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

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[1]*Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California;* [2]*Department of Neuropsychiatry, Osaka City University Medical School, Osaka, Japan;* [3]*Institute of Physiology, Pecs University Medical School, Pecs, Hungary; and* [4]*Laboratoire EA 3666 Homéostasie-Allostasie-Pathologie, Université de Bordeaux 2, Bordeaux, France*

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Zorrilla, Eric P., Koki Inoue, Éva M. Fekete, Antoine Tabarin, Glenn R. Valdez, and George F. Koob. Measuring meals: structure of prandial food and water intake of rats. *Am J Physiol Regul Integr Comp Physiol* 288: R1450–R1467, 2005. First published January 6, 2005; doi:10.1152/ajpregu.00175.2004.—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). The spontaneous nocturnal intake of male Wistar rats was studied. The meal breakpoint was defined as the interval between feeding or drinking events providing the most stable estimate of meal structure. Alternative breakpoints derived from prevailing methodology, log-survivorship, or frequency histogram analysis of interfeeding intervals without respect to drinking were compared (drinking-naive meals). Threshold interfeeding 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 were also studied. Under the drinking-explicit definition, rats averaged nine or ten 13-min meals/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-naive, 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-naive definitions 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 the consideration of drinking in meal definitions can provide a better estimate of meal structure.

feeding or drinking; food-associated drinking; meal size or duration; eating rate; intermeal interval; behavioral satiety sequence; bout microstructure analysis; meal pattern analysis; satiation

THE MEAL IS A PROPOSED, PHYSIOLOGICALLY relevant unit of intake. Ingestive behavior can also be described in smaller, more useful microstructural units, such as bouts, bites, or 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 patterns have often been analyzed, there has been little uniformity in how meals have been defined (e.g., Refs. 7, 10, 19, 42, 58, 59). Typically, a meal has been conceptualized with two-process models, whereby a cluster of feeding events (i.e., a "meal") is separated from other clusters by a nonfeeding interval (i.e., "intermeal interval") that is long compared with the intervals between feeding events within clusters (i.e., "intrameal intervals"). Drinking has generally not been considered in rodent meal definitions (i.e., drinking-naive 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). Second, 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-to-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 (≥73%) daily water intake in rats occurs in drinking bouts shortly (10 min) before or after 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

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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 intrameal (i.e., interbout) drinking, provide better fits for interfeeding 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), observed as an initially low likelihood of resuming feeding immediately after completing a meal, which monotonically increases with time. Although the ingestive physiology of ruminants differs in important respects from that of single-stomached animals, we hypothesize that prandial drinking is a shared, integral component of meal taking for many species.

The method to determine the precise threshold meal interval, or breakpoint, that best distinguishes intrameal intervals from intermeal intervals also has been discussed (see Ref. 19 for a review). Many studies have used arbitrary breakpoints (from $<$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 interfeeding intervals, rather than on the resulting estimated meal patterns. Therefore, investigators 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) has also 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 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 after meal completion and grow with the passage of time. Evidence supporting the predictions of satiety for postmeal 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 interfeeding 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 analyses of estimated meal size and of estimated meal duration. The procedure was to determine the interevent 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 *1*) the existence of stable individual differences, *2*) the latent structure of prandial food and water intake, *3*) whether the subsequent likelihood of initiating a new meal over time conformed to predictions of satiety, *4*) 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 *5*) the emission of the behavioral satiety sequence in relation to estimated meal termination. The validities of alternate meal breakpoints derived from log-survivorship or frequency histogram analyses of interfeeding intervals were compared (i.e., drinking-naive meal definitions). Because of the possible difficulty of accurately measuring drinking behavior and to allow comparisons to studies that have used breakpoints based only on interfeeding intervals, threshold interfeeding intervals that sought to account for drinking indirectly were also evaluated as potential surrogate breakpoints ("drinking-implicit meals").

