Measuring meals: Structure of prandial food and water intake of rats

Eric P. Zorrilla¹, Koki Inoue¹², Éva M. Fekete¹³, Antoine Tabarin¹⁴, Glenn R. Valdez¹, George F. Koob¹

¹ Department of Neuropharmacology, The Scripps Research Institute, 10550 N.Torrey Pines Rd., La Jolla, CA 92037, U.S.A.
² Department of Neuropsychiatry, Osaka City University Medical School, Osaka 545-8585, Japan
³ Institute of Physiology, Pecs University Medical School, Pecs Hungary
⁴ Laboratoire EA 3666 "Homéostasie - Allostasie - Pathologie", Université de Bordeaux 2, 33000 Bordeaux, France

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Address correspondence to:

Eric P. Zorrilla, Ph.D.
Department of Neuropharmacology, CVN-7
The Scripps Research Institute
10550 N. Torrey Pines Rd.
La Jolla, CA 92037
Phone: (858) 784-7416
Fax: (858) 784-7405
e-mail: ezorrilla@scripps.edu
ABSTRACT

Attempts to understand ingestion have sought to understand the control of meals. The present study evaluated a meal definition that included prandial drinking (drinking-explicit meals). Spontaneous nocturnal intake of male Wistar rats was studied. The meal breakpoint was defined as the interval between feeding or drinking events that provided the most stable estimate of meal structure. Alternative breakpoints derived from prevailing methodology, log-survivorship or frequency histogram analysis of inter-feeding intervals without respect to drinking, were compared (drinking-naïve meals). Threshold inter-feeding intervals that accounted for drinking indirectly were evaluated as surrogate breakpoints (drinking-implicit meals). Definitions were compared by determining which criterion better conformed to predictions of satiety. Microstructural differences resulting from the definitions also were studied. Under the drinking-explicit definition, rats averaged 9-10, 13 min meals per night, during which they consumed food and water equally in duration (5-6 min) and quantity (2.3 g). Individual differences were observed in microstructure measures. Meals defined by drinking-informed, but not drinking-naïve, methods were followed by the behavioral satiety sequence and by an initially low likelihood of resuming feeding that monotonically increased with time. The drinking-explicit definition uniquely revealed preprandial and postprandial correlations of similar magnitude. Under drinking-informed definitions, food restriction increased meal size, whereas drinking-naïve definitions reported increased meal frequency. Drinking-implicit definitions provided workable approximations of meal frequency and size, but inferior estimates of feeding duration, eating rate and the preprandial correlation. Thus, log-survivorship analysis is not appropriate for identifying meal breakpoints, and considering drinking in meal definitions can provide a better estimate of meal structure.

KEY WORDS: food intake, feeding or drinking, food-associated drinking, meal size or duration, eating rate, inter-meal interval, behavioral satiety sequence, bout microstructure analysis, meal pattern analysis.
Introduction

The meal is a proposed, physiologically relevant unit of intake. Ingestive behavior also can be described usefully in smaller microstructural units, such as bouts, bites and licks (34). Historically, however, many attempts to understand the control of food intake have focused on understanding the initiation, maintenance and termination of meals (6, 51, 64).

Although meal pattern analysis is common, there has been little uniformity in how meals have been defined (e.g., 7, 10, 19, 42, 58, 59). Typically, a meal has been conceptualized using two-process models, whereby a cluster of feeding events (i.e., a “meal”) is separated from other clusters by a non-feeding interval (i.e., “inter-meal interval”) that is long compared to the intervals between feeding events within clusters (i.e., “intra-meal interval”). Drinking has generally not been considered in rodent meal definitions (i.e., drinking-naïve meal definitions).

Most species, however, show a close relationship between eating and drinking (17, 46, 52, 56). In the rat, 70-85% of spontaneous daily water intake is temporally associated with feeding (21, 32). In intact rats, the relation of feeding and drinking is reflected in alternating bursts of feeding and drinking, hereafter referred to as “bouts.” Several findings indicate that this temporal contiguity is partly regulatory and is not just a by-product of coincident behavioral activity. First, between individuals, rats maintain a consistent food-to-water ratio across feeding episodes (9). Accordingly, within individual rats, the correlation between the amount of food and water consumed in the 40 min surrounding feeding episodes is extremely high (average $r=0.76$ calculated from Ref. (21)). Third, rats gradually increase their “mealwise” water/food ratios to accommodate changes in diet composition that impose greater fluid intake requirements for homeostasis (21, 50). Fourth, restricting food access to the diurnal cycle not only increases diurnal drinking in a stable “mealwise” fashion, but also markedly decreases the amount drunk during the nocturnal cycle, during which rats remain active (21). Fifth, conditions that increase “dry mouth” (e.g., desalivation, decreased food hydration) markedly reduce food intake when water is not available and strongly motivate presumed compensatory increases in prandial drinking when water becomes available (32). Most ($\geq73\%$) daily water
intake in rats occurs in drinking bouts shortly (10 min) prior to or following feeding bouts but a significant
amount (8-10%) occurs in intervals between closely spaced feeding bouts (11, 21, 48). That drinking
precedes, follows and, especially, links bouts of feeding raises the fundamental question of whether
individual feeding bouts as opposed to conjoined clusters of feeding and drinking bouts are better regarded
as meals.

Supporting the latter possibility, three-process models which include a process thought to reflect
intra-meal (i.e., inter-bout) drinking provide better fits for inter-feeding intervals than do two-process models
(4, 46, 65). Moreover, meals, replete with drinking interruptions, but not the individual feeding bouts that
constitute meals, conform to predictions of satiety in the cow (63), that is an initially low likelihood of
resuming feeding immediately after completing a meal that then monotonically increases with time.
Although the ingestive physiology of ruminants differs in important respects from that of single-stomached
animals, we hypothesized that prandial drinking is a shared, integral component of meal-taking.

The method to determine the **precise** threshold meal interval, or breakpoint, that best distinguishes
intra-meal intervals from inter-meal intervals also has been discussed (see (19) for a review). Many studies
have used arbitrary breakpoints (from less than 1 to as many as 40 min). More recently, investigators have
used several forms of empirical analysis to estimate the threshold meal interval (19, 58, 59, 65). Existing
methods focus on the constituent inter-feeding intervals, rather than on the resulting estimated meal patterns.
Thereby, they may not adequately weigh the relative costs of misassigning between-meal intervals as
opposed to within-meal intervals.

One mathematical approach that frequently is used to derive breakpoints for meal and lick pattern
studies -- log-survivorship analysis -- also has recently been challenged (61, 62). A core assumption of
breakpoint methods based on negative exponentials, like log-survivorship analysis, is that the probability of
an event occurring (e.g., starting a meal), does not change as a function of the time elapsed from the previous
event (i.e., feeding) (20). This premise directly contradicts the concept of satiety, which suggests that the
likelihood to initiate feeding would be very low immediately following meal completion and grow with the
passage of time. Evidence supporting the predictions of satiety for post-meal intervals has been observed in feeding records of farm animals (47, 65) and indirectly in lick patterns of rodents (31). However, a recent rebuttal concluded that visual inspection of inter-feeding intervals in rodents provided no strong evidence that precludes the appropriateness of log-survivorship analysis for breakpoint analysis (10). Resolving this issue is critical. If the instantaneous likelihood of initiating a meal in fact grows with time since an animal has last eaten, then the log-survivorship method is not valid for the study of ingestion because it would inherently split meals.

In light of the preceding discussion, the purpose of the present study was to estimate the threshold meal interval in rats through direct analysis of estimated meal size and of estimated meal duration. The procedure was to determine the inter-event interval(s) between feeding- and drinking-directed behavior (i.e., a drinking-explicit meal definition) that provided the most stable joint estimates of meal size and meal duration. Subsequent analyses validated the resultant meal structure by determining a) the existence of stable individual differences, b) the latent structure of prandial food and water intake, c) whether the subsequent likelihood of initiating a new meal over time conformed to predictions of satiety, d) whether how much a rat ate during a meal was related to how long it previously had not eaten or would not subsequently eat, and e) the emission of the behavioral satiety sequence in relation to estimated meal termination. The validity of alternate meal breakpoints derived from log-survivorship or frequency histogram analysis of inter-feeding intervals was compared (i.e., drinking-naïve meal definitions). Because of the possible difficulty of accurately measuring drinking behavior and to allow comparison to studies that have used breakpoints based only on inter-feeding intervals, threshold inter-feeding intervals that sought to account for drinking indirectly also were evaluated as potential surrogate breakpoints (“drinking-implicit meals”).
Materials and Methods

Subjects

Mature (401-522 g, 13-16 weeks of age at the time of testing), male Wistar rats (n=52; Charles River, Hollister, CA) were used. On arrival, subjects were group-housed in a 12h:12h reverse-lit, humidity- (60%) and temperature-controlled (22°C) vivarium. Outside of nosepoke testing, standard rodent chow (LM-485 Diet 7012, Harlan Teklad, Madison, WI) and water were available ad libitum unless otherwise stated. Subjects were acclimated to the vivarium for at least one week prior to the start of experiments. Surgical and experimental procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication number 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Microstructural Analysis of Ingestion

Apparatus

For microstructural analysis of ingestion, rats were tested individually in Plexiglas test cages (22 x 22 cm). Cages had a wire-mesh floor and were located in ventilated, sound-attenuating enclosures equipped with a 1.1 W miniature bulb synchronized to the vivarium’s light/dark cycle. Subjects were allowed to obtain palatable chow pellets (45 mg precision food pellets, Formula A/I; Carbohydrate 60.0%, Fat 3.7%, Protein 24.1%, Ash 7.0%, Moisture 5.2%, 370 Cal/100 g; P.J. Noyes Company Inc., Lancaster, NH) from a trough replenished by an automated pellet dispenser (Med Associates Inc., St. Albans, VT). The acquisition of individual pellets was detected by photobeams that were broken when the rat displaced a freely swinging door to access the pellet in the trough. An additional pellet was not delivered until the door returned to a neutral position, thereby allowing resolution of food-directed behavior at the unit of an individual pellet, similar to a classical “eatometer” (33) or “panel-push” system (2). From a hole on the opposite wall of the test cage, rats could make nosepoke responses, detected by photobeams, to obtain delivery of 100 µl aliquots of water governed by a solenoid water valve (W.W. Grainger, Lincolnshire, IL).
into an adjacent reservoir. Responses, defined as photobeam breaks of at least 0.5 sec duration, were recorded automatically by an IBM PC-compatible microcomputer with 10 ms resolution.

