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Establishing meal patterns by lickometry in the marmoset monkey (*Callithrix jacchus*): translational applications from the bench to the field and the clinic

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Abstract

The ability to measure and interpret variables associated with feeding behavior and food intake is essential to a variety of nonhuman primate study modalities. The development of a technique to accurately and efficiently measure food intake and meal patterning in captivity will enhance both the interpretation of foraging behavior in the wild as well as our ability to model clinically relevant human feeding pathologies. In this study we successfully developed the use of a rodent lickometer system to monitor meal patterning in captive common marmosets. We describe the modifications necessary for this type of instrumentation to be used successfully with marmosets. We define variables of interest that relate to both previous rodent literature and human clinical measures. Finally, we relate our findings to potential translational value for both primate field research and biomedical applications.

Keywords

Meal pattern; diet induced obesity; ingestion rate; microstructure

INTRODUCTION

Feeding behavior and food intake are central variables for a variety of nonhuman primate studies. In a natural setting, the search for and ingestion of food is a primary feature in understanding the ecology of any species. Foraging ecology studies are limited in most cases by a non-defined relationship between the feeding behaviors being observed and the food intake that takes place. The captive setting offers the opportunity to examine meal patterns in relation to food intake in ways that may inform field studies. In addition, food intake is often a central variable in biomedical studies, particularly those aimed at understanding how food intake behaviors determine clinical relevant phenotypes, such as anorexia and obesity.

A number of automated devices have been developed since 1950 in order to examine animals' feeding patterns and food choices. These devices include drinkometers,

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gustometers and lickometers, and their use has mostly been validated in rodent species [Weijnen, 1998]. The lickometer is specifically designed to examine meal patterning and microstructure of the feeding pattern throughout short and daily trials in rodents [Davis & Smith, 1992; Weijnen, 1989; Weijnen, 1998]. The lickometer offers a great deal of flexibility, typically using electronic circuits to measure the changes in the current due to the animal consuming food. Lickometers have been used to examine hypotheses regarding meal protection [Kaplan et al., 2001], central drivers of lick frequency [Davis & Smith, 1992], satiety [Baird et al., 1999], and postingestional triggers [Davis, 1999] by measuring daily consumption, daily meal patterns, and microstructural variables such as lick length, interlick length and bursting events [Rushing et al., 1997; Houpt & Frankmann, 1996].

While not widely used in nonhuman primates a few studies have modified electronic data collection units to specifically monitor feeding phenotypes in primates. The use of drinkometers and gustometers in macaque species has focused on the examination of alcohol consumption and the ability to differentiate taste [Pritchard et al., 1994; Reilly et al., 1994]. A number of studies with macaques have explored meal patterning with the usage of modified pellet distribution systems to define intake in relation to obesity [Bello et al., 2008; Scott & Moran, 2007]. A liquid feeding pump delivery system has successfully been developed in the past to deliver Ensure to chaired macaques and successfully monitored food intake and meal patterns [Hansen et al., 1981; Jen & Hansen, 1984]. The ability to examine meal patterning and daily intake in nonhuman primates allows further elucidation of mechanisms underlying food choice and satiety.

Marmosets are small new world primates in the family Callitrichinae and are especially suited to an experimental paradigm which includes the consumption of a liquid diet source. One of the primary dietary sources for marmosets in the wild is gum exudate, and they are behaviorally adapted to gouge holes in tree limbs, lick, and consume the flowing sap from the tree wounds [Rylands, 1996]. Field studies examining the feeding ecology and daily activity budgets for a variety of marmoset species suggest that the percentage of the daily consumption made up of exudate (gum) feeding ranges from 3% in *Callithrix intermedia* [Rylands, 1982] to 14.3% *C. geoffroyi* [Passamani, 1998]. Overall time spent feeding during the day ranges from 13% *C. flaviceps* [Ferrari, 1988] to 34% for *C. geoffroyi* [Passamani, 1998]. Daily feeding time budgets have revealed two peaks of consumption during the day, the largest peak being early morning with a smaller one in the afternoon [Rylands, 1982]; for *C. geoffroyi* this peak was specifically in gum exudate feeding [Passamani, 1998]. Marmosets have been found to readily consume liquid diet sources provided in captivity [McGrew et al., 1986]. The standard gel based diet used at the marmoset colony of the Southwest National Research Primate Center (SNPRC) can be modified to a liquid form which allows the use of a rodent lickometer system in order to assess daily feeding patterns and examine the microstructure of feeding and satiety.

A method to reliably and efficiently monitor food intake patterns in captive marmosets may ultimately be applied to both enhanced interpretation of foraging behavior data in the wild as well as to refining our ability to use these species as models of clinical relevant food intake pathologies in humans. We here describe the modification and use of a lickometer system to determine meal patterning in captive common marmosets (*Callithrix jacchus*). In addition to presenting descriptive findings on meal patterns in this species, we relate these findings to foraging patterns in wild callitrichid primates and to development of obese phenotypes. The goals of this project were to define meal structure and patterning in the marmoset monkey through use of a modified lickometer system, develop protocols for assessment of the data comparable to those previously used in the rodent literature, and define the variability in feeding measures between individuals in order to establish variables that might be of the most interest for translation to future field and clinical studies.

METHODS

Lickometer

A Columbus Instruments DM-8 lick counter linked to a PC on which the Columbus instruments multidevice interface software was used to collect data from up to eight channels during a session. Each channel included a lead attached to the bottle sipper tube and a lead connected to the cage surface; the animal completed the less than 1×10^{-6} ampere circuit when contacting the cage wire with a foot and the sipper with its tongue. The interface software batched the counts in 10 second intervals which were saved continuously throughout the experimental procedure. Data was then exported as a .csv file to Excel for further analysis.

