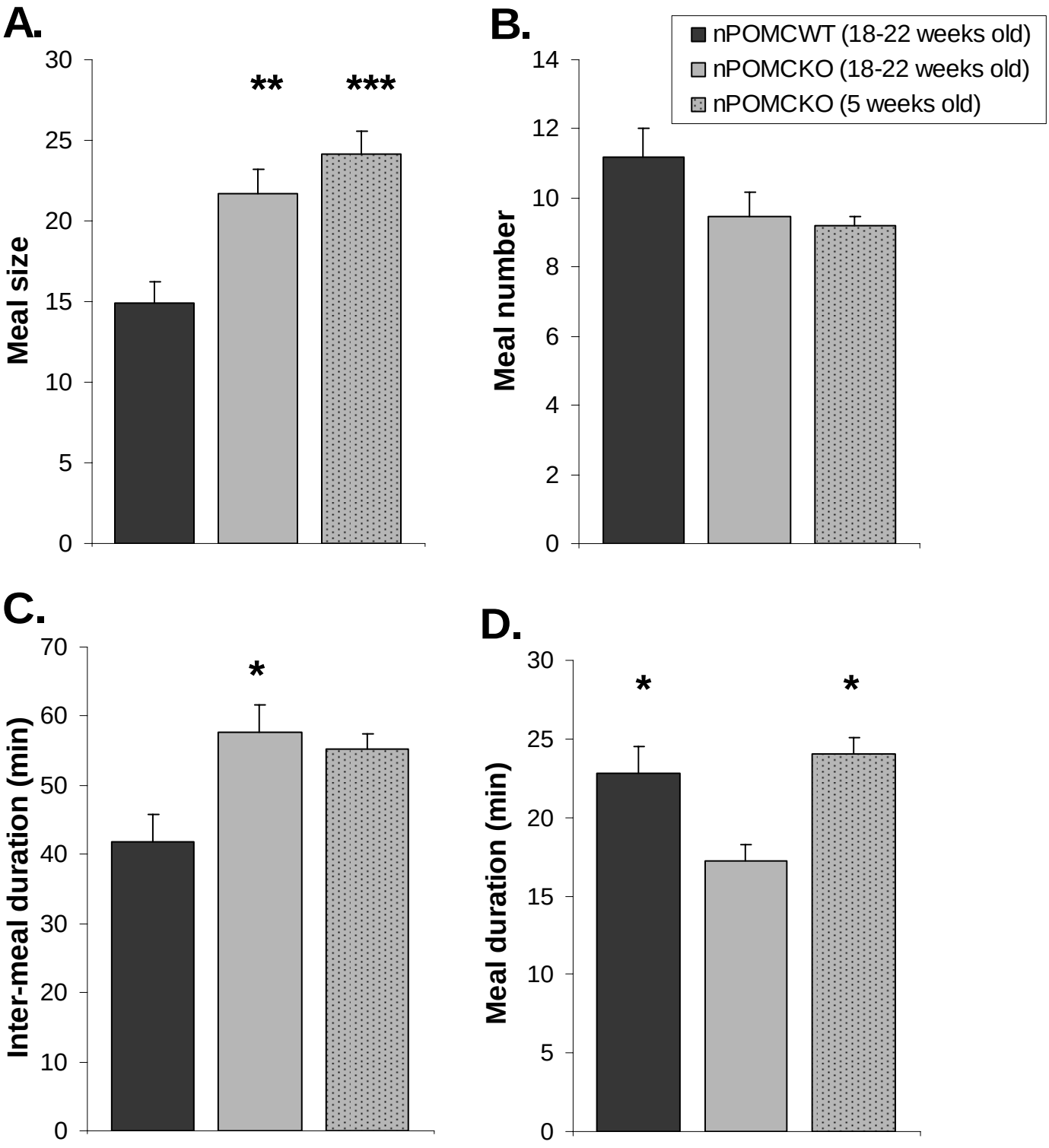

Figure 3.4 Average nocturnal meal values in young and adult nPOMCKO mice, and adult nPOMCWT controls. * $P < 0.05$, ** $P < 0.01$, ** $P < 0.001$.

Figure 3.5

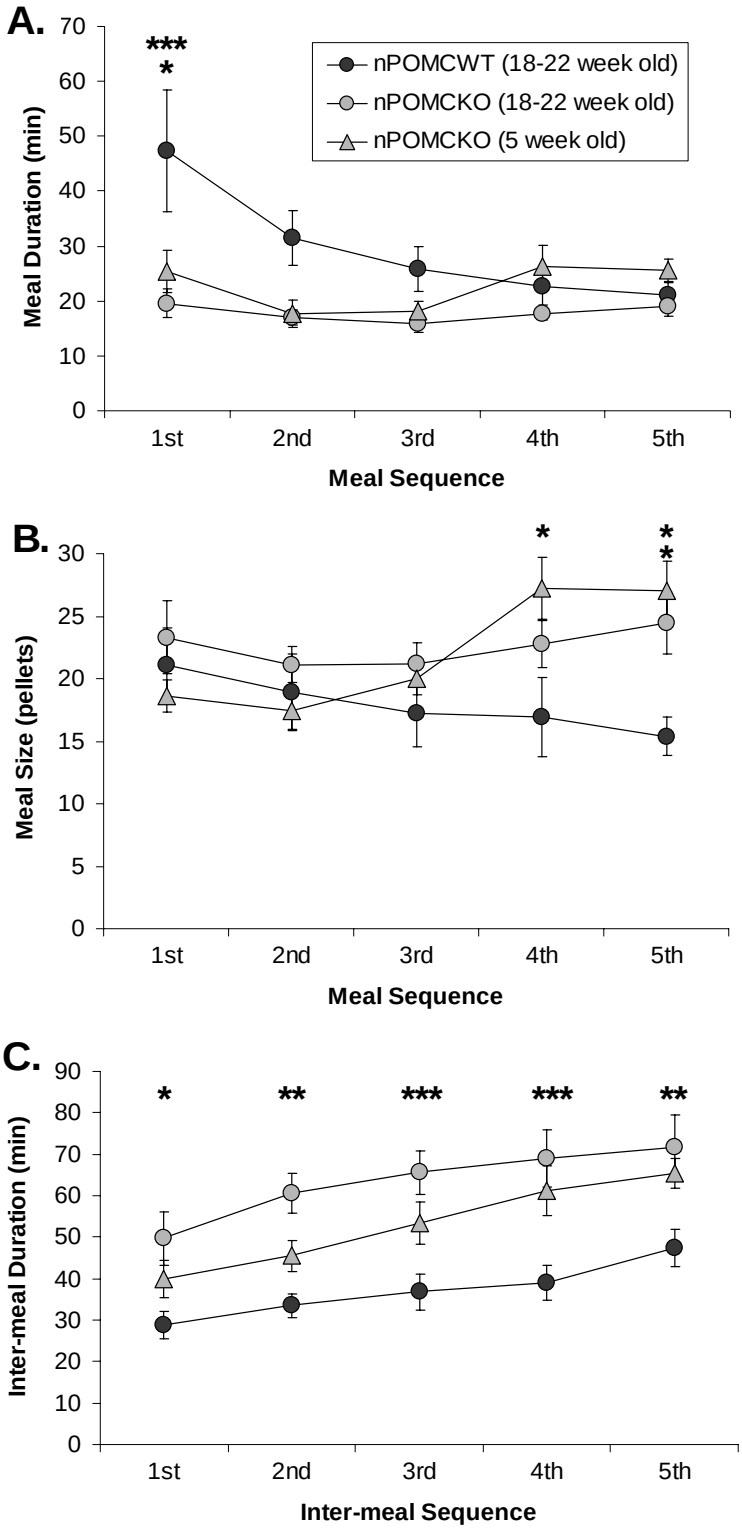

Figure 3.5 Comparisons of sequential meal pattern during the nocturnal period in young and adult nPOMCKO mice, and adult nPOMCWT controls.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.4 *DISCUSSION*

Characterization of the nocturnal meal pattern of adult nPOMCKO mice showed that their hyperphagic phenotype was realized via significant increases in the quantity of food they consumed during each meal. Moreover, the absence of genotype differences in the number of meals that these animals initiated indicates that nPOMCKO hyperphagia results exclusively from increases in meal size. If anything, average meal number for the nPOMCKO mice tended to be lower than nPOMCWT. Two other prominent features of nPOMCKO mouse nocturnal meal pattern were the differences in meal and inter-meal interval durations. The amount of time nPOMCKO mice took to eat these large meals was significantly shorter than the nPOMCWT mice indicating that nPOMCKO mice were eating at a faster rate. The nPOMCKO mice appeared to compensate for these large meals by following them with significantly longer inter-meal interval durations.

Analysis of average nocturnal meals in young nPOMCKO mice showed that these mice were exhibiting patterns similar to those seen in adults of the same genotype. Young nPOMCKO mice not only ate larger meals than controls, their meal sizes were comparable to adult nPOMCKO meal sizes. As was seen in adult nPOMCKO mice, there were no differences in meal number from nPOMCWT mice. The similarities extended into the pattern of sequential nocturnal meals. Regardless of age, the first meal was shorter in nPOMCKO than in nPOMCWT mice; nPOMCKO meal sizes diverged from nPOMCWT mice by the fifth meal, and by the fourth meal in the young nPOMCKO mice.

Despite the similarities in the meal patterns of young and adult nPOMCKO mice, it may be argued that the comparisons with nPOMCWT mice do not control for body

weight. Meal pattern results of the young nPOMCKO mice represent measurements of 23 hour feeding and drinking behavior between 43 and 53 days old. During these 10 days the average body weights of young nPOMCKO mice increased from 34.4 ± 1.0 to 41.1± 1.2 g, surpassing the average body weight of 31.0± 0.6 g of adult nPOMCWT controls during the same time period. While this may be the case, Smart et al. (Smart *et al.*, 2006) reported that 4-5 week old nPOMCKO mice, while being only slightly more obese than nPOMCWT littermates, still ate 50% more food per day. Our data showed that young nPOMCKO mice 37-39 days old were eating over 6 grams of food per day as early as day 1 when they had body weights at or below the nPOMCWT control mice. If body weight affects meal pattern at all, it is to account for the differences in meal duration between young and adult nPOMCKO mice. The encumbering effects of the profound obesity in adult nPOMCKO mice could indirectly lead to shorter meals by favoring immobility in front of the food lever.

Mice with monogenic mutations leading to an obese phenotype have been shown to express similar meal patterns as seen in nPOMCKO mice. Mice homozygous for the *ob* gene variant are unable to express functional leptin (Campfield *et al.*, 1995; Pelleymounter *et al.*, 1995). Meal pattern analysis of *ob*/*ob* mice, a strain exhibiting with an obese phenotype resulting from homozygosity for the non-functional *ob* allele of the leptin gene, had the same number of meals as lean controls (*ob*/*ob* mice 6.4 ± 0.7 vs. lean mice 7.3 ± 0.8) but ate significantly more during meals (Ho and Chin, 1988). The meal parameter values relied on a TMI of 12 minutes and they used a ‘complete meal’ definition which, like our own study, included both eating and drinking events.

The similarities in the respective meal patterns of *ob*/*ob* mice and nPOMCKO mice suggest the possibility that the primary causal factor behind both is a profound disruption of POMC neuron signaling. Leptin receptors are expressed on POMC neurons in the ARC and NTS (Ellacott *et al.*, 2006), and leptin increases action potentials in POMC neurons (Cowley *et al.*, 2001). Dysfunction of POMC signaling should decrease the sensitivity of these mice to peripheral signals responsible for meal termination, while the compensatory increases in the time between meals suggests that intermeal satiety signaling is intact and not regulated by POMC.

Alternatively, POMC deficiency may be directly increasing appetitive motivation. POMC is known to be centrally involved in a number of appetitive behaviors including grooming, sex and feeding (Spruijt *et al.*, 1992; Van der Ploeg *et al.*, 2002). Several studies have implicated POMC in drug reward (Alvaro *et al.*, 1996; Alvaro *et al.*, 1997; Alvaro *et al.*, 2003; Hsu *et al.*, 2005). Furthermore, melanocortin receptors are expressed in several brain regions important to natural and drug reward (Alvaro *et al.*, 1996; Adan and Gispen, 1997). A recent meal pattern study was conducted using mice lacking functional melanocortin-4 receptor alleles (MC4RKO). When subjected to a progressive ratio schedule where the response cost in lever presses escalated by 1 for each consecutive food pellet, the MC4RKO mice continued to respond for food at costs significantly higher than controls (Vaughan *et al.*, 2006).

In conclusion, the results of this study provide new details about the effects of neuronal POMC deficiency on the development of meal pattern phenotype. The primary findings were that adult nPOMCKO mice ate significantly more food in less time than controls, exhibiting compensatory increases in the duration of their inter-meal interval

durations. Young nPOMCKO mice shared elements of the aberrant meal pattern phenotype seen in adults by eating significantly larger meals with a trend towards longer inter-meal interval durations, while having average nocturnal meal durations comparable in length to nPOMCWT mice, possibly the result of not yet being encumbered by their own obesity. It remains to be seen whether the aberrant meal pattern phenotype of nPOMCKO mice may be the result of desensitization to satiety signals, an enhancement of appetite, or some other mechanism yet to be specified.

CHAPTER 4.0

GENERAL DISCUSSION

4.1 *SUMMARY OF RESEARCH*

The methodological study of meal patterns described in Chapter 2 provided evidence to validate the operant method used to gather feeding and drinking data, and the drinking-explicit model for adult male C57BL/6 mice. Video analysis provided evidence to allow interpretation of food pellet deliveries as being equivalent to food pellet consumption. These mice were able to maintain energy balance under an FR40 schedule for food pellets. Furthermore, the mice were able to learn the operant task in a single 24 hour training session without the need for prior food deprivation.

The results of this study also indicate that the inclusion of drinking along with feeding when measuring meals in mice is justified because it accurately predicts the post-meal period when the behavioral satiety sequence occurs. Drinking-explicit meals also met expectations of the satiety concept that predicts a very low probability of new meal initiation immediately after the end of a meal, which increases proportionally to the amount of time since the last meal. Neither of these conditions was satisfied with meal definitions derived from feeding events alone. The circadian feeding rhythm was conserved in this paradigm, and evidence was found for differences between nocturnal and diurnal meal pattern. First, nocturnal meals were larger and longer than subsequent ones, and meal sizes and durations decreased in successive diurnal meals. Finally, I produced and validated a software suite to automate analysis of meal pattern from raw data to final results, providing a useful tool for the scientific community.

The application of this methodology to analyze the feeding behavior of nPOMCKO mice provided important clues to the functional role of the central melanocortin system in regulating meal pattern. The meal pattern phenotype associated

with neuron-specific POMC deficiency was characterized by striking alterations in meal size, and in meal and inter-meal interval durations. The hyperphagic component of their phenotype was entirely the result of increased meal sizes rather than meal number. The nPOMCKO mice maintained the same number of meals by having longer inter-meal interval duration than controls. Interestingly, the meals of adult nPOMCKO mice were much shorter than those of the control mice, which meant that the mutant mice were eating significantly more food in a shorter amount of time. Establishing hypothetical mechanisms that regulate the initiation and termination of meals is a prerequisite for any attempts at interpretation of these results. Luckily, valuable groundwork has already been laid by a cadre of behavioral neuroscientists over the past several decades.

4.2 *MEAL CONTROL MECHANISMS*

Richter (Richter, 1927) was the first to quantitatively show that an assortment of behaviors including feeding, drinking, activity, rest and elimination, occur periodically in discrete episodes, or bouts. His investigation of salt appetite led to the discovery that an animal could alter its behavior as a strategy to maintain an internal physiological variable around a closely defended set-point; adrenalectomized rats compensated for the resulting over-excretion of NaCl with dramatic increases in salt intake (Richter, 1941). It was not difficult to extend this logic and envision that deflections away from some nutritional set-point could motivate an organism to initiate and terminate feeding bouts in the service of maintaining its ‘internal milieu’ within homeostatic boundaries (Moran and Schulkin, 2000).

The depletion-repletion hypothesis represented the application of a homeostatic mechanism to explain meal patterns. According to this hypothesis, the meal pattern of an animal was primarily driven by its "momentary physiological state" reflected in the availability of circulating macronutrients (Le Magnen and Devos, 1970; Collier *et al.*, 1972; Le Magnen and Devos, 1980). Circulating macronutrient levels would be subject to continuous reductions as they were siphoned off to maintain metabolic demands of the animal. When these levels fell below a certain threshold a motivational process would drive the animal to initiate a new meal that would continue until macronutrient repletion was sufficient to terminate the motivational process and the meal. Thus the meal is the basic regulatory mechanism of energy homeostasis. If this hypothesis was true, then there should be a direct correlation between the size of a meal and elapsed time before the next meal. Several researchers reported positive correlations between meal size and the duration of the post-prandial interval (Snowdon, 1969; Le Magnen and Devos, 1970), however, others did not (Baker, 1953; Levitsky, 1970; Collier *et al.*, 1972).

Advocates of the depletion-repletion hypothesis encountered additional problems reconciling their theory with the powerful effects of environmental contingencies on meal pattern. In one study, rats were trained to bar press on a fixed-ratio schedule to gain unrestricted access to a food bin (Collier *et al.*, 1972). Once the rat spent more than 10 minutes outside the bin the meal was considered over, the bin closed and would only open if the rat completed another set of bar presses. The schedule doubled every few days to see how it affected meal size and number. Meal number fell and meal size rose as the schedule became more demanding, but no correlations were found between meal size and post-prandial duration and rats were able to maintain total daily food intake and body

weight. These results indicated that rats were able to exert long term controls over food intake in ways not easily accounted for by meal-to-meal regulation predicted by the depletion-repletion model.

The regulation of body weight and food intake has since been reconceptualized in terms of short-term controls, combined with *tonic* signals of energy stores underlying long-term controls (Woods *et al.*, 1974; Bray and Campfield, 1975; Kissileff and Van Itallie, 1982; Havel, 2001). Short-term controls, mediated by *episodic* signals accompanying meal-to-meal ingestive activity, are proposed to underlie psychological constructs like hunger and satiety. The term "satiety" is frequently treated as a monolithic concept in the literature, however, when food intake is resolved into meals it is clear that at least two processes can be distinguished: prandial satiety and postprandial satiety, or more commonly, satiation and satiety respectively (Blundell, 1991; Gerstein *et al.*, 2004). Satiation (*prandial satiety*) is what terminates a meal; satiety (*postprandial satiety*) temporarily suppresses the motivation to initiate a new meal, colloquially referred to as "hunger". The physiological underpinnings of both operational constructs involve sensorimotor interactions with the ingested material as it enters the oral cavity and passes through the alimentary canal (Chaudhri *et al.*, 2006). Satiation (meal size control) and satiety (inter-meal interval control) represent valid meal control mechanisms whose physiological concomitants are gradually coming into focus (Blundell *et al.*, 2001).

Another mechanism affiliated with short-term food intake controls is palatability, or the hedonic value of food (Berridge, 1996). Grill & Norgren devised an ingenious behavioral assay of taste reactivity that provided valid measurements of an animal's hedonic evaluation of gustatory stimuli, allowing researchers to distinguish food

palatability from feeding motivation (Grill and Norgren, 1978). Investigations of numerous primate species, rodents and newborn human infants have revealed phylogenetic conservation of facial reactions to sweet and bitter tastes (Berridge, 2000). Orofacial reactions to palatable tastants reliably elicit tongue protrusions, lip-smacking and paw-licks in rodents, while gustatory stimulation from unpalatable substances like quinine provoke gapes and head shakes. Measurements of the positive, neutral, and negative affective orofacial reactions to tastants have provided researchers with behavioral access to otherwise unobservable affective states.

4.3 *INTERPRETING THE MEAL PATTERN PHENOTYPE OF nPOMCKO MICE*

The large meal sizes of nPOMCKO mice indicate that central POMC peptides are primarily involved in satiation mechanisms responsible for meal termination, especially given that the prolonged durations of inter-meal intervals in nPOMCKO mice following these large meals indicate that postprandial satiety mechanisms are functionally intact. The fact that these results were also seen in juvenile mutants indicated that the meal pattern phenotype was independent of body weight. It could be further argued that the phenotypic indistinguishability of the young nPOMCKO from adult nPOMCWT mice make it unlikely that there are any differences in gastrointestinal capacity that might otherwise explain the significant increases in meal size seen in all nPOMCKO mice. This should be easy enough to test in future experiments by comparing measurements of the stomach weights and intestinal lengths of nPOMCKO, nPOMCWT and wild-type C57BL/6 mice.

The NTS represents a neuroanatomical locus where satiation mechanisms are likely to act and is also a primary target for POMC modulation. The presence of MC4 receptors and opioid receptors in brainstem regions receiving and sending signals to and from the gut provide a substrate that would permit POMC modulation (Mountjoy *et al.*, 1994; Kishi *et al.*, 2003). MC4R knockout mice are insensitive to the food decreasing effects of the short-term satiety peptide, CCK, as are mice treated with 4^{th} ventricular microinjections of the MC3/4 receptor antagonist, SHU9119 (Fan *et al.*, 2004). Furthermore, CCK has been shown to stimulate c-fos expression in green fluorescent labeled POMC neurons of the NTS. Solitary tract stimulation elicits EPSCs in this same population of NTS POMC neurons (Appleyard *et al.*, 2005). CCK increases the amplitude of these EPSCs, which are blocked by non-NMDA glutamate receptor antagonists and attenuated by opioid receptor agonists.

Another intriguing possibility, for which there appears to be no published data, is that the loss of central POMC activity in the brainstem may lead to acceleration of gastric emptying and/or intestinal motility with the same end result of diminished satiation. In one pilot study that I conducted to estimate the maximum capacity of a mouse stomach I subjected a group of wild-type C57BL/6 mice to a 24 hour period of food deprivation followed by access to food for 1 hour before measuring the full and empty stomach weight. Much to my surprise, the stomachs of all the mice were completely empty despite their having consumed ~2g of food, almost half of their average daily food intake (unpublished results). This could be interpreted as the result of an acceleration of gastric motility, an adaptive means to get needed nutrients where they can be absorbed quickly. If neuronal POMC deficiency in the nPOMCKO mice is interpreted physiologically as

urgent nutritional need similar to what was observed in 24-hour food deprived mice, then perhaps accelerated gastric emptying could be the cause of the large meals.

The other notable meal pattern feature, most apparent in older nPOMCKO mice, was the significantly increased eating rate as these mice consumed significantly larger meals in shorter time periods than controls. One possible explanation for the increased rate is an enhancement of the hedonic evaluation of gustatory stimuli. Neuronal POMC deficiency may exert its effects at several points along the neural pathways involved in processing gustatory stimuli arising from the oral cavity during food mastication. These orosensory signals first reach the nucleus of the solitary tract (NTS), converging with vagal projections conveying viscerosensory information, then to the parabrachial nucleus before progressing on to thalamocortical gustatory areas as well as to the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST), regions that have been associated with affective regulation (Scott and Mark, 1986). MC4 receptors are expressed at each of these major relays in gustatory processing, as well as in regions involved in motivational aspects of food intake including the ventral tegmental area and nucleus accumbens (Mountjoy *et al.*, 1994; Alvaro *et al.*, 1996; Kishi *et al.*, 2003; Liu *et al.*, 2003; Hsu *et al.*, 2005). Central melanocortin signaling appears to be functionally involved in reward-related circuits. Microinjections of α-MSH into the ventral tegmental area increase dopamine metabolites in the nucleus accumbens (Lindblom *et al.*, 2001; Lindblom *et al.*, 2002), and the behavioral effects of cocaine are blocked in MC4 receptor knockout mice and reduced in yellow agouti mice, a strain that ectopically expresses an endogenous melanocortin antagonist (Hsu *et al.*, 2005).