Videotape analysis ($n=12$ rats for 2 hr each) revealed that almost all (97.2%) nosepoke responses for food were followed by immediate consumption of a pellet. The vast majority (96.2%) of “false-positive” (not immediate) trials occurred when the rat responded twice in rapid succession after the feeder failed to deliver a pellet, suggesting that the “false-positive” response was food-directed. Similarly, 96.8% of nosepokes for water were followed by drinking within 5 sec. All “false-positive” (>5 sec latency) water responses observed were followed by drinking within 1 min. Spillage of food pellets in this system was low ($M \pm SEM: 1.2 \pm 0.2 \%$ of total responses; $n=105$ sessions). The results suggest that the present procedures allow highly reliable study of spontaneous feeding and drinking with excellent quantitative and temporal resolution (see also (2)).

Behavioral Testing

Experiment 1

The purpose of Experiment 1 was to establish the threshold interval between inter-ingestive events (i.e., feeding or drinking) that defined the inter-meal interval and to apply this interval to determine the topography, consistency, individual differences and factor structure in spontaneous prandial intake. Rats in Experiment 1 received daily 15-hr sessions spanning their active cycle (-1 hr lights off through +2 hr lights on) until responding had stabilized ($\pm 20\%$ responding for initial 12-hr food intake for 3 consecutive days). To determine the threshold meal interval, data from 4 subsequent treatment-free sessions of naive rats ($n=25$) were analyzed. Sessions began at 1700 hr (-1 hr lights off, the time at which anticipatory nocturnal feeding often begins; (19, 21)), and consecutive test days were ($M \pm SEM$) 5 $\pm$ 0.5 days apart. Thus, stable individual differences were assessed across 3 weeks.

Experiment 2

Experiment 2 had five objectives. The first objective was to derive alternative meal breakpoint estimates using prevailing approaches for defining meals. Drinking-naïve breakpoints were derived from
calculated (20) and subjective (10) applications of log-survivorship analysis of inter-feeding intervals and from subjective analysis of the frequency histogram of inter-feeding intervals under the assumption of a 2-process model (i.e., within-meal vs. between-meal inter-feeding intervals) (45). Drinking-implicit breakpoints also were identified as the threshold inter-feeding breakpoint that would classify between and within-meal intervals most similarly to the proposed 3-process drinking-explicit definition (i.e., within-meal intervals without drinking vs. within-meal intervals with drinking vs. between-meal intervals). A drinking-implicit inter-feeding breakpoint, if valid, could be used in the absence of fluid intake data to perform meal pattern analysis that indirectly accounts for prandial drinking. The second objective was to describe the frequency and duration of prandial drinking pauses as well as the volume of and latency to begin water intake within each pause. For testing, rats in Experiment 2 \( n=6 \) lived in the test cages continuously (24 hr/day) except for brief periods (30-60 min) immediately prior to the onset of their dark cycle during which they were removed to permit maintenance and cleaning. Once stable responding was attained, records of nocturnal inter-feeding intervals from two consecutive sessions were used to estimate alternative meal breakpoints.

The third objective was to test the hypothesis that the meal breakpoints led to a unimodal distribution of estimated post-meal intervals. A bimodal or otherwise heterogeneous distribution of post-meal intervals would suggest that a breakpoint was inappropriately splitting or merging meals.

The fourth objective was to test the hypothesis that the time since a rat had last eaten was associated with its moment-to-moment likelihood of initiating a meal. Specifically, it was determined whether meal definition(s) produced nocturnal post-meal intervals that conformed to predictions of satiety (i.e., an initially very low probability of initiating a meal following completion of a meal that monotonically increases thereafter) or, in contrast, whether nocturnal meal onsets had a constant starting probability across time, a core assumption of log-survivorship analysis.

The fifth objective was to test the hypotheses that the time since a rat had last eaten was associated with the size of its next meal (i.e., the “prandial correlation”) and also that the size of a rat’s meal was
associated with the duration for which it subsequently would not eat again (i.e., the “postprandial correlation”). These correlations are postulated to reveal how rats flexibly regulate their intake from meal-to-meal. A significant preprandial correlation is hypothesized to reflect the influence of a positive drive state of short-term insufficiency (colloquially “hunger”) on subsequent meal size. A significant postprandial correlation is hypothesized to reflect the influence of the amount consumed within a meal on the subsequent persistence of the state of not eating (colloquially “satiety”). Since the initial studies of Le Magnen and Tallon (43, 44), the magnitude of these correlations have been examined frequently. The prevailing dogma is that rats do not exhibit a preprandial correlation, but that they perhaps exhibit a postprandial correlation (15, 16, 18, 19, 43, 57, 60). Some (but not all) investigators have reported postprandial correlations, but such correlations have been suggested to be artifacts of excessively long meal breakpoints or of the inappropriate grouping of data.

For objectives 3-5, records from four sessions within a 7-day period were used to analyze the distribution of post-meal intervals. The first meal from each session was excluded because of the potentially confounding influence of recent experimenter intervention, the uncertainty of the pre-meal interval and the inaccess to food during cage cleaning. The final nocturnal post-meal interval was excluded because of the confounding influence of the switch to the diurnal cycle.

Experiment 3

Experiment 3 determined which criteria defined meal terminations that were reliably followed by emission of a behavioral satiety sequence (1, 24), in which sated rats transition from termination of feeding to increased behavioral activity (e.g., grooming, sniffing) and finally to rest. The behavioral satiety sequency has most often been studied in food-deprived rats that are provided renewed access to food during their light cycle. However, postprandial resting also occurs following meal offset during the dark cycle in free-feeding rats (10, 15, 48). Nocturnal postprandial resting reportedly occurs in direct proportion to meal size even in the absence of separate sleeping niches (5).
For testing, rats \((n=10)\) in Experiment 3 resided in test cages as did rats in Experiment 2 (23.5 hr/day). Following attainment of stable intake, rats were individually videotaped for 2 hr from the onset of their daily nosepoke session concurrent with automated recording of responses for food and water. To compare the construct validity of the definitions, meals were defined using the 3-process drinking-explicit (i.e., IRI-300 Food or Water) or drinking-implicit criteria (i.e., IRI-840 or 1065 Food) or using the 2-process, drinking-naïve criteria (i.e., IRI-19 or 120 Food). To assess the behavioral satiety sequence, a reliable, hypothesis-naïve rater (ÉMF) coded each rat’s behavior from videotape at 5 sec intervals as resting (defined to include standing inactivity, but more often observed as the rat lying on the wire-mesh floor), active, eating or drinking.

**Experiment 4**

The purpose of Experiment 4 was to compare the effects of 22-hr food deprivation and chronic food restriction on the estimated meal structure that resulted from the competing meal definitions. If the definitions led to different estimates of the microstructural effects of food restriction, then it would demonstrate the practical relevance of defining meals as outlined here. Rats \((n=11)\) were provided daily 6 hr nocturnal nosepoke sessions, outside of which food and water were available *ad libitum*, until stable responding for food was achieved. Thereafter, rats were limited to 2 hr of daily nosepoke access (Day 0) with *ad libitum* water, but not food, access outside of nosepoke testing. The microstructure of 2-hr intake under *ad libitum* conditions (Day 0) was compared to the microstructure of intake following a single 22 hr food deprivation (Day 1) and also following chronic, scheduled food restriction (Day 7) using the proposed drinking-explicit meal definition (IRI-300 Food or Water) and alternative 2-process, drinking-naïve (IRI-19 or 120 Food) and 3-process, drinking-implicit (IRI-840 or 1065 Food) meal definitions.

**Statistical Analyses**

Logarithmic scales were used for graphical presentation of frequencies and rates of change involving time, as has been recommended to resolve meal structure (62)

**Experiment 1**
Estimation of drinking-explicit threshold meal interval. The drinking-explicit threshold meal interval was estimated by determining the inter-event interval(s) between feeding- and drinking-directed nosepokes that provided the most stable, joint estimates of meal size for food and total meal duration, thereby minimizing the negative consequences of misassigned events and time. This method is related to previous approaches in which transitions or stabilities in the slope of a function were identified through first-derivative analysis (14). The current approach relies on the assumption that if the distributions of within- and between-meal intervals overlap, they do so on descending and ascending portions of their distributions, respectively, as has been shown previously (10, 65). Intervals close to the true meal threshold criterion misassign relatively few events and, therefore, are associated with a local stability in the rate of change in both measures of estimated meal structure. Intervals further from the “true” threshold interval will misassign varying quantities of events determined by the underlying distributions of within- and between-meal intervals. Consequently, for first-derivative functions of estimated meal size and duration, the threshold interval is marked not only as a stability, but also as a local minimum inflection point (or range of points).

A meal for rats was defined as any burst of responses for food or water that contained at least 5 food-directed responses, or 0.225 g, a value more than 2-fold lower than empirically estimated lower bounds for meal size (0.457-0.617 g (19)). Average meal characteristics (i.e., meal size for food and total duration) were estimated using a series of maximum interresponse (IRI) intervals ranging from 30 sec to 30 min, where 30 min was used as the upper bound because it was the first interval at which all nocturnal ingestion for any subject was characterized as a single meal, thereby creating an artificial floor effect for longer intervals. Having determined the zero-order functions for the estimated meal characteristics, local rates of change in the slope were calculated as the difference in the value of the zero-order function for consecutive intervals per standardized unit of time (30 sec).

Three approaches were used to define the minimum inflection point(s) that marked the threshold meal interval in the “first-order” function. First, the absolute minimum was identified visually. Second, linear regression was performed on mean values spanning the candidate minimum to determine whether the
average “first-order” function was fit significantly better in this range by one line as opposed to two or more lines intersecting at the minimum (i.e., segmented, multi-phase, or “hockey stick” regression; (25)). The latter solution would suggest a threshold meal interval at the minimum(s) that provided the best joint fit. Finally, multivariate adaptive regression splines (MARS), a brute force segmented regression procedure (22), was applied to the aggregate, first-order individual data. MARS, an objective, exhaustive, stepwise regression method, conjointly seeks to maximize the variance explained and minimize model overfitting by identifying incrementally significant breakpoints in the slope of the predictor space. The specific question of interest was whether MARS identified the candidate minimum as a statistically relevant inflection point. Analyses were limited to the nocturnal hours of observation since the microstructures of diurnal and nocturnal intake differ (47). Day was the unit of analysis.