Cage and bottle modification

Stainless steel cages were used to house and test the marmosets. With the dimensions of approximately 1m × 1.5m × 1.5m and a central divider option that allowed the cage to be split in half (see Fig 1a). The cage could be used to either test two singly housed animals with one on each side separated with a solid divider, or to separate an individual from a group using a mesh divider allowing visual and limited tactile contact with the group, but limiting food exchange between group members. The front wall of the cage is made up of several panels of stainless steel and each forms its own circuit when connected to a lickometer channel. Multiple bottles could be set up for each animal to examine animal preference for higher or lower fat diet (Figure 1a). The standard water bottles used for marmosets, a 16 oz. macrolon bottle with rubber stopper, and 5/16th inch tube ball stopper with 5 inch center bend (Ancare) were used with minor modifications. In order to prevent typical marmoset behaviors from affecting the lick counts being accurately registered the outer casing for a 1ml syringe (Monoject plastic syringe, rigid pack) was secured around the sipper tube with electrical tape (Figure 1b). This modification allowed only the marmoset's tongue to make contact with the sipper tube and prevented sucking, contact via scent marking, and hand contact.

Liquid diet

The animals at the Southwest National Primate Center are typically maintained on a diet which includes a purified gel based diet (Teklad™) [Tardif et al., 1998]. The Teklad diet was formulated such that 15.6 % of the kcal are from protein, 70.4 % from carbohydrate and 14% from fat. A higher fat diet has also been formulated in which all of the vitamin and nutrient concentrations are maintained but the percentage of fat is increased such that 15.7% of the kcal are from protein, 44.6% are from carbohydrate and 39.6 % are from fat. These diets were reconfigured to produce a diet that would remain suspended in water with the addition of xanthum gum. All of the nutrient information is the same for the liquid forms of the diet as the solid forms. A mixture of 20g of diet powder with 100 ml of water produces a lower fat diet with an estimated metabolizable energy density of 0.6 kcal per gram, and a higher fat diet with 0.72 kcal per gram wet weight.

Subjects

The common marmosets (*Callithrix jacchus*) were housed at the Southwest National Primate Research Center for this study. All protocols were approved by the Animal Care and Use Committee, and adhered to the American Society of Primatologists (ASP) principles for the ethical treatment of non-human primates. Eighteen adult marmosets (10 females: 8 males) ranging in age from 5.2 to 10.3 years of age that had previously (2008) been examined as part of a study on diet induced obesity (DIO) were tested with the lickometer system. The DIO subjects were all maintained on a high fat diet during that study (2008) and at its

conclusion they were transitioned back to the lower fat colony base diet. At the time of the lickometer study none of the animals differed in body weight, body fat or age regardless of whether they had previously gained or maintained their weight during the DIO study. These adult subjects were singly housed in caging 0.5m × 0.5m × 0.8m with nest box, branches and environmental enrichment [Layne et al., 2003] and were moved in to the stainless steel test cages prior to testing. The test cage was placed in their housing room, so that the location and identity of neighboring animals did not change. A further 33 infants (20 females: 13 males) were followed through development as part of a study to examine the impact of maternal obesity on infant development. Lickometer trials were done for each infant at the ages of three, six and twelve months. Infants were housed with their family group throughout the study in cages of the same approximate design and size as the lickometer stainless steel test cages. The entire family was moved to the stainless steel cages one week prior to testing to allow acclimation to the cage. All test subjects were habituated to the liquid diet for at least three days prior to testing. During habituation the subjects had ad lib access to water and their standard gel based solid diet, and were observed consuming the liquid diet during periodic visual checks. The animals were considered to be habituated if they were observed to readily approach and consume the diet upon placement of the bottles by the third day. In the case of the infant subjects the bottles of liquid diet were placed on the cage for the entire family group. Young infants were more likely to try the diet if parents and older siblings tested it first (unpublished observation). All food was removed from the entire group at 1630 the evening before the lickometer trial was to begin. The infant of interest was then separated from the family group to one side of the test cage at the beginning of the lickometer trial.

Trial design

Each animal participated in a two bottle choice test lickometer trial. The lickometer recorded two channels of data for each animal, one channel registered data from a bottle of lower fat liquid diet and the other from a bottle of higher fat liquid diet. The position of the bottles was assigned randomly and was reversed on day two in order to control for side bias. Lickometer trials began between 0800 and 0900 in the morning with all bottles being weighed before placement on the cage. The experimenter verified the circuitry of lickometer by completing the circuit manually; these counts were later removed from the analysis. Bottles were replaced between 1200 and 1330 to prevent separation of the diet, and verify that the lickometer circuitry was intact; all bottles were weighed upon removal and replacement. Bottles were removed between 1630 and 1700 and weighed. Animals had water ad lib during the lickometer trial, but no access to solid food. At the end of the second day of testing all animals were returned to their standard housing and feeding regime. Trials were aborted if the animal failed to consume any diet prior to 1200 on either day; an abort on Day 1 terminated the trial for Day 2. One trial was aborted due to a clogged sipper tube noted during the mid-day replacement, and another trial was aborted due to spillage from the sipper tube. Both trials were rerun at a later time. No other instances of clogging or spillage from the tube occurred.

Data File Coding

As this is the first study that we are aware of to examine daily meal patterning in the marmoset it was necessary for us to derive definitions and develop methods for data coding and analysis. Meals were defined as bouts of at least two licks separated by a minimum intermeal interval (IMI) of three minutes (18 intervals, 10 seconds each). The three minute IMI criterion was developed by varying the IMI length from 10 seconds to 20 minutes and calculating the number of meals [Rushing et al., 1997]. The number of meals reaching criterion was graphed by the intermeal interval criterion and the asymptote of the line was determined. For the majority of the trial data examined the asymptote was found to be at 18

intervals, or three minutes (Figure 2). Defining burst patterning allows a fine grained analysis of activity during a meal, thus bursts were defined as bouts of at least two licks separated by a minimum interburst interval (IBI) of 30 seconds (3 intervals). The criterion for the interburst interval was determined based on personal observation of breaks due to distraction of approximately thirty seconds during drinking and licking bouts but not leaving the vicinity of the drinking bottles (Ross).