It remains unclear which of the POMC-derived neuropeptides, whether melanocortins or the opioid β-endorphin, contribute to the aberrant meal pattern phenotype found in nPOMCKO mice, nor in what way. However, deficiency in one or more of the melanocortin agonists appears to be the most likely explanation. Several studies using β-endorphin knockout mice have shown no alterations in their preference for palatable solutions (Appleyard *et al.*, 2003; Hayward *et al.*, 2006), which suggests that putative enhancement of the hedonic value of food is not readily attributable to the absence of β-endorphin. A recent study of the effects of each of the endogenous melanocortin agonists on food intake in globally POMC-deficient mice indicates that α-MSH most potently increases food intake (Tung *et al.*, 2006). One cannot rule out, however, the possibility that the loss of endogenous expression of the POMC-derived opioid β-endorphin is an important contributing factor. Meal pattern analyses of β-endorphin knockout mice could help identify which of any effects β-endorphin exerts over the temporal organization of ingestive behavior.

4.4 *ETHOLOGICAL PERSPECTIVE ON MEAL PATTERN*

Behavioral control systems act in real-time to regulate the initiation, maintenance and termination of behaviors. Like the analysis of meal pattern, the organization of other behaviors across time is central to ethological approaches. Ethology is the branch of biology concerned with the comparative study of animal behavior (Lorenz, 1981). Noteworthy commonalities between contemporary meal pattern models and ethology are their shared interest in the behavior of animals framed by their ecological niche and how these behaviors are temporally organized (Collier, 1985). In contrast to experimental and

physiological psychologists, ethologists commit considerable time and effort compiling detailed observations of the entire set of a species' behaviors prior to any theory building (Hinde, 1982). These inventories of an animal's behavioral repertoire, or *ethograms*, include the duration, frequency and order of all behavioral types found in a given species. Meal pattern measurements can properly be regarded as a subset of a species' complete ethogram.

Among the theoretical developments derived from ethograms were animal models of behavioral control and motivation. Observations of the ways in which animals switch between different behavioral sequences over time led to the postulation of mechanisms for selecting situation-appropriate behaviors from those available in an animal's repertoire. Some mechanism to select between competing behaviors must exist since many behaviors, like approach and avoidance, are mutually exclusive in that both can't be performed simultaneously. Contemporary ethological models employ variations of some process by which motivational values assigned to each behavioral option in the repertoire are determined by both external and internal factors, and can be dynamically updated to reflect changing circumstances. Depending on the model, these motivational values interact in a selection process involving cooperative, competitive, inhibitory and/or disinhibitory mechanisms where the "winner" at any given moment is the one that gets expressed, and all other behaviors are temporarily suppressed (McFarland and Sibly, 1975; Hinde, 1982; Redgrave *et al.*, 1999).

Unlike ethological investigations, however, most meal pattern studies have analyzed meals independently from the context of other behaviors simultaneously competing for expression in the animal. Investigation of meal pattern in isolation ignores

the important fact that the initiation of a meal also represents the termination of whatever behavior preceded meal commencement; likewise, meal termination is always a transition to a new behavior. Ethological observations of such behavioral switches have revealed complexities that imply the existence of selection mechanisms, influenced by both environmental and physiological causal factors, that mediate which one of the host of competing behaviors within an animal's behavioral repertoire is instantiated at any given moment (McFarland and Sibly, 1975; Redgrave *et al.*, 1999). Meal pattern analysis framed within the context of the entirety of behavioral options available to an animal should provide a more realistic picture of the regulation of ingestive behavior (de Ruiter *et al.*, 1969; Wiepkema, 1971; Heinrichs, 2001).

4.5 *FUTURE DIRECTIONS*

Recommendations for future experiments can be divided into two broad categories: (1) further elucidation of the contributions of POMC to meal pattern, and (2) further methodological developments of meal pattern analysis. Investigation of meal patterns of other mouse strains with compromised melanocortin systems can add to the story. Obvious candidates would be A(y) mice, a strain that ectopically expresses the endogenous melanocortin receptor antagonist, agouti, and MC4 receptor knockout mice. The hyperphagia and obesity of these strains and the nPOMCKO mice arise from complementary variations of melanocortin dysfunction: MC4 receptor knockout mice have selective inability to transduce melanocortin signaling, A(y) mice have chronic blockade of central melanocortin receptors, and nPOMCKO mice lack melanocortin agonists to bind melancortin receptors. Comparisons of these mice with wild-type mice

chronically treated with either exogenous or endogenous melanocortin receptor antagonists would help to disentangle developmental effects of melanocortin dysfunction. In addition to the proposed experiments briefly described above, the results of a β-endorphin knockout mouse study could help to establish the loss of melanocortins as the functional cause behind the results reported in Chapter 3, or reveal a more complex causal relationship. Novel mouse strains selectively deficient in melanocortins but not β-endorphin, when generated, would be an excellent complement to meal pattern studies of β-endorphin knockout mice. The effort to behaviorally phenotype the meal pattern of β-endorphin mice highlights the utility of conducting similar meal pattern phenotyping of mouse strains with mutations in genes that are functionally related to POMC, whether upstream like *ob*/*ob* and *db*/*db* mice, or downstream of POMC activity, for example δ- , μ- or κ-opioid receptor deficient mice.

The value of meal pattern phenotyping studies can be enhanced by parallel methodological developments in the dual processes of extracting the full complement of dependent measures immanent in the raw data sets, and operationalizing meal pattern measures. With respect to the first process, the methodological approach of ethology as a scientific practice provides two insights of particular relevance to meal pattern analysis: framing ingestive behavior as part of a larger behavioral control system already discussed in detail above, and the explicit inclusion of time in behavioral measurements. The temporal dimension of ingestive behavior in meal pattern analysis permits not only the quantification of any behavior that has the properties of duration, size, rate and number, but quantification at several time-scales could provide details to facilitate advances in short-term versus long-term controls of food intake and energy homeostasis. A

comprehensive meal pattern phenotype would include within-meal measures of feeding and drinking bout quantities, rates, durations, number and order as well as the duration and number of within-meal pauses. Furthermore, utilization of an operant method allows additional measures of post-reinforcement pause and work bouts durations, and lever press rates. In addition to calculating overall averages of daily meal pattern measures, the day-to-day alterations in these measures allow tracking of infradian variability, for example, in the effects of estrus on meal pattern, or of variability between individuals, something that has only been conducted in rats (Glendinning and Smith, 1994).

A comprehensive meal pattern phenotype will provide an ideal resource for any operationalization attempts. A study to operationalize “hunger” could be undertaken by determining how meal pattern measures change after 24-hour food deprivation. Operationalization of “hedonic value” or “palatability” could be accomplished in meal pattern studies where the food available to mice was replaced with a more palatable one. The value of meal pattern analyses is the enlargement of the set of dependent measures. In comparison to measurements of cumulative food intake alone, meal pattern measurements of size, duration, rate, latency, and frequency greatly improve the likelihood that subtle treatment effects may be detected. As a closing note, there appear to be almost no behavioral genetic studies of meal pattern (Petersen and McCarthy, 1981); the benefits of comprehensive meal pattern phenotypes could be used to remedy this, offering the prospect of identifying genes that are contributing to the current epidemic of obesity and new targets for pharmaceutical intervention.

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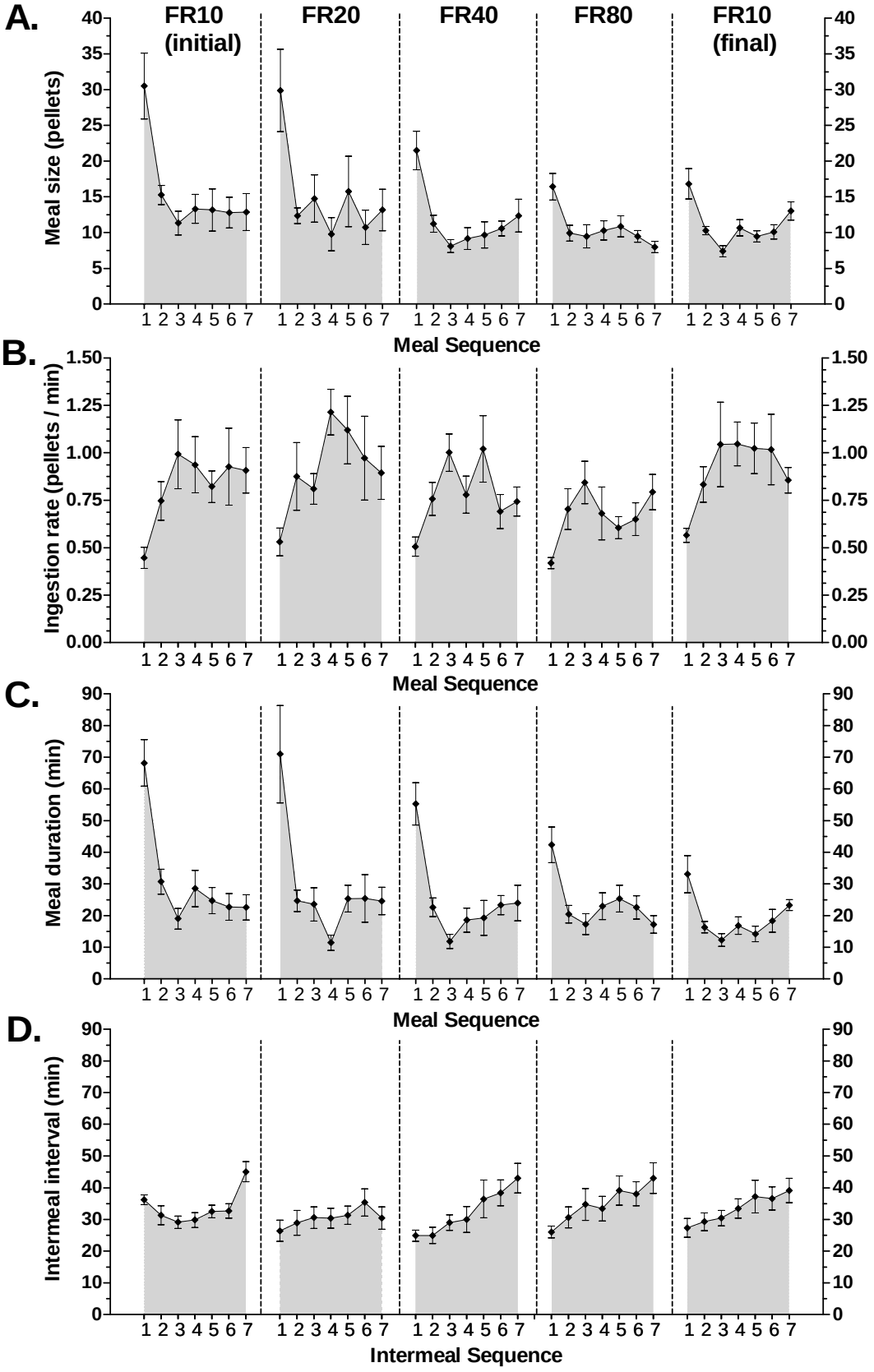
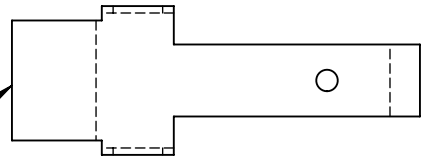
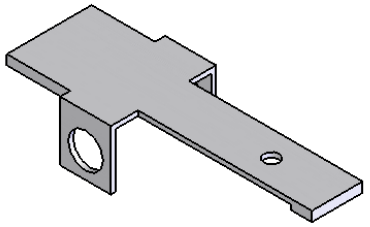
APPENDIX


A.
FR10 (initial)
FR20
FR40
FR80
FR10 (final)
Meal size (pellets)
Meal Sequence
B.
Ingestion rate (pellets / min)
Meal Sequence
C.
Meal duration (min)
Meal Sequence
D.
Intermeal interval (min)
Intermeal Sequence

Meal parameter values for the first seven nocturnal meals and intermeal intervals split by pellet reinforcement schedule. Individual meals (A-C) and intermeal intervals (D) are numbered in their sequence of occurrence. Schedule progression from left to right: initial FR10, FR20, FR40, FR80, and final FR10. (A) Meal size, (B) Within-meal ingestion rate, (C) Meal duration, and (D) Intermeal interval duration. Values are within-subject means ± SEM during four consecutive nocturnal periods at each schedule, n = 8 mice.

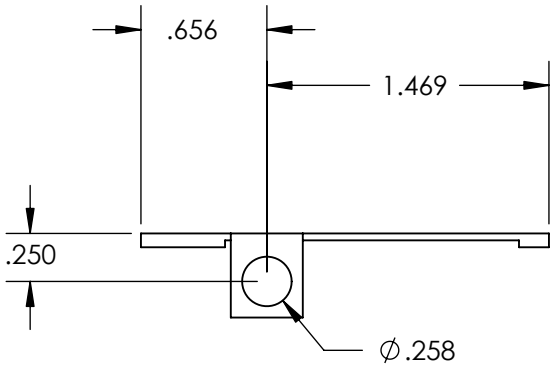




NOTE: Empericle estimate

Estimated mass to displace lever: 2g
Estimated travel: 3.175 mm

Force = 2g*9.81m/(s*s) = 0.01962N
Work: 0.0196 N * 3.175 mm= 6.22935 × 10-5 Joules



Mass properties of ENV-310-01 (Part Configuration - Default)

Density = 0.289 pounds per cubic inch

Mass = 0.016 pounds

Volume = 0.057 cubic inches

Surface area = 2.954 inches^2

Center of mass: (inches)
X = 0.799
Y = 0.386
Z = -0.388

Principal axes of inertia and principal moments of inertia: (pounds * square inches)
Taken at the center of mass.
Ix = (1.000, 0.016, 0.000) Px = 0.001
Iy = (0.000, 0.000, -1.000) Py = 0.006
Iz = (-0.016, 1.000, 0.000) Pz = 0.007

Moments of inertia: (pounds * square inches)
Taken at the center of mass and aligned with the output coordinate system.
Lxx = 0.001 Lxy = 0.000 Lxz = 0.000
Lyx = 0.000 Lyy = 0.007 Lyz = 0.000
Lzx = 0.000 Lzy = 0.000 Lzz = 0.006

Moments of inertia: (pounds * square inches)
Taken at the output coordinate system.
Ixx = 0.006 Ixy = 0.005 Ixz = -0.005
Iyx = 0.005 Iyy = 0.020 Iyz = -0.002
Izx = -0.005 Izy = -0.002 Izz = 0.019

Frictional Properties of shaft/lever interface

Lever is 304 stainless steel
Coefficient of friction is 0.78

Shaft is Nylon
Coefficient of friction is 0.15-0.25

Bearing surface area is unknown

NOTE: THIS IS NOT A PRODUCTION PRINT
FOR REFERENCE ONLY

VISUAL BASIC FOR APPLICATIONS CODE FOR MEAL PATTERN ANALYSIS CONTROL SOFTWARE

Written, tested and debugged entirely by Christian Richard

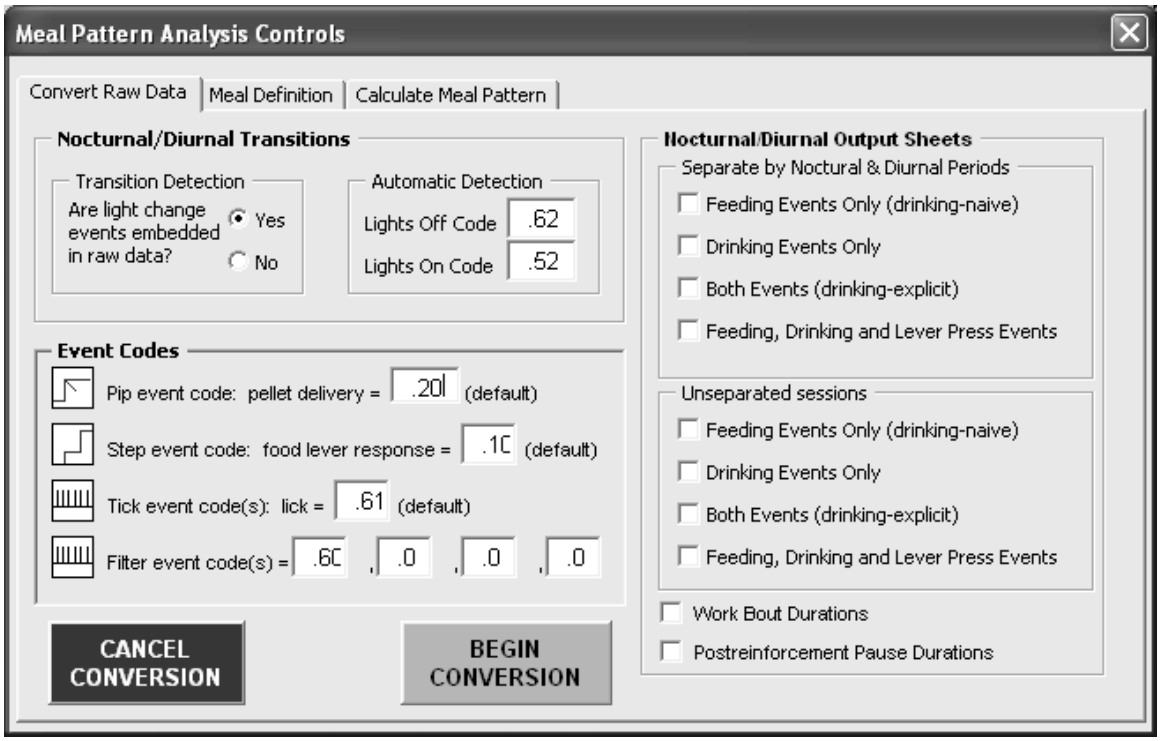


Figure 1. Converts med-associate formatted data, and extracts selected interval data and writes to new sheet.

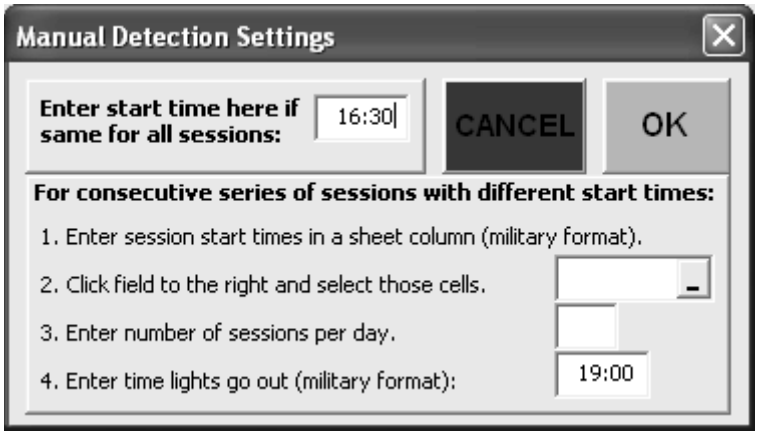


Figure 2. Used to manually tell software where the nocturnal-diurnal boundaries are; also allows selection of multiple times if analysing data from several sessions.

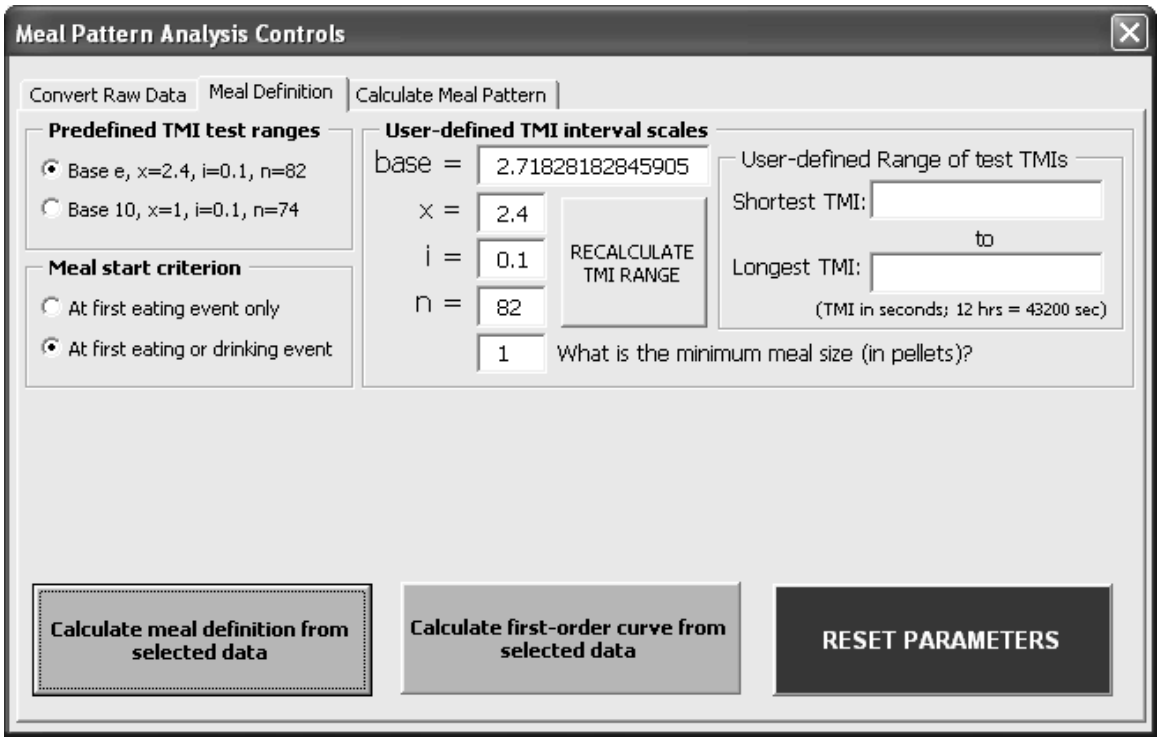


Figure 3. This calculates zero-order data values, automatically generates first-order curves from selected data. Can derive meal definitions with user-specified values.