**Meal structure parameters.** The estimated threshold meal interval was used to calculate descriptive statistics of average nocturnal meal structure. Parameters included the number of meals; the average size, duration and response rate of meals; the average intermeal interval; the food/water ratio; and the satiety ratio. Meal duration was calculated as the total time from the first to last response of a meal, and duration of eating and drinking within the meal was calculated as the duration of consecutive responses for food or water, respectively. Thus, transitions between eating and drinking were included in total meal duration, but not in the specific durations of eating or drinking. Meal sizes for eating and drinking were calculated separately as the average number of food or water-directed responses during meals. Rates of eating and drinking were calculated by dividing each meal size with its respective duration. The intermeal interval was defined as the interval from the last feeding response of a meal to the first feeding response of the next meal. The food/water ratio, an index of the balance between food and fluid intake, was defined as the ratio between the quantities of food and water consumed per meal. Finally, satiety ratio, an index of the non-eating (i.e., “satiety”) time produced by each gram of food consumed, was calculated as the average intermeal interval divided by the average meal size for food.
Individual differences. To determine whether stable individual differences existed in the microstructure of ingestion and to determine whether group means were consistent over time (23), a mixed-model analysis of variance (ANOVA) was performed with Subject as a grouping factor and Day of testing as a within-subject factor. The magnitude of individual differences, indicated by significant effects of Subject, were quantified with two-way random effect intraclass correlations (ICC; Shrout and Fleiss 1979), indicating the reliability of absolute differences between single observations (ICC[2,1]) or the means of the subjects’ 4 observations (ICC[2,4]). To allow comparison to individual differences, significant Day effects, indicating varying group means, were calculated as an $\eta$ measure of effect size from the following simplified formula (Equation 1):

$$\sqrt{\frac{(df_1 \times F)}{((df_1 \times F) + df_2)}}$$

(David C. Howell, University of Vermont, personal communication) (1)

Factor structure of the microstructure of ingestion. To identify constructs underlying differences in the microstructure of daily, nocturnal ingestion, meal parameters were subjected to a principal components factor analysis with varimax rotation. Five orthogonal factors with eigenvalues greater than 1 were retained (30), a threshold that corresponded with visual identification of the scree plot (8). Factor loadings were computed and interpreted.

Experiment 2

Objective 1 – Alternative drinking-naïve and drinking-implicit breakpoints. Log-survivorship analysis was performed as described previously (20). Briefly, the attrition of inter-feeding intervals (log Y-axis) was plotted as a function of increasing duration (X-axis). The following double negative exponential (Equation 2) was fit to the resulting semi-logarithmic scatterplot:

$$y(x) = (1 - p)e^{-wx} + pe^{-bx}$$

where $y(x)$ represented the proportion of interfeeding intervals longer than duration $x$, $p$ represented the proportion of interfeeding intervals that were alleged between-meal intervals, $1 - p$ represented the proportion of interfeeding intervals that were alleged within-meal intervals, and $w$ and $b$ represented the
initiation rates of within- and between-meal responses, respectively. The associated threshold meal criterion \((T)\), reflecting the intersection of the more vertical, fast process (intra-meal intervals) with the more horizontal, slow process (inter-meal intervals), was calculated as follows (Equation 3):

\[
T = \left(\frac{1}{w-b}\right) \times \log\left(\frac{(1-p) \times w}{p \times b}\right)
\]

To identify a 2-process, drinking-naïve, frequency histogram-based breakpoint, the frequency histogram of inter-feeding intervals was constructed using equal-sized logarithmic time bins, and the trough between the alleged distributions of within- and between-meal intervals was estimated.

To identify a 3-process, drinking-implicit breakpoint, separate probability functions were fit to the frequency histograms of (1) intra-meal feeding intervals that did not contain drinking, (2) intra-meal feeding intervals that contained drinking, and (3) inter-meal feeding intervals, as determined from the proposed drinking-explicit definition. For each distribution, the peak distribution function with the greatest \(r^2\) was accepted (TableCurve 2D 5.01; Systat Software, Point Richmond, CA). The possibility of non-Gaussian functions (i.e., log-normal, Pearson family, or Weibull) was included to permit the possibility of finite/semi-infinite (as opposed to infinite) as well as time-dependent, asymmetric distributions, both of which would be consistent with the constructs of within-meal satiation and between-meal waning of satiety. The fit of the combined mixed distribution model was assessed using PeakFit 4.12 (Systat Software, Point Richmond, CA).

**Objective 2 – Description of prandial drinking pauses.** Descriptive statistics were calculated for the frequency and duration of prandial drinking pauses as estimated under the drinking-explicit definition and for the quantity of and latency to initiate water intake within each pause. The frequency distribution of the latency to initiate drinking within each pause was graphed as raw data and as a logarithmic function of time to determine if a single distribution described the initiation of drinking between presumed bouts of feeding.
The relation of the duration of drinking pauses to the amount drunk during the pause was calculated as a Pearson correlation.

Objective 3 - Distribution of post-meal intervals. The frequency histograms of estimated post-meal intervals under each breakpoint were graphed as raw data and as a logarithmic function of time to visualize the underlying distribution(s).

Objective 4 – Probability of meal initiation. To compare the estimated likelihood of initiating a meal across time under each meal definition, the “instantaneous” probability of meal initiation was calculated as 100*(the incremental number of rats that initiated their second meal within the time bin of interest / the number of rats that had not yet initiated a second meal at the onset of the time bin of interest). Meal initiation probabilities were examined on short (0-16 min; starting within 4 min) and long (0-4.5 hr; starting within 15 min) post-meal time-scales through an instantaneous starting probability of 100%. For comparison, the random slow process that purportedly governs meal initiation under the assumptions of log-survivorship analysis was depicted as a function of the initiation rate $b$, as calculated in Objective 1.

Objective 5 – Preprandial and postprandial correlations. To determine the magnitude of the pre- and post-prandial correlations, separate Pearson correlations were performed for each rat to determine the relation between the sizes of all the nocturnal meals it consumed during the test sessions with the log-transformed duration of its contiguous pre- or post-meal intervals, respectively. To determine the average pre- and post-prandial correlation across rats, *individual* Pearson correlations were combined across animals using *df*-weighted fixed effect meta-analysis as described elsewhere (66). This procedure eliminates the artifacts introduced from performing correlations on raw inter-meal intervals, which have a highly positively skewed distribution (see Results), from averaging each subject’s data prior to performing the correlation, or from performing the correlation on all meals across circadian phases or across all subjects (i.e., “ecological
fallacies”; (53)). Averaging and pooling distorted estimates of the true pre- and post-prandial correlations in much previous work (see also (7, 15, 49, 60)). A modification of the Stouffer method was used to calculate associated $p$-values (54). The relative magnitudes of the pre- and post-prandial correlations were then compared using $df$-weighted fixed effect meta-analysis. The software packages used were Excel 2003 (Microsoft, Redmond, WA) and Comprehensive Meta-Analysis 1.0 (Biosoft, Englewood, NJ).

**Experiment 3**

Behaviors were analyzed for 15-min beginning from the first full minute after the last food response of the estimated meal. A behavioral satiety sequence was judged to be present if the rat initially exhibited primarily active (i.e., grooming, rearing, sniffing, locomotion) or drinking behaviors that were subsequently replaced by a predominance of resting behavior in the absence of feeding. To determine whether the meal definitions influenced the frequency with which a behavioral satiety sequence was observed shortly following estimated meal completion, a chi-square analysis was performed. In addition, the average frequency of each behavior category was compared using separate 5 (Meal Definition) X 15 (Time: 1-min bins) repeated-measure ANOVAs.

**Experiment 4**

To determine whether the meal definitions gave rise to different interpretations of the effects of acute or chronic food restriction on the microstructure of feeding, 2-hr meal pattern measures were subjected to a 5 (Meal Definition) X 3 (Restriction: Ad lib vs. 1 or 7 days of restricted feeding) repeated-measures ANOVA, and post hoc within-subject Newman-Keuls tests were used to interpret significant effects. The focus of the present analyses was on the effects of meal definition, so detailed analysis and interpretation of the microstructural effects of food restriction are discussed elsewhere (26).
Additional software packages used were Systat 10.0 (SPSS, Chicago, IL), SPSS 10.0 (SPSS), InStat 3.0 (GraphPad, San Diego, CA), DataFit 8.0 (Oakdale Engineering, Oakdale, PA), and MARS 2.0 (Salford Systems, San Diego, CA).
Results

Experiment 1

Estimation of Drinking-Explicit Threshold Meal Interval

Figure 1 shows the zero- and first-order functions of estimated average meal duration for rats as a function of the maximum IRI between food and water responses defined to continue a meal (i.e., drinking-explicit meal definition). A large range (4-62 min) was evident in the estimated average meal duration depending on the maximum IRI used (Figure 1, top). Linear regression revealed that the resulting first-order scatterplot (Figure 1, middle) was fit better by a two-line solution than by a single regression line ($p<0.0001$). IRI values in the range of 210 to 720 showed a relative stability in the rate of change, with an absolute minimum at 300 sec. Linear regression on mean values revealed that two lines intersecting at this minimum provided the best joint fit of any two-line solutions. Values to the left of the minimum indicated a decreasing rate of change ($y=120 - 0.324x$, $r^2=0.91$), consistent with the predicted decrease in misassigned time. Values to the right of the minimum indicated an increasing rate of change ($y=16.6 + 0.35x$, $r^2=0.77$), consistent with the predicted increase in misassigned time. MARS analysis of the aggregate individual data revealed breakpoints in the slope of the first-order function at the following 4 intervals: IRI 90, 210, 300, 1200 sec (Figure 1, bottom; basis functions [BF]: BF1=max[0, IRI-300], BF2=max[0, 300-IRI], BF3=max[0, IRI-90], BF4=max[0, IRI-210], BF5=max[0, IRI-1200]; $y=240.82 + 0.78BF1 - 0.68BF2 - 1.15BF3 + 0.39BF4 + 0.06BF5$). Of these, the IRI 300 knot was the only minimum inflection point. Similar results were obtained from inspection of the first-order function of estimated meal size (data not shown). Accordingly, the maximum IRI for rats was defined as 300 sec between feeding or drinking events.

Temporal Probability of Meal Initiation

Figure 2 shows the probability distribution of the average intermeal interval as a function of the time since completion of the previous meal. Consistent with predictions of satiety, very few meals were initiated
shortly after a meal terminated, with the average probability of imminent meal initiation subsequently increasing to a maximum likelihood of approximately 50-60% at 35-65 min after the last meal.

Characteristics of Nocturnal Prandial Intake

Table 1 shows descriptive statistics for selected characteristics of nocturnal prandial intake in the rat using the IRI 300 sec definition. On average, rats consumed 9-10, 13-minute meals per night. Under this diet, food and water were consumed equally in quantity, duration and rate. More variability was evident in drinking-related measures (SD’s comprising 36-45% of means) than in eating-related measures (SD’s comprising 15-31% of means). Under this meal definition, a large separation was evident between the largest observed average within-meal intervals (for water, 27.3 sec; for food, 9.2 sec) and the smallest observed average between-meal interval (34.1 min).

Sources of Variability in Nocturnal Prandial Intake

Individual differences were a large source of variability in the microstructure of spontaneous nocturnal prandial intake, accounting for 26-88% ($M \pm SEM: 57 \pm 5\%$) of the variance in subjects’ average scores across 3 weeks of observation, as reflected in significant Subject effects (average $r=0.77$, $p<0.0001$). In contrast, not one of the group means differed across Days of testing during the 3 week sampling period. The findings testify to the reliability and consistency of these measures and indicate the existence of substantial, stable individual differences in the meal microstructure of ingestion (see Supplemental Online Table 1).