All data were downloaded as .csv files and imported into Excel for further data analysis. The operator induced test counts were removed from each file prior to coding. Data files were coded by scoring the presence of a meal, and when there was a series of at least 18 intervals with no counts registered the intermeal interval associated with that meal was scored. All meals and intermeal intervals were scored consecutively. Bursts were scored within a meal as burst 1.1, followed by the IBI of 1.1 if there was a series of at least 3 but less than 18 intervals with no count. Each burst and IBI were scored consecutively within a meal. An interval with a single count surrounded by no count intervals was scored as a zero count interval. No meals or IMI were scored at the end of the daily collection if they did not meet criteria for beginning the next meal or IMI. The criteria for scoring were developed into a java based program developed by Yung Lai and Zhiwei Wang at the University of Texas San Antonio Computational Biology Initiative High Performance Computing Center. This program automatically scored an entire session data file for meals, intermeal intervals, bursts and interburst intervals. All automated data coding was verified by visual assessment prior to further analysis.

The scored data including interval number, count, meal, intermeal interval, bursts and interburst interval were imported into SPSS 13.0. Compare means was used to calculate the maximum value, minimum value, mean, and percent of total interval and count for each meal, intermeal interval, burst and interburst interval. These values were used to calculate further variables of interest that are defined in Table 1, as well as to verify that all scoring met criteria for all meals (i.e.: intermeal intervals at least 18 intervals in length, and a maximum count value of 1). Values were average across the two day data collection to determine the average intake per day.

RESULTS

Idiosyncrasies of marmosets and lickometers

During the development of the lickometer for use with the marmosets several things were noted. First and foremost it was necessary to use caging made of stainless steel. Typical caging for the captive marmosets is made with PVC coated wire mesh, which does not transmit the electrical signal, while caging made with uncoated aluminum wire mesh transmits the electrical signal too weakly to accurately register lick counts. Secondly, modification of the sipper tube with a non-conductive covering (we used a syringe cap) is essential in order to prevent the animals from making contact with the sipper and registering false counts, or preventing licks from being registered. Finally, we found that taping the leads to the cage and to the bottle helped prevent the removal of the wiring by the animals; this was particularly important when testing the younger subjects who were very persistent in chewing on and playing with the attachments. One adult female and five young infants were not able to be habituated to the lickometer and liquid diet and were not included in the study. It is possible that further longer habituation periods may make the process more universal.

Daily meal patterning

In developing the lickometer technology for use in a nonhuman primate we felt it to be extremely important to be able to relate this data and the definitions of variables to output already derived in rodent studies. A great deal of the early work with rodent lickometer data emphasized the variability between individuals as well as the types of data one could examine [Davis & Smith, 1992; Rushing et al., 1997; Houpt & Frankmann, 1996]. Table 1 provides our definitions for meals and bursts and descriptive statistics for the number of meals consumed, total number of counts, average meal length, and average intermeal interval length, and lists the comparative definitions for rodents. A great deal of variation was found between animals in their daily feeding as can be seen in Figure 3, which illustrates the output from the lickometer interface. In this case one animal shows many counts throughout the day, whereas the other animal shows bouts of feeding activity separated by long bouts of inactivity. For the developing marmosets the amount of time that was associated with a meal ranged from 2.5 hours in 3 month old infants to 3.2 hours in 12 month old marmosets; while adults spent on average 1.4 hours of their day in meals. The average meal length was the longest for the 12 month old marmosets at 5.3 minutes, with the adults having the shortest average meal length of 2.6 minutes. While the number of meals each day varied between individuals, on average the marmosets engaged in 35 meals per day. The structure of bursts within a meal varied greatly between individuals. Specifically, when surveying the adult marmoset with the most bursts within a single meal we find a high frequency of very short bursts (Figure 4A), and a high frequency of very short IBI's (Figure 4B) in his meals throughout the day. Whereas the animal with the least bursts in a single meal displays fewer short IBI's (Figure 4C & 4D).

The intake of liquid diet during the lickometer trial for the subjects is depicted in Table 2. Subjects did not prefer the high fat liquid diet. Overall the total grams consumed and therefore the kilocalories ingested increased with body size. Three month old marmosets consumed about 38g liquid diet per trial on average (25 kcal) while adult marmosets on average consumed 56 grams of liquid diet or 36 kcalories. The difference in diet intake with body size was not linear, with the mean kcalories per gram of body weight decreasing from 0.15 in three month olds to 0.09 in adults (Table 2). A regression of the natural log transformed mean values for energy intake and body mass estimated an allometry of 0.4 (SEM = 0.065). Mean kcal ingested per body mass raised to the 0.4 power did not differ among the four age groups (Table 2).

In order to determine whether the lickometer was able to detect satiety during feeding, the counts in the first meal of the day were graphed over the length of the meal for the infant and adult marmosets (Figure 5). We found that with the exception of a few animals the marmosets displayed a deceleration of consumption during the first meal of the day, although there was a great deal of variability in the rate and trajectory of the meal between individuals.

In order to determine whether marmosets in captivity follow a similar daily feeding pattern to that described in the wild the percentage of the daily counts that occurred during each hour over the eight hour trial were graphed in Figure 6. All of the subject groups displayed higher rates of feeding early in the day with another mild peak at midday, with approximately 24% of their daily count occurring in the first hour. Most of this is associated with the length of the first meal of the day which ranged from an average of 12 minutes in the adult animals to 21 minutes in the 12 month old animals. The fact that infant marmosets that are tested repetitively throughout their development continue to display this pattern and that this pattern so closely resembles that previously reported both in captivity and in the field [Passamani, 1998] suggest that the high rate of intake early in the day is likely not due to a novelty effect of the liquid diet.