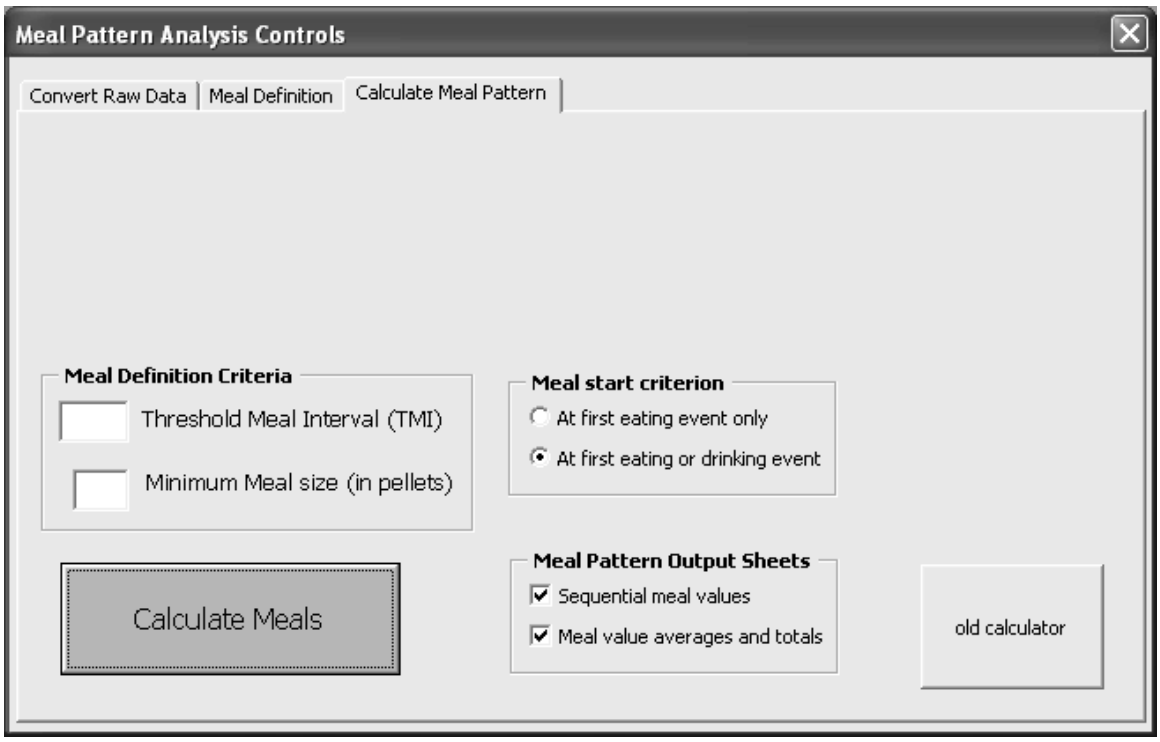


Figure 4. Calculates individual meal values at any desired meal definition. Also automatically generates averages and totals that are otherwise a pain in the ass to calculate.

```
‘MODULE 1
Option Base 1

Type typeDiurnal
   Diurnal() As Variant
End Type

Type typeNocturnal
   Nocturnal() As Variant
End Type


Sub MealPatternAnalysis()

   MealAnalysisControls.Show 0

End Sub
```

```
‘FORM ManualDetectionForm
‘The code below controls the Manual Detection Settings (Figure 2)
Sub cbCancelManualDetect_Click()
   Unload Me
End Sub

Public Sub cbStoreManualStartTimes_Click()

Select Case tbSingleStartTime
Case Is <> ""
   Select Case reStartTimes.Text
   Case Is <> ""
      MsgBox prompt:="You cannot select both single and multiple start times", _
      Title:="Conflicting Requests", _
      Buttons:=vbCritical
      StartTimes = ""
      reStartTimes.Text = ""
      tbSingleStartTime = ""
   Case Is = ""
      ManualDetectionForm.Hide
   End Select 'case resStartTimes.text
End Select 'tbSingleStartTime

Select Case tbSingleStartTime
Case Is = ""
   Select Case reStartTimes.Text
   Case Is <> ""
```

```
      If tbSessionsPerDay = "" Then
         MsgBox _
         prompt:="Enter the number of consecutive sessions sharing the same start time" _
         & vbCr & "(e.g. sessions per day).", _
         Title:="Almost There..."
      Else: ManualDetectionForm.Hide
      End If
   End Select ' case reStartTimes.Text
End Select ' case tbSingleStartTime
```

```
‘FORM MealAnalysisControls
‘The code below controls the Manual Detection Settings (Figures 1, 3, 4)

Private Sub cbCancel_Click()
   Unload Me
End Sub

Private Sub cbCancelDefineMeal_Click()
   tbTMIbase = ""
   tbTMIstart = ""
   tbIncrement = ""
   tbTMInum = ""
   tbMinMeal = 1

End Sub

Private Sub cbDefineMeal_Click()
   Call MealEstimate
End Sub

Private Sub cbFirstOrderCalc_Click()
   Call FirstOrderCurve 'produces a quick first-order derivative curve for zero-order meal
calculations
End Sub

Private Sub cbOld_Click()
   Call OldSequentialMealCalculator
End Sub

Private Sub cbQuickMealDefCalc_Click()
   Call MealEstimate 'calls program to calculate meal values using multiple possible meal definitions
End Sub

Private Sub cbSequentialMeals_Click()
   Call SequentialMealsCalculator
```

```
End Sub

Private Sub cbUpdate_Click()
'this code helps user pick TMI range to test by showing lowest and highest possible TMI

Dim NewMin As Double
Dim NewMax As Double
Dim TMIbase As Currency 'base for exponent
Dim FirstTMI As Currency 'power that base is raised to for the starting TMI
Dim LastTMI As Currency 'power base is raised for final TMI; if too high it will crash program

TMIbase = tbTMIbase.Value
FirstTMI = tbTMIstart.Value
LastTMI = FirstTMI + (tbIncrement * tbTMInum)
NewMin = TMIbase ^ FirstTMI
NewMax = TMIbase ^ LastTMI

tbMinTMI.Value = NewMin
tbMaxTMI.Value = NewMax

End Sub

Private Sub obAutomaticDetect_Click()
   tbSingleStartTime = ""
   tbSessionsPerDay = ""
   reStartTimes = ""
   tbFilterCode3 = 0
   tbFilterCode4 = 0
   obManualDetect = False
   obAutomaticDetect = True

   fAutomatic.Enabled = True
   lLightsOff.Enabled = True
   lLightsOn.Enabled = True
   tbLightsOnCode.Enabled = True
   tbLightsOffCode.Enabled = True
End Sub

Sub obManualDetect_Click()
   ManualDetectionForm.Show
   obManualDetect = True
   obAutomaticDetect = False
   tbFilterCode3 = tbLightsOffCode
   tbFilterCode4 = tbLightsOnCode
```

```
    fAutomatic.Enabled = False
    lLightsOff.Enabled = False
    lLightsOn.Enabled = False
    tbLightsOnCode.Enabled = False
    tbLightsOffCode.Enabled = False
    tbLightsOnCode = 0
    tbLightsOffCode = 0

End Sub

Private Sub obNoExponent_Click()

    obYesExponent = False
    obNoExponent = True

    tbTMIbase = 10
    tbTMIstart = 1
    tbIncrement = 0.05
    tbTMInum = 74
    tbMinMeal = 1
End Sub

Sub obYesExponent_Click()

    obYesExponent = True
    obNoExponent = False

    tbTMIbase = Exp(1)
    tbTMIstart = 2.4
    tbIncrement = 0.1
    tbTMInum = 83
    tbMinMeal = 1

End Sub

Sub DataCheck()
If ActiveSheet.Name <> "RawData" Then _
    RightSheet = MsgBox(Title:="Caution!", _
    prompt:="Is this is raw data sheet", _
    Buttons:=vbYesNo + vbQuestion)

Select Case RightSheet
    Case Is = vbNo
        MsgBox Title:="Analysis aborted", _
        prompt:="Please select raw data sheet before continuing."
        Call cbCancel_Click
```

```
   Case Is = vbYes
      ActiveSheet.Name = "RawData"
End Select

End Sub

Sub cbConvert_Click()

Call DataCheck
Call ProcessIntervals

End Sub

Sub ProcessIntervals()
'this creates an array of sequential intervals
'for licks, pellets and the two combined
'for pellets intervals with and without drinking
'default internal codes for events are:
'0.00001 = lights off
'0.00002 = lights on
'0.00003 = pellet delivery event
'0.00004 = lick event
'0.00005 = PRP ending with lever press
'0.00006 = PRP ending with lick

      'Array variables for raw data
         Dim RawData As Variant
         Dim TotalSessions As Integer
         Dim ThisSession As Integer
         Dim ThisInterval As Currency
         Dim TotalIntervals As Variant

      'Interval Type Array increments
         Dim PNum As Long
         Dim LNum As Long
         Dim PLNum As Long
         Dim PRPnum As Long
         Dim WorkBoutNum As Long
         Dim AllEventsNum As Long

      'Arrays for each interval set
         Dim Pellet() As Variant
         Dim Lick() As Variant
         Dim PellLick() As Variant
         Dim PRParray() As Variant
         Dim WorkBoutArray() As Variant
```

```
'Holding variables
   Dim PelletHold As Variant
   Dim LickHold As Variant
   Dim PellLickHold As Variant
   Dim PRP As Currency
   Dim WorkBout As Currency
   Dim AllEvents As Currency

'Variables to determine how values are treated
   Dim ThisIntervalValue As Currency
   Dim ThisEvent As Currency

'Variables to prevent mis-analysis
   Dim RightSheet As Integer
   Dim PNumBound As Long
   Dim LNumBound As Long
   Dim PLNumBound As Long
   Dim PRPNumBound As Long
   Dim AllEventsNumBound As Long
   Dim WorkNumBound As Long
   Dim BeginPRP As Byte
   Dim BeginWB As Byte
   Dim TransferArray() As Variant
   Dim WriteArray() As Currency

'Variables to assign nocturnal-diurnal boundaries
   Dim Lights As Currency
   Dim CumulativeTime As Currency
   Dim TimeToNextLightChange As Currency
   Dim StartTimesArray As Variant
   Dim SessionStartsArray As Variant
   Dim StartTime As Date
   Dim DayLeft As Date
   Dim NocturnalStart As Date
   Dim Times As Integer
   Dim SessionsPerDay As Integer
   Dim SessionStartTime As Integer
   Dim TotalSecs As Currency
   Dim TotalSessionStarts As Long
   Dim LightDarkCycles As Boolean
   Dim IntervalBeforeLightChange As Currency
   Dim TotalTime As Currency

RawData = Range(ActiveCell.Address).CurrentRegion.Value
If IsEmpty(RawData) Then
```

```
    MsgBox Title:="Analysis aborted", _
    prompt:="Must click on any cell in data set to continue."
    Unload Me
    Exit Sub
End If

TotalSessions = UBound(RawData, 2)
TotalIntervals = UBound(RawData, 1)


If cbAllPellets = True Or cbPellets = True Then _
    ReDim Pellet(1 To TotalIntervals + 10, 1 To TotalSessions) ' added 10 to include OFF and ON
If cbAllLicks = True Or cbLicks = True Then _
    ReDim Lick(1 To TotalIntervals + 10, 1 To TotalSessions)
If cbAllPellLicks = True Or cbPellLicks = True Then _
    ReDim PellLick(1 To TotalIntervals + 10, 1 To TotalSessions)
If cbPRP = True Then _
    ReDim PRParray(1 To TotalIntervals + 10, 1 To TotalSessions)
If cbWB = True Then _
    ReDim WorkBoutArray(1 To TotalIntervals + 10, 1 To TotalSessions)

    PNum = 1
    LNum = 1
    PLNum = 1
    PRPnum = 1
    WorkBoutNum = 1
    AllEventsNum = 1
    BeginPRP = 0
    BeginWB = 0

'-----------------------------------------------

If obManualDetect = True Then 'IMPORTANT - Manual detection of light-dark transitions
                              'assumes session starts during day
    If ManualDetectionForm.reStartTimes <> "" Then
        StartTimesArray = Range(ManualDetectionForm.reStartTimes.Text)
        SessionsPerDay = CInt(ManualDetectionForm.tbSessionsPerDay)
        TotalSessionStarts = (SessionsPerDay * UBound(StartTimesArray, 1))
        ReDim SessionStartsArray(1 To TotalSessionStarts, 1 To 1)
        ThisSession = 1

        For Times = 1 To UBound(StartTimesArray, 1)
            StartTime = StartTimesArray(Times, 1)
            NocturnalStart = ManualDetectionForm.tbNocturnalStart
            DayLeft = StartTime - TimeValue(NocturnalStart)
            'DayLeft = h:m until start of dark cycle
```

```
            TotalSecs = DayLeft * (-86400)
            'TotalSecs is the total number of seconds for the first diurnal period
            For SessionStartTime = ThisSession To (SessionsPerDay + (ThisSession - 1))
               SessionStartsArray(SessionStartTime, 1) = TotalSecs
            Next SessionStartTime
            ThisSession = ThisSession + SessionsPerDay
         Next Times
      End If ' for multiple start times for consecutive sets of sessions

      If ManualDetectionForm.tbSingleStartTime <> "" Then
         StartTime = ManualDetectionForm.tbSingleStartTime
         NocturnalStart = ManualDetectionForm.tbNocturnalStart
         DayLeft = StartTime - TimeValue(NocturnalStart)
         TotalSecs = DayLeft * (-86400)

         Set rng = Range(ActiveCell.Address).CurrentRegion
         TotalSessionStarts = rng.Columns.Count
         ReDim SessionStartsArray(1 To TotalSessionStarts, 1 To 1)
         For SessionStartTime = 1 To TotalSessionStarts
            SessionStartsArray(SessionStartTime, 1) = TotalSecs
         Next SessionStartTime
      End If ' for same start time for all sessions
   End If 'for obManualDetect

   '---------------------------------------------

   For ThisSession = 1 To TotalSessions
      If obManualDetect = True Then TimeToNextLightChange = SessionStartsArray(ThisSession, 1)
      CumulativeTime = 0
      TotalTime = 0
      Lights = 0.0001 ' value added to interval to indicate that lights have turned off
                  ' adding 0.0002 indicates lights on
      For ThisInterval = 1 To TotalIntervals
         If RawData(ThisInterval, ThisSession) = "" Then Exit For
         ThisEvent = RawData(ThisInterval, ThisSession) _
                  - Int(RawData(ThisInterval, ThisSession))
         ThisIntervalValue = (RawData(ThisInterval, ThisSession) - ThisEvent) / 100 'assumes 10ms
   resolution

   '----------- only if light-dark cycle boundaries were manually entered
         If obManualDetect = True Then
            CumulativeTime = CumulativeTime + ThisIntervalValue
            If CumulativeTime >= TimeToNextLightChange Then
               IntervalBeforeLightChange = TimeToNextLightChange - (CumulativeTime -
   ThisIntervalValue)
```

```
            If cbAllLicks = True Or cbLicks = True Then
               LickHold = LickHold + IntervalBeforeLightChange
               Lick(LNum, ThisSession) = LickHold + Lights
               LickHold = 0
               LNum = LNum + 1
            End If

            If cbAllPellets = True Or cbPellets = True Then
               PelletHold = PelletHold + IntervalBeforeLightChange
               Pellet(PNum, ThisSession) = PelletHold + Lights
               PelletHold = 0
               PNum = PNum + 1
            End If

            If cbAllPellLicks = True Or cbPellLicks = True Then
               PellLickHold = PellLickHold + IntervalBeforeLightChange
               PellLick(PLNum, ThisSession) = PellLickHold + Lights
               PellLickHold = 0
               PLNum = PLNum + 1
            End If

            If cbPRP = True Then
               PRP = PRP + IntervalBeforeLightChange
               PRParray(PRPnum, ThisSession) = PRP + Lights
               PRPnum = PRPnum + 1
               PRP = 0
            End If

            If cbWB = True Then
               WorkBout = WorkBout + IntervalBeforeLightChange
               WorkBoutArray(WorkBoutNum, ThisSession) = WorkBout + Lights
               WorkBoutNum = WorkBoutNum + 1
               WorkBout = 0
            End If

            If Lights = 0.0001 Then Lights = 0.0002 Else Lights = 0.0001
            ThisIntervalValue = ThisIntervalValue - IntervalBeforeLightChange
            IntervalBeforeLightChange = 0
            TimeToNextLightChange = 43200 'assumes 12-12 cycle
            CumulativeTime = ThisIntervalValue
         End If 'for CumulativeTime test
      End If ' for obManualDetect
'----------------------------------------

      'this section of code is used to detect events in data
      'in order to generate user-requested interval lists
```

```
   If cbWB = True Then
      If BeginWB = 1 Then WorkBout = WorkBout + ThisIntervalValue
   End If

'-----------------------------------------------
'signal for lights on event
Case Is = tbLightsOnCode
   Select Case obManualDetect
      Case Is = False
      Lights = 0.0002
         If cbAllLicks = True Or cbLicks = True Then
            LickHold = LickHold + ThisIntervalValue
            Lick(LNum, ThisSession) = LickHold + Lights
            LickHold = 0
            LNum = LNum + 1
         End If

         If cbAllPellets = True Or cbPellets = True Then
            PelletHold = PelletHold + ThisIntervalValue
            Pellet(PNum, ThisSession) = PelletHold + Lights
            PelletHold = 0
            PNum = PNum + 1
         End If

         If cbAllPellLicks = True Or cbPellLicks = True Then
            PellLickHold = PellLickHold + ThisIntervalValue
            PellLick(PLNum, ThisSession) = PellLickHold + Lights
            PellLickHold = 0
            PLNum = PLNum + 1
         End If

         If cbPRP = True Then
            Select Case ThisIntervalValue
               Case Is <= 665
                  If BeginPRP = 1 Then PRP = PRP + ThisIntervalValue
               Case Is > 665
                  PRParray(PRPnum, ThisSession) = -1
                  If BeginPRP = 1 Then
                     PRP = PRP + ThisIntervalValue
                     PRPnum = PRPnum + 2
                  Else
                     PRPnum = PRPnum + 1
                  End If
            End Select
         End If 'for when cbPRP is true
```

```
                If cbWB = True Then
                   If BeginWB = 1 Then WorkBout = WorkBout + ThisIntervalValue

'                     WorkBout = WorkBout + ThisIntervalValue
'                     WorkBoutArray(WorkBoutNum, ThisSession) = WorkBout + Lights
'                     WorkBoutNum = WorkBoutNum + 1
'                     WorkBout = 0
                   End If

                Lights = 0.0001
             End Select ' for obManualDetect

          '-----------------------------------------------
          'signal for lights out
          Case Is = tbLightsOffCode
             Select Case obManualDetect
                Case Is = False
                Lights = 0.0001
                   If cbAllLicks = True Or cbLicks = True Then
                      LickHold = LickHold + ThisIntervalValue
                      Lick(LNum, ThisSession) = LickHold + Lights
                      LickHold = 0
                      LNum = LNum + 1
                   End If

                   If cbAllPellets = True Or cbPellets = True Then
                      PelletHold = PelletHold + ThisIntervalValue
                      Pellet(PNum, ThisSession) = PelletHold + Lights
                      PelletHold = 0
                      PNum = PNum + 1
                   End If

                   If cbAllPellLicks = True Or cbPellLicks = True Then
                      PellLickHold = PellLickHold + ThisIntervalValue
                      PellLick(PLNum, ThisSession) = PellLickHold + Lights
                      PellLickHold = 0
                      PLNum = PLNum + 1
                   End If

                   If cbPRP = True Then
                      Select Case ThisIntervalValue
                         Case Is <= 665
                            If BeginPRP = 1 Then PRP = PRP + ThisIntervalValue
                         Case Is > 665
```

```
                        PRParray(PRPnum, ThisSession) = -1
                        If BeginPRP = 1 Then
                           PRP = PRP + ThisIntervalValue
                           PRPnum = PRPnum + 2
                        Else
                           PRPnum = PRPnum + 1
                        End If
                     End Select
                  End If 'for when cbPRP is true

                  If cbWB = True Then
                     If BeginWB = 1 Then WorkBout = WorkBout + ThisIntervalValue

'                     WorkBout = WorkBout + ThisIntervalValue
'                     WorkBoutArray(WorkBoutNum, ThisSession) = WorkBout + Lights
'                     WorkBoutNum = WorkBoutNum + 1
'                     WorkBout = 0
                  End If

               Lights = 0.0002
            End Select ' for obManualDetect

         '-----------------------------------------------
         'signal for food-oriented response event (e.g. lever press event)
         Case Is = tbFoodStepCode
            If cbAllLicks = True Or cbLicks = True Then
               LickHold = LickHold + ThisIntervalValue
            End If
            If cbAllPellets = True Or cbPellets = True Then
               PelletHold = PelletHold + ThisIntervalValue
            End If
            If cbAllPellLicks = True Or cbPellLicks = True Then
               PellLickHold = PellLickHold + ThisIntervalValue
            End If

            If cbPRP = True Then
               Select Case ThisIntervalValue
                  Case Is < 0.3
                     If BeginPRP = 1 Then PRP = PRP + ThisIntervalValue
                  Case 0.3 To 665
                     If BeginPRP = 1 Then
                        PRP = PRP + ThisIntervalValue
                        PRParray(PRPnum, ThisSession) = PRP + 0.0005
                        PRPnum = PRPnum + 1
                        PRP = 0
                        BeginPRP = 0
```

```
            End If
         Case Is > 665
            PRParray(PRPnum, ThisSession) = -1
            If BeginPRP = 1 Then
               PRP = PRP + ThisIntervalValue
               PRParray(PRPnum + 1, ThisSession) = PRP + 0.0005
               PRPnum = PRPnum + 2
               PRP = 0
               BeginPRP = 0
            Else
               PRPnum = PRPnum + 1
            End If
      End Select
   End If 'for when cbPRP is true

   If cbWB = True Then
      Select Case BeginWB
         Case Is = 0
            BeginWB = 1
         Case Is = 1
            WorkBout = WorkBout + ThisIntervalValue
      End Select
   End If

'-------------------------------------------------------------
'signal for reinforcement event (e.g. pellet delivery event)
Case Is = tbPipCode '0.0003 added to indicate pellet delivery event
   'includes all inter-pellet intervals
   If cbAllPellets = True Or cbPellets = True Then
      Pellet(PNum, ThisSession) = PelletHold + 0.0003
      PNum = PNum + 1
      PelletHold = 0
   End If
   'includes inter-event interval ending in pellet
   If cbAllPellLicks = True Or cbPellLicks = True Then
      PellLick(PLNum, ThisSession) = PellLickHold + 0.0003
      PLNum = PLNum + 1
      PellLickHold = 0
   End If

   If cbPRP = True Then
      'pellet delivery begins postreinforcement pause
      BeginPRP = 1
   End If

   If cbWB = True Then
```

```
        'pellet delivery ends work bout
        WorkBoutArray(WorkBoutNum, ThisSession) = WorkBout + 0.0003
        WorkBoutNum = WorkBoutNum + 1
        WorkBout = 0
        BeginWB = 0
    End If

'-------------------------------------------------------------
'signal for irrelevant event (e.g. non-food lever press event)
Case Is = tbFilterCode1
    If cbAllLicks = True Or cbLicks = True Then
        LickHold = LickHold + ThisIntervalValue
    End If
    If cbAllPellets = True Or cbPellets = True Then
        PelletHold = PelletHold + ThisIntervalValue
    End If
    If cbAllPellLicks = True Or cbPellLicks = True Then
        PellLickHold = PellLickHold + ThisIntervalValue
    End If
    If cbPRP = True Then
        'any post-ingestive event signals termination of PRP
        If BeginPRP = 1 Then
            PRP = PRP + ThisIntervalValue
            PRParray(PRPnum, ThisSession) = PRP + 0.0007
            PRPnum = PRPnum + 1
            PRP = 0
            BeginPRP = 0
        End If
    End If
    If cbWB = True Then
        If BeginWB = 1 Then WorkBout = WorkBout + ThisIntervalValue
    End If
'-------------------------------------------------------------
'signal for irrelevant event
Case Is = tbFilterCode2
    If cbAllLicks = True Or cbLicks = True Then