Factor Structure of Nocturnal Prandial Intake

Table 2 shows the factor structure of the measures of nocturnal prandial intake as revealed by principal components factor analysis. Five interpretable, orthogonal factors, each accounting for 13.5-26.2% of the total variance, were retained. Factor 1, a “meal patterning” factor, described whether subjects had
few, but large, meals or many, but small, meals. Factor 2, which included the food/water ratio measure and was termed “prandial thirst,” supported the hypothesis that meal-related drinking is partly determined by individual-specific, regulatory needs related to feeding. Prandial thirst was dissociable from food intake per se, however, evidenced by the absence of large loadings for the feeding measures. Factor 3, a “satiety” factor, was the only factor predictive of the rats’ total nocturnal food intake and associated strongly with the aptly named satiety ratio. Factors 4 and 5 reflected the rats’ drinking and eating rates, respectively. In sum, constructs underlying “how” subjects ate and drank (Factors 1, 4 and 5) were largely dissociable from “how much” (Factors 2 and 3). The only “how” measures that specifically loaded on the factors describing “how much” was consumed were the food/water ratio, for fluid, and the satiety ratio, for food.

Insert Table 2 about here

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**Experiment 2**

**Objective 1. Estimation of Alternative Drinking-Naïve and Drinking-Implicit Threshold Meal Intervals**

To compare the validity of other methods used to estimate the meal threshold criterion, drinking-naïve meal breakpoints were identified using prevailing approaches – log-survivorship and frequency histogram analysis. Figure 3 depicts the aggregate frequency histogram of all inter-feeding intervals \( n=6361 \) from 12 nocturnal feeding sessions of 6 rats (bottom panel) and the corresponding log-survivorship breakpoint analysis (top). An inverse \( y \)-weighted double exponential function fit the interval attrition scatterplot \( y=0.9693 e^{-0.2295x} + 0.0307 e^{-0.0003977x} ; r^2=0.96 \) and indicated an intermeal breakpoint of 18.6 sec between feeding events (see Figure 3, top). However, the aggregate frequency histogram (bottom panel) clearly showed that many intervals beyond the purported meal threshold (e.g., 18-47 sec) were continuous with the large distribution of presumed intra-meal intervals and discontinuous from the distributions of much less frequent, longer intervals. The results suggested that the calculated breakpoint misclassified intervals at the vertex of the log-survivor function and thereby split meals near the shoulder of the fast (i.e., intra-meal) distribution. Therefore, we also identified an intermeal breakpoint of 120 sec based on the subjective convention of defining the threshold just to the right of the rapid acceleration in slope, an approach still in
practice and which gave a value consistent with rat feeding studies that used log-survivorship analysis in this manner (10, 23).

As a second approach, a breakpoint was determined from inspection of the frequency histogram of inter-feeding intervals under the assumption of a 2-process, drinking-naive model (i.e., within-meal vs. between-meal inter-feeding intervals) (Figure 3, bottom). Although a formal maximum likelihood 2-model function could have been fit to this distribution (e.g., (65)), all published studies in rodents have adopted a subjective approach (see (19)), which we therefore adopted for comparative interest. Potential arguments could be made for a subjective breakpoint at 60 sec or anywhere along a trough from 96-317 sec (Figure 3, bottom). Smoothing the function by aggregating data into larger bins suggested an arbitrary breakpoint between 76 and 317 sec (see Figure 4, top panel). Thus, the subjective log-survivorship based estimate of 120 sec also was an acceptable estimate for the subjective, 2-process histogram approach.

As a third general approach, we estimated a meal threshold criterion under a 3-process model composed of 2 within-meal processes (i.e., intra-meal sustained feeding and intra-meal pauses to drink) and 1 between-meal process (i.e., meal initiation) (65). A practical motivation for this was to determine if a breakpoint based only on inter-feeding intervals that implicitly recognized intra-meal drinking pauses could discriminate within-meal intervals from between-meal intervals as effectively as the proposed drinking-explicit definition. An equivalent drinking-implicit criterion would be valuable because it would allow meals to be defined properly in the absence of fluid intake data. To do this, separate frequency histograms were constructed of (1) intra-meal inter-feeding intervals that did not contain drinking, (2) intra-meal inter-feeding intervals that contained drinking and (3) inter-meal feeding intervals as defined by the drinking-explicit definition (see Figure 4). Peak probability functions were individually fit to each of these histograms (see Methods for details). Excellent fits were observed for each distribution, supporting the proposed classification of inter-feeding intervals ($r^2$'s=0.9999997, 0.95, 0.98 for intra-meal feeding intervals without drinking, intra-meal intervals with drinking and inter-meal intervals, respectively; see Figure 4).
combined mixed distribution model also fit the aggregate frequency histogram well, further supporting the 3-process model ($r^2=0.999993; F[1,13]=386229; \text{Figure 4, top left overlay}$). Under this model, the intersection of the intermediate and slowest functions (Figure 4, bottom left and bottom right distributions, respectively) represents the threshold meal criterion that minimizes the expected number of misassigned events (65) and was interpolated to be an inter-feeding interval of 1065 sec (17.7 min). As a second empirical criterion under the 3-process model, the inter-feeding interval was identified that minimized the number of observed (as opposed to expected) inter-feeding intervals which were classified differently from the drinking-explicit definition. This breakpoint (826-852 sec) only classified 6 intervals (0.09%) differently from the drinking-explicit definition, in each case merging estimated meals, and was treated as 840 sec (14 min).

**Objective 2. Description of Prandial Drinking Pauses**

Under the drinking-explicit definition, each of the 6 rats exhibited prandial drinking pauses during their nocturnal meals ($M+SEM: 4.7 \pm 1.0$ pauses per night). Individual pauses averaged slightly longer than 5 min ($5.7 \pm 0.6$; range: 0.4 - 12.7 min) and contained approximately 2 ml of water intake ($1.9 \pm 0.3$; range: 0.1 – 6.2 ml), with a strong correlation present between the duration of the pause and the quantity drunk ($r[28]=0.64, p<0.0001$). Latencies to initiate drinking within the pause were distributed unimodally as a log-normal function of time (not shown), with a mean transformed interval of 45 sec (range: 7 - 269 sec) from the prior feeding response.

**Objective 3. Effect of Meal Definition on the Estimated Post-Meal Interval**

Figure 5 shows the effects of the meal definition used on the estimated distribution of post-meal intervals. The left panels depict post-meal intervals on a linear time scale as has been conventionally done. All breakpoints suggested a highly positively skewed, non-Gaussian distribution for post-meal intervals. Logarithmic transformation of time (right panels) revealed a homogenous, unimodal distribution of post-
meal intervals for the drinking-explicit breakpoint (IRI-300 Food or Water), indicating that post-meal intervals are approximately log-normally distributed. Thus, under the drinking-explicit breakpoint, meals were estimated to be spontaneously initiated as an exponential function of time since the prior meal, entirely consistent with the predictions of satiety.

In contrast, breakpoints derived from drinking-naïve log-survivorship and frequency histogram analysis (IRI-19 or IRI-120 Food) resulted in heterogeneous, non-unimodal log-transformed distributions. Inter-feeding intervals of <7.5-10 min duration appeared to belong to different functions, hypothesized to be the inadvertent splitting of meals by the breakpoints.

The drinking-implicit breakpoints (IRI-840 or IRI-1065 Food) yielded unimodal log-normal distributions. However, the drinking-implicit log-transformed distributions were slightly truncated at the left tail relative to the drinking-explicit definition (see Figure 5).

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Objective 4. Effect of Meal Definition on the Estimated Instantaneous Probability of Initiating a Meal

Figure 6 shows the effect of the meal definition used on the estimated instantaneous probability of initiating a meal as a function of the time since the rat had last eaten. Starting probabilities are shown on short (left panels, 0-16 min) and long (right panels, 0-4.5 hr) post-meal time scales. As shown in Figure 6, none of the observed meal initiation functions fit the basic assumption of log-survivorship analysis (dashed line, top panels), which is that the meal initiation rate was constant \( b \) irrespective of the time since prior meal completion.

Rather, the drinking-explicit definition (IRI-300 Food or Water, Fig. 6, top) indicated that the likelihood of initiating a meal increased monotonically as a function of time since the prior meal. Relative to the log-survivorship null hypothesis, this reflected a lower probability of resuming feeding following brief post-meal intervals and a higher probability of resuming feeding following longer post-meal intervals.
The drinking-naive log-survivorship and frequency histogram definitions (IRI-19 or IRI-120 Food, Fig. 6, middle) did not suggest a monotonically increasing likelihood of initiating a meal following meal completion. Rather, at brief post-meal intervals (<12 min), instantaneous probabilities of starting a meal within 4 min were as high as 30% and then decreased substantially to levels that resembled the drinking-explicit definition (Fig. 6, left). The relative excess of starting probabilities at brief post-meal intervals is reflected in the bimodal distribution of post-meal intervals in Figure 5 and supports the hypothesis that the definitions systematically (but inadvertently) split meals.

On long time scales (Fig. 6, right), the 3-process drinking-implicit definitions (IRI-840 or IRI-1065 Food, bottom) suggested monotonically increasing starting probabilities which closely resembled those that resulted from the drinking-explicit definition. However, at short post-meal intervals (Fig. 6, left), the drinking-implicit definitions yielded smaller starting probabilities than the drinking-explicit definition. The hypothesized underestimation of early starting probabilities is reflected in the truncated distribution of post-meal intervals in Figure 5 and is hypothesized to reflect that the drinking-implicit definitions systematically overlooked the briefest post-meal intervals and thereby merged some meals.

Objective 5. Effect of Meal Definition on the Estimated Preprandial and Postprandial Correlations

Table 3 summarizes the observed relation of meal size with the contiguous preprandial and postprandial intervals. The drinking-explicit meal definition yielded small-to-moderate, but significant, average preprandial and postprandial correlations that were of comparable magnitude (see Table 3). Within every individual subject, the correlations were positive. Thus, as shown in Figure 7, subjects reliably ate larger meals when they had not eaten for longer periods of time (a preprandial correlation) and went longer without eating following larger meals (a postprandial correlation). No other meal definition led to the identical pattern of findings. Only one of the drinking-implicit definitions (IRI-1065 Food) also led to the conclusion that the magnitude of the preprandial and postprandial correlations did not differ reliably, but it
suggested correlations which were less robust than those obtained from the drinking-explicit definition (see Table 3).

Performing correlations across all rats on decile averages of meal size and intermeal intervals as was done historically (44), greatly inflated estimates of the preprandial and postprandial relations (e.g., $r’s=0.83$ and 0.94, respectively, under the drinking-explicit definition).