DISCUSSION

Development of the lickometer for marmosets

Marmosets readily consumed the liquid purified diet developed for use with the lickometer studies, and most subjects habituated easily to the lickometer setup and testing paradigm. We were able to collect data on daily meal patterning and consumption and develop variables from the lickometer data that relate to the rodent lickometer literature. Rats, a rodent of similar size to the marmoset, allowed to freely feed on a milk diet connected to a lickometer for 24 hours consumed an average of 12 meals per day, with meals defined by a five minute intermeal interval, each meal was approximately 1500 counts and five minutes in length [Rushing et al., 1997]. We used the same criteria to derive an appropriate intermeal interval length for the marmosets which was slightly shorter at three minutes. Defining meals in this way we found that marmosets on average consumed 35 meals per day, with an average meal length of three minutes. During the 24 hour period the rats consumed an average of 85 ml of the milk diet and the adult marmosets consumed approximately 56 g of the liquid diet. Thus our defined meal lengths and daily consumption results are consistent with consumption rates previously reported for the rat, a well characterized animal of similar size to the marmoset.

In the rodent literature it is often only the first meal of the dark cycle that is analyzed to determine taste preference, and to examine post ingestion satiety factors [Spector & Smith, 1984; Davis & Smith, 1988]. It has long been held that the first meal after a fast, including following the daily sleeping time, is the most important meal for setting the rate of consumption throughout the day. In humans not only do diet and behavioral interventions often focus on the first meal of the day, but long held mythology supports that breakfast is the most important meal of the day. However, in terms of modeling human behavior rodents differ significantly from humans as they tend to eat throughout the 24 hour period with more meals focused during the night active cycle than during the day [Rushing et al., 1997]. Marmosets much more closely resemble humans in the fact that they have a true night time fast, with no consumption occurring at night [Rylands, 1996; Sri Kantha & Suzuki, 2006]. Our data suggests that the importance of the first meal of the day is relevant for marmosets as well, with the first meal of the day following the overnight fast setting the pace for overall consumption of the day with longer first meals being associated with both higher total number of counts for the day and higher counts per hour.

The mean value for estimated metabolizable energy intake of the liquid diet by adult marmosets when expressed on a dry matter basis (36.4 + 10.7 kcal) was lower than the estimated metabolizable energy intake previously reported for 13 adult marmosets fed the solid version of the diet (47.9 + 10.7 kcal) [Power & Myers, 2009]. This difference warrants further investigation as we can hypothesize several explanations for this difference. The solid food intake trials were done as 24 hour consumption trials, whereas the lickometer trials were set up as 8 hour feeding experiments each day. Although marmosets do not typically eat during their sleeping phase, it is possible that limiting them to 8 hours rather than the entire light cycle (12 h) limited the data collection for daily consumption. The other possibility is that while the animals differed in dry mass consumption we do not currently know how the volume consumptions differ between the solid and liquid forms of this diet. The increased water weight and the presence of xanthum gum may alter the perception of intake due to volume cues. Further research will be needed to determine what factors are involved and whether this difference has a biological consequence.

Translation to field research

Quantification of meal patterning, consumption and intake in captive marmosets brings new insight to the feeding ecology of marmosets in general. While feeding behaviors in captivity will not be identical to those in the wild; we believe that general trends may still hold universal especially when measuring liquid consumption by a primate that is primarily an exudate feeder. Some of the variables that may be informative from the captive lickometer data in regards to feeding ecology field studies relate to the time of the day of the study, the interval length of focal sampling and the relationship between satiation and patch dispersal.

As was previously reported for wild *Callithrix geoffroyi* [Passamani, 1998] we found that for the captive marmosets the first hour of the day accounted for a large portion of the feeding. The lickometer data revealed that on average 24 % of the daily licks were done within the first hour of the day and feeding plateaued off throughout the day with only a minor peak at mid-day. Researchers interested in questions like food choice and use of patch space might be well served to focus upon the first hour of the day for more tightly defined sampling as this may account for a more significant portion of the intake for the day. Focal sampling lengths for Callitrichines are typically reported as 3 to 5 minute intervals [Passamani, 1998; Garber, 1980; Garber, 1984, Martin & Setz, 2000]. These intervals fit well with what we found with the lickometer data in terms of both the length of a minimum intermeal interval, defined as three minutes, and the average meal length, also approximately three minutes. Thus, it is likely that significant data regarding meal structuring can be gleaned using this sampling technique in the field. However, the lickometer data from captivity reveals that not only does meal length vary a great deal between individuals but the length of the first meal of the day can be a great deal longer with the average for the 12 month old juveniles being 21 minutes.

Callitrichine primates have been the fruitful subjects of numerous studies of the effects of social and spatial factors upon foraging decisions [Peres, 1996; Bicca-Marques & Garber, 2003; Bicca-Marques & Garber, 2004]. Many of the ecological foraging models being tested in these studies involve an assessment of benefits (e.g. amount of food an individual animal procures) relative to costs (e.g. searching time and lost access to food via competition). These models frequently contain assumptions regarding the ability of a given “patch” of food to satiate a given animal and the relations of time spent eating to amount of food consumed. Captive studies such as the one we describe here could be helpful in defining relations between time feeding and satiety and the variance in intake that may be associated with a given time spent feeding. Garber [1993] reports that groups of mixed species troops of tamarins (*Saguinus*) typically spent 5-8 minutes exploiting a naturally occurring food patch while the average time that group members spent at baited feeding platforms was 5-6 minutes [Bicca-Marques & Garber, 2003]. The similarity of these values suggests that they represent some limit to the time which these animals will devote to a given food patch, but whether this limit is driven by satiation or by other factors cannot be determined in the field setting. Captive studies offer the opportunity to determine the relation of feeding patterns to satiety with limited intervening factors. While the data from our study, using a liquid food source, cannot be directly compared to the studies cited here, the meal lengths we documented in the captive setting (averaging 2.7 minutes overall and 7.45 minutes for the first and last meal of the day) suggests that satiation within 5-6 minutes of consumption is reasonable for the saddle-backed tamarin (*Saguinus fuscicollis*), as species roughly similar in size to *Callithrix jacchus*. Future captive studies could be designed to more clearly link the satiation findings from the captive setting to foraging studies in the wild.