MATERIALS AND METHODS

Subjects

Mature (401–522 g, 13–16 wk of age at the time of testing), male Wistar rats (n = 52; Charles River, Hollister, CA) were used. On arrival, animals were group housed in a 12:12-h 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. Animals were acclimated to the vivarium for at least 1 wk before the start of experiments. Surgical and experimental procedures adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (publication no. 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Microstructural Analyses of Ingestion

For microstructural analyses of ingestion, rats were tested individually in Plexiglas test cages (22 × 22 × 35 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. Animals were allowed to obtain palatable chow pellets (45-mg precision food pellets, Formula A/I: 60.0% carbohydrate, 3.7% fat, 24.1% protein, 7.0% ash, 5.2% moisture, 370 cal/100 g; P.J. Noyes, Lancaster, NH) from a trough replenished by an automated pellet dispenser (Med Associates, 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-s duration, were recorded automatically by an IBM PC-compatible microcomputer with 10-ms resolution.

Videotape analyses (n = 12 rats for 2 h 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 s. All false-positive ($>$5 s latency) water responses observed were followed by drinking within 1 min. Spillage of food pellets in this system was low (mean ± SE: 1.2 ± 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 resolutions (see also Ref. 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 intermeal 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-h sessions spanning their active cycle (−1 h lights off through +2 h lights on) until responses had stabilized (±20% responding for initial 12-h food intake for 3 consecutive days). To determine the threshold meal interval, data from four subsequent treatment-free sessions of naive rats ($n = 25$) were analyzed. Sessions began at 1700 [−1 h lights off, the time at which anticipatory nocturnal feeding often begins (19, 21)], and consecutive test days were 5 ± 0.5 (SE) days apart. Thus stable individual differences were assessed across 3 wk.

Experiment 2. Experiment 2 had five objectives. The first objective was to derive alternative meal breakpoint estimates using prevailing approaches for defining meals. Drinking-naive breakpoints were derived from calculated (20) and subjective (10) applications of log-survivorship analyses of interfeeding intervals and from subjective analyses of the frequency histograms of interfeeding intervals under the assumption of a two-process model (i.e., within-meal vs. between-meal interfeeding intervals) (45). Drinking-implicit breakpoints were also identified as the threshold interfeeding breakpoint that would classify between- and within-meal intervals most similarly to the proposed three-process drinking-explicit definition (i.e., within-meal intervals without drinking vs. within-meal intervals with drinking vs. between-meal intervals). A drinking-implicit interfeeding breakpoint, if valid, could be used in the absence of fluid intake data to perform meal pattern analyses that would indirectly account 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 h/day) except for brief periods (30–60 min) immediately before the onset of their dark cycle, during which they were removed to permit maintenance and cleaning. Once stable responding was attained, records of nocturnal interfeeding 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 postmeal intervals. A bimodal or otherwise heterogeneous distribution of postmeal 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 postmeal intervals that conformed to predictions of satiety (i.e., an initially very low probability of initiating a meal after 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 "preprandial 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 postmeal intervals. The first meal from each session was excluded because of the potentially confounding influence of recent experimenter intervention, the uncertainty of the premeal interval, and the lack of access to food during cage cleaning. The final nocturnal postmeal 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 transitioned from termination of feeding to increased behavioral activity (e.g., grooming, sniffing) and finally to rest. The behavioral satiety sequence 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 after 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 h/day). After attainment of stable intake, rats were individually videotaped for 2 h 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 three-process drinking-explicit [i.e., interresponse interval of 300 s (IRI-300) food or water] or drinking-implicit criteria (i.e., IRI-840 or IRI-1065 food) or using the two-process, drinking-naive criteria (i.e., IRI-19 or IRI-120 food). To assess the behavioral satiety sequence, a reliable, hypothesis-naive rater (É. M. Fekete) coded each rat's behavior from videotape at 5-s 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-h 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-h 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 h of daily nosepoke access (*day 0*) with ad libitum water, but not food, access outside of nosepoke testing. The microstructure of 2-h intake under ad libitum conditions (*day 0*) was compared with the microstructure of intake after a single 22-h food deprivation (*day 1*) and also after chronic, scheduled food restriction (*day 7*) using the proposed drinking-explicit meal definition (IRI-300 food or water) and alternative two-process, drinking-naive (IRI-19 or IRI-120 food) and three-process, drinking-implicit (IRI-840 or IRI-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 interevent 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 analyses (14). The present 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 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 five food-directed responses, or 0.225 g, a value more than twofold 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 by using a series of maximum IRIs ranging from 30 s to 30 min, where 30 min was used as the upper limit 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, we calculated local rates of change in the slope as the difference in the value of the zero-order function for consecutive intervals per standardized unit of time (30 s).