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**Insert Figure 7 and Table 3 about here**

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**Experiment 3**

**Effect of Meal Definition on Estimated Emission of the Behavioral Satiety Sequence**

Meal definition also significantly influenced the frequency with which a behavioral satiety sequence was observed shortly following estimated meal termination ($\chi^2[4]=35.4$, $p<0.0001$). A behavioral satiety sequence was evident within the 15 min analysis period in 9 of 10 rats under each of the drinking-inclusive definitions, with the IRI-300 Food or Water and IRI-840 Food definitions yielding identical results. Under each of the drinking-inclusive definitions, the lone rat not to exhibit a behavioral satiety sequence did not resume feeding within the observation window, but simply remained active. In contrast, a subsequent behavioral satiety sequence was very rare under the drinking-naïve, 2-process meal definitions (0/10 and 1/10 for the IRI-19 and 120 Food definitions, respectively). Under the drinking-naïve definitions, 60% of rats ate during more than one-third of the rating samples within 6 min of meal completion.

Figure 8 shows the average time-sampled behavior of rats following estimated meal termination as judged by each definition. Meal definition significantly influenced the frequency with which eating (Definition: $F[4,36]=17.55$; Definition X Time: $F[56,504]=1.85$, $p’s<0.001$), active behaviors ($F[4,36]=5.78$; $F[56,504]=3.13$, $p’s<0.001$), drinking ($F[56,504]=2.26$, $p<0.001$) and rest ($F[4,36]=30.43$; $F[56,504]=5.56$, $p’s<0.001$) were observed following estimated meal termination. Under the drinking-inclusive definitions, primarily active and drinking behaviors initially followed meal completion, but subsequently diminished until resting vastly predominated after 6 min. Under the drinking-naïve definitions, activity and drinking
also initially predominated, but they were rapidly supplanted by resumption of feeding after 3-5 min. At no
time under the drinking-naïve definitions did resting comprise more than one-third of behavioral samples.

Experiment 4

Effect of Meal Definition on Estimated Effect of Food Restriction on Meal Patterning

Meal definition also significantly influenced estimates of meal frequency, meal size, duration of
feeding within meals, and eating rate in rats under *ad libitum* or time-restricted feeding schedules. This was
reflected in highly significant Meal Definition and, more importantly, Meal Definition X Restriction effects
on meal frequency ($F[4,40]=159.21$, $p<0.0001$; $F[8,80]=54.84$, $p<0.0001$), meal size ($F[4,40]=55.94$,
$p<0.0001$; $F[8,80]=12.72$, $p<0.0001$), and meal duration ($F[4,40]=33.62$, $p<0.0001$; $F[8,80]=5.43$,
$p<0.0001$). Meal Definition also affected the estimated rate of eating within meals ($F[4,40]=164.82$,
$p<0.0001$). As shown in Figure 9, the drinking-explicit meal definition (IRI-300 Food or Water) led to the
perception that food restriction selectively increased meal size and duration (Figs. 9B and 9C) and tended to
slow the average sustained rate of eating (Fig. 9D) without altering meal frequency (Fig. 9A).

No other meal definition yielded an estimated meal structure statistically equivalent to that obtained
from the drinking-explicit definition. The drinking-implicit definitions (IRI-1065 and IRI-840 Food) led to
the most similar general conclusions regarding changes in meal frequency and meal size. Still, the drinking-
implicit definitions did not as reliably detect the effects of 1 day restriction on meal size or duration. In
addition, the drinking-implicit definitions consistently overestimated the average duration of feeding within
meals (Fig. 9C), underestimated the sustained eating rate (Fig. 9D) and did not show the restriction-induced
slowing of sustained feeding seen under the drinking-explicit definition (Fig. 9D).

In contrast to all of the drinking-inclusive definitions, the drinking-naïve definitions (IRI-19 and IRI-
120 Food) led to the perception that food restriction selectively increased meal frequency (see Fig. 9A)
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without altering the size or duration of meals (Fig. 9B and 9C). Across feeding conditions, the drinking-naïve definitions also tended to overestimate meal frequency and underestimate meal size and duration.

To illustrate the basis for these differences, Figure 10 depicts the event records of feeding and drinking for two representative rats under *ad lib* and food-restricted conditions. As can be seen, the log-survivorship and frequency histogram-based drinking-naïve definitions (IRI-19 and 120 Food) did not consider meals to continue across bouts of drinking that were interposed between feeding bouts. In contrast, both the drinking-explicit (IRI-300 Food or Water) and drinking-implicit definitions (IRI-840 and IRI-1065 Food) characterized meals as continuous periods of ingestion, with alternating bouts of feeding and drinking. These differences underlie the contrasting effects of meal definition on meal frequency (Fig. 9A) and meal size (Fig. 9B).

However, the drinking-explicit definition differed from the drinking-implicit definitions in that the former distinguished between components of the meal that were spent in sustained feeding vs. sustained drinking. As a result, only the drinking-explicit definition produced estimates of the duration of feeding (Fig. 9C) and the sustained local feeding rate within meals (Fig. 9D) that were not confounded by intra-meal drinking. In addition, as shown in one of the representative cases, the drinking-implicit definitions merged briefly spaced, but apparently discrete meals that were not linked by prandial drinking (Fig. 10, Rat #68, 1 Day Restriction, 40-60 min into the session). The results support the hypotheses that the drinking-implicit breakpoints confounded drinking and feeding within meals and merged meals across the briefest post-meal intervals.

The event records also illustrate that the quantity of prandial drinking (i.e., beginning or terminating water intake within 5 min of feeding) increased markedly in association with the restriction-induced hyperphagia, despite the fact that water was available *ad libitum* throughout food deprivation. From baseline levels (6.7 ± 1.7 ml), prandial drinking during the 2-hr period doubled (13.1 ± 1.2 ml) and more than tripled...
tripled (22.8 ± 2.0 ml) following acute and chronic food restriction, respectively (p’s<0.00005). The finding further supports the hypothesis that food-associated drinking is regulatory and not coincidental.
Discussion

The present study validated a novel basis for measuring meals in rats. Conceptually, prandial drinking was considered to be a part of meals. Mathematically, the threshold meal interval was estimated by identifying the interresponse interval that provided the most stable joint estimates of meal size and meal duration and confirming this inflection point with two forms of segmented regression. With this definition, post-meal intervals were unimodally distributed as a log-normal function, reflecting that the likelihood of initiating a new meal was initially very low and subsequently increased as an exponential function of time, findings that fulfill predictions of satiety. In contrast, drinking-naïve meal definitions that were derived from log-survivorship analysis or subjective inspection of the frequency histogram of inter-feeding intervals resulted in a bimodal, heterogeneous distribution of post-meal intervals with excessively high meal starting probabilities within 12 min of estimated meal completion. The pattern of postprandial resting also supported the novel definition as meal termination was typically followed by a behavioral satiety sequence, more often than following meals defined without knowledge of prandial drinking. The drinking-explicit meal definition uniquely revealed that rats reliably exhibit preprandial and postprandial correlations of similar magnitude, indicating that rats adjust meal size in relation to how long they have not eaten and subsequently do not eat in relation to the size of their most recent meal. Individual differences and factor structure further supported the reliability and validity of the associated measures of meal microstructure.

The inclusion of drinking had dramatic effects on the interpretation of changes in feeding patterns. Under the drinking-explicit definition, food restriction was observed to increase meal size and duration selectively. In contrast, the drinking-naïve meal definitions did not consistently link feeding bouts across interposed prandial drinking bouts. Consequently, drinking-naïve definitions reported that food restriction selectively increased meal frequency. The findings support the hypothesis that food-associated drinking is a behaviorally integrated component of meals that should be allowed for in meal definitions. Furthermore, they demonstrate the utility of identifying transitions in the slope of a function through first-derivative
analysis, a method that can be applied to understand better many behaviors regulated by multiple underlying processes.

**Relevance for Log-Survivorship Analysis**

As discussed earlier, prior rodent meal pattern studies have used arbitrary or, less often, mathematically-derived meal definitions. The most prevalent mathematical method has been log-survivorship analysis (20, 59). As discussed, however, survivorship analysis assumes that the probability of initiating a meal is independent of the time from the last feeding event. The present data forcefully show that meal initiation does not follow the assumed time course in the rat. None of the observed meal initiation functions (not even that derived from log-survivorship analysis itself) suggested a constant probability of meal initiation. Rather, the drinking-inclusive definitions indicated that the likelihood of initiating a meal increased monotonically as a function of time since the prior meal. Relative to the calculated log-survivorship null hypothesis, this reflected a lower probability of resuming feeding following brief post-meal intervals and a higher probability of resuming feeding following longer post-meal intervals. Taken with similar observations in pigs and cows (47, 65), results supports the hypothesis that a construct like satiety regulates meal initiation across diverse species. As important, the present results indicate that log-survivorship analysis is not valid for identifying breakpoints in meal or lick pattern analysis. Because the instantaneous likelihood of initiating a meal in fact grows with time since an animal has last eaten, the log-survivorship method will inherently split meals at brief post-meal intervals.

**Relevance for Drinking-Naïve Meal Definitions**

Previous studies that derived intermeal breakpoints in the rat using log-survivorship or frequency histogram analysis have relied on inter-feeding intervals without regard to drinking behavior. With the present dataset, a similar 2-process (drinking-naïve) analysis of frequency histograms would have suggested a meal breakpoint of 1 to 5 min between feeding responses, thresholds that systematically split clustered bouts of feeding (i.e., meals) at brief prandial drinking pauses. This was evident in the present study as (1) the non unimodal distribution of post-meal intervals and meal starting probabilities, (2) the relative lack of a
behavioral satiety sequence following estimated meal termination, and (3) the failure to consider meals to continue across interposed drinking bouts under *ad libitum* and restricted feeding conditions. Inspection of frequency histograms under the drinking-explicit definition indicated a distribution of intermediate duration drinking pauses which extended beyond the drinking-naive breakpoint of inter-feeding intervals. During pauses to drink, draughts were initiated rapidly (45 sec latency) as log-normal function of time and averaged 1.9 ml. The substantially greater influence of more frequent intra-bout intervals (in the *y*-dimension) and of longer inter-meal intervals (in the *x*-dimension) overwhelmed the intermediate distribution of inter-bout drinking pauses and made them difficult to recognize without knowledge of drinking behavior.

The results do not appear to be peculiar to the current methodology. Inspection of frequency histograms reported by another group (see Figure 2 in (10)) of inter-feeding intervals from spontaneously feeding rats suggests a distribution of inter-feeding intervals with a mode of 2 min intermediate to a “faster”, much larger distribution with a mode of 9 sec (presumed intra-bout intervals during sustained feeding) and a “slower” distribution with a mode of 40 min (presumed between-meal intervals). The intermediate duration intervals (presumed inter-bout drinking pauses) also are visible as a deviation from the “vertex” of the fitted double-exponential function from 2-9 min (see Figure 1 in (10)). Very much like the present results, a discontinuity between intermediate and longer duration inter-feeding intervals is evident as an approximately 30-fold increase in the frequency of inter-feeding intervals from the absolute minimum at 14-23 min to a peak at 23-39 min. The timecourse presented in (10) corresponds well with the progressive loss of satiety in the present study that began accelerating steeply 12-16 min following meal completion (Figure 6) with the corresponding distribution of post-meal intervals (Figure 5).