Translation to the clinic

The understanding of how initiation, maintenance and termination of eating are patterned throughout the day has been central to the exploration of appetite. In humans, studies of meal structure have recently challenged commonly held beliefs regarding feeding differences in obese versus lean individuals. Historically, it was believed that obese humans consumed not only larger meals, but they consumed these meals faster [Ferster et al., 1962], leading to interventions built around training slower food consumption [Brownell, 1990]. However, recent studies using a variety of monitoring methods suggest little difference in rates of consumption or speed of meals between obese and nonobese humans [see reviews Spiegal, 2000; Guss & Kissileff, 2000]. Interpretation of these results may be complicated by the possibility that humans – particularly females – modify their feeding behavior in a research setting. The development of animal models would be one tool to use in circumventing the problems associated with the confounding effects of such modification of feeding patterns that may occur in humans.

A number of studies have found that overall values for rate of consumption did not differ between obese and nonobese humans; however, the results were not consistent across experimental techniques. The use of universal eating monitor to covertly measure consumption of semisolid casseroles by humans appears to most closely resemble lickometer data collection; however, there have been a number of technical issues raised by the experimenters when testing obese humans [Guss & Kissileff, 2000; Kissileff & Guss, 2001]. The universal eating monitor has primarily been used to monitor satiation curves and defining abnormal eating patterns rather than measuring individual bites. The satiation curves in marmosets depicted in Figure 5 closely resemble those reported for humans, in that there was a great deal of individual variation with some individuals reaching satiation quickly, whereas others never showed a decline in the rate of consumption throughout the meal. However, one of the major concerns is that obese women fail to eat what they describe as a normal meal when eating from the monitor, typically because they dislike the food choices and are less likely to comply with study protocol [Guss & Kissileff, 2000]. Several researchers have proposed that research on human women, especially obese women, is extremely difficult in a laboratory setting as social cues and stigma act to inhibit more natural behavior, whereas this may have little impact on men [Guss & Kissileff, 2000; Spiegal, 2000].

There is no reason to think that marmosets will vary their performance due to sex, obesity status or particular anxiety regarding the task; and as one of the shortest lived (average lifespan of 6 years) and fastest reproducing (producing two litters per year with an average of two infants per litter) anthropoid primates, they are an ideal model for translational biomedical questions [Tardif et al., 2003]. Marmosets have been found to develop spontaneous obesity, as well as diet induced obesity in a captive setting [Tardif et al., 2009; Wachtman et al., 2011]. Although the marmoset lickometer data differs in its focus on licks from human data focusing on bites, it is possible that the data will more truly reflect differences between individuals than what can be found in human laboratory collection, especially as it reflects a complete daily pattern rather than a single meal as is often collected for humans.

CONCLUSIONS

We were successfully able to develop a method to reliably and efficiently monitor food intake patterns in captive nonhuman primates, and define variables that relate to previous rodent meal patterning descriptions. This technology can ultimately be applied to both enhanced interpretation of feeding ecology data as well as to refining our ability to model and interpret the development of obesity.

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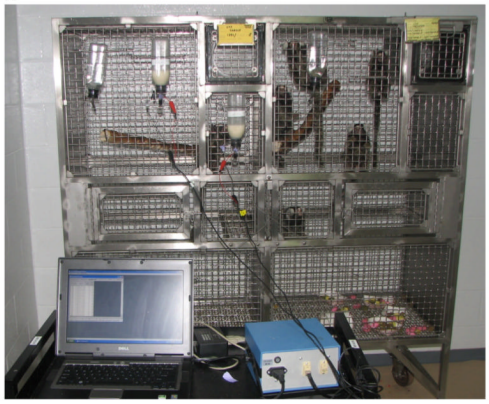

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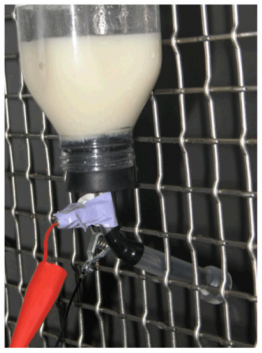


Figure 1.

A) Lickometer setup on a stainless steel marmoset breeder cage. B) The setup of the bottle sipper tube to prevent counts from extraneous contact.

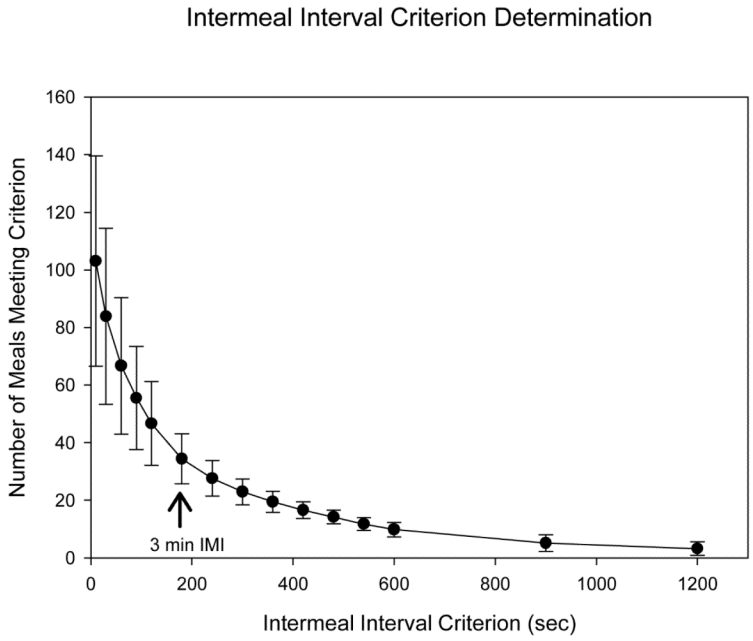


Figure 2.