        LickHold = LickHold + ThisIntervalValue
    End If
    If cbAllPellets = True Or cbPellets = True Then
        PelletHold = PelletHold + ThisIntervalValue
    End If
    If cbAllPellLicks = True Or cbPellLicks = True Then
        PellLickHold = PellLickHold + ThisIntervalValue
    End If
    If cbPRP = True Then
        'any post-ingestive event signals termination of PRP
```

```
Select Case ThisEvent
    'signal for lick events; adding 0.0004 to indicate event is lick
    Case Is = tbLickCode
      If cbAllLicks = True Or cbLicks = True Then
        LickHold = LickHold + ThisIntervalValue
        Lick(LNum, ThisSession) = LickHold + 0.0004
        LNum = LNum + 1
        LickHold = 0
      End If

      If cbAllPellets = True Or cbPellets = True Then
        PelletHold = PelletHold + ThisIntervalValue
      End If

      If cbAllPellLicks = True Or cbPellLicks = True Then
        PellLickHold = PellLickHold + ThisIntervalValue
        PellLick(PLNum, ThisSession) = PellLickHold + 0.0004
        PLNum = PLNum + 1
        PellLickHold = 0
      End If

      If cbPRP = True Then
        Select Case ThisIntervalValue
          Case Is < 1
            If BeginPRP = 1 Then PRP = PRP + ThisIntervalValue
          Case 1 To 665
            If BeginPRP = 1 Then
              PRP = PRP + ThisIntervalValue
              PRParray(PRPnum, ThisSession) = PRP + 0.0006
              PRPnum = PRPnum + 1
              PRP = 0
              BeginPRP = 0
            End If
          Case Is > 665
            PRParray(PRPnum, ThisSession) = -1
            If BeginPRP = 1 Then
              PRP = PRP + ThisIntervalValue
              PRParray(PRPnum + 1, ThisSession) = PRP + 0.0006
              PRPnum = PRPnum + 2
              PRP = 0
              BeginPRP = 0
            Else
              PRPnum = PRPnum + 1
            End If
        End Select
      End If 'for when cbPRP is true
```

```
      If BeginPRP = 1 Then
         PRP = PRP + ThisIntervalValue
         PRParray(PRPnum, ThisSession) = PRP + 0.0007
         PRPnum = PRPnum + 1
         PRP = 0
         BeginPRP = 0
      End If
   End If
   If cbWB = True Then
      If BeginWB = 1 Then WorkBout = WorkBout + ThisIntervalValue
   End If

'-------------------------------------------------------------
'signal for irrelevant event
Case Is = tbFilterCode3 'tbFilterCode3
   If cbAllLicks = True Or cbLicks = True Then
      LickHold = LickHold + ThisIntervalValue
   End If
   If cbAllPellets = True Or cbPellets = True Then
      PelletHold = PelletHold + ThisIntervalValue
   End If
   If cbAllPellLicks = True Or cbPellLicks = True Then
      PellLickHold = PellLickHold + ThisIntervalValue
   End If
   If cbPRP = True Then
      'any post-ingestive event signals termination of PRP
      If BeginPRP = 1 Then
         PRP = PRP + ThisIntervalValue
         PRParray(PRPnum, ThisSession) = PRP + 0.0007
         PRPnum = PRPnum + 1
         PRP = 0
         BeginPRP = 0
      End If
   End If
   If cbWB = True Then
      If BeginWB = 1 Then WorkBout = WorkBout + ThisIntervalValue
   End If

'-------------------------------------------------------------
'signal for irrelevant event
Case Is = tbFilterCode4
   If cbAllLicks = True Or cbLicks = True Then
      LickHold = LickHold + ThisIntervalValue
   End If
   If cbAllPellets = True Or cbPellets = True Then
      PelletHold = PelletHold + ThisIntervalValue
```

```
    End If
    If cbAllPellLicks = True Or cbPellLicks = True Then
       PellLickHold = PellLickHold + ThisIntervalValue
    End If
    If cbPRP = True Then
       'any post-ingestive event signals termination of PRP
       If BeginPRP = 1 Then
          PRP = PRP + ThisIntervalValue
          PRParray(PRPnum, ThisSession) = PRP + 0.0007
          PRPnum = PRPnum + 1
          PRP = 0
          BeginPRP = 0
       End If
    End If
    If cbWB = True Then
       If BeginWB = 1 Then WorkBout = WorkBout + ThisIntervalValue
    End If

'-----------------------------------------------------------------
'signal for end of session
Case Is = 0.31
    If cbAllLicks = True Or cbLicks = True Then
       LickHold = LickHold + ThisIntervalValue
       Lick(LNum, ThisSession) = LickHold + Lights
       LickHold = 0
    End If

    If cbAllPellets = True Or cbPellets = True Then
       PelletHold = PelletHold + ThisIntervalValue
       Pellet(PNum, ThisSession) = PelletHold + Lights
       PelletHold = 0
    End If

    If cbAllPellLicks = True Or cbPellLicks = True Then
       PellLickHold = PellLickHold + ThisIntervalValue
       PellLick(PLNum, ThisSession) = PellLickHold + Lights
       PellLickHold = 0
    End If

    If cbPRP = True Then
       PRP = PRP + ThisIntervalValue
       PRParray(PRPnum, ThisSession) = PRP
       PRPnum = PRPnum + 1
       PRP = 0
       BeginPRP = 0
    End If
```

```
                If cbWB = True Then
                   WorkBout = WorkBout + ThisIntervalValue
                   WorkBoutArray(WorkBoutNum, ThisSession) = WorkBout + Lights
                   WorkBoutNum = WorkBoutNum + 1
                   WorkBout = 0
                   BeginWB = 0
                End If

                If PNumBound < PNum Then PNumBound = PNum
                If LNumBound < LNum Then LNumBound = LNum
                If PLNumBound < PLNum Then PLNumBound = PLNum
                If PRPNumBound < PRPnum Then PRPNumBound = PRPnum
                If WorkNumBound < WorkBoutNum Then WorkNumBound = WorkBoutNum

                PNum = 1
                LNum = 1
                PLNum = 1
                PRPnum = 1
                WorkBoutNum = 1
             End Select 'for case is 0.31
             '-------------------------------------------------------------
   Next ThisInterval
Next ThisSession

'---------------------------------------

If cbAllPellets = True Then
   If UBound(Pellet, 1) > 65536 Then
      MsgBox prompt:="One or more sessions are too long to write.", _
            Title:="Cancelling conversion", _
            Buttons:=vbExclamation
   Else: ' copy the values in Pellet array to the transfer array
       ' to increase speed of algorithm, and to prevent 'out of memory' errors
      ReDim WriteArray(1 To PNumBound, 1 To TotalSessions)
      For y = 1 To TotalSessions
         For x = 1 To PNumBound
            WriteArray(x, y) = Pellet(x, y)
         Next x
      Next y
   Call SimpleArrayToSheets(WriteArray(), "All-F")
   End If ' for UBound() > 65536 test
End If ' for cbAllPellets

If cbAllLicks = True Then
   If UBound(Lick, 1) > 65536 Then
```

```
      MsgBox prompt:="One or more sessions are too long to write.", _
            Title:="Cancelling conversion", _
            Buttons:=vbExclamation
   Else: ' copy the values in Lick array to the transfer array
      ' to increase speed of algorithm, and to prevent 'out of memory' errors
      ReDim WriteArray(1 To LNumBound, 1 To TotalSessions)
      For y = 1 To TotalSessions
         For x = 1 To LNumBound
            WriteArray(x, y) = Lick(x, y)
         Next x
      Next y
      Call SimpleArrayToSheets(WriteArray(), "All-D")
   End If ' for UBound() > 65536 test
End If ' for cbAllLicks

If cbAllPellLicks = True Then
   If UBound(PellLick, 1) > 65536 Then
      MsgBox prompt:="One or more sessions are too long to write.", _
            Title:="Cancelling conversion", _
            Buttons:=vbExclamation
   Else: ' copy the values in Pellet & Lick array to the write array
      ' to increase speed of algorithm, and to prevent 'out of memory' errors
      ReDim WriteArray(1 To PLNumBound, 1 To TotalSessions)
      For y = 1 To TotalSessions
         For x = 1 To PLNumBound
            WriteArray(x, y) = PellLick(x, y)
         Next x
      Next y
      Call SimpleArrayToSheets(WriteArray(), "AllF+D")
   End If ' for UBound() > 65536 test
End If ' for cbAllPellLicks

If cbPellets = True Then
   ReDim TransferArray(1 To PNumBound, 1 To TotalSessions)
   For y = 1 To TotalSessions
      For x = 1 To PNumBound
         If Pellet(x, y) = 0 Then Exit For
         TransferArray(x, y) = Pellet(x, y)
      Next x
   Next y
   Call EventArrayToSheets(TransferArray(), "F")
End If

If cbLicks = True Then
   ReDim TransferArray(1 To LNumBound, 1 To TotalSessions)
   For y = 1 To TotalSessions
```

```
      For x = 1 To LNumBound
         If Lick(x, y) = 0 Then Exit For
         TransferArray(x, y) = Lick(x, y)
      Next x
   Next y
   Call EventArrayToSheets(TransferArray(), "D")
End If

If cbPellLicks = True Then
   ReDim TransferArray(1 To PLNumBound, 1 To TotalSessions)
   For y = 1 To TotalSessions
      For x = 1 To PLNumBound
         If PellLick(x, y) = 0 Then Exit For
         TransferArray(x, y) = PellLick(x, y)
      Next x
   Next y
   Call EventArrayToSheets(TransferArray(), "F+D")
End If

If cbPRP = True Then
   ReDim WriteArray(1 To PRPNumBound, 1 To TotalSessions)
   For y = 1 To TotalSessions
      For x = 1 To PRPNumBound
         If PRParray(x, y) = 0 Then Exit For
         WriteArray(x, y) = PRParray(x, y)
      Next x
   Next y
   Call SimpleArrayToSheets(WriteArray(), "PRPs")
End If

If cbWB = True Then
   ReDim WriteArray(1 To WorkNumBound, 1 To TotalSessions)
   For y = 1 To TotalSessions
      For x = 1 To WorkNumBound
         If WorkBoutArray(x, y) = 0 Then Exit For
         WriteArray(x, y) = WorkBoutArray(x, y)
      Next x
   Next y
   Call SimpleArrayToSheets(WriteArray(), "WorkBouts")
End If

End Sub
Sub SimpleArrayToSheets(SimpleArray() As Currency, SimpleName)

Dim Color As Range
Dim ColorCode As Currency 'equivalent to EventType
```

```
Dim ColorRegion As Range
Dim ColorSheet As Worksheet
Dim x As Long
Dim y As Integer

x = UBound(SimpleArray, 1)
y = UBound(SimpleArray, 2) 'total sessions

Set ColorSheet = Sheets.Add
With ColorSheet
   .Name = SimpleName
   .Range(ActiveCell, Cells(x, y)) = SimpleArray
   .Range(ActiveCell, Cells(x, y)).NumberFormat = "0.00"
End With

Set ColorRegion = Range(ActiveCell.Address).CurrentRegion
For Each Color In ColorRegion
   ColorCode = Color.Value - Round(Color.Value, 2)
   Select Case ColorCode
      Case Is = 0.0006 'postreinforcement pause ending with lick event
         Color.Interior.ColorIndex = 34
      Case Is = 0.0005 'postreinforcement pause ending with lever press event
         Color.Interior.ColorIndex = 44
      Case Is = 0.0004 ' lick event
         Color.Interior.ColorIndex = 8
      Case Is = 0.0003 ' pellet delivery event
         Color.Interior.ColorIndex = 40
      Case Is = 0.0002 ' lights on
         With Color
            .Font.Bold = True
            .Interior.ColorIndex = 6
            .Font.ColorIndex = 46
         End With
      Case Is = 0.0001 ' lights off
         With Color
            .Font.Italic = True
            .Interior.ColorIndex = 32
            .Font.ColorIndex = 2
         End With
   End Select 'for EventType proxy AKA ColorCode
   If Color.Value <= 0 Then Color.Value = ""
Next Color

End Sub

Sub EventArrayToSheets(EventArray(), ArrayName)
```

```
'this sub takes the two-dimensional array containing
'unseparated sequences of diurnal and nocturnal intervals
'separates them out into three-dimensional arrays
'made up of n 2-dimensional arrays containing
'first, second...nth diurnal (or nocturnal) period
'interval data so that they can be written into
'separate worksheets

Dim LightChange As Currency
Dim Interval As Currency
Dim EventType As Currency
Dim Cell As Variant

Dim Session As Integer
Dim Diurnal() As Variant
Dim Nocturnal() As Variant
Dim TransferArray() As Currency

Dim DiurnalSheets As Worksheet
Dim NocturnalSheets As Worksheet
Dim FirstInterval As Long
Dim LastInterval As Long

Dim LightOn As Integer
Dim LightOff As Integer
Dim LightOffBound As Integer
Dim LightOnBound As Integer

TotalIntervals = UBound(EventArray, 1)
TotalSessions = UBound(EventArray, 2)

If TotalIntervals > 65536 Then TotalIntervals = 65536

ReDim Diurnal(1 To TotalIntervals, 1 To TotalSessions, 1 To 5)
ReDim Nocturnal(1 To TotalIntervals, 1 To TotalSessions, 1 To 5)

For Session = 1 To TotalSessions
FirstInterval = 0
LastInterval = 0
LightOn = 0
LightOff = 0

   For Interval = 1 To TotalIntervals
      If EventArray(Interval, Session) = 0 Then Exit For
      LightChange = EventArray(Interval, Session) - Round((EventArray(Interval, Session)), 2)
      LastInterval = LastInterval + 1
```

```
      If LightChange < 0.0003 Then
         Select Case LightChange
            Case Is = 0.0001
               LightOff = LightOff + 1
               For i = 1 To (LastInterval)
                  If EventArray((FirstInterval + i), Session) = 0 Then Exit For
                  Diurnal(i, Session, LightOff) = EventArray((FirstInterval + i), Session)
               Next i
               FirstInterval = FirstInterval + LastInterval
               LastInterval = 0
               i = 0

            Case Is = 0.0002
               LightOn = LightOn + 1
               For i = 1 To (LastInterval)
                  If EventArray((FirstInterval + i), Session) = 0 Then Exit For
                  Nocturnal(i, Session, LightOn) = EventArray((FirstInterval + i), Session)
               Next i
               FirstInterval = FirstInterval + LastInterval
               LastInterval = 0
               i = 0
         End Select 'for LightChange check
      End If
   Next Interval

   If LightOffBound < LightOff Then LightOffBound = LightOff
   If LightOnBound < LightOn Then LightOnBound = LightOn

Next Session

Application.ScreenUpdating = False

'these lines of code take each successive 2-D layer of the diurnal (or nocturnal)
'3-dimensional arrays and transfer that layers' data to a generic transfer array
'used to write to a worksheet

For z = 1 To LightOffBound
   ReDim TransferArray(1 To UBound(Diurnal, 1), 1 To UBound(Diurnal, 2))
   For y = 1 To UBound(Diurnal, 2)
      For x = 1 To UBound(Diurnal, 1)
         If Diurnal(x, y, z) = 0 Then
            Diurnal(x, y, z) = Empty
         End If
         TransferArray(x, y) = Diurnal(x, y, z)
      Next x
```

```
    Next y
    Set DiurnalSheets = Sheets.Add
    With DiurnalSheets
        .Name = "d#" & z & "-" & ArrayName
        .Range(ActiveCell, Cells(UBound(TransferArray, 1), UBound(TransferArray, 2))) =
TransferArray
        .Range(ActiveCell, Cells(UBound(TransferArray, 1), UBound(TransferArray,
2))).NumberFormat = "0.00"
        .Range(ActiveCell, Cells(UBound(TransferArray, 1), UBound(TransferArray, 2))).Select
    End With

    Cells.Replace What:="0", Replacement:="", LookAt:=xlWhole, SearchOrder _
        :=xlByRows, MatchCase:=False, SearchFormat:=True, ReplaceFormat:=False

    For Each Cell In Selection
        EventType = Cell.Value - Round(Cell.Value, 2)
        Select Case EventType
            Case Is = 0.0006 'postreinforcement pause ending with lick event
                Cell.Interior.ColorIndex = 34
            Case Is = 0.0005 'postreinforcement pause ending with lever press event
                Cell.Interior.ColorIndex = 44
            Case Is = 0.0004 ' lick event or drinking bout
                Cell.Interior.ColorIndex = 8
            Case Is = 0.0003 ' pellet delivery event or workbout
                Cell.Interior.ColorIndex = 40
            Case Is = 0.0002 ' lights on
                With Cell
                    .Font.Bold = True
                    .Interior.ColorIndex = 6
                    .Font.ColorIndex = 46
                End With
            Case Is = 0.0001 ' lights off
                With Cell
                    .Font.Italic = True
                    .Interior.ColorIndex = 32
                    .Font.ColorIndex = 2
                End With
        End Select 'for EventType
    Next Cell
Next z

For z = 1 To LightOnBound
    ReDim TransferArray(1 To UBound(Diurnal, 1), 1 To UBound(Diurnal, 2))
    For y = 1 To UBound(Nocturnal, 2)
        For x = 1 To UBound(Nocturnal, 1)
            If Nocturnal(x, y, z) = 0 Then
```

```
            Nocturnal(x, y, z) = Empty
          End If
          TransferArray(x, y) = Nocturnal(x, y, z)
        Next x
    Next y
    Set NocturnalSheets = Sheets.Add
    With NocturnalSheets
      .Name = "n#" & z & "-" & ArrayName
      .Range(ActiveCell, Cells(UBound(TransferArray, 1), UBound(TransferArray, 2))) =
TransferArray
      .Range(ActiveCell, Cells(UBound(TransferArray, 1), UBound(TransferArray,
2))).NumberFormat = "0.00"
      .Range(ActiveCell, Cells(UBound(TransferArray, 1), UBound(TransferArray, 2))).Select
    End With

    Cells.Replace What:="0", Replacement:="", LookAt:=xlWhole, SearchOrder _
      :=xlByRows, MatchCase:=False, SearchFormat:=True, ReplaceFormat:=False

    For Each Cell In Selection
      EventType = Cell.Value - Round(Cell.Value, 2)
      Select Case EventType
        Case Is = 0.0006 'postreinforcement pause ending with lick event
          Cell.Interior.ColorIndex = 34
        Case Is = 0.0005 'postreinforcement pause ending with lever press event
          Cell.Interior.ColorIndex = 44
        Case Is = 0.0004 ' lick event or drinking bout
          Cell.Interior.ColorIndex = 8
        Case Is = 0.0003 ' pellet delivery event or workbout
          Cell.Interior.ColorIndex = 40
        Case Is = 0.0002 ' lights on
          With Cell
            .Font.Bold = True
            .Interior.ColorIndex = 6
            .Font.ColorIndex = 46
          End With
        Case Is = 0.0001 ' lights off
          With Cell
            .Font.Italic = True
            .Interior.ColorIndex = 32
            .Font.ColorIndex = 2
          End With
      End Select 'for EventType
    Next Cell
Next z

Application.ScreenUpdating = True
```

```
End Sub

Private Sub MealEstimate()

Dim EventData As Variant
Dim ZeroOrderMD() As Variant
Dim ZeroOrderMS() As Variant
Dim ZeroOrderMN() As Variant
Dim MSbyTotalPell() As Variant

Dim Interval As Long
Dim TotalIntervals As Long
Dim Session As Integer
Dim TotalSessions As Long

Dim EventType As Currency
Dim IntervalLength As Currency
Dim ThisIntervalValue As Currency

Dim TMI As Currency
Dim Increment As Currency
Dim PossibleTMI As Integer
Dim PossibleTMIs As Integer
Dim MinMeal As Byte
Dim FirstPelletStartsMeal As Boolean
Dim MealStart As String
Dim Specs As String

Dim SumMealSizes As Integer
Dim SumMealDurations As Currency
Dim MealNumber As Integer
Dim TempMD As Currency
Dim TempMS As Currency
Dim AvgMD As Currency
Dim AvgMS As Currency
Dim PercentFoodAccounted As Currency
Dim TotalPellets As Integer

EventData = Range(ActiveCell.Address).CurrentRegion.Value
TotalIntervals = UBound(EventData, 1)
TotalSessions = UBound(EventData, 2)
ReDim ZeroOrderMD(1 To tbTMInum, 1 To TotalSessions)
ReDim ZeroOrderMS(1 To tbTMInum, 1 To TotalSessions)
ReDim ZeroOrderMN(1 To tbTMInum, 1 To TotalSessions)
ReDim MSbyTotalPell(1 To tbTMInum, 1 To TotalSessions)
```

```
Application.ScreenUpdating = False
MinMeal = tbMinMeal
FirstPelletStartsMeal = obPelletOnly
If FirstPelletStartsMeal = True Then MealStart = "PelStart" Else MealStart = "AnyStart"
PossibleTMIs = tbTMInum

Specs = Round(tbTMIbase, 2) & ",inc=" & tbIncrement & ",MM" & MinMeal & "," & MealStart

"----------------------------
For Session = 1 To TotalSessions
Increment = tbTMIstart

"-------------------------------------
For PossibleTMI = 1 To PossibleTMIs
   TMI = tbTMIbase ^ Increment
   Increment = Increment + tbIncrement

   TempMD = 0
   TempMS = 0
   SumMealDurations = 0
   SumMealSizes = 0
   MealNumber = 0
   AvgMD = 0
   AvgMS = 0
   PercentFoodAccounted = 0
   TotalPellets = 0

"-----------------------------------------------
      For Interval = 1 To TotalIntervals 'in this session
         ThisIntervalValue = EventData(Interval, Session)
         If ThisIntervalValue = 0 Then Exit For

         EventType = ThisIntervalValue - Round(ThisIntervalValue, 2)
         If EventType = 0.0003 Then TotalPellets = TotalPellets + 1
         IntervalLength = ThisIntervalValue - EventType

"-------------------------------------------------------------
         Select Case IntervalLength
            Case Is < TMI
               Select Case FirstPelletStartsMeal
               'does meal start with eating only, or either drinking or eating?
                  Case Is = True 'eating only
                     Select Case EventType
                        Case Is = 0.0003 'only pellet delivery event starts meal
                           If TempMS = 0 Then
```

```
                        TempMS = 1

                        TempMD = 0 'if MM=1 then there can be meal durations of 0 seconds
                     Else:
                        TempMS = TempMS + 1
                        TempMD = TempMD + IntervalLength
                     End If
                  Case Is <> 0.0003 'event is not a pellet delivery event
                     If TempMS = 0 Then
                        TempMD = 0