The results collectively suggest that drinking-naïve analysis of inter-feeding intervals in the rat may lead to a bout-like definition of meals under which meals are not uniformly continued across prandial drinking pauses. Associated measures of meal microstructure (e.g., intermeal interval) would then be an erroneous mixture of inter-bout and inter-meal variability. Such splitting is also problematic because meals,
not food bouts, appear to be the unit of behavior relevant to understanding satiation (which terminates feeding) and satiety (which postpones resumption of feeding).

Relevance for Drinking-Implicit Meal Definitions

Threshold inter-feeding intervals that *implicitly* accounted for drinking were identified, as observed (14 min) or modeled (17.7 min). The drinking-implicit definitions provided the closest approximation of the drinking-explicit definition with respect to discriminating meals from one another. However, while the drinking-implicit breakpoints accounted for inter-bout drinking, they did not differentiate a 14-min inter-feeding interval bridged by drinking from a 14-min interval without drinking. As a result, they mistook short, non-drinking between-meal intervals for long, intra-meal drinking pauses in the present study, such that meals were merged across the briefest post-meal intervals (e.g., Figures 5, 6 and 10). Reflecting this, the drinking-implicit definitions underestimated the magnitude of the preprandial correlation, which was not detected reliably in the rat until now. The drinking-implicit definitions also did not distinguish between portions of the meal that are spent in sustained feeding vs. drinking (e.g., Figure 10). Consequently, drinking-implicit definitions overestimated the average duration of feeding within meals and underestimated the sustained eating rate (e.g., Figure 9). Finally, the drinking-implicit definitions did not as sensitively detect the restriction-induced slowing of sustained feeding or increase in meal size and duration that was seen under the drinking-explicit definition, suggesting increased variability of results. In sum, if it is not possible to measure drinking behavior *under similar test conditions* then a breakpoint of 14-17.7 min is likely to provide an imperfect, but workable, approximation of meal size and meal frequency, but not of the duration of feeding or eating rate. Breakpoints outside this range that are common in the rat feeding literature (e.g., 10 or 20 min) are not supported by the present study, as they would misclassify even more meals. It is important to emphasize that the current breakpoints should not be overgeneralized to studies that differ substantively in their procedures. For example, we have observed that breakpoints differ between mice of different genetic backgrounds (wildtype littermates of mu opiate receptor knockout mice as compared to wildtype littermates of corticotropin-releasing factor receptor deficient mice) (unpublished
observations), and other methodological factors may be relevant (e.g., strain, age or sex of subjects, diet, feeding apparatus, procurement costs to feed or drink).

On the other hand, the similarity of the present observed drinking-implicit breakpoint (14 min) to that previously suggested by an entirely different form of mathematical analysis, is striking. Point autocorrelation analysis was used to identify regularities in the time course of feeding behavior of free-feeding rats under slightly different experimental conditions and suggested an identical threshold breakpoint of 14 min between feeding events (19). In the same manner that stable, individual differences were observed for every measure of meal microstructure in the present study, the study using autocorrelation analysis also observed reliable individual differences in the eating rate (19). That similar conclusions were obtained through different methods supports the generality of the present findings and their implications for the ingestive physiology of the rat. However, with respect to applying findings to future studies, the current method has several advantages over autocorrelation analysis. First, the present method can obtain a drinking-explicit definition, which avoids the reviewed possible shortcomings of drinking-implicit definitions in describing meal microstructure. Second, autocorrelation analysis has several general weaknesses. These include the need to set lag interval widths and significance levels subjectively, decisions that can influence the estimated threshold meal interval (61). Also, autocorrelation plots are difficult to interpret when multiple lags are significant (20), as is often true (19).

**Drinking is Part of the Meal**

Several findings further support the hypothesis that drinking is a part of meal-taking. Stable individual differences were observed in the nightly and mealwise quantity, duration and rate of prandial drinking as well as the prandial food/water ratio, a measure of the degree to which eating was accompanied by drinking. Also, two coherent drinking-related constructs were observed in factor analysis of meal microstructure measures, one reflecting prandial thirst and a second reflecting prandial drinking rate. In addition, meals defined to include prandial drinking (explicitly or implicitly) were more likely to be followed by a behavioral satiety sequence than meals defined naïve to drinking. Finally, acute and chronic food
restriction not only produced hyperphagia, but also respectively doubled and tripled the quantity of prandial drinking, despite the fact that water continued to be available *ad libitum* during food deprivation.

Other studies also have shown that food-associated drinking is evident throughout the day. The link has a circadian component (3, 29) and is considerably stronger during the animals’ predominant feeding cycle (28, 56), when the organism adaptively should be most sensitive to dipsogenic feeding cues. Supporting the regulatory nature of this relation, rats also drink more in response to experimental cellular and extracellular osmotic challenges during their feeding cycles (27). Food-associated drinking is stimulated by both pregastric, preabsorptive (and possibly conditioned) feeding signals (21, 36, 37) as well as by post-absorptive consequences of feeding, including dehydration (35, 41). Food associated-drinking appears to be partly mediated by the renal renin-angiotensin system, since renal denervation (40), inhibition of peripheral angiotensin II synthesis (37, 38), and administration of angiotensin receptors antagonists into the periphery (AT₁, AT₂) or brain (AT₁) inhibit meal-related drinking (41). Accordingly, lesions of periventricular tissue of the anteroventral third ventricle, which responds to humoral signals that motivate drinking, including angiotensin II, abolish the quantitative, but not temporal, association of drinking with feeding. Peripheral (39, 55) and possibly brain (41) histamine receptors as well as the vagus nerve (36) also mediate prandial drinking.

The relationship between feeding and drinking is not obligatory. Rats eat (albeit substantially less) in the absence of water, and non-prandial drinking occurs both in the presence and absence of food. Likewise, certain stimuli elicit opposite changes in food and water intake (e.g., increased ambient temperature) or increase fluid intake without concomitant changes in feeding (e.g., cellular hypovolemic challenges). These dissociations simply indicate that not *all* drinking is food-stimulated and that different biological mechanisms underlie the regulation of energy and osmotic balance. However, the behavioral integration of some drinking with feeding is critical for body-fluid homeostasis. Feeding leads to hypovolemia, elevated plasma renin activity and increased gastrointestinal, hepatic-portal and systemic plasma osmolality (see (39)). Thus, individual-specific anticipated and realized osmoregulatory needs motivate prandial drinking.
How vs. How Much

Whether a rat nibbled (i.e., ate many, short, small meals), gorged (i.e., ate few, extended, large meals), or ate quickly was independent of its total nocturnal food intake. Thus, in contrast to conclusions from a study that used log-survivorship analysis of inter-feeding intervals to derive the meal breakpoint (23), how a rat ate (i.e., meal frequency, size and eating rate) was dissociable from how much it ate during the evening. The results from factor analysis were supported by the presence of both preprandial and postprandial correlations of a meal’s size with its contiguous intermeal intervals in Experiment 2. Such correlations indicate that, given the opportunity, rats mutually accommodate their intermeal intervals and meal sizes, presumably to serve regulated constraints (e.g., perceived gastrointestinal load, feedback from signals of energy balance). Such adaptive, flexible control of daily food intake would reduce the univariate relations of meal frequency or meal size to total intake. Consequently, across healthy rats that are able to feed at will, the average size of meals during an evening is not related to how much will be consumed during the course of the entire night. The results do not support the prevailing view (e.g., (64)) that meal size per se is the primary determinant of longer-term variations in intake between free-feeding animals.

Prandial correlations in rats were reliable but accounted for a small minority of variability in a free feeding rat’s nocturnal meal size and inter-meal intervals (7% and 14% for preprandial and postprandial relations, respectively). The preprandial correlation might be interpreted to mean that how much a rat eats is relatively independent of how long it previously had gone without eating. However, in the food restriction study, a rat very predictably ($r=0.83$) ate much more in its first meal after a lengthy imposed pre-meal interval (>22 hr) than in its first meal after a much briefer enforced pre-meal interval (30-60 min for cage cleaning) (7.3+0.9 vs. 3.9+0.5 g, $p<0.0008$). Thus, under a different range of environmental conditions, the premeal interval was responsible for the substantial majority (69%) of differences in the size of a rat’s meal. A solution to this paradox may be found in the example of a thermostatically-controlled air conditioner cooling a house, with the duration of cooling cycles being crudely analogous to meal size and the intervals between cycles analogous to intermeal intervals. Across daytime hours in the summer, the best predictor of
the variance in how long a free-running intermittent air conditioner must run to return a home’s temperature from a specified departure from the desired temperature would be the time of day, reflecting the variable heat stress from the external environment. That is, in a free-running system, the intervals between cooling cycles would not be strongly positively correlated to the duration of cooling cycles (and might even be negatively correlated as the air conditioner would not only have to work longer, but also more often to counter rising external temperatures). However, if the air conditioner was disabled until mid-afternoon (analogous to an extended imposed pre-meal interval) it would then have to work much more (larger meal size) to achieve the thermostat set point than an air conditioner that had until then been freely regulating. We hypothesize that it is precisely because the feeding control system is allowed to initiate meals freely in a dynamic environment (e.g., circadian changes in metabolic demands) that spontaneous departures from regulation (e.g., inappropriately sized inter-meal intervals) account for a small proportion of the variance in meal size in free-feeding rats relative to other predictors (e.g., time of day; (16, 18)). Moreover, if a thermostat controlled an intermittent air conditioner perfectly (without error) to oppose a constant, elevated external temperature, there would be no correlation of the time between cooling cycles with the duration of the cooling cycles because there would be no variance to correlate. That is, under conditions in which departures from regulation (i.e., variance from the mean values) are rare and small, correlations also would be small because they relate variance rather than mean scores, which jointly would be held constant within a very restricted range. Only by temporarily disabling the air conditioner (preventing meal initiation) would the strong relation of the inter-cooling interval to the subsequent required duration of cooling become fully apparent.

Limitations and Caveats

Although the present evidence suggests that meals may be a unit more relevant to satiety than are bouts, it would be incorrect to dismiss the usefulness of bout microstructure analysis (with bouts again referring to the individual, sometimes alternating, bursts of feeding and drinking that collectively comprise meals; Figure 10). First, the factors that govern the maintenance of and transitions between bouts of feeding and drinking are of interest in and of themselves. For example, rats with hippocampal or “recovered lateral”
hypothalamic lesions show marked fragmentation of the bout-like structure of feeding and drinking. Such lesions produce more than 4-fold increases in the number of transitions between feeding and drinking (10, 32) without changing total daily food intake, suggesting a disruption of meal syntax rather than of satiation or satiety mechanisms. Also, other important microstructural information can be revealed by bout analysis including intra-bout changes in the rate of feeding. For bout analysis to be most effective, however, it would be appropriate to distinguish intra-bout feeding intervals from inter-bout drinking pauses. In the dataset of Experiment 2, such a breakpoint was approximately 143 sec, since not one intra-bout inter-feeding interval was longer than 143 sec, whereas 94% of inter-bout drinking pauses were longer than 143 sec. The similarity of this breakpoint to that obtained from subjective 2-process analysis of frequency histograms (IRI-120 Food) supports the assertion that the drinking-naïve breakpoints were resolving bout, rather than meal, microstructure.