Defining the intermeal interval criterion. Mean number of meals per marmoset (n = 15) with criteria for the intermeal interval ranging from 10 sec to 20 minutes. As the IMI increases the number of meals decreases. The 3 min IMI criterion is indicated by the arrow.

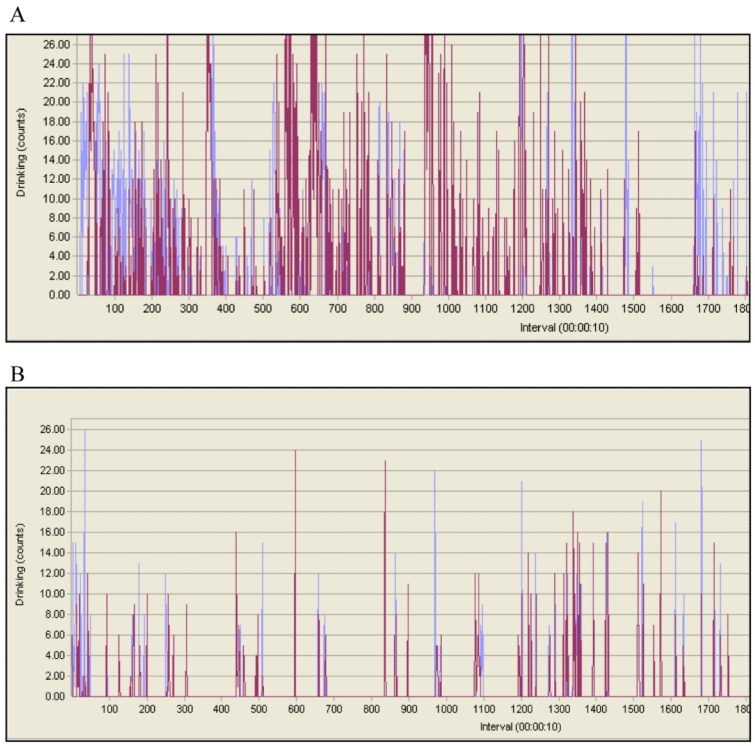
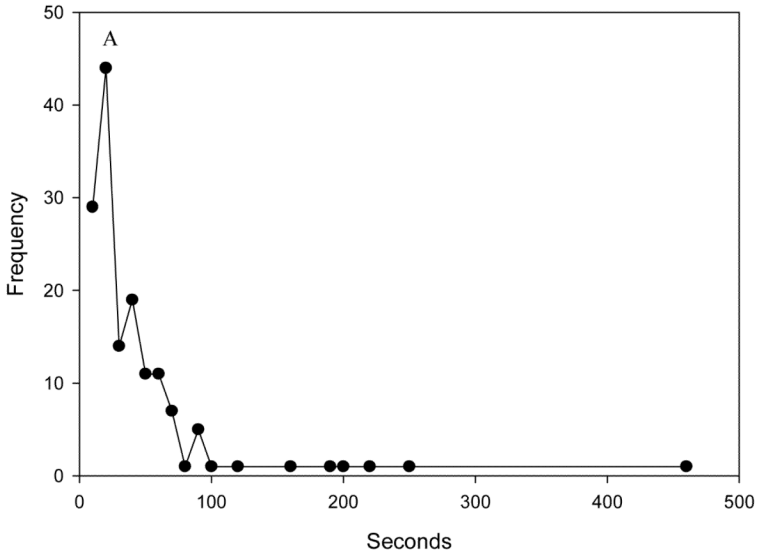
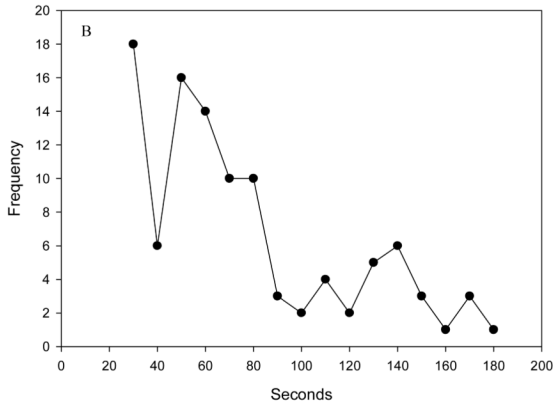
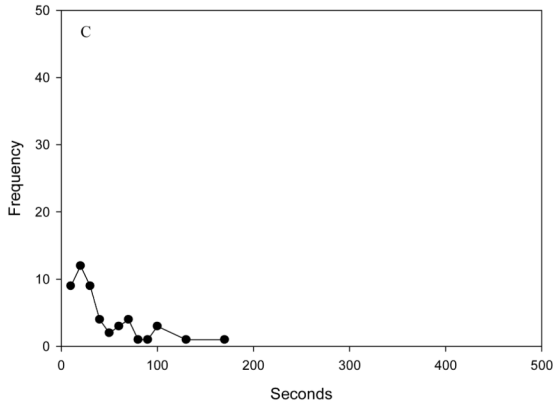


Figure 3.
Lickometer output from the Columbus instruments interface depicting the variation in the eating patterns of individual marmosets, purple indicates the high fat diet and blue indicates the low fat diet. A) This marmoset displayed many counts throughout the day with very few long breaks, B) while this marmoset displayed brief intake periods throughout the day separated by long time periods of no consumption.