                     Else:
                        TempMD = TempMD + IntervalLength
                     End If
               End Select ' to see if event is pellet delivery or not

            Case Is = False 'either drinking or eating can start meal
               TempMD = TempMD + IntervalLength
               If EventType = 0.0003 Then TempMS = TempMS + 1
         End Select 'for events considered start of meal

      Case Is >= TMI
         If TempMS >= MinMeal Then
            SumMealDurations = SumMealDurations + TempMD
            SumMealSizes = SumMealSizes + TempMS
            MealNumber = MealNumber + 1
         End If

      TempMD = 0
      TempMS = 0

      If EventType = 0.0003 Then TempMS = 1
   End Select 'for IntervalLength

"-------------------------------------------------------------
   Next Interval

      If TempMS >= MinMeal Then
         SumMealDurations = SumMealDurations + TempMD
         SumMealSizes = SumMealSizes + TempMS
         MealNumber = MealNumber + 1
      End If

      TempMD = 0
      TempMS = 0

"-----------------------------------------------
```

```
    Select Case MealNumber 'prevents division by zero error
        Case Is = 0
            AvgMD = 0
            AvgMS = 0
        Case Is > 0
            AvgMD = SumMealDurations / MealNumber
            AvgMS = SumMealSizes / MealNumber
            PercentFoodAccounted = SumMealSizes / TotalPellets
    End Select

    ZeroOrderMD(PossibleTMI, Session) = AvgMD
    ZeroOrderMS(PossibleTMI, Session) = AvgMS
    ZeroOrderMN(PossibleTMI, Session) = MealNumber
    MSbyTotalPell(PossibleTMI, Session) = PercentFoodAccounted

Next PossibleTMI 'to change the possible TMI

Next Session 'to begin calculation of zero-order curve for next session

"----------------------------
Call ArrayWriter(ZeroOrderMD(), "MD", Specs)
Call ArrayWriter(ZeroOrderMS(), "MS", Specs)
Call ArrayWriter(ZeroOrderMN(), "MN", Specs)
Call ArrayWriter(MSbyTotalPell(), "%", Specs)

End Sub

Private Sub ArrayWriter(TransferArray(), n As String, Specs)

Dim ArrayToWrite As Worksheet
Dim RangeRow As Long
Dim RangeCol As Integer

RangeRow = UBound(TransferArray, 1)
RangeCol = UBound(TransferArray, 2)

Set ArrayToWrite = Sheets.Add
With ArrayToWrite
    .Range("a1", Cells(RangeRow, RangeCol)).Value = TransferArray
    .Range("a1", Cells(RangeRow, RangeCol)).NumberFormat = "general"
    .Name = n & "," & Specs
End With

End Sub
```

```
Private Sub FirstOrderCurve()
'this subroutine calculates first-order curve from zero-order curve

Dim Increment As Currency
Dim Avg As String
Dim Avg1 As Currency
Dim Avg2 As Currency
Dim FirstOrder As Variant
Dim FirstOrderCurve As Integer
Dim ZeroOrderCurve As Integer
Dim PossibleTMI As Integer
Dim TMI As Currency
Dim XAxis As Range
Dim YAxis As Range
Dim SheetName As String
Dim AllSessions As Long
Dim AllIntervals As Long

SheetName = ActiveSheet.Name
Data = Range(ActiveCell.Address).CurrentRegion.Value
AllSessions = UBound(Data, 2)
AllIntervals = UBound(Data, 1)
Application.ScreenUpdating = False

Selection.End(xlToLeft).Select
Range(ActiveCell, ActiveCell.End(xlUp)).Select
ActiveCell.Offset(0, (AllSessions + 1)).Select


For ZeroOrderCurve = 1 To AllIntervals
   Avg = "=AVERAGE(RC[" & -(AllSessions + 1) & "]:RC[-2])"
   ActiveCell.FormulaR1C1 = Avg
   Selection.Font.Bold = True
   Selection.NumberFormat = "0.000000"
   ActiveCell.Offset(1, 0).Select
Next ZeroOrderCurve

ActiveCell.Offset(-1, 0).Select
Selection.End(xlUp).Select
ActiveCell.Offset(1, 1).Select


Increment = 0
For FirstOrderCurve = 1 To AllIntervals - 1
   Avg1 = ActiveCell.Offset(0, -1).Value
   Avg2 = ActiveCell.Offset(-1, -1).Value
```

```
   FirstOrder = (Avg1 - Avg2) / tbTMIbase ^ Increment
   ActiveCell.Value = FirstOrder
   Selection.Font.ColorIndex = 3
   Selection.NumberFormat = "0.000000"
   Increment = Increment + tbIncrement
ActiveCell.Offset(1, 0).Select
Next FirstOrderCurve

ActiveCell.Offset(-1, 0).Select
Range(Selection, Selection.End(xlUp)).Select
Set YAxis = Selection
ActiveCell.Offset(0, 1).Select

Increment = tbTMIstart
For PossibleTMI = 1 To AllIntervals - 1
   TMI = tbTMIbase ^ Increment
   ActiveCell.Value = TMI
   Increment = Increment + tbIncrement
   Selection.Font.ColorIndex = 5
   Selection.Font.Italic = True
   Selection.NumberFormat = "0.0"
   ActiveCell.Offset(1, 0).Select
Next PossibleTMI

ActiveCell.Offset(-1, 0).Select
Range(Selection, Selection.End(xlUp)).Select
Set XAxis = Selection


Charts.Add
   With ActiveChart
      .ChartType = xlLineMarkers
      .SeriesCollection(1).Values = YAxis
      .SeriesCollection(1).XValues = XAxis
      .HasLegend = False
      .HasTitle = True
      .ChartTitle.Characters.Text = "First-order curve"
      .Axes(xlCategory, xlPrimary).HasTitle = True
      .Axes(xlCategory, xlPrimary).AxisTitle.Characters.Text = "Possible TMIs"
      .Axes(xlValue, xlPrimary).HasTitle = True
      .Axes(xlValue, xlPrimary).AxisTitle.Characters.Text = "Rate of change"
      .Axes(xlValue).TickLabels.NumberFormat = "general"
      .Location Where:=xlLocationAsObject, Name:=SheetName
   End With

'   ActiveChart.HasLegend = False
```

```
    ActiveChart.PlotArea.Select
        With Selection.Border
            .ColorIndex = 16
            .Weight = xlThin
            .LineStyle = xlContinuous
        End With

    Selection.Interior.ColorIndex = xlNone
    ActiveChart.Axes(xlCategory).Select

    Selection.TickLabels.NumberFormat = "0"
    With Selection.TickLabels
        .Alignment = xlCenter
        .Offset = 100
        .ReadingOrder = xlContext
        .Orientation = xlUpward
    End With

'      ActiveChart.Axes(xlValue).Select
'    Selection.TickLabels.NumberFormat = "General"
'
    ActiveChart.ChartArea.Select
'resets session counter
AllSessions = 0

ActiveChart.SeriesCollection(1).Select
    With Selection.Border
        .Weight = xlThin
        .LineStyle = xlAutomatic
    End With
    With Selection
        .MarkerBackgroundColorIndex = xlAutomatic
        .MarkerForegroundColorIndex = xlAutomatic
        .MarkerStyle = xlNone
        .Smooth = False
        .MarkerSize = 5
        .Shadow = False
    End With

End Sub

Sub SequentialMealsCalculator()

Dim EventData As Variant
Dim MDarray() As Variant
```

```
Dim MSarray() As Variant
Dim IMIarray() As Variant
Dim MealLickArray() As Variant
Dim IMILickArray() As Variant
Dim AvgMeals() As Variant

Dim MDnum As Integer
Dim MSnum As Integer
Dim IMInum As Integer
Dim MealLickNum As Integer
Dim IMILickNum As Integer

Dim Interval As Long
Dim TotalIntervals As Long
Dim Session As Integer
Dim TotalSessions As Long

Dim EventType As Currency
Dim IntervalLength As Currency
Dim ThisIntervalValue As Currency

Dim ThresholdInterval As Currency
Dim MinMeal As Byte
Dim FirstPelletStartsMeal As Boolean
Dim MealStart As String
Dim Specs As String

Dim MealDuration As Currency
Dim MealSize As Integer
Dim IMI As Currency
Dim EatingRate As Currency
Dim IMILicks As Integer
Dim MealLicks As Integer

Dim TotalMD As Currency
Dim TotalMS As Currency
Dim TotalIMI As Currency
Dim TotalIMILicks
Dim TotalMealLicks
Dim TotalLicks As Integer
Dim TotalPellets As Integer

EventData = Range(ActiveCell.Address).CurrentRegion.Value
TotalIntervals = UBound(EventData, 1)
TotalSessions = UBound(EventData, 2)
ReDim MDarray(1 To TotalIntervals, 1 To TotalSessions)
```

```
ReDim MSarray(1 To TotalIntervals, 1 To TotalSessions)
ReDim IMIarray(1 To TotalIntervals, 1 To TotalSessions)
ReDim MealLickArray(1 To TotalIntervals, 1 To TotalSessions)
ReDim IMILickArray(1 To TotalIntervals, 1 To TotalSessions)
ReDim AvgMeals(1 To 12, 1 To TotalSessions + 1)

Application.ScreenUpdating = False
ThresholdInterval = tbThresholdInterval
MinMeal = tbMM
FirstPelletStartsMeal = obFoodMealStart
If FirstPelletStartsMeal = True Then MealStart = "FoodOnly" Else MealStart = "AnyStart"
Specs = ThresholdInterval & "-" & MinMeal & "," & MealStart

"----------------------------
For Session = 1 To TotalSessions
'all variables reset to zero for new session
   MealSize = 0
   MealDuration = 0
   IMI = 0
   EatingRate = 0
   MealLicks = 0
   IMILicks = 0
   TotalMD = 0
   TotalMS = 0
   TotalIMI = 0
   TotalIMILicks = 0
   TotalMealLicks = 0
   TotalLicks = 0
   TotalPellets = 0

   MDnum = 0
   MSnum = 0
   IMInum = 0
   MealLickNum = 0
   IMILickNum = 0
"-----------------------------------------------
      For Interval = 1 To TotalIntervals 'in this session
         ThisIntervalValue = EventData(Interval, Session)
         If ThisIntervalValue = 0 Then Exit For

         EventType = ThisIntervalValue - Round(ThisIntervalValue, 2)
         IntervalLength = ThisIntervalValue - EventType

         Select Case EventType
            Case Is = 0.0003: TotalPellets = TotalPellets + 1
            Case Is = 0.0004: TotalLicks = TotalLicks + 1
```

```
        End Select

"-------------------------------------------------------------
        Select Case IntervalLength

           Case Is < ThresholdInterval
              Select Case FirstPelletStartsMeal
              'does meal start with eating only (pellet delivery event),
              'or can drinking (lick event) also start a meal as in Zorrilla et al. 2005?
                 Case Is = True 'only eating event starts a meal
                    Select Case EventType
                       Case Is = 0.0003 'an eating (pellet delivery) event
                          If MealSize = 0 Then
                             MealSize = 1
                             IMI = IMI + MealDuration + IntervalLength
                             MealDuration = 0 'if MM=1 then there can be meal durations of 0 seconds
                             IMILicks = IMILicks + MealLicks
                             MealLicks = 0
                          Else: 'if mouse has acquired at least 1 pellet
                             MealSize = MealSize + 1
                             MealDuration = MealDuration + IntervalLength
                          End If 'for eating event
                       Case Is <> 0.0003
                          If EventType = 0.0004 Then 'a drinking (lick) event
                             MealLicks = MealLicks + 1
                          End If 'since interval is < TMI lick event is provisionally within-meal

                          If MealSize = 0 Then 'unless mouse has not yet eaten a food pellet
                             IMI = IMI + MealDuration + IntervalLength
                             MealDuration = 0
                             'provisional within-meal lick(s) are also delegated to preceding IMI
                             IMILicks = IMILicks + MealLicks
                             MealLicks = 0
                          Else: 'if mouse has eaten (acquired) at least 1 food pellet
                             MealDuration = MealDuration + IntervalLength
                          End If
                    End Select ' to see if event is pellet delivery or not

                 Case Is = False 'either drinking or eating can start meal
                 'n.b. - if very first interval is < TMI then it is written
                 '      to IMI array and represents latency to first meal
                    If Interval = 1 Then
                       IMI = IntervalLength
                    Else
                       MealDuration = MealDuration + IntervalLength
                    End If
```

```
            If EventType = 0.0003 Then MealSize = MealSize + 1
            If EventType = 0.0004 Then MealLicks = MealLicks + 1
        End Select 'for events considered start of meal

    Case Is >= TMI
        If MealSize >= MinMeal Then
            'a 'real' meal has ended and meal values are written to meal arrays
            MDnum = MDnum + 1
            MDarray(MDnum, Session) = MealDuration / 60
            TotalMD = TotalMD + MealDuration

            MSnum = MSnum + 1
            MSarray(MSnum, Session) = MealSize
            TotalMS = TotalMS + MealSize

            EatingRate = EatingRate + (MealSize / (MealDuration / 60))

            MealLickNum = MealLickNum + 1
            MealLickArray(MealLickNum, Session) = MealLicks
            TotalMealLicks = TotalMealLicks + MealLicks

            'confirmed 'real' meal also confirms that preceding IMI values are final
            IMInum = IMInum + 1
            IMIarray(IMInum, Session) = IMI / 60 'only converted from sec to min
            TotalIMI = TotalIMI + IMI           'just before writing to array
            IMI = IntervalLength                'otherwise only values in sec are manipulated

            IMILickNum = IMILickNum + 1
            IMILickArray(IMILickNum, Session) = IMILicks
            TotalIMILicks = TotalIMILicks + IMILicks
            IMILicks = 0

        Else: 'meal was not 'real' so its values are added to IMI
            IMI = IMI + MealDuration + IntervalLength
            IMILicks = IMILicks + MealLicks

        End If 'for test of whether putative meal is 'real' or not

        'regardless of whether a meal was achieved or not
        'all meal-related values are zeroed
        MealDuration = 0
        MealSize = 0
        MealLicks = 0

        'pellet delivery or lick events "innocent until proven guilty"
        'thus attributed to possible nascent meal
```

```
            If EventType = 0.0003 Then MealSize = 1
            If EventType = 0.0004 Then MealLicks = MealLicks + 1
        End Select 'for IntervalLength

    Next Interval
"-------------------------------------------------------------
        'to tie up loose ends for final meal and/or IMI
        If MealSize >= MinMeal Then 'last real meal has ended
            MDnum = MDnum + 1
            MDarray(MDnum, Session) = MealDuration / 60 'to convert MD from sec to min before
writing to array
            TotalMD = TotalMD + MealDuration

            MSnum = MSnum + 1
            MSarray(MSnum, Session) = MealSize
            TotalMS = TotalMS + MealSize

            EatingRate = EatingRate + (MealSize / (MealDuration / 60))

            MealLickNum = MealLickNum + 1
            MealLickArray(MealLickNum, Session) = MealLicks
            TotalMealLicks = TotalMealLicks + MealLicks

            IMInum = IMInum + 1
            IMIarray(IMInum, Session) = IMI / 60
            TotalIMI = TotalIMI + IMI

            IMILickNum = IMILickNum + 1
            IMILickArray(IMILickNum, Session) = IMILicks
            TotalIMILicks = TotalIMILicks + IMILicks

        Else: 'period ends with IMI not meal
            IMI = IMI + MealDuration
            IMInum = IMInum + 1
            IMIarray(IMInum, Session) = IMI / 60    'to convert IMI from sec to min before writing to
array
            TotalIMI = TotalIMI + IMI

            IMILickNum = IMILickNum + 1
            IMILicks = IMILicks + MealLicks
            IMILickArray(IMILickNum, Session) = IMILicks
            TotalIMILicks = TotalIMILicks + IMILicks
        End If

If cbAvgMeals = True Then
```

```
    'embarrassingly kludgy way to prevent division errors
    If TotalLicks = 0 Then TotalLicks = 1
    If TotalPellets = 0 Then TotalPellets = 1
    If MDnum = 0 Then MDnum = 1
    If MSnum = 0 Then MSnum = 1
    If TotalMD = 0 Then TotalMD = 1
    If TotalMD = 0 Then TotalMD = 1
    If IMILickNum = 0 Then IMILickNum = 1
    If MealLickNum = 0 Then MealLickNum = 1

    AvgMeals(1, Session + 1) = TotalPellets
    AvgMeals(2, Session + 1) = TotalLicks
    AvgMeals(3, Session + 1) = MDnum                    'same as meal number
    AvgMeals(4, Session + 1) = (TotalMD / MDnum) / 60   'average meal duration
    AvgMeals(5, Session + 1) = TotalMS / MSnum          'average meal size
    AvgMeals(6, Session + 1) = (TotalIMI / IMInum) / 60  'average intermeal interval
    AvgMeals(7, Session + 1) = EatingRate / MDnum       'average eating rate
    AvgMeals(8, Session + 1) = TotalMealLicks / MealLickNum 'average prandial licks
    AvgMeals(9, Session + 1) = TotalIMILicks / IMILickNum   'average non-prandial licks
    AvgMeals(10, Session + 1) = (1 - (TotalMS / TotalPellets)) * 100 '% non-meal food
    AvgMeals(11, Session + 1) = (TotalMealLicks / TotalLicks) * 100 '% meal licks
    AvgMeals(12, Session + 1) = (TotalIMILicks / TotalLicks) * 100 '% IMI licks
End If

"-----------------------------------------------

Next Session 'to begin calculation of zero-order curve for next session

"----------------------------
'Meal values have been calculated for every session

If cbSequential = True Then
    Call MealWriter(MDarray(), "MD", Specs)
    Call MealWriter(MSarray(), "MS", Specs)
    Call MealWriter(MealLickArray(), "MealLicks", Specs)
    Call MealWriter(IMIarray(), "IMI", Specs)
    Call MealWriter(IMILickArray(), "IMILicks", Specs)
End If

If cbAvgMeals = True Then
    AvgMeals(1, 1) = "TotalPellets"
    AvgMeals(2, 1) = "TotalLicks"
    AvgMeals(3, 1) = "Meal Number"
    AvgMeals(4, 1) = "Avg Meal Duration (min)"
    AvgMeals(5, 1) = "Avg Meal Size (pellets)"
    AvgMeals(6, 1) = "Avg Intermeal Interval (min)"
```

```
    AvgMeals(7, 1) = "Avg Eating Rate (pellets/min)"
    AvgMeals(8, 1) = "Avg Prandial Licks"
    AvgMeals(9, 1) = "Avg Non-prandial licks"
    AvgMeals(10, 1) = "% Non-meal food"
    AvgMeals(11, 1) = "% Meal licks"
    AvgMeals(12, 1) = "% IMI licks"

    Call MealWriter(AvgMeals(), "Avgs&Totals", Specs)
End If

End Sub

Private Sub MealWriter(TransferArray(), n As String, Specs)

Dim ArrayToWrite As Worksheet
Dim RangeRow As Long
Dim RangeCol As Integer

RangeRow = UBound(TransferArray, 1)
RangeCol = UBound(TransferArray, 2)

Set ArrayToWrite = Sheets.Add
With ArrayToWrite
    .Range("a1", Cells(RangeRow, RangeCol)).Value = TransferArray
    .Range("a1", Cells(RangeRow, RangeCol)).NumberFormat = "0.0"
    .Name = n & "," & Specs
End With

End Sub

Private Sub OldSequentialMealCalculator()

'FINDS SEQUENTIAL MEAL SIZES, MEAL DURATIONS AND INTERMEAL INTERVALS
'AT A USER-DEFINED THRESHOLD MEAL INTERVAL AND MINIMUM MEAL
'FOR ALL SELECTED SESSIONS

'Version 1.0    - Wrote code to calculate sequential MS and MD values; IMI not yet functional.
'Version 2.0    - Debugged code that calculates sequantial IMIs.
'               -Accuracy of all values calculated with code confirmed by hand-calculation.
'Version 3.0    - Wrote and debugged code to calculate meal-intermeal sequence.
'Version 3.1    - Added TotalPell variable to count all nocturnal pellets whether meal or "snack"
'               - Also automatically labels meal tabs with TMI and MM.
'Version 3.2    - Rem'd the total pellet write and moved code to convert all formats from currency to
general
'               -Also rem'd the subroutine that writes order of meal-intermeal intervals.
'               -Can be activated by removing ' before subroutine Call command.
```

```
'Version 4.0    -Replacing with tailored version of MealEstimate algorithm; faster, more elegant
'               -keeping this macro in case I need to troubleshoot or debug as this is the most
'               -validated algorithm for calculating sequential meals that I've written to date

Dim MealDur As Currency 'Meal Duration estimations based on MinMeal pellets considered a
minimum meal
Dim TempMealDur As Currency 'Temporary meal duration that holds duration values until MM is
realized
Dim MealNum As Integer 'Meal number
Dim MealSize As Integer 'number of pellet events within putative meal
Dim TotalPell As Integer 'all nocturnal pellets delivered
Dim LickNum As Integer 'to count within-meal licks
Dim Licks As Integer
Dim TotalLicks As Integer
Dim IMI As Currency 'Intermeal interval
Dim IMInum As Integer 'Inter meal interval array counter
Dim MealIMINum As Integer 'counter for meal-IMI array
Dim MealIMITest As Integer 'to test if meal or IMI comes first

'sets upper limit to allowable duration of initial IMI
'this is to prevent IMIs of ~30 second generated when AllSessions
'immediately lever press to criterion for food pellet
Dim FirstIMICutoff As Integer
'set to 0 if you want to get ESTIMATES of latency to first meal
'ESTIMATES because the first interval in PellLicks is not starting
'from exactly when the lights go out, but from the first nocturnal
'event
'FirstIMICutoff = 0

Dim MealSizeArray() As Variant 'array to hold sequential meal sizes
Dim MealDurArray() As Variant 'array to hold sequential meal durations
Dim IMIarray() As Variant 'array to hold sequential intermeal interval durations
Dim MealIMIArray() As Variant 'array to hold sequential meal-intermeal durations
Dim MealLickArray() As Variant
Dim ThisIntervalLength As Currency
Dim EventType As Currency
Dim AllSessions As Integer 'number of sessions in data set

'to easily change which sheet the program reads interval data from to calculate meal values
Dim SessionData As String
'Default worksheet name from "ProcessDataForMealDefinition" algorithm is PellLicks