The current experimental methodology differed in several ways from those used in certain laboratories that employ meal pattern analysis. Relevant procedural differences that potentially could influence the distributions of intra-bout, inter-bout and inter-meal feeding intervals (and meal syntax generally), include the strains used (23), the test cage size, and the procurement costs of consuming food and water (12, 13). However, the similarity of the log-survivorship breakpoint and frequency histograms of inter-feeding intervals observed herein to those published elsewhere (10, 19) tend to discount the argument that methodological differences account for the present results.

Still, the mode of delivery and location of water in the present experiments may have increased the duration of intra-meal drinking pauses relative to those observed when a lickable water source is located in closer proximity to the food source. This procedural factor may have allowed intra-meal drinking intervals to be distinguished more easily from intra-bout feeding intervals in the present study, but thereby also increased the potentially confounding influence of prandial drinking pauses. Conversely, a drinking-naïve (or implicit) breakpoint would be more accurate if the duration of prandial drinking pauses was shorter than the duration of inter-meal intervals and comparable to the duration of intra-bout feeding intervals. For example, unlike
what was found in the present study, Tolkamp and colleagues (61, 65), observed that the log-transformed distribution of presumed prandial pauses to drink in cows do not substantially overlap with the distribution of presumed inter-meal intervals, but rather are of similar duration to the intra-bout intervals between episodes of feeding at a trough. Under such conditions, prandial drinking pauses are less likely to be confounded with inter-meal intervals, and drinking-naïve analysis of frequency histograms could provide reliable estimates of meal size and meal frequency. Furthermore, if methodology and physiology allow bouts of sustained feeding to be distinguished from interposed pauses to drink (e.g., discerning a cow’s comings and goings from a feeding trough with transponders (61)), then a drinking-implicit definition might also provide reliable estimates of feeding duration and eating rate by considering only the portions of the meal spent at the food source. To the degree that prandial drinking pauses in the rat could be shortened (e.g., facilitating the speed and ease of water access), intra-bout feeding intervals could be lengthened (e.g., allowing larger quantities of food to be consumed per feeder visit), and the rat’s sustained location at a feeding source could be determined (e.g., photobeams surveying a niche that contained a powdered food trough) then a more valid breakpoint based only on the distribution of interfeeding intervals might also be possible in rats.

Conclusion

In sum, a conceptually and mathematically novel basis for defining meals in rodents was validated. Unlike drinking-naïve definitions, meals defined to include both eating and drinking (a minimum of 5 min between feeding or drinking events) conformed to predictions of satiety. How a rat consumed was largely dissociable from how much it consumed, in part because rats flexibly regulated their daily nocturnal intake through mutual accommodation of meal size and periods of not eating. Stable, individual differences were seen in every meal-related measure of eating and drinking, supporting the reliability of the measures. Because the probability of initiating a meal is not independent from the time a rat has last eaten, log-survivorship analysis is not valid for identifying breakpoints in meal or lick pattern analysis because it will inherently split meals. Whereas the novel method of defining meals revealed that acute and chronic food restriction selectively increased meal size and duration, drinking-naïve definitions reported a selective
increase in “meal” (in reality, bout) frequency. Drinking-implicit breakpoints (~14 min between feeding events) provided imperfect, but workable, approximations of meal size and meal frequency, but inferior estimates of the preprandial correlation, feeding duration and sustained eating rate. The findings confirm the power of meal pattern analysis. Accurate laboratory- and even experiment-specific meal definitions that account for drinking are recommended for future studies.
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References


FIGURE LEGENDS

Figure 1. Zero- (top panel) and first-order (middle, bottom) functions of estimated average meal duration for rats as a function of the maximum interresponse interval (IRI) between nosepoke responses for food or water considered to continue a meal. The first-order function, which depicts local rates of change in the slope of the zero-order function, has an absolute minimum at an IRI of 300 sec (dashed line). Two-segment linear regression of mean values (middle panel) converging on this point provided good fit (total $r^2=0.86$; $F[2,14]=41.45$, $p<0.0001$; see text), significantly better than the one line solution ($r^2=0.00$, not shown) and equivalent to or better than other two-segment solutions. Multivariate adaptive regression spline analysis (MARS) (bottom panel) provided adequate fit of individual values ($F[5,1694]=43.24$, $p<1x10^{-14}$, $r^2=0.49$; linear GCV=3516). The resulting segmented function had 4 reliable breakpoints in the slope, the most predictive of which (IRI 300) was a minimum inflection point (see text). Error bars reflect SEM’s for observed values. Data were obtained from 100 12-hour nocturnal nosepoke sessions of 25 mature, male Wistar rats with Day as the unit of analysis. Note that time is depicted on a logarithmic scale, as has been recommended for similar applications (62).

Figure 2. Average probability of initiating a meal within the next 10 minutes as a function of the average time since completion of the prior meal. Data were obtained from 100 12-hour nocturnal nosepoke sessions of 25 mature, male Wistar rats with Day as the unit of analysis.

Figure 3. Comparison of log-survivorship analysis of inter-feeding intervals (top panel) with the log-normal frequency histogram of inter-feeding intervals (bottom). The calculated log-survivorship analysis suggested a breakpoint of 18.6 sec between inter-feeding intervals, represented as the intersection of the extensions of the purported fast intra-meal (more vertical) and slow inter-meal (more horizontal) feeding functions illustrated in the top panel. This breakpoint appears to interrupt a coherent distribution of inter-feeding intervals (bottom, long dashed line). An alternative 2-process, drinking naïve breakpoint of approximately
120 sec (bottom, short dashed line) was suggested both by selecting an inter-feeding interval just to the right of the rapid acceleration in slope of the log-survivorship function (top) and by subjectively identifying a natural breakpoint between alleged intra-meal and inter-meal feeding intervals (bottom panel). Data represent all \((n=6361)\) nocturnal inter-feeding intervals from 12 nocturnal tests sessions of 6 male Wistar rats. Note that time in the bottom panel is depicted on a logarithmic scale, as has been recommended (62).

Figure 4. Identity of inter-feeding intervals under the 3-process, drinking-explicit meal definition. Under the drinking-explicit meal definition, the aggregate distribution of feeding intervals (top left) resolves to two forms of intra-meal intervals (top right, bottom left) and one type of inter-meal intervals (bottom right). The intra-meal intervals purportedly represent bouts of sustained feeding (top right, intra-bout intervals without drinking) and prandial drinking pauses (bottom left, inter-bout intervals with drinking). From these distributions, drinking-implicit meal inter-response interval (IRI) breakpoints were calculated by minimizing the number of observed (frequency histogram bars; IRI-840 sec) or modeled (dashed lines; IRI-1065) inter-feeding intervals that were assigned differently from the drinking-implicit definition with respect to being intra-meal vs. inter-meal intervals. A 2-process, drinking-naive analysis of all inter-feeding intervals would suggest a much shorter breakpoint (top left; ~IRI-120) than the 3-process, drinking-informed analyses. Data represent all \((n=6361)\) nocturnal inter-feeding intervals from 12 nocturnal test sessions of 6 male Wistar rats. Note that time in the bottom panel is depicted on a logarithmic scale, as has been recommended (62). Frequency is presented on a logarithmic scale to allow continuous display of relative interval frequencies.

Figure 5. Frequency histogram of post-meal intervals on linear (left panels) or logarithmic (right) time scales under candidate meal definitions. Post-meal intervals are unimodal, homogeneous and log-normally distributed under a meal definition that explicitly accounts for prandial drinking (top; interresponse interval [IRI]-300 sec food or water). Drinking-implicit definitions produce similar log-normal distributions, but that are truncated at the left tail (right, bottom panels; IRI-840, 1065 Food), suggesting that they omit the briefest
post-meal intervals. Drinking-naïve definitions produce much more heterogeneous and even bimodal post-meal distributions (right, middle panels; IRI-19, 120 Food), suggesting that they split meals. Data represent 24 nocturnal test sessions of 6 male Wistar rats.

Figure 6. Moment-to-moment probability of initiating a meal as a function of time since food was last eaten under candidate meal definitions. Panels depict the likelihood of starting a meal within a specified unit of time (4 or 15 min, see y-axis labels) beginning from certain post-meal time intervals (see x-axis). Starting probabilities are shown on short (left panels, min) and long post-meal time scales (right panels, hr). The meal definition that explicitly accounts for prandial drinking (top, interresponse interval [IRI]-300 food or water) perceives initially very low meal starting probabilities that monotonically increase with time. This contrasts sharply with the core assumption of log-survivorship analysis that the probability of meal initiation is constant and independent of the time for which a rat has not eaten (top, dashed line). Drinking-implicit meal definitions (bottom, IRI-840, 1065 food) also yielded monotonically increasing starting probabilities, but had a relative dearth of brief post-meal time scales (bottom left), suggesting that they omitted the briefest post-meal intervals. Drinking-naïve meal definitions (middle, IRI-19, 120 food) did not exhibit monotonically increasing starting probabilities, because of an excess likelihood of resuming feeding at short post-meal time scales. Data represent 24 nocturnal test sessions of 6 male Wistar rats.

Figure 7. Correlations between nocturnal meal size and contiguous intermeal intervals in the rat. Representative scatterplots and correlations (dashed lines) from one rat illustrate the comparable magnitudes of the preprandial correlation (left panel) and the postprandial correlation (right) during nocturnal feeding, as revealed by the drinking-explicit meal definition (see also Table 3). The preprandial correlation indicates that the longer the rat went without eating, the larger its next meal was. The postprandial correlation indicates that the more the rat ate during a meal, the longer it then subsequently went without resuming feeding. The existence of both correlations suggests that rats flexibly regulate their daily nocturnal intake by
mutually accommodating how much (meal size) and how frequently (meal initiation) they eat. Note that the duration of intermeal intervals (y-axis) was log-transformed to achieve Gaussian and homogeneous distributions appropriate for correlation analysis.