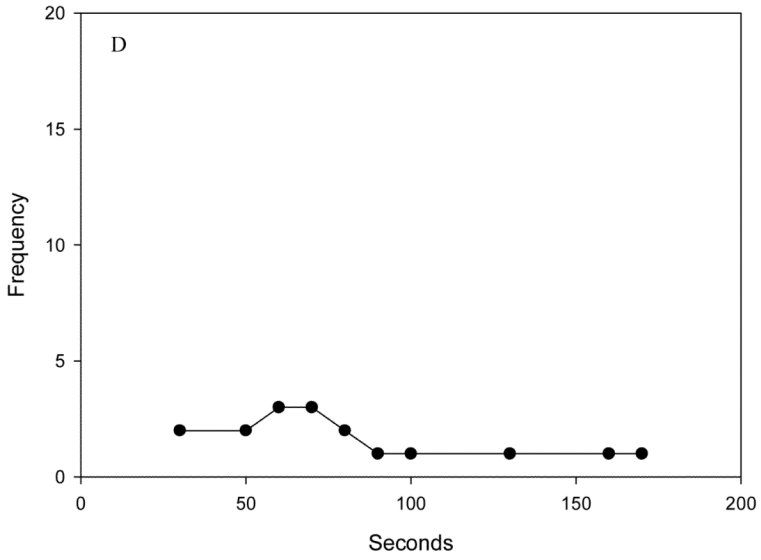


Figure 4.

The frequency of bursts with lengths of 10 seconds to 8 minutes and interburst intervals with lengths of 30 seconds to 3 minutes. A) Bursts for adult animal with the most number of bursts within a meal B) IBI's for adult animal with the most number of bursts within a meal C) Bursts for the adult animal with the least number of bursts within a meal D) IBI's for the adult animal with the least number of bursts within a meal

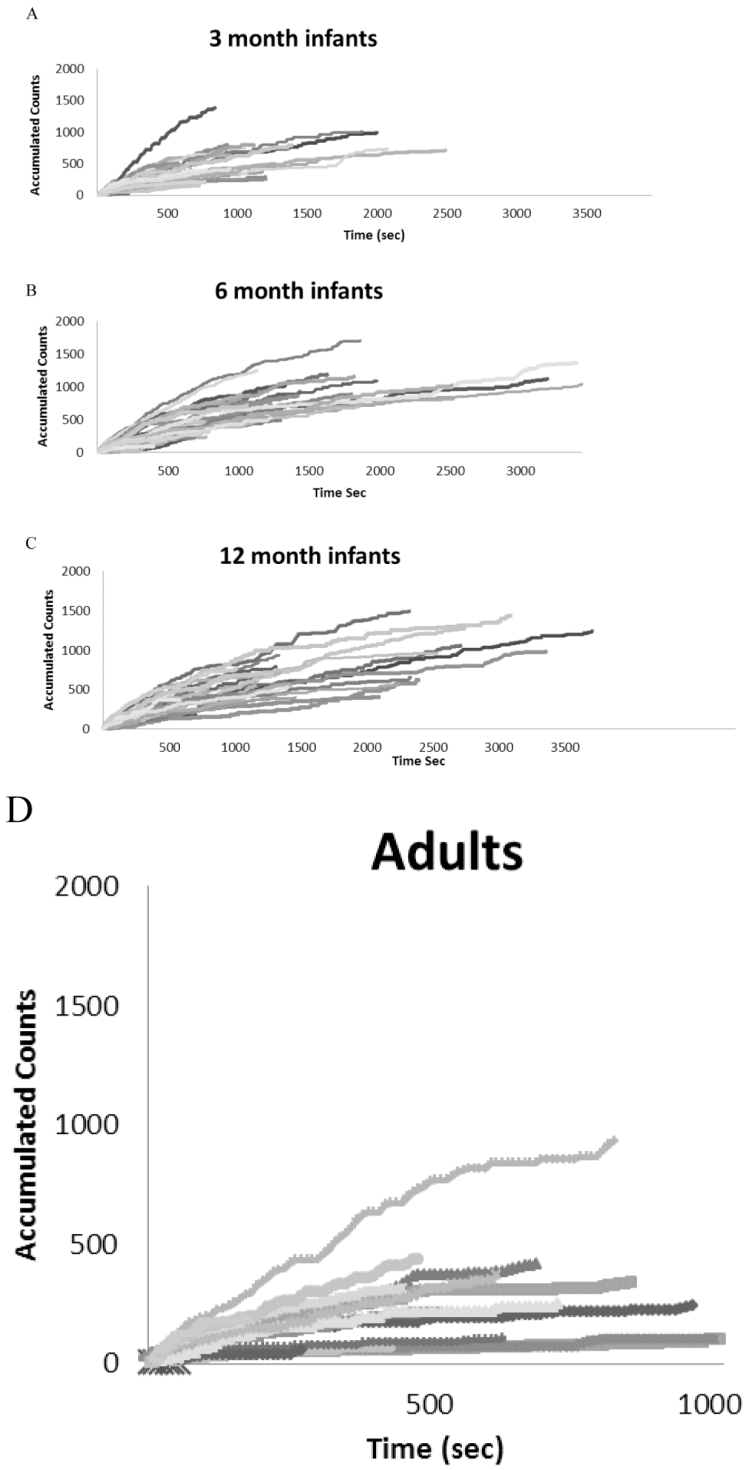


Figure 5.

Cumulative lick counts during the first meal for subjects showing a deceleration in consumption over time for most animals A) 3 month old infants, B) 6 month old infants, C) 12 month old infants, and D) adults.