SessionData = ActiveSheet.Name


Dim MinMeal As Integer 'minimum meal criteria
Dim ThresholdInterval As Currency 'changing assumed threshold interval for meal definition
```

```
Dim MealSheet As Object 'name of worksheet with sequential meal parameter values

rg = Range(ActiveCell.Address).CurrentRegion.Value
AllSessions = UBound(rg, 2)
ThresholdInterval = tbThresholdInterval
MinMeal = tbMM

Application.ScreenUpdating = False

Set MealSheet = Worksheets.Add
MealSheet.Name = "Meals@TMI" & ThresholdInterval & "-MM" & MinMeal

Sheets(SessionData).Select
Range("a1").Select

For Session = 1 To AllSessions

    Selection.End(xlUp).Select
    Range(Selection, Selection.End(xlDown)).Select

        For Each Cell In Selection
          EventType = Cell.Value - Round(Cell.Value, 2)
          ThisIntervalLength = Cell.Value - EventType

          Select Case ThisIntervalLength
            Case Is < ThresholdInterval
            'These lines of code make sure that first pellet interval of a possible meal
            'is not included in the next meal duration.
            'They also make sure that first pellet intervals are included in the preceding IMI

              If EventType = 0.0003 Then MealSize = MealSize + 1
                Select Case EventType
                  Case Is = 0.0003
                    Select Case MealSize
                      Case Is = 1
                        IMI = IMI + ThisIntervalLength + TempMealDur
                        TempMealDur = 0
                      Case Is > 1
                        TempMealDur = TempMealDur + ThisIntervalLength
                    'end of mealsize select case
                    End Select
                  'color index case
                  Case Is = 0.0004
                    TempMealDur = TempMealDur + ThisIntervalLength
                    Licks = Licks + 1
                  Case Is < 3
```

```
            TempMealDur = TempMealDur + ThisIntervalLength
        'end of cell color select case
        End Select

Case Is >= ThresholdInterval

    'minimum meal criteria is MinMeal pellets
    Select Case MealSize
    'MM IS SATISFIED
    Case Is >= MinMeal
          LickNum = LickNum + 1
          MealNum = MealNum + 1
          MealDur = TempMealDur
          TempMealDur = 0
          'Writing meal size and meal durations for bona fide meal
          ReDim Preserve MealSizeArray(MealNum)
             MealSizeArray(MealNum) = MealSize
          ReDim Preserve MealDurArray(MealNum)
             MealDurArray(MealNum) = MealDur
          ReDim Preserve MealLickArray(LickNum)
             MealLickArray(LickNum) = Licks
           Licks = 0

          'Writing intermeal interval values and resetting for next IMI
          Select Case IMI
             'This case is satisfied when an IMI happens first
             Case Is > FirstIMICutoff
                'writes the IMI to its array
                IMInum = IMInum + 1
                ReDim Preserve IMIarray(IMInum)
                IMIarray(IMInum) = IMI

                'writes the IMI and MD to the Meal-IMI array in IMI->MD order
                MealIMINum = MealIMINum + 1
                   ReDim Preserve MealIMIArray(MealIMINum)
                      MealIMIArray(MealIMINum) = IMI + 0.001
                MealIMINum = MealIMINum + 1
                   ReDim Preserve MealIMIArray(MealIMINum)
                      MealIMIArray(MealIMINum) = MealDur


             'This case is satisfied when meal happens first,
             'e.g. the initial IMI is 60 seconds or less
             '(or whatever FirstIMICutoff is set to).
             'This is done to filter initial IMIs that reflect
             'the mouse essentially eating immediately after
```

```
            'the beginning of the nocturnal period.
            'This case should only happen at the beginning of
            'the period of measurement except in instances
            'where the TMI is < 60 seconds
            'by default it is set to 0 to include all putative IMIs
            Case Is <= FirstIMICutoff
               Select Case MealNum
                  'doesn't write initial IMIs that are formally correct,
                  'but experimentally incorrect.
                  Case Is = 1
                     MealIMINum = MealIMINum + 1
                     ReDim Preserve MealIMIArray(MealIMINum)
                     MealIMIArray(MealIMINum) = MealDur
                     'also don't want to reset IMI to 0 in case
                     'subsequent intervals also sum with IMI

                  'if the IMI is < FirstIMICutoff and it isn't the first
                  'meal anymore, than the unusually low IMI should NOT
                  'be discarded
                  Case Is > 1
                     'writes the IMI to its array
                     IMInum = IMInum + 1
                     ReDim Preserve IMIarray(IMInum)
                     IMIarray(IMInum) = IMI

                     'writes the IMI and MD to the Meal-IMI array in IMI->MD order
                     MealIMINum = MealIMINum + 1
                     ReDim Preserve MealIMIArray(MealIMINum)
                     MealIMIArray(MealIMINum) = IMI + 0.001

                     MealIMINum = MealIMINum + 1
                     ReDim Preserve MealIMIArray(MealIMINum)
                     MealIMIArray(MealIMINum) = MealDur
               'this is end select for IMIs less than FirstMealCutoff
               End Select
         'This is end select for IMI case
         End Select

      'guarantees that current interval is included in next IMI
      'also guarantees that all subsequent IMI cases will <>0
      IMI = ThisIntervalLength
      'resets MS and MD for next meal
      MealSize = 0
      MealDur = 0
      Licks = 0
```

```
                    'MM IS *NOT* SATISFIED
                    Case Is < MinMeal
                        IMI = IMI + ThisIntervalLength + TempMealDur
                        MealSize = 0
                        TempMealDur = 0
                        Licks = 0

                    'This is end select for case that tests MM satisfaction
                    End Select

                'count pellet for next (possible) meal
                If EventType = 0.0003 Then MealSize = MealSize + 1
                'this interval is an inter-meal interval, as such
                'doesn't belong in the preceding or (maybe) proceding meal

            'This is the end select to see how the current interval compares to the TMI
            End Select

        'This will tally all meal AND non-meal food pellets dispensed during the evening
        If EventType = 0.0003 Then TotalPell = TotalPell + 1
        If EventType = 0.0004 Then TotalLicks = TotalLicks + 1
        Next Cell

'to get (possible) final meal of day for given mouse
Select Case MealSize
   Case Is >= MinMeal
      LickNum = LickNum + 1
      MealNum = MealNum + 1
      MealDur = TempMealDur
      TempMealDur = 0

      'Writing meal size and meal durations for bona fide meal
      ReDim Preserve MealSizeArray(MealNum)
      MealSizeArray(MealNum) = MealSize
      ReDim Preserve MealDurArray(MealNum)
      MealDurArray(MealNum) = MealDur
      ReDim Preserve MealLickArray(LickNum)
      MealLickArray(LickNum) = Licks
      Licks = 0

      'writes the IMI to its array
      IMInum = IMInum + 1
      ReDim Preserve IMIarray(IMInum)
      IMIarray(IMInum) = IMI

      'writes the IMI and MD to the Meal-IMI array in IMI->MD order
```

```
        MealIMINum = MealIMINum + 1
        ReDim Preserve MealIMIArray(MealIMINum)
        MealIMIArray(MealIMINum) = IMI + 0.001
        MealIMINum = MealIMINum + 1
        ReDim Preserve MealIMIArray(MealIMINum)
        MealIMIArray(MealIMINum) = MealDur

    Case Is < MinMeal
        IMInum = IMInum + 1
        ReDim Preserve IMIarray(IMInum)
        IMI = IMI + TempMealDur
        IMIarray(IMInum) = IMI

        MealIMINum = MealIMINum + 1
        ReDim Preserve MealIMIArray(MealIMINum)
        MealIMIArray(MealIMINum) = IMI + 0.001
End Select

    'writes avg meal duration for desired threshold interval
    Call WriteMealDurArray(MealDurArray, MealNum, Session, ThresholdInterval, MinMeal,
SessionData, MealSheet)
    'writes number of pellet and lick events avg for desired threshold interval
    Call WriteMealSizesArray(MealSizeArray, MealNum, Session, TotalPell, ThresholdInterval,
MinMeal, SessionData, MealSheet)
    Call WriteLickSizesArray(MealLickArray, LickNum, Session, TotalLicks, ThresholdInterval,
MinMeal, SessionData, MealSheet)

    'writes each IMI in sequential order as added to IMI array for desired threshold interval
    Call WriteIMIArray(IMIarray, IMInum, Session, ThresholdInterval, MinMeal, SessionData,
MealSheet)
    'writes MDs and IMIs in order that they occur to determine .
    Call WriteMealIMIArray(MealIMIArray, MealIMINum, Session, ThresholdInterval, MinMeal,
SessionData, MealSheet)

        ReDim MealDurArray(1)
        ReDim MealSizeArray(1)
        ReDim IMIarray(1)
        ReDim MealIMIArray(1)
        ReDim MealLickArray(1)

            MealDur = 0
            TempMealDur = 0
            MealNum = 0
            LickNum = 0
            Licks = 0
            IMI = 0
```

```
        IMInum = 0
        MealIMINum = 0
        MealSize = 0
        TotalPell = 0
        TotalLicks = 0
        ThisIntervalLength = 0
        EventType = 0

      Sheets(SessionData).Select

   'increments over to next mouse
   ActiveCell.Offset(0, 1).Select

Next Session

ScreenUpdating = True
Sheets(MealSheet.Name).Select

End Sub
Sub WriteMealDurArray(MealDurArray, MealNum, Session, ThresholdInterval, MinMeal,
SessionData, MealSheet)

Sheets(MealSheet.Name).Select
Cells(1, Session).Activate

For i = 1 To MealNum
   ActiveCell.Value = MealDurArray(i)
   ActiveCell.NumberFormat = "0"
   ActiveCell.Offset(1, 0).Select
Next i


Sheets(SessionData).Select

End Sub

Sub WriteMealSizesArray(MealSizeArray, MealNum, Session, TotalPell, ThresholdInterval,
MinMeal, SessionData, MealSheet)

Sheets(MealSheet.Name).Select
Cells(101, Session).Activate

'These lines of code will write total number of pellets consumed in bold
ActiveCell.Value = TotalPell
Selection.Font.Bold = True
Selection.Interior.ColorIndex = 40
```

```
ActiveCell.Offset(1, 0).Select

For i = 1 To MealNum
   ActiveCell.Value = MealSizeArray(i)
   ActiveCell.NumberFormat = "0"
   ActiveCell.Offset(1, 0).Select
Next i

Sheets(SessionData).Select

End Sub
Sub WriteLickSizesArray(MealLickArray, LickNum, Session, TotalLicks, ThresholdInterval,
MinMeal, SessionData, MealSheet)

Sheets(MealSheet.Name).Select
Cells(201, Session).Activate

'These lines of code will write total number of pellets consumed in bold
ActiveCell.Value = TotalLicks
Selection.Font.Bold = True
Selection.Interior.ColorIndex = 8
ActiveCell.Offset(1, 0).Select

For i = 1 To LickNum
   ActiveCell.Value = MealLickArray(i)
   ActiveCell.NumberFormat = "0"
   ActiveCell.Offset(1, 0).Select
Next i

Sheets(SessionData).Select

End Sub
Sub WriteIMIArray(IMIarray, IMInum, Session, ThresholdInterval, MinMeal, SessionData,
MealSheet)

Sheets(MealSheet.Name).Select
Cells(301, Session).Activate

For i = 1 To IMInum
   ActiveCell.Value = IMIarray(i)
   ActiveCell.Interior.ColorIndex = 6
   ActiveCell.NumberFormat = "0"
   ActiveCell.Offset(1, 0).Select
Next i

Sheets(SessionData).Select
```

```
End Sub

Sub WriteMealIMIArray(MealIMIArray, MealIMINum, Session, ThresholdInterval, MinMeal,
SessionData, MealSheet)

Dim IMITest1 As Currency
Dim IMITest2 As Currency

Sheets(MealSheet.Name).Select
Cells(401, Session).Activate

For i = 1 To MealIMINum
   IMITest1 = MealIMIArray(i) * 100
   IMITest2 = (IMITest1) - Int(IMITest1)
   If IMITest2 > 0 Then ActiveCell.Interior.ColorIndex = 6
   If IMITest2 > 0 Then ActiveCell.Value = MealIMIArray(i) - 0.001 Else ActiveCell.Value =
MealIMIArray(i)
   ActiveCell.NumberFormat = "0"
   ActiveCell.Offset(1, 0).Select
Next i

Sheets(SessionData).Select

End Sub
```

```
\ FR1-FRN-TLN-LEY : FIXED RATIO 1
\ FRN- Food lever Retract NO
\ TLN- Training Light changes No
\ LEY- Light events recorded YES

\ HOUSELIGHT stays on after end of session
\ WILL ONLY RECORD 999,918 DATA POINTS WHEN USING NEW SOFTWARE/HARDWARE
\ *** NOTE: TIME COUNT HAS CHANGED TO 10ms TO CATCH ALL DURATIONS BETWEEN MOUSE
LICKS
\ source program was l8FR.MPC FIXED RATIO SOURCE PROGRAM FOR MEDLAB8
\ code rewritten and edited by Chris Richard
\  CONSTANTS USED IN THIS PROGRAM
\  Edit input and output #'s if different for your system

                  \  Inputs ****************************
^Lever1 = 1       \  Food lever
^Lever2 = 2       \  Water Lever (dummy)
^licks = 3        \  Lickometer for sipper lick counts

                  \  Outputs *****************************
^foodlev = 1      \  Food lever
^waterlev = 2     \  Water lever
^Pellet = 5       \  Food hopper
^sipper = 4       \  Retractable sipper
^foodlite = 3     \  Food stimulus light
^waterlite = 6    \  Water stimulus light
^houselight = 7   \  House light
^M = 1380         \  Time of session in minutes

DIM C = 999918   \  Dimension Array C for x data points.
DIM Z = 1         \  Z(0) = 0, no reinforcement lighting, Z(0)=1, reinforcement
lighting
LIST F = 7,19     \  Military hour for lights on, lights off

\  VARIABLES USED IN THIS PROGRAM

\   B  =  Counter for water Lever
\  (C) =  Inter-Response Time (IRT) Array
\   D  =  Reinforcement Counter
\  (F) =  Array containing day/night cycle hour (military hours)
\   H  =  Hour     (DOS time)
\   I  =  Subscript for the IRT Array C.
\   J  =  Minutes variable for TIME command (not used)
\   K  =  Seconds variable for TIME command (not used)
\   M  =  Session Time in Minutes. If not set program will run continuously.
\   L  =  Counter for food lever
\   T  =  Clock Ticks for IRT's.  Resolution = 0.1 second.
\   X  =  Response Ratio set by User.   Default = 1
```

```
\   Y  =  Response Ratio used by program. Updates to new value
\         at Start and following reinforcement.
\   S  =  Counter for sips (licks)
\  (Z) =  Array to indicate reinforcement lighting

\   Z-PULSES USED IN THIS PROGRAM
\   Z1 =  Reinforcement Pulse
\   Z2 =  Reset Pulse for IRT Timer/Counter
\   Z3 =  Light hours reinforcement lighting pattern
\   Z4 =  Dark hours reinforcement lighting pattern
\   Z5 =  Resets (Z) to non-reinforcement status, i.e. 0
\   ************************************************
\                   FIXED RATIO SCHEDULE
\   Default value = 1
\   Change Variable X to change ratio
\   Changes after procedure is started only take effect
\       after current ratio has been satisfied.
\   ************************************************

S.S.1,
S1,
   #start:ON ^foodlev,^sipper,^houselight;IF X = 0 [@True,@False]
             @True:SET X = 1,Y=X--->S2
             @False:SET Y = X--->S2
S2,
   Y#R^Lever1:on ^pellet;Z1--->S3
S3,
   .05":off ^pellet;SET Y = X--->S2



S.S.2,  \food lever COUNTER
S1,
  #start:SHOW 1,Lever1,L--->S2
S2,
   #R^Lever1:ADD L;SHOW 1,Lever1,L--->SX



S.S.3,    \ Food REINFORCEMENT COUNTER
S1,
   #start:SHOW 2,Rein,D--->S2
S2,
   #Z1:ADD D;SHOW 2,Rein,D;SET Z(0)=1--->SX


S.S.5,   \ DISPLAY FIXED RATIO VALUE AND SESSION TIME
S1,
```

```
  #Start:--->S2
S2,
   .1":SHOW 6,FR=,Y,5,SESS_N,M--->SX



S.S.6,  \TIME T IS INCREMENTED IN UNITS OF 0.01 SECONDS
S1,
  #Start:--->S2
S2,
   #Z2--->S2         \ Z-PULSE FOR Z2 OCCURS AT THE BEGINNING OF ANY EVENT
   0.01":ADD T--->SX  \EVERY 0.01" TIME T INCREMENTS UP BY 1



S.S.7,   \ ARRAY SET WITH IRT, INCREMENTED TO NEXT EMPTY POINT AND SET WITH -
987.987
S1,
   #Start:--->S2
S2,           \ TRACE for food lever (0.1)
   #R^Lever1:SET C(I) = T+0.1,T = 0;Z2;ADD I;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX

S.S.8,   \ SET REINFORCEMENT MARK (0.2) FOR FOOD LEVER
S1,
   #start:--->S2
S2,
   #Z1:SET C(I) = 0.2;ADD I;IF I = 999918 [@True,@False]
            @True:--->STOPabortflush
            @False:SET C(I) = -987.987--->SX

\EACH TIME REINFORCEMENT Z1 SATISFIED, 0.2 MARKER PUT IN C(I),
\ARRAY INCREMENTED C(I+1)AND -987.987 PUT TEMPORARILY INTO C(I+1)


S.S.9,   \WATER LEVER PRESS
S1,      \ SETS EVENT PEN TO BASELINE
  #Start:SHOW 4,Lever2,B--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME WATER LEVER PRESSED
   #R^Lever2:ADD B;SHOW 4,Lever2,B;
             SET C(I) = T + 0.60, C(I+1) = 0.50, T=0, I=I+2;Z2;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX
```

```
S.S.10,   \ MOUSE LICK EVENTS
S1,
   #START: SHOW 3,Licks,S--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME MOUSE LICKS
   #R^licks:ADD S;SHOW 3,Licks,S;
            SET C(I) = T + 0.61, C(I+1) = 0.51, T=0, I=I+2;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX

S.S.11,       \Records lights out event
S1,
   #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(1)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowNight:SET C(I) = T + 0.62, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
         @StillDay:--->S2



S.S.12,       \Recored lights on event
S1,
     #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(0)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowDay:SET C(I) = T + 0.52, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
        @StillNight:--->S2



S.S.13,        \SESSION CLOCK
S1,
   #start:SET M=^M;IF M =0  [@True,@False]
            @True:--->S2
            @False:--->S3
S2,
   .1":IF M >0[@True,@False]
          @True:--->S3
          @False:--->SX
S3,
   1':SUB M;IF M <= 0 [@True,@False]
```

```
          @True:SET C(I) = T + 0.31, I=I+1, C(I)=-987.987--->S4
          @False:--->SX
S4,
   .01":lockon ^houselight--->stopabortflush
\IF THERE IS NO MORE TIME LEFT, THE FOOD LEVER TRACES GO TO BASELINE FOR EASIER
VISUALIZATION

\***********************************************
\             HOUSE LIGHT CONTROL 1 - NO TRAINING LIGHTS
\***********************************************
S.S.14,
S1,
     #START--->S2
\F(0) HOUR LIGHT LOCKON
\F(1) HOUR LIGHT LOCKOFF

S2,
     0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
     @LightHours:LOCKON ^HOUSELIght--->S2
        @DarkHours:LOCKOFF ^houselight--->S2


\***********************************************
\             HOUSE LIGHT CONTROL 2 - TRAINING LIGHTS
\***********************************************
\S.S.12,
\DURING DARK HOURS FOODLIGHT ON FOR 1" W/ LEVERPRESS
\DURING LIGHT HOURS FOODLIGHT ON AND HOUSELIGHT OFF FOR 1" W/ LEVERPRESS
\S1,
\    #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

\S2,
\    0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
\    @LightHours:IF z(0) = 1[@LitRein,@LitNoRein]
\         @LightRein:Z3--->SX
\         @LightnonRein:ON^HOUSELIght--->S2
 \       @DarkHours:IF Z(0)= 1[@DrkRein, @DrkNRein]
\           @DrkRein:Z4--->SX
\         @DrkNRein:OFF ^houselight --->S2
\
\
\S.S.13, \LIGHT HOURS
\S1,
\    #Z3:OFF ^houselight ;ON^FOODLITE--->S2
\S2,
\    0.5":ON ^houselight ; OFF^FOODLITE;Z5--->S1
```

```
\
\S.S.14, \DARK HOURS
\S1,
\         #Z4:ON^FOODLITE--->S2
\S2,
\         0.5":OFF^FOODLITE;Z5--->S1

\S.S.15,
\S1,
\         #Z5:SET Z(0)=0--->SX
```

```
\ FR5-FRY-TLN-LEY : FIXED RATIO 5
\ FRY- Food lever Retract Yes
\ TLN- Training Light changes No
\ LEY- Light events recorded YES

\ HOUSELIGHT stays on after end of session
\ WILL ONLY RECORD 999,918 DATA POINTS WHEN USING NEW SOFTWARE/HARDWARE
\ *** NOTE: TIME COUNT HAS CHANGED TO 10ms TO CATCH ALL DURATIONS BETWEEN MOUSE
LICKS
\ source program was l8FR.MPC FIXED RATIO SOURCE PROGRAM FOR MEDLAB8
\ code rewritten and edited by Chris Richard
\  CONSTANTS USED IN THIS PROGRAM
\  Edit input and output #'s if different for your system

                  \  Inputs ****************************
^Lever1 = 1       \  Food lever
^Lever2 = 2       \  Water Lever (dummy)
^licks = 3        \  Lickometer for sipper lick counts

                  \  Outputs *****************************
^foodlev = 1      \  Food lever
^waterlev = 2     \  Water lever
^Pellet = 5       \  Food hopper
^sipper = 4       \  Retractable sipper
^foodlite = 3     \  Food stimulus light
^waterlite = 6    \  Water stimulus light
^houselight = 7   \  House light
^M = 1380         \  Time of session in minutes

DIM C = 999918   \  Dimension Array C for x data points.
DIM Z = 1         \  Z(0) = 0, no reinforcement lighting, Z(0)=1, reinforcement
lighting
LIST F = 7,19     \  Military hour for lights on, lights off

\  VARIABLES USED IN THIS PROGRAM

\   B  =  Counter for water Lever
\  (C) =  Inter-Response Time (IRT) Array
\   D  =  Reinforcement Counter
\  (F) =  Array containing day/night cycle hour (military hours)
\   H  =  Hour     (DOS time)
\   I  =  Subscript for the IRT Array C.
\   J  =  Minutes variable for TIME command (not used)
\   K  =  Seconds variable for TIME command (not used)