Figure 8. Emission of a behavioral satiety sequence following estimated meal termination under candidate meal definitions. Panels show the time-sampled (5-sec) counts (M±SEM) of eating, active, resting and drinking behavior for rats during the first full 15 min following estimated meal completion as judged by different meal definitions. Drinking-explicit (top left panels; interresponse interval [IRI]-300 sec between consecutive feeding or drinking responses) and drinking-implicit meal breakpoints (bottom; IRI-840, 1065 food) provided evidence of a post-ingestive behavioral satiety sequence following estimated meal termination. Rats initially showed primarily post-prandial drinking and active behaviors that were supplanted by resting after 6 min. Eating remained very low. Meals defined naïve to drinking (middle; IRI-19, 120 Food) were not typically followed by a behavioral satiety sequence. Rather, after an initial burst of drinking and activity, feeding resumed as the most common behavior 3 min after meal termination. Resting remained very low. Data were obtained from 10 mature, male Wistar rats from the onset of their nocturnal nosepoke session.

Figure 9. Effect of meal definition on the estimated 2-hr nocturnal meal pattern during ad libitum and restricted feeding. Panels depict mean (± SEM) meal frequency (A), average meal size (B), average meal feeding duration (C) and average sustained eating rate (D) for 11 mature, male Wistar rats fed ad libitum or under acute (1 day) or chronic (7-day) restricted (only 2-hr daily access) feeding schedules. Water was available ad libitum in all conditions. Meals were defined using meal definition that explicitly accounted for prandial drinking (interresponse interval [IRI] of 300 sec between consecutive feeding or drinking responses), that were naïve to prandial drinking (IRI-19, IRI-120 food) or that implicitly accounted for
prandial drinking (IRI-840, 1065 food). *p<0.05 vs. respective IRI-300 Food and water condition, #p<0.05 vs. respective ad libitum-fed condition (within-subject Student Newman-Keuls tests).

Figure 10. Event records of feeding and drinking during 2-hr nocturnal access. The graphs show typical alternating bouts of feeding (upper vertical ticks) and drinking (lower vertical ticks) in two representative mature, male Wistar rats under non-deprived and acutely restricted (22 hr food deprivation) feeding conditions. Water was available ad libitum in all conditions. Each vertical tick represents a response for food (45 mg pellet) or water (100 µl aliquot). Food restriction increased food intake and food-associated water intake. The drinking-naïve inter-response interval (IRI) breakpoints (19 or 120 sec between feeding responses; IRI-19, 120 F) tended to split ingestive episodes at prandial drinking breaks. In contrast, the drinking-explicit (IRI-300 F&W) and drinking-implicit definitions (IRI-840, 1065 F) considered meals to continue across pauses to drink. However, the drinking-implicit definitions also considered meals to continue across brief intervals that were not linked by drinking (e.g., Rat #63, 1 day restriction, 40-60 min) and did not distinguish between portions of the meal spent in sustained feeding vs. drinking. These differences resulted in different perceptions of the effects of food restriction on meal patterning (right side and Figure 9).
Table 1

Selected Characteristics of Nocturnal Meal Structure in Nondeprived Wistar Rats

<table>
<thead>
<tr>
<th>Measure</th>
<th>Range</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Meals</td>
<td></td>
<td>9.4</td>
<td>4</td>
<td>15</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Average intermeal interval, min</td>
<td></td>
<td>65.5</td>
<td>34.1</td>
<td>218.7</td>
<td>26.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Satiety ratio, min/g food eaten</td>
<td></td>
<td>28.6</td>
<td>18.8</td>
<td>117.1</td>
<td>10.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Food/water ratio, g/ml</td>
<td></td>
<td>1.2</td>
<td>0.5</td>
<td>3.7</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Average meal size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food, g</td>
<td></td>
<td>2.3</td>
<td>1.1</td>
<td>4.8</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Water, ml</td>
<td></td>
<td>2.3</td>
<td>0.7</td>
<td>6.6</td>
<td>1.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Average meal duration, min</td>
<td></td>
<td>13.1</td>
<td>6.3</td>
<td>25.1</td>
<td>3.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Food</td>
<td></td>
<td>5.4</td>
<td>2.2</td>
<td>10.8</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>5.3</td>
<td>0.9</td>
<td>12.5</td>
<td>2.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Average within-meal rate of intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food, g/min</td>
<td></td>
<td>0.4</td>
<td>0.3</td>
<td>0.7</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Water, ml/min</td>
<td></td>
<td>0.5</td>
<td>0.2</td>
<td>1.0</td>
<td>0.2</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Supplemental Online Table 1

Stability of Individual Differences and Group Means in the Microstructure of Prandial Intake in Rats

<table>
<thead>
<tr>
<th>Measure</th>
<th>Stability of Individual Differences</th>
<th>Instability of Group Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stability of Single Observation</td>
<td>Stability of Average$^2$</td>
</tr>
<tr>
<td></td>
<td>$F(24,72)$ p-value</td>
<td>$F(3,72)$ p-value</td>
</tr>
<tr>
<td>Total meal-related</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>5.47 &lt;0.0001 0.54 0.82 0.12 0.95</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>6.09 &lt;0.0001 0.37 0.70 1.04 0.38</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>6.13 &lt;0.0001 0.55 0.83 1.81 0.15</td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>11.61 &lt;0.0001 0.73 0.91 0.87 0.46</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>6.45 &lt;0.0001 0.57 0.84 1.91 0.14</td>
<td></td>
</tr>
<tr>
<td>Meal Structure</td>
<td></td>
<td></td>
</tr>
<tr>
<td># of Meals</td>
<td>3.11 &lt;0.0001 0.35 0.68 0.35 0.79</td>
<td></td>
</tr>
<tr>
<td>Intermeal interval</td>
<td>3.72 &lt;0.0001 0.41 0.74 0.42 0.74</td>
<td></td>
</tr>
<tr>
<td>Satiety ratio</td>
<td>2.29 &lt;0.005 0.25 0.57 0.04 0.99</td>
<td></td>
</tr>
<tr>
<td>Food/water balance</td>
<td>3.82 &lt;0.0001 0.40 0.73 2.59 0.06</td>
<td></td>
</tr>
<tr>
<td>Average meal size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>3.03 &lt;0.0005 0.34 0.68 0.35 0.79</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>3.35 &lt;0.0001 0.38 0.71 0.37 0.77</td>
<td></td>
</tr>
<tr>
<td>Average meal duration</td>
<td>2.01 &lt;0.05 0.20 0.51 0.59 0.62</td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>2.94 &lt;0.0005 0.33 0.67 0.36 0.78</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>1.89 &lt;0.05 0.32 0.66 1.03 0.39</td>
<td></td>
</tr>
<tr>
<td>Average rate of intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>18.91 &lt;0.0001 0.81 0.94 2.48 0.07</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>11.08 &lt;0.0001 0.72 0.91 1.10 0.36</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Calculated as an intraclass correlation(2,1) of the absolute agreement between single observations

$^2$Calculated as an intraclass correlation(2,4) of the absolute agreement of the mean
Table 2

Factor Loadings of Meal-related Feeding and Drinking Parameters in Rats

<table>
<thead>
<tr>
<th>Measure</th>
<th>Factor 1 “Meal Patterning”</th>
<th>Factor 2 “Prandial Thirst”</th>
<th>Factor 3 “Satiety Ratio”</th>
<th>Factor 4 “Drinking Rate”</th>
<th>Factor 5 “Eating Rate”</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Meals</td>
<td>-0.90</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Intermeal interval</td>
<td>0.75</td>
<td>----</td>
<td>-0.51</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Satiety ratio</td>
<td>----</td>
<td>----</td>
<td>-0.91</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Food / water ratio</td>
<td>----</td>
<td>-0.89</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

**Food**

- Total responses: 0.90
- Total duration: 0.53
- Responses per meal: 0.95
- Duration per meal: 0.88
- Response rate: 0.97

**Water**

- Total responses: 0.91
- Total duration: 0.55
- Responses per meal: 0.51
- Duration per meal: 0.56
- Response rate: 0.93

Variance explained (%): 26.2, 22.1, 17.7, 13.8, 13.5

Note: Blank entries represent loadings <0.40.
Table 3

Influence of Meal Definition on the Estimated Relations of Meal Size to Pre- and Postprandial Intervals in Rats

<table>
<thead>
<tr>
<th>Meal Definition</th>
<th>Meals</th>
<th>Rats showing a positive relation</th>
<th>Weighted mean $r$</th>
<th>SEM</th>
<th>Rats showing a positive relation</th>
<th>Weighted mean $r$</th>
<th>SEM</th>
<th>Effect</th>
<th>$Q$-value</th>
<th>$p$-value</th>
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<tr>
<td><strong>3-process models</strong></td>
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<td>Drinking explicit</td>
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<tr>
<td>IRI-300 Food &amp; Water</td>
<td>215</td>
<td>$6/6^\dagger$</td>
<td>0.26***</td>
<td>0.07</td>
<td>$6/6^\dagger$</td>
<td>0.37****</td>
<td>0.07</td>
<td>Post = Pre</td>
<td>1.50</td>
<td>.22 (ns)</td>
</tr>
<tr>
<td>Drinking implicit</td>
<td></td>
<td></td>
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<tr>
<td>IRI-840 Food</td>
<td>197</td>
<td>$6/6^\dagger$</td>
<td>0.13</td>
<td>0.07</td>
<td>$6/6^\dagger$</td>
<td>0.36****</td>
<td>0.07</td>
<td>Post &gt; Pre</td>
<td>5.48</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IRI-1065 Food</td>
<td>183</td>
<td>$5/6$</td>
<td>0.17*</td>
<td>0.08</td>
<td>$5/6$</td>
<td>0.25**</td>
<td>0.08</td>
<td>Post = Pre</td>
<td>0.60</td>
<td>.60 (ns)</td>
</tr>
<tr>
<td><strong>2-process models</strong></td>
<td></td>
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<tr>
<td>IRI-19 Food</td>
<td>365</td>
<td>$6/6^\dagger$</td>
<td>0.50****</td>
<td>0.05</td>
<td>$5/6$</td>
<td>0.14**,#</td>
<td>0.05</td>
<td>Pre &gt; Post</td>
<td>28.64</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>IRI-120 Food</td>
<td>273</td>
<td>$6/6^\dagger$</td>
<td>0.12</td>
<td>0.06</td>
<td>$5/6$</td>
<td>0.31***</td>
<td>0.06</td>
<td>Post &gt; Pre</td>
<td>5.15</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

$p<0.05$ more likely than chance (sign-rank test); *$p<0.05$, **$p<0.005$, ***$p<0.0005$, ****$p<0.000001$ vs. 0 (df-weighted fixed effect meta-analysis); #$p<0.01$ vs. drinking explicit definition (df-weighted fixed effect meta-analysis).
Drinking-Explicit
IRI-300 Food / Water

Behavior Counts

Post-Meal Interval (Min)

Drinking-Naive
IRI-19 Food

Behavior Counts

Post-Meal Interval (Min)

Drinking-Naive
IRI-120 Food

Drinking-Implicit
IRI-840 Food

Behavior Counts

Post-Meal Interval (Min)

Drinking-Implicit
IRI-1065 Food