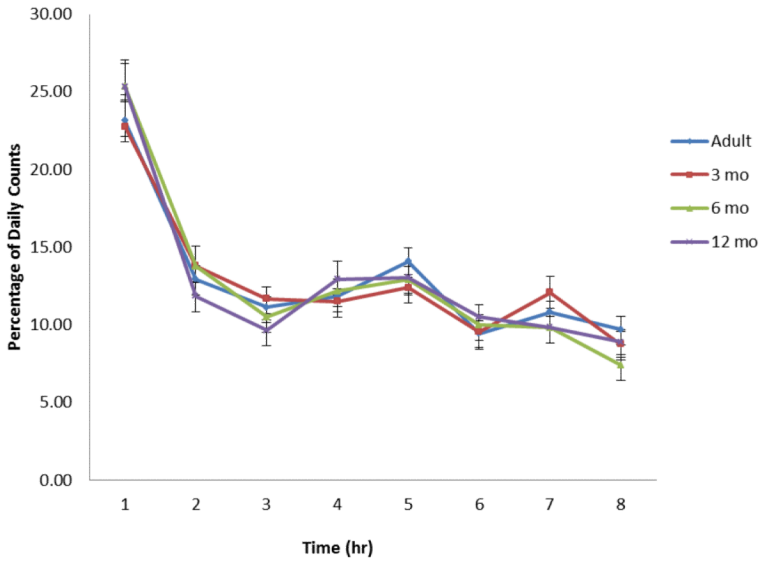


Figure 6.
The average percentage of the daily counts that were accrued during each hour throughout a daily trial for the adult subjects and the infants at 3 months, 6 months, and 12 months (± standard error).

Table 1

Variables derived and analyzed from the lickometer count data, their definitions and how they compare to variables typically used in the rodent literature. For each variable the mean and standard deviation are provided for the adult subjects, and the infants at age 3 months, 6 months, and 12 months.

Variable	Definition	Rodent variable (reference)	Adults X ± SD	3 month X ± SD	6 month X ± SD	12 month X ± SD
Meal	Minimum of two counts, separated by > 3 min (daily time assigned to meal in sec)	Meal (Rushing 1997)	5111.67 ± 3181.76	8956.51 ± 3747.64	10710.38 ± 5061.13	11477.7 ± 5223.88
Burst	Minimum of two counts, separated by > 30 seconds but < 3 min (daily time assigned to burst in sec)	Burst (~250 ms) (Rushing 1997, Houpt 1996, Davis 1992)	2535.28 ± 1341.58	3404.52 ±1380.25	4168.08 ± 1732.51	4567.21 ± 1799.67
Intermeal interval	No interval with > 1 count, > 3 minutes in length (daily time assigned to IMI in sec)	IMI (Houpt 1996, Davis 1992)	19083.75 ± 6597.35	18817.06 ± 3788.43	17920.54 ± 5463.22	17812. 79 ± 6819.45
Interburst interval	No interval with >1 count, > 30 sec (daily time assigned to IBI in sec)	IBI (Rushing 1997, Houpt 1996, Davis 1992)	2561.67 ± 2183.7	5469.84 ± 2500.26	6542.31 ± 3578.13	6909.51 ± 3734.53
Sum Count	Total number of counts for the day	Cumulative licks (Houpt 1996)	2214.64 ± 1250.6	3176.38 ± 1751.45	3768.45 ± 1745.56	3922.92 ± 1706.1
Total Meal Number	Total number of meals for the day		33.73 ± 8.87	37.87 ± 10.39	36.6 ± 9.42	37.61 ± 10.56
Average meal length	Average time (sec) of all meals of the day for an individual	Meal duration (Rushing 1997, Houpt 1996)	159.57 ± 54.58	239.6 ± 98.93	296.58 ± 152.65	320.37 ± 185.65
Average intermeal interval length	IMI Duration (Houpt 1996, Davis 1992)	675.5 ± 250.74	564.41 ± 342.16	562.11 ± 398.5	531.93 ± 399.55	
Meal one length	First meal of the day (sec)	Most studied (Davis 1992)	714.24 ± 498.08	844.76 ± 797.62	1094.92 ± 971.44	1296.56 ± 959.84
Meal one IMI	IMI following first meal of the day (sec)		532.58 ± 733.57	542.06 ± 693.5	450 ± 714.76	504.1 ± 1448.83
Last meal length	Last complete meal of day (sec)		180 ± 198.62	186.83 ± 377.03	354.31 ± 710.13	499.67 ± 1205.52
Last meal IMI	Last complete IMI of the day (sec)		913.33 ± 1405.61	568.65 ± 675.89	835.08 ± 1614.01	1052.62 ± 2784.04

Variable	Definition	Rodent variable (reference)	Adults X ± SD	3 month X ± SD	6 month X ± SD	12 month X ± SD
Average Lick	Counts per meal		70.32 ± 43.14	83.24 ± 41.37	105.03 ± 55.14	111.71 ± 69.12

Table 2

Consumption of liquid diet during lickometer trials for adult marmosets, and infants throughout development at 3 months, 6 months, and 12 months of age.

	Adults X ± SD	3 month X ± SD	6 month X ± SD	12 month X ± SD
Total (g)	55.79 ± 16.27	38.4 ± 13.4	45.05 ± 18.05	48.16 ± 20.36
Hi Fat (g)	24.64 ± 14.19	15.48 ± 9.56	16.09 ± 10.72	13.98 ± 10.26
Low Fat (g)	31.15 ± 15.54	23.24 ± 10.5	28.95 ± 13.45	34.18 ± 18.53
Gram/meal	1.75 ± 0.7	1.06 ± 0.46	1.25 ± 0.53	1.37 ± 0.77
Kcal total	36.43 ± 10.69	25.09 ± 8.81	28.96 ± 11.73	30.58 ± 12.78
Kcal/body mass (g)	0.09 ± 0.03	0.15 ± 0.06	0.12 ± 0.07	0.11 ± 0.06
Body mass (g)	405.9 ± 39.7	168.48 ± 30.28	257.15 ± 65.32	319.92 ± 97.2
Kcal/body mass$^{0.4}$	3.30	3.23	3.15	3.04