\   M  =  Session Time in Minutes. If not set program will run continuously.
\   L  =  Counter for food lever
\   T  =  Clock Ticks for IRT's.  Resolution = 0.1 second.
\   X  =  Response Ratio set by User.   Default = 1
```

```
\   Y  =  Response Ratio used by program. Updates to new value
\         at Start and following reinforcement.
\   S  =  Counter for sips (licks)
\  (Z) =  Array to indicate reinforcement lighting

\   Z-PULSES USED IN THIS PROGRAM
\   Z1 =  Reinforcement Pulse
\   Z2 =  Reset Pulse for IRT Timer/Counter
\   Z3 =  Light hours reinforcement lighting pattern
\   Z4 =  Dark hours reinforcement lighting pattern
\   Z5 =  Resets (Z) to non-reinforcement status, i.e. 0
\   ************************************************
\                   FIXED RATIO SCHEDULE
\   Default value = 1
\   Change Variable X to change ratio
\   Changes after procedure is started only take effect
\       after current ratio has been satisfied.
\   ************************************************

S.S.1,
S1,
   #start:ON ^foodlev,^sipper,^houselight;IF X = 0 [@True,@False]
             @True:SET X = 5,Y=X--->S2
             @False:SET Y = X--->S2
S2,
   Y#R^Lever1:on ^pellet; off ^foodlev;Z1--->S3
S3,
   .05":off ^pellet;SET Y = X--->S4
S4,
   10":on ^foodlev--->S2


S.S.2,  \food lever COUNTER
S1,
  #start:SHOW 1,Lever1,L--->S2
S2,
   #R^Lever1:ADD L;SHOW 1,Lever1,L--->SX



S.S.3,    \ Food REINFORCEMENT COUNTER
S1,
   #start:SHOW 2,Rein,D--->S2
S2,
   #Z1:ADD D;SHOW 2,Rein,D;SET Z(0)=1--->SX


S.S.5,   \ DISPLAY FIXED RATIO VALUE AND SESSION TIME
```

```
S1,
  #Start:--->S2
S2,
   .1":SHOW 6,FR=,Y,5,SESS_N,M--->SX



S.S.6,  \TIME T IS INCREMENTED IN UNITS OF 0.01 SECONDS
S1,
  #Start:--->S2
S2,
   #Z2--->S2         \ Z-PULSE FOR Z2 OCCURS AT THE BEGINNING OF ANY EVENT
   0.01":ADD T--->SX  \EVERY 0.01" TIME T INCREMENTS UP BY 1



S.S.7,   \ ARRAY SET WITH IRT, INCREMENTED TO NEXT EMPTY POINT AND SET WITH -
987.987
S1,
   #Start:--->S2
S2,           \ TRACE for food lever (0.1)
   #R^Lever1:SET C(I) = T+0.1,T = 0;Z2;ADD I;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX

S.S.8,   \ SET REINFORCEMENT MARK (0.2) FOR FOOD LEVER
S1,
   #start:--->S2
S2,
   #Z1:SET C(I) = 0.2;ADD I;IF I = 999918 [@True,@False]
           @True:--->STOPabortflush
           @False:SET C(I) = -987.987--->SX

\EACH TIME REINFORCEMENT Z1 SATISFIED, 0.2 MARKER PUT IN C(I),
\ARRAY INCREMENTED C(I+1)AND -987.987 PUT TEMPORARILY INTO C(I+1)


S.S.9,   \WATER LEVER PRESS
S1,      \ SETS EVENT PEN TO BASELINE
  #Start:SHOW 4,Lever2,B--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME WATER LEVER PRESSED
   #R^Lever2:ADD B;SHOW 4,Lever2,B;
             SET C(I) = T + 0.60, C(I+1) = 0.50, T=0, I=I+2;Z2;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX
```

```
S.S.10,   \ MOUSE LICK EVENTS
S1,
   #START: SHOW 3,Licks,S--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME MOUSE LICKS
   #R^licks:ADD S;SHOW 3,Licks,S;
            SET C(I) = T + 0.61, C(I+1) = 0.51, T=0, I=I+2;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX

S.S.11,       \Records lights out event
S1,
   #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(1)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowNight:SET C(I) = T + 0.62, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
         @StillDay:--->S2



S.S.12,       \Recored lights on event
S1,
     #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(0)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowDay:SET C(I) = T + 0.52, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
        @StillNight:--->S2



S.S.13,        \SESSION CLOCK
S1,
   #start:SET M=^M;IF M =0  [@True,@False]
            @True:--->S2
            @False:--->S3
S2,
   .1":IF M >0[@True,@False]
          @True:--->S3
          @False:--->SX
S3,
```

```
   1':SUB M;IF M <= 0 [@True,@False]
          @True:SET C(I) = T + 0.31, I=I+1, C(I)=-987.987--->S4
          @False:--->SX
S4,
   .01":lockon ^houselight--->stopabortflush
\IF THERE IS NO MORE TIME LEFT, THE FOOD LEVER TRACES GO TO BASELINE FOR EASIER
VISUALIZATION

\***********************************************
\             HOUSE LIGHT CONTROL 1 - NO TRAINING LIGHTS
\***********************************************
S.S.14,
S1,
     #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

S2,
     0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
     @LightHours:ON ^HOUSELIght--->S2
        @DarkHours:OFF ^houselight--->S2


\***********************************************
\             HOUSE LIGHT CONTROL 2 - TRAINING LIGHTS
\***********************************************
\S.S.12,
\DURING DARK HOURS FOODLIGHT ON FOR 1" W/ LEVERPRESS
\DURING LIGHT HOURS FOODLIGHT ON AND HOUSELIGHT OFF FOR 1" W/ LEVERPRESS
\S1,
\    #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

\S2,
\    0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
\    @LightHours:IF z(0) = 1[@LitRein,@LitNoRein]
\         @LightRein:Z3--->SX
\         @LightnonRein:ON^HOUSELIght--->S2
 \       @DarkHours:IF Z(0)= 1[@DrkRein, @DrkNRein]
\           @DrkRein:Z4--->SX
\         @DrkNRein:OFF ^houselight --->S2
\
\
\S.S.13, \LIGHT HOURS
\S1,
\    #Z3:OFF ^houselight ;ON^FOODLITE--->S2
\S2,
```

```
\    0.5":ON ^houselight ; OFF^FOODLITE;Z5--->S1
\
\S.S.14, \DARK HOURS
\S1,
\        #Z4:ON^FOODLITE--->S2
\S2,
\        0.5":OFF^FOODLITE;Z5--->S1

\S.S.15,
\S1,
\        #Z5:SET Z(0)=0--->SX
```

```
\ FR10-FRY-TLN-LEY : FIXED RATIO 10
\ FRY- Food lever Retract YES
\ TLN- Training Light changes No
\ LEY- Light events recorded YES

\ HOUSELIGHT stays on after end of session
\ WILL ONLY RECORD 999,918 DATA POINTS WHEN USING NEW SOFTWARE/HARDWARE
\ *** NOTE: TIME COUNT HAS CHANGED TO 10ms TO CATCH ALL DURATIONS BETWEEN MOUSE
LICKS
\ source program was l8FR.MPC FIXED RATIO SOURCE PROGRAM FOR MEDLAB8
\ code rewritten and edited by Chris Richard
\  CONSTANTS USED IN THIS PROGRAM
\  Edit input and output #'s if different for your system

                  \  Inputs ****************************
^Lever1 = 1       \  Food lever
^Lever2 = 2       \  Water Lever (dummy)
^licks = 3        \  Lickometer for sipper lick counts

                  \  Outputs *****************************
^foodlev = 1      \  Food lever
^waterlev = 2     \  Water lever
^Pellet = 5       \  Food hopper
^sipper = 4       \  Retractable sipper
\^foodlite = 3     \  Food stimulus light (note- not active in the FR10 program)
^waterlite = 6    \  Water stimulus light
^houselight = 7   \  House light
^M = 1380         \  Time of session in minutes

DIM C = 999918   \  Dimension Array C for x data points.
DIM Z = 1         \  Z(0) = 0, no reinforcement lighting, Z(0)=1, reinforcement
lighting
LIST F = 7,19     \  Military hour for lights on, lights off

\  VARIABLES USED IN THIS PROGRAM

\   B  =  Counter for water Lever
\  (C) =  Inter-Response Time (IRT) Array
\   D  =  Reinforcement Counter
\  (F) =  Array containing day/night cycle hour (military hours)
\   H  =  Hour     (DOS time)
\   I  =  Subscript for the IRT Array C.
\   J  =  Minutes variable for TIME command (not used)
\   K  =  Seconds variable for TIME command (not used)
\   M  =  Session Time in Minutes. If not set program will run continuously.
\   L  =  Counter for food lever
\   T  =  Clock Ticks for IRT's.  Resolution = 0.1 second.
\   X  =  Response Ratio set by User.   Default = 1
```

```
\   Y  =  Response Ratio used by program. Updates to new value
\         at Start and following reinforcement.
\   S  =  Counter for sips (licks)
\  (Z) =  Array to indicate reinforcement lighting

\   Z-PULSES USED IN THIS PROGRAM
\   Z1 =  Reinforcement Pulse
\   Z2 =  Reset Pulse for IRT Timer/Counter
\   Z3 =  Light hours reinforcement lighting pattern
\   Z4 =  Dark hours reinforcement lighting pattern
\   Z5 =  Resets (Z) to non-reinforcement status, i.e. 0
\   ************************************************
\                   FIXED RATIO SCHEDULE
\   Default value = 1
\   Change Variable X to change ratio
\   Changes after procedure is started only take effect
\       after current ratio has been satisfied.
\   ************************************************

S.S.1,
S1,
   #start:ON ^foodlev,^sipper,^houselight;IF X = 0 [@True,@False]
             @True:SET X = 10,Y=X--->S2
             @False:SET Y = X--->S2
S2,
   Y#R^Lever1:on ^pellet; off ^foodlev;Z1--->S3
S3,
   .05":off ^pellet;SET Y = X--->S4
S4,
   10":on ^foodlev--->S2



S.S.2,  \food lever COUNTER
S1,
  #start:SHOW 1,Lever1,L--->S2
S2,
   #R^Lever1:ADD L;SHOW 1,Lever1,L--->SX



S.S.3,    \ Food REINFORCEMENT COUNTER
S1,
   #start:SHOW 2,Rein,D--->S2
S2,
   #Z1:ADD D;SHOW 2,Rein,D;SET Z(0)=1--->SX
```

```
S.S.5,   \ DISPLAY FIXED RATIO VALUE AND SESSION TIME
S1,
  #Start:--->S2
S2,
   .1":SHOW 6,FR=,Y,5,SESS_N,M--->SX



S.S.6,  \TIME T IS INCREMENTED IN UNITS OF 0.01 SECONDS
S1,
  #Start:--->S2
S2,
   #Z2--->S2         \ Z-PULSE FOR Z2 OCCURS AT THE BEGINNING OF ANY EVENT
   0.01":ADD T--->SX  \EVERY 0.01" TIME T INCREMENTS UP BY 1



S.S.7,   \ ARRAY SET WITH IRT, INCREMENTED TO NEXT EMPTY POINT AND SET WITH -
987.987
S1,
   #Start:--->S2
S2,           \ TRACE for food lever (0.1)
   #R^Lever1:SET C(I) = T+0.1,T = 0;Z2;ADD I;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX

S.S.8,   \ SET REINFORCEMENT MARK (0.2) FOR FOOD LEVER
S1,
   #start:--->S2
S2,
   #Z1:SET C(I) = 0.2;ADD I;IF I = 999918 [@True,@False]
          @True:--->STOPabortflush
          @False:SET C(I) = -987.987--->SX

\EACH TIME REINFORCEMENT Z1 SATISFIED, 0.2 MARKER PUT IN C(I),
\ARRAY INCREMENTED C(I+1)AND -987.987 PUT TEMPORARILY INTO C(I+1)


S.S.9,   \WATER LEVER PRESS
S1,      \ SETS EVENT PEN TO BASELINE
  #Start:SHOW 4,Lever2,B--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME WATER LEVER PRESSED
   #R^Lever2:ADD B;SHOW 4,Lever2,B;
             SET C(I) = T + 0.60, C(I+1) = 0.50, T=0, I=I+2;Z2;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
```

```
                @False:SET C(I) = -987.987--->SX

S.S.10,   \ MOUSE LICK EVENTS
S1,
   #START: SHOW 3,Licks,S--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME MOUSE LICKS
   #R^licks:ADD S;SHOW 3,Licks,S;
            SET C(I) = T + 0.61, C(I+1) = 0.51, T=0, I=I+2;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX

S.S.11,       \Records lights out event
S1,
   #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(1)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowNight:SET C(I) = T + 0.62, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
         @StillDay:--->S2



S.S.12,       \Recored lights on event
S1,
     #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(0)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowDay:SET C(I) = T + 0.52, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
        @StillNight:--->S2



S.S.13,        \SESSION CLOCK
S1,
   #start:SET M=^M;IF M =0  [@True,@False]
            @True:--->S2
            @False:--->S3
S2,
   .1":IF M >0[@True,@False]
          @True:--->S3
          @False:--->SX
```

```
S3,
   1':SUB M;IF M <= 0 [@True,@False]
         @True:SET C(I) = T + 0.31, I=I+1, C(I)=-987.987--->S4
         @False:--->SX
S4,
   .01":lockon ^houselight--->stopabortflush
\IF THERE IS NO MORE TIME LEFT, THE FOOD LEVER TRACES GO TO BASELINE FOR EASIER
VISUALIZATION

\***********************************************
\             HOUSE LIGHT CONTROL 1 - NO TRAINING LIGHTS
\***********************************************
S.S.14,
S1,
     #START--->S2
\F(0) HOUR LIGHT LOCKON
\F(1) HOUR LIGHT LOCKOFF

S2,
     0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
     @LightHours:LOCKON ^HOUSELIght--->S2
        @DarkHours:LOCKOFF ^houselight--->S2


\***********************************************
\             HOUSE LIGHT CONTROL 2 - TRAINING LIGHTS
\***********************************************
\S.S.12,
\DURING DARK HOURS FOODLIGHT ON FOR 1" W/ LEVERPRESS
\DURING LIGHT HOURS FOODLIGHT ON AND HOUSELIGHT OFF FOR 1" W/ LEVERPRESS
\S1,
\    #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

\S2,
\    0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
\    @LightHours:IF z(0) = 1[@LitRein,@LitNoRein]
\         @LightRein:Z3--->SX
\         @LightnonRein:ON^HOUSELIght--->S2
 \       @DarkHours:IF Z(0)= 1[@DrkRein, @DrkNRein]
\           @DrkRein:Z4--->SX
\         @DrkNRein:OFF ^houselight --->S2
\
\
\S.S.13, \LIGHT HOURS
\S1,
\    #Z3:OFF ^houselight ;ON^FOODLITE--->S2
```

```
\S2,
\    0.5":ON ^houselight ; OFF^FOODLITE;Z5--->S1
\
\S.S.14, \DARK HOURS
\S1,
\        #Z4:ON^FOODLITE--->S2
\S2,
\        0.5":OFF^FOODLITE;Z5--->S1

\S.S.15,
\S1,
\        #Z5:SET Z(0)=0--->SX
```

```
\ FR20-FRY-TLN-LEY : FIXED RATIO 20
\ FRY- Food lever Retract Yes
\ TLN- Training Light changes No
\ LEY- Light events recorded YES

\ HOUSELIGHT stays on after end of session
\ WILL ONLY RECORD 999,918 DATA POINTS WHEN USING NEW SOFTWARE/HARDWARE
\ *** NOTE: TIME COUNT HAS CHANGED TO 10ms TO CATCH ALL DURATIONS BETWEEN MOUSE
LICKS
\ source program was l8FR.MPC FIXED RATIO SOURCE PROGRAM FOR MEDLAB8
\ code rewritten and edited by Chris Richard
\  CONSTANTS USED IN THIS PROGRAM
\  Edit input and output #'s if different for your system

                  \  Inputs ****************************
^Lever1 = 1       \  Food lever
^Lever2 = 2       \  Water Lever (dummy)
^licks = 3        \  Lickometer for sipper lick counts

                  \  Outputs *****************************
^foodlev = 1      \  Food lever
^waterlev = 2     \  Water lever
^Pellet = 5       \  Food hopper
^sipper = 4       \  Retractable sipper
\^foodlite = 3     \  Food stimulus light (note- not active in the FR20 program)
^waterlite = 6    \  Water stimulus light
^houselight = 7   \  House light
^M = 1380         \  Time of session in minutes

DIM C = 999918   \  Dimension Array C for x data points.
DIM Z = 1         \  Z(0) = 0, no reinforcement lighting, Z(0)=1, reinforcement
lighting
LIST F = 7,19     \  Military hour for lights on, lights off

\  VARIABLES USED IN THIS PROGRAM

\   B  =  Counter for water Lever
\  (C) =  Inter-Response Time (IRT) Array
\   D  =  Reinforcement Counter
\  (F) =  Array containing day/night cycle hour (military hours)
\   H  =  Hour     (DOS time)
\   I  =  Subscript for the IRT Array C.
\   J  =  Minutes variable for TIME command (not used)
\   K  =  Seconds variable for TIME command (not used)
\   M  =  Session Time in Minutes. If not set program will run continuously.
\   L  =  Counter for food lever
\   T  =  Clock Ticks for IRT's.  Resolution = 0.1 second.
\   X  =  Response Ratio set by User.   Default = 1
```

```
\   Y  =  Response Ratio used by program. Updates to new value
\         at Start and following reinforcement.
\   S  =  Counter for sips (licks)
\  (Z) =  Array to indicate reinforcement lighting

\   Z-PULSES USED IN THIS PROGRAM
\   Z1 =  Reinforcement Pulse
\   Z2 =  Reset Pulse for IRT Timer/Counter
\   Z3 =  Light hours reinforcement lighting pattern
\   Z4 =  Dark hours reinforcement lighting pattern
\   Z5 =  Resets (Z) to non-reinforcement status, i.e. 0
\   ************************************************
\                   FIXED RATIO SCHEDULE
\   Default value = 1
\   Change Variable X to change ratio
\   Changes after procedure is started only take effect
\       after current ratio has been satisfied.
\   ************************************************

S.S.1,
S1,
   #start:ON ^foodlev,^sipper,^houselight;IF X = 0 [@True,@False]
             @True:SET X = 20,Y=X--->S2
             @False:SET Y = X--->S2
S2,
   Y#R^Lever1:on ^pellet; off ^foodlev;Z1--->S3
S3,
   .05":off ^pellet;SET Y = X--->S4
S4,
   10":on ^foodlev--->S2


S.S.2,  \food lever COUNTER
S1,
  #start:SHOW 1,Lever1,L--->S2
S2,
   #R^Lever1:ADD L;SHOW 1,Lever1,L--->SX



S.S.3,    \ Food REINFORCEMENT COUNTER
S1,
   #start:SHOW 2,Rein,D--->S2
S2,
   #Z1:ADD D;SHOW 2,Rein,D;SET Z(0)=1--->SX


S.S.5,   \ DISPLAY FIXED RATIO VALUE AND SESSION TIME
```

```
S1,
  #Start:--->S2
S2,
   .1":SHOW 6,FR=,Y,5,SESS_N,M--->SX



S.S.6,  \TIME T IS INCREMENTED IN UNITS OF 0.01 SECONDS
S1,
  #Start:--->S2
S2,
   #Z2--->S2         \ Z-PULSE FOR Z2 OCCURS AT THE BEGINNING OF ANY EVENT
   0.01":ADD T--->SX  \EVERY 0.01" TIME T INCREMENTS UP BY 1



S.S.7,   \ ARRAY SET WITH IRT, INCREMENTED TO NEXT EMPTY POINT AND SET WITH -
987.987
S1,
   #Start:--->S2
S2,           \ TRACE for food lever (0.1)
   #R^Lever1:SET C(I) = T+0.1,T = 0;Z2;ADD I;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX

S.S.8,   \ SET REINFORCEMENT MARK (0.2) FOR FOOD LEVER
S1,
   #start:--->S2
S2,
   #Z1:SET C(I) = 0.2;ADD I;IF I = 999918 [@True,@False]
           @True:--->STOPabortflush
           @False:SET C(I) = -987.987--->SX

\EACH TIME REINFORCEMENT Z1 SATISFIED, 0.2 MARKER PUT IN C(I),
\ARRAY INCREMENTED C(I+1)AND -987.987 PUT TEMPORARILY INTO C(I+1)


S.S.9,   \WATER LEVER PRESS
S1,      \ SETS EVENT PEN TO BASELINE
  #Start:SHOW 4,Lever2,B--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME WATER LEVER PRESSED
   #R^Lever2:ADD B;SHOW 4,Lever2,B;
             SET C(I) = T + 0.60, C(I+1) = 0.50, T=0, I=I+2;Z2;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX
```

```
S.S.10,   \ MOUSE LICK EVENTS
S1,
   #START: SHOW 3,Licks,S--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME MOUSE LICKS
   #R^licks:ADD S;SHOW 3,Licks,S;
            SET C(I) = T + 0.61, C(I+1) = 0.51, T=0, I=I+2;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX

S.S.11,       \Records lights out event
S1,
   #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(1)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowNight:SET C(I) = T + 0.62, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
         @StillDay:--->S2



S.S.12,       \Recored lights on event
S1,
     #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(0)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowDay:SET C(I) = T + 0.52, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
        @StillNight:--->S2



S.S.13,        \SESSION CLOCK
S1,
   #start:SET M=^M;IF M =0  [@True,@False]
            @True:--->S2
            @False:--->S3
S2,
   .1":IF M >0[@True,@False]
          @True:--->S3
          @False:--->SX
S3,
```

```
   1':SUB M;IF M <= 0 [@True,@False]
          @True:SET C(I) = T + 0.31, I=I+1, C(I)=-987.987--->S4
          @False:--->SX
S4,
   .01":lockon ^houselight--->stopabortflush
\IF THERE IS NO MORE TIME LEFT, THE FOOD LEVER TRACES GO TO BASELINE FOR EASIER
VISUALIZATION

\***********************************************
\             HOUSE LIGHT CONTROL 1 - NO TRAINING LIGHTS
\***********************************************
S.S.14,
S1,
     #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

S2,
     0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
     @LightHours:ON ^HOUSELIght--->S2
        @DarkHours:OFF ^houselight--->S2


\***********************************************
\             HOUSE LIGHT CONTROL 2 - TRAINING LIGHTS
\***********************************************
\S.S.12,
\DURING DARK HOURS FOODLIGHT ON FOR 1" W/ LEVERPRESS
\DURING LIGHT HOURS FOODLIGHT ON AND HOUSELIGHT OFF FOR 1" W/ LEVERPRESS
\S1,
\    #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

\S2,
\    0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
\    @LightHours:IF z(0) = 1[@LitRein,@LitNoRein]
\         @LightRein:Z3--->SX
\         @LightnonRein:ON^HOUSELIght--->S2
 \       @DarkHours:IF Z(0)= 1[@DrkRein, @DrkNRein]
\           @DrkRein:Z4--->SX
\         @DrkNRein:OFF ^houselight --->S2
\
\
\S.S.13, \LIGHT HOURS
\S1,
\    #Z3:OFF ^houselight ;ON^FOODLITE--->S2
\S2,
```

```
\    0.5":ON ^houselight ; OFF^FOODLITE;Z5--->S1
\
\S.S.14, \DARK HOURS
\S1,
\        #Z4:ON^FOODLITE--->S2
\S2,
\        0.5":OFF^FOODLITE;Z5--->S1

\S.S.15,
\S1,
\        #Z5:SET Z(0)=0--->SX
```

```
\ FR30-FRY-TLN-LEY : FIXED RATIO 30
\ FRY- Food lever Retract Yes
\ TLN- Training Light changes No
\ LEY- Light events recorded YES

\ HOUSELIGHT stays on after end of session
\ WILL ONLY RECORD 999,918 DATA POINTS WHEN USING NEW SOFTWARE/HARDWARE
\ *** NOTE: TIME COUNT HAS CHANGED TO 10ms TO CATCH ALL DURATIONS BETWEEN MOUSE
LICKS
\ source program was l8FR.MPC FIXED RATIO SOURCE PROGRAM FOR MEDLAB8
\ code rewritten and edited by Chris Richard
\  CONSTANTS USED IN THIS PROGRAM
\  Edit input and output #'s if different for your system

                  \  Inputs ****************************
^Lever1 = 1       \  Food lever
^Lever2 = 2       \  Water Lever (dummy)
^licks = 3        \  Lickometer for sipper lick counts

                  \  Outputs *****************************
^foodlev = 1      \  Food lever
^waterlev = 2     \  Water lever
^Pellet = 5       \  Food hopper
^sipper = 4       \  Retractable sipper
\^foodlite = 3     \  Food stimulus light (note- not active in the FR30 program)
^waterlite = 6    \  Water stimulus light
^houselight = 7   \  House light
^M = 1380         \  Time of session in minutes

DIM C = 999918   \  Dimension Array C for x data points.
DIM Z = 1         \  Z(0) = 0, no reinforcement lighting, Z(0)=1, reinforcement
lighting
LIST F = 7,19     \  Military hour for lights on, lights off

\  VARIABLES USED IN THIS PROGRAM

\   B  =  Counter for water Lever
\  (C) =  Inter-Response Time (IRT) Array
\   D  =  Reinforcement Counter
\  (F) =  Array containing day/night cycle hour (military hours)
\   H  =  Hour     (DOS time)
\   I  =  Subscript for the IRT Array C.
\   J  =  Minutes variable for TIME command (not used)
\   K  =  Seconds variable for TIME command (not used)
\   M  =  Session Time in Minutes. If not set program will run continuously.
\   L  =  Counter for food lever
\   T  =  Clock Ticks for IRT's.  Resolution = 0.1 second.
\   X  =  Response Ratio set by User.   Default = 1
```

```
\   Y  =  Response Ratio used by program. Updates to new value
\         at Start and following reinforcement.
\   S  =  Counter for sips (licks)
\  (Z) =  Array to indicate reinforcement lighting

\   Z-PULSES USED IN THIS PROGRAM
\   Z1 =  Reinforcement Pulse
\   Z2 =  Reset Pulse for IRT Timer/Counter
\   Z3 =  Light hours reinforcement lighting pattern
\   Z4 =  Dark hours reinforcement lighting pattern
\   Z5 =  Resets (Z) to non-reinforcement status, i.e. 0
\   ************************************************
\                   FIXED RATIO SCHEDULE
\   Default value = 1
\   Change Variable X to change ratio
\   Changes after procedure is started only take effect
\       after current ratio has been satisfied.
\   ************************************************

S.S.1,
S1,
   #start:ON ^foodlev,^sipper,^houselight;IF X = 0 [@True,@False]
             @True:SET X = 30,Y=X--->S2
             @False:SET Y = X--->S2
S2,
   Y#R^Lever1:on ^pellet; off ^foodlev;Z1--->S3
S3,
   .05":off ^pellet;SET Y = X--->S4
S4,
   10":on ^foodlev--->S2


S.S.2,  \food lever COUNTER
S1,
  #start:SHOW 1,Lever1,L--->S2
S2,
   #R^Lever1:ADD L;SHOW 1,Lever1,L--->SX



S.S.3,    \ Food REINFORCEMENT COUNTER
S1,
   #start:SHOW 2,Rein,D--->S2
S2,
   #Z1:ADD D;SHOW 2,Rein,D;SET Z(0)=1--->SX


S.S.5,   \ DISPLAY FIXED RATIO VALUE AND SESSION TIME
```

```
S1,
  #Start:--->S2
S2,
   .1":SHOW 6,FR=,Y,5,SESS_N,M--->SX



S.S.6,  \TIME T IS INCREMENTED IN UNITS OF 0.01 SECONDS
S1,
  #Start:--->S2
S2,
   #Z2--->S2         \ Z-PULSE FOR Z2 OCCURS AT THE BEGINNING OF ANY EVENT
   0.01":ADD T--->SX  \EVERY 0.01" TIME T INCREMENTS UP BY 1



S.S.7,   \ ARRAY SET WITH IRT, INCREMENTED TO NEXT EMPTY POINT AND SET WITH -
987.987
S1,
   #Start:--->S2
S2,           \ TRACE for food lever (0.1)
   #R^Lever1:SET C(I) = T+0.1,T = 0;Z2;ADD I;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX

S.S.8,   \ SET REINFORCEMENT MARK (0.2) FOR FOOD LEVER
S1,
   #start:--->S2
S2,
   #Z1:SET C(I) = 0.2;ADD I;IF I = 999918 [@True,@False]
           @True:--->STOPabortflush
           @False:SET C(I) = -987.987--->SX

\EACH TIME REINFORCEMENT Z1 SATISFIED, 0.2 MARKER PUT IN C(I),
\ARRAY INCREMENTED C(I+1)AND -987.987 PUT TEMPORARILY INTO C(I+1)


S.S.9,   \WATER LEVER PRESS
S1,      \ SETS EVENT PEN TO BASELINE
  #Start:SHOW 4,Lever2,B--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME WATER LEVER PRESSED
   #R^Lever2:ADD B;SHOW 4,Lever2,B;
             SET C(I) = T + 0.60, C(I+1) = 0.50, T=0, I=I+2;Z2;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX
```

```
S.S.10,   \ MOUSE LICK EVENTS
S1,
   #START: SHOW 3,Licks,S--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME MOUSE LICKS
   #R^licks:ADD S;SHOW 3,Licks,S;
            SET C(I) = T + 0.61, C(I+1) = 0.51, T=0, I=I+2;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX

S.S.11,       \Records lights out event
S1,
   #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(1)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowNight:SET C(I) = T + 0.62, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
         @StillDay:--->S2



S.S.12,       \Recored lights on event
S1,
     #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(0)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowDay:SET C(I) = T + 0.52, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
        @StillNight:--->S2



S.S.13,        \SESSION CLOCK
S1,
   #start:SET M=^M;IF M =0  [@True,@False]
            @True:--->S2
            @False:--->S3
S2,
   .1":IF M >0[@True,@False]
          @True:--->S3
          @False:--->SX
S3,
```

```
   1':SUB M;IF M <= 0 [@True,@False]
          @True:SET C(I) = T + 0.31, I=I+1, C(I)=-987.987--->S4
          @False:--->SX
S4,
   .01":lockon ^houselight--->stopabortflush
\IF THERE IS NO MORE TIME LEFT, THE FOOD LEVER TRACES GO TO BASELINE FOR EASIER
VISUALIZATION

\***********************************************
\             HOUSE LIGHT CONTROL 1 - NO TRAINING LIGHTS
\***********************************************
S.S.14,
S1,
     #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

S2,
     0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
     @LightHours:ON ^HOUSELIght--->S2
        @DarkHours:OFF ^houselight--->S2


\***********************************************
\             HOUSE LIGHT CONTROL 2 - TRAINING LIGHTS
\***********************************************
\S.S.12,
\DURING DARK HOURS FOODLIGHT ON FOR 1" W/ LEVERPRESS
\DURING LIGHT HOURS FOODLIGHT ON AND HOUSELIGHT OFF FOR 1" W/ LEVERPRESS
\S1,
\    #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

\S2,
\    0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
\    @LightHours:IF z(0) = 1[@LitRein,@LitNoRein]
\         @LightRein:Z3--->SX
\         @LightnonRein:ON^HOUSELIght--->S2
 \       @DarkHours:IF Z(0)= 1[@DrkRein, @DrkNRein]
\           @DrkRein:Z4--->SX
\         @DrkNRein:OFF ^houselight --->S2
\
\
\S.S.13, \LIGHT HOURS
\S1,
\    #Z3:OFF ^houselight ;ON^FOODLITE--->S2
\S2,
```

```
\    0.5":ON ^houselight ; OFF^FOODLITE;Z5--->S1
\
\S.S.14, \DARK HOURS
\S1,
\        #Z4:ON^FOODLITE--->S2
\S2,
\        0.5":OFF^FOODLITE;Z5--->S1

\S.S.15,
\S1,
\        #Z5:SET Z(0)=0--->SX
```

```
\ FR40-FRY-TLN-LEY : FIXED RATIO 40
\ FRY- Food lever Retract YES
\ TLN- Training Light changes No
\ LEY- Light events recorded YES

\ HOUSELIGHT stays on after end of session
\ WILL ONLY RECORD 999,918 DATA POINTS WHEN USING NEW SOFTWARE/HARDWARE
\ *** NOTE: TIME COUNT HAS CHANGED TO 10ms TO CATCH ALL DURATIONS BETWEEN MOUSE
LICKS
\ source program was l8FR.MPC FIXED RATIO SOURCE PROGRAM FOR MEDLAB8
\ code rewritten and edited by Chris Richard
\  CONSTANTS USED IN THIS PROGRAM
\  Edit input and output #'s if different for your system

                  \  Inputs ****************************
^Lever1 = 1       \  Food lever
^Lever2 = 2       \  Water Lever (dummy)
^licks = 3        \  Lickometer for sipper lick counts

                  \  Outputs *****************************
^foodlev = 1      \  Food lever
^waterlev = 2     \  Water lever
^Pellet = 5       \  Food hopper
^sipper = 4       \  Retractable sipper
\^foodlite = 3     \  Food stimulus light (note- not active in the FR40 program)
^waterlite = 6    \  Water stimulus light
^houselight = 7   \  House light
^M = 1380         \  Time of session in minutes

DIM C = 999918   \  Dimension Array C for x data points.
DIM Z = 1         \  Z(0) = 0, no reinforcement lighting, Z(0)=1, reinforcement
lighting
LIST F = 7,19     \  Military hour for lights on, lights off

\  VARIABLES USED IN THIS PROGRAM

\   B  =  Counter for water Lever
\  (C) =  Inter-Response Time (IRT) Array
\   D  =  Reinforcement Counter
\  (F) =  Array containing day/night cycle hour (military hours)
\   H  =  Hour     (DOS time)
\   I  =  Subscript for the IRT Array C.
\   J  =  Minutes variable for TIME command (not used)
\   K  =  Seconds variable for TIME command (not used)
\   M  =  Session Time in Minutes. If not set program will run continuously.
\   L  =  Counter for food lever
\   T  =  Clock Ticks for IRT's.  Resolution = 0.1 second.
\   X  =  Response Ratio set by User.   Default = 1
```

```
\    Y  =  Response Ratio used by program. Updates to new value
\          at Start and following reinforcement.
\    S  =  Counter for sips (licks)
\   (Z) =  Array to indicate reinforcement lighting

\    Z-PULSES USED IN THIS PROGRAM
\    Z1 =  Reinforcement Pulse
\    Z2 =  Reset Pulse for IRT Timer/Counter
\    Z3 =  Light hours reinforcement lighting pattern
\    Z4 =  Dark hours reinforcement lighting pattern
\    Z5 =  Resets (Z) to non-reinforcement status, i.e. 0
\    ************************************************
\                    FIXED RATIO SCHEDULE
\    Default value = 1
\    Change Variable X to change ratio
\    Changes after procedure is started only take effect
\        after current ratio has been satisfied.
\    ************************************************

S.S.1,
S1,
   #start:ON ^foodlev,^sipper,^houselight;IF X = 0 [@True,@False]
             @True:SET X = 40,Y=X--->S2
             @False:SET Y = X--->S2
S2,
   Y#R^Lever1:on ^pellet; off ^foodlev;Z1--->S3
S3,
   .05":off ^pellet;SET Y = X--->S4
S4,
   10":on ^foodlev--->S2



S.S.2,  \food lever COUNTER
S1,
  #start:SHOW 1,Lever1,L--->S2
S2,
   #R^Lever1:ADD L;SHOW 1,Lever1,L--->SX



S.S.3,    \ Food REINFORCEMENT COUNTER
S1,
   #start:SHOW 2,Rein,D--->S2
S2,
   #Z1:ADD D;SHOW 2,Rein,D;SET Z(0)=1--->SX
```

```
S.S.5,   \ DISPLAY FIXED RATIO VALUE AND SESSION TIME
S1,
  #Start:--->S2
S2,
   .1":SHOW 6,FR=,Y,5,SESS_N,M--->SX



S.S.6,  \TIME T IS INCREMENTED IN UNITS OF 0.01 SECONDS
S1,
  #Start:--->S2
S2,
   #Z2--->S2         \ Z-PULSE FOR Z2 OCCURS AT THE BEGINNING OF ANY EVENT
   0.01":ADD T--->SX  \EVERY 0.01" TIME T INCREMENTS UP BY 1



S.S.7,   \ ARRAY SET WITH IRT, INCREMENTED TO NEXT EMPTY POINT AND SET WITH -
987.987
S1,
   #Start:--->S2
S2,           \ TRACE for food lever (0.1)
   #R^Lever1:SET C(I) = T+0.1,T = 0;Z2;ADD I;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX

S.S.8,   \ SET REINFORCEMENT MARK (0.2) FOR FOOD LEVER
S1,
   #start:--->S2
S2,
   #Z1:SET C(I) = 0.2;ADD I;IF I = 999918 [@True,@False]
          @True:--->STOPabortflush
          @False:SET C(I) = -987.987--->SX

\EACH TIME REINFORCEMENT Z1 SATISFIED, 0.2 MARKER PUT IN C(I),
\ARRAY INCREMENTED C(I+1)AND -987.987 PUT TEMPORARILY INTO C(I+1)


S.S.9,   \WATER LEVER PRESS
S1,      \ SETS EVENT PEN TO BASELINE
  #Start:SHOW 4,Lever2,B--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME WATER LEVER PRESSED
   #R^Lever2:ADD B;SHOW 4,Lever2,B;
             SET C(I) = T + 0.60, C(I+1) = 0.50, T=0, I=I+2;Z2;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
```

```
                @False:SET C(I) = -987.987--->SX

S.S.10,   \ MOUSE LICK EVENTS
S1,
   #START: SHOW 3,Licks,S--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME MOUSE LICKS
   #R^licks:ADD S;SHOW 3,Licks,S;
            SET C(I) = T + 0.61, C(I+1) = 0.51, T=0, I=I+2;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX

S.S.11,       \Records lights out event
S1,
   #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(1)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowNight:SET C(I) = T + 0.62, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
         @StillDay:--->S2



S.S.12,       \Recored lights on event
S1,
     #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(0)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowDay:SET C(I) = T + 0.52, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
        @StillNight:--->S2



S.S.13,        \SESSION CLOCK
S1,
   #start:SET M=^M;IF M =0  [@True,@False]
            @True:--->S2
            @False:--->S3
S2,
   .1":IF M >0[@True,@False]
          @True:--->S3
          @False:--->SX
```

```
S3,
   1':SUB M;IF M <= 0 [@True,@False]
         @True:SET C(I) = T + 0.31, I=I+1, C(I)=-987.987--->S4
         @False:--->SX
S4,
   .01":lockon ^houselight--->stopabortflush
\IF THERE IS NO MORE TIME LEFT, THE FOOD LEVER TRACES GO TO BASELINE FOR EASIER
VISUALIZATION

\***********************************************
\             HOUSE LIGHT CONTROL 1 - NO TRAINING LIGHTS
\***********************************************
S.S.14,
S1,
     #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

S2,
     0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
     @LightHours:lockON ^HOUSELIght--->S2
        @DarkHours:lockOFF ^houselight--->S2


\***********************************************
\             HOUSE LIGHT CONTROL 2 - TRAINING LIGHTS
\***********************************************
\S.S.12,
\DURING DARK HOURS FOODLIGHT ON FOR 1" W/ LEVERPRESS
\DURING LIGHT HOURS FOODLIGHT ON AND HOUSELIGHT OFF FOR 1" W/ LEVERPRESS
\S1,
\    #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

\S2,
\    0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
\    @LightHours:IF z(0) = 1[@LitRein,@LitNoRein]
\         @LightRein:Z3--->SX
\         @LightnonRein:ON^HOUSELIght--->S2
 \       @DarkHours:IF Z(0)= 1[@DrkRein, @DrkNRein]
\           @DrkRein:Z4--->SX
\         @DrkNRein:OFF ^houselight --->S2
\
\
\S.S.13, \LIGHT HOURS
\S1,
\    #Z3:OFF ^houselight ;ON^FOODLITE--->S2
```

```
\S2,
\    0.5":ON ^houselight ; OFF^FOODLITE;Z5--->S1
\
\S.S.14, \DARK HOURS
\S1,
\        #Z4:ON^FOODLITE--->S2
\S2,
\        0.5":OFF^FOODLITE;Z5--->S1

\S.S.15,
\S1,
\        #Z5:SET Z(0)=0--->SX
```

```
\ FR40-FRY-TLN-LEY.2day : FIXED RATIO 40
\ FRY- Food lever Retract YES
\ TLN- Training Light changes No
\ LEY- Light events recorded YES

\ HOUSELIGHT stays on after end of session
\ WILL ONLY RECORD 999,918 DATA POINTS WHEN USING NEW SOFTWARE/HARDWARE
\ *** NOTE: TIME COUNT HAS CHANGED TO 10ms TO CATCH ALL DURATIONS BETWEEN MOUSE
LICKS
\ source program was l8FR.MPC FIXED RATIO SOURCE PROGRAM FOR MEDLAB8
\ code rewritten and edited by Chris Richard
\  CONSTANTS USED IN THIS PROGRAM
\  Edit input and output #'s if different for your system

                  \  Inputs ****************************
^Lever1 = 1       \  Food lever
^Lever2 = 2       \  Water Lever (dummy)
^licks = 3        \  Lickometer for sipper lick counts

                  \  Outputs *****************************
^foodlev = 1      \  Food lever
^waterlev = 2     \  Water lever
^Pellet = 5       \  Food hopper
^sipper = 4       \  Retractable sipper
\^foodlite = 3     \  Food stimulus light (note- not active in the FR40 program)
^waterlite = 6    \  Water stimulus light
^houselight = 7   \  House light
^M = 2820         \  Time of session in minutes

DIM C = 999918   \  Dimension Array C for x data points.
DIM Z = 1         \  Z(0) = 0, no reinforcement lighting, Z(0)=1, reinforcement
lighting
LIST F = 7,19     \  Military hour for lights on, lights off

\  VARIABLES USED IN THIS PROGRAM

\   B  =  Counter for water Lever
\  (C) =  Inter-Response Time (IRT) Array
\   D  =  Reinforcement Counter
\  (F) =  Array containing day/night cycle hour (military hours)
\   H  =  Hour     (DOS time)
\   I  =  Subscript for the IRT Array C.
\   J  =  Minutes variable for TIME command (not used)
\   K  =  Seconds variable for TIME command (not used)
\   M  =  Session Time in Minutes. If not set program will run continuously.
\   L  =  Counter for food lever
\   T  =  Clock Ticks for IRT's.  Resolution = 0.1 second.
\   X  =  Response Ratio set by User.   Default = 1
```

```
\   Y  =  Response Ratio used by program. Updates to new value
\         at Start and following reinforcement.
\   S  =  Counter for sips (licks)
\  (Z) =  Array to indicate reinforcement lighting

\   Z-PULSES USED IN THIS PROGRAM
\   Z1 =  Reinforcement Pulse
\   Z2 =  Reset Pulse for IRT Timer/Counter
\   Z3 =  Light hours reinforcement lighting pattern
\   Z4 =  Dark hours reinforcement lighting pattern
\   Z5 =  Resets (Z) to non-reinforcement status, i.e. 0
\   ************************************************
\                   FIXED RATIO SCHEDULE
\   Default value = 1
\   Change Variable X to change ratio
\   Changes after procedure is started only take effect
\       after current ratio has been satisfied.
\   ************************************************

S.S.1,
S1,
   #start:ON ^foodlev,^sipper,^houselight;IF X = 0 [@True,@False]
             @True:SET X = 40,Y=X--->S2
             @False:SET Y = X--->S2
S2,
   Y#R^Lever1:on ^pellet; off ^foodlev;Z1--->S3
S3,
   .05":off ^pellet;SET Y = X--->S4
S4,
   10":on ^foodlev--->S2



S.S.2,  \food lever COUNTER
S1,
  #start:SHOW 1,Lever1,L--->S2
S2,
   #R^Lever1:ADD L;SHOW 1,Lever1,L--->SX



S.S.3,    \ Food REINFORCEMENT COUNTER
S1,
   #start:SHOW 2,Rein,D--->S2
S2,
   #Z1:ADD D;SHOW 2,Rein,D;SET Z(0)=1--->SX
```

```
S.S.5,   \ DISPLAY FIXED RATIO VALUE AND SESSION TIME
S1,
  #Start:--->S2
S2,
   .1":SHOW 6,FR=,Y,5,SESS_N,M--->SX



S.S.6,  \TIME T IS INCREMENTED IN UNITS OF 0.01 SECONDS
S1,
  #Start:--->S2
S2,
   #Z2--->S2         \ Z-PULSE FOR Z2 OCCURS AT THE BEGINNING OF ANY EVENT
   0.01":ADD T--->SX  \EVERY 0.01" TIME T INCREMENTS UP BY 1



S.S.7,   \ ARRAY SET WITH IRT, INCREMENTED TO NEXT EMPTY POINT AND SET WITH -
987.987
S1,
   #Start:--->S2
S2,           \ TRACE for food lever (0.1)
   #R^Lever1:SET C(I) = T+0.1,T = 0;Z2;ADD I;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
                @False:SET C(I) = -987.987--->SX

S.S.8,   \ SET REINFORCEMENT MARK (0.2) FOR FOOD LEVER
S1,
   #start:--->S2
S2,
   #Z1:SET C(I) = 0.2;ADD I;IF I = 999918 [@True,@False]
          @True:--->STOPabortflush
          @False:SET C(I) = -987.987--->SX

\EACH TIME REINFORCEMENT Z1 SATISFIED, 0.2 MARKER PUT IN C(I),
\ARRAY INCREMENTED C(I+1)AND -987.987 PUT TEMPORARILY INTO C(I+1)


S.S.9,   \WATER LEVER PRESS
S1,      \ SETS EVENT PEN TO BASELINE
  #Start:SHOW 4,Lever2,B--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME WATER LEVER PRESSED
   #R^Lever2:ADD B;SHOW 4,Lever2,B;
             SET C(I) = T + 0.60, C(I+1) = 0.50, T=0, I=I+2;Z2;
             IF I = 999918 [@True,@False]
                @True:--->STOPABORTflush
```

```
                @False:SET C(I) = -987.987--->SX

S.S.10,   \ MOUSE LICK EVENTS
S1,
   #START: SHOW 3,Licks,S--->S2

S2,      \MAKES EVENT PEN MARK EACH TIME MOUSE LICKS
   #R^licks:ADD S;SHOW 3,Licks,S;
            SET C(I) = T + 0.61, C(I+1) = 0.51, T=0, I=I+2;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX

S.S.11,       \Records lights out event
S1,
   #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(1)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowNight:SET C(I) = T + 0.62, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
         @StillDay:--->S2



S.S.12,       \Recored lights on event
S1,
     #start:--->S2
S2,
1":TIME H,J,K; IF (H=F(0)) AND (J=0) AND (K=0)[@Day,@Night]
     @NowDay:SET C(I) = T + 0.52, T=0, I=I+1;Z2;
            IF I = 999918 [@True,@False]
               @True:--->STOPABORTflush
               @False:SET C(I) = -987.987--->SX
        @StillNight:--->S2



S.S.13,        \SESSION CLOCK
S1,
   #start:SET M=^M;IF M =0  [@True,@False]
            @True:--->S2
            @False:--->S3
S2,
   .1":IF M >0[@True,@False]
          @True:--->S3
          @False:--->SX
```

```
S3,
   1':SUB M;IF M <= 0 [@True,@False]
         @True:SET C(I) = T + 0.31, I=I+1, C(I)=-987.987--->S4
         @False:--->SX
S4,
   .01":lockon ^houselight--->stopabortflush
\IF THERE IS NO MORE TIME LEFT, THE FOOD LEVER TRACES GO TO BASELINE FOR EASIER
VISUALIZATION

\***********************************************
\             HOUSE LIGHT CONTROL 1 - NO TRAINING LIGHTS
\***********************************************
S.S.14,
S1,
     #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

S2,
     0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
     @LightHours:lockON ^HOUSELIght--->S2
        @DarkHours:lockOFF ^houselight--->S2


\***********************************************
\             HOUSE LIGHT CONTROL 2 - TRAINING LIGHTS
\***********************************************
\S.S.12,
\DURING DARK HOURS FOODLIGHT ON FOR 1" W/ LEVERPRESS
\DURING LIGHT HOURS FOODLIGHT ON AND HOUSELIGHT OFF FOR 1" W/ LEVERPRESS
\S1,
\    #START--->S2
\F(0) HOUR LIGHT ON
\F(1) HOUR LIGHT OFF

\S2,
\    0.5":TIME H,J,K; IF (H>=F(0)) AND (H<F(1))[@Day,@Night]
\    @LightHours:IF z(0) = 1[@LitRein,@LitNoRein]
\         @LightRein:Z3--->SX
\         @LightnonRein:ON^HOUSELIght--->S2
 \       @DarkHours:IF Z(0)= 1[@DrkRein, @DrkNRein]
\           @DrkRein:Z4--->SX
\         @DrkNRein:OFF ^houselight --->S2
\
\
\S.S.13, \LIGHT HOURS
\S1,
\    #Z3:OFF ^houselight ;ON^FOODLITE--->S2
```

```
\S2,
\    0.5":ON ^houselight ; OFF^FOODLITE;Z5--->S1
\
\S.S.14, \DARK HOURS
\S1,
\        #Z4:ON^FOODLITE--->S2
\S2,
\        0.5":OFF^FOODLITE;Z5--->S1

\S.S.15,
\S1,
\        #Z5:SET Z(0)=0--->SX
```