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, multiphase, 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 because 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-to-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-to-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 noneating (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 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; 57a), indicating the reliability of absolute differences between single observations [ICC(2,1)] or the means of the subjects' four observations [ICC(2,4)]. To allow comparison to individual differences, significant day effects, indicating varying group means, were calculated as an η measure of effect size from the following simplified formula (*Eq. 1*)

$$\sqrt{(df_1 \times F)/[(df_1 \times F) + df_2]} \quad (1)$$

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

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 component factor analysis with varimax rotation. Five orthogonal factors with eigenvalues >1 were retained (30), a threshold that corresponded to visual identification of the scree plot (8). Factor loadings were computed and interpreted.

Experiment 2. OBJECTIVE 1: ALTERNATIVE DRINKING-NAIVE AND DRINKING-IMPLICIT BREAKPOINTS. Log-survivorship analysis was performed as described previously (20). Briefly, the attrition of interfeeding intervals (log *y*-axis) was plotted as a function of increasing duration (*x*-axis). The following double negative exponential (*Eq. 2*) was fit to the resulting semi-logarithmic scatterplot

$$y(x) = (1 - p)e^{-wx} + pe^{-bx} \quad (2)$$

where $y(x)$ represents the proportion of interfeeding intervals longer than duration x, p represents the proportion of interfeeding intervals that were alleged between meal intervals, $1 - p$ represents the proportion of interfeeding intervals that were alleged within-meal intervals, and w and b represent 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 (intrameal intervals) with the more horizontal, slow process (intermeal intervals), was calculated as follows (*Eq. 3*)

$$T = \left(\frac{1}{w - b}\right) \times \log\left[\frac{(1 - p) \times w}{p \times b}\right] \quad (3)$$

To identify a two-process, drinking-naive, frequency histogram-based breakpoint, the frequency histogram of interfeeding 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 three-process, drinking-implicit breakpoint, separate probability functions were fit to the frequency histograms of *1*) intrameal feeding intervals that did not contain drinking, *2*) intrameal feeding intervals that contained drinking, and *3*) intermeal 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. We assessed the fit of the combined mixed distribution model with PeakFit 4.12 (Systat Software).

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 whether 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's correlation.

OBJECTIVE 3: DISTRIBUTION OF POSTMEAL INTERVALS. The frequency histograms of estimated postmeal 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 h; starting within 15 min) postmeal 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 postprandial correlations, separate Pearson's correlations were performed for each rat to determine the relation between the sizes of all the nocturnal meals consumed during the test sessions and the log-transformed duration of the rat's contiguous pre- or postmeal intervals, respectively. To determine the average pre- and postprandial correlations across rats, individual Pearson's 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 intermeal intervals, which have a highly positively skewed distribution (see RESULTS), from averaging each animal's data before the correlation was performed, or from performing the correlation on all meals across circadian phases or across all subjects [i.e., "ecological fallacies" (53)]. Averaging and pooling have distorted estimates of the true pre- and postprandial correlations in previous studies (see also Refs. 7, 15, 49, 60). A modification of the Stouffer's method was used to calculate associated *P* values (54). The relative magnitudes of the pre- and postprandial correlations were then compared by use of *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 after estimated meal completion, a χ^2 analysis was performed. In addition, we compared the average frequency of each behavior category using separate 5 (meal definition) × 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-h meal pattern measures were subjected to a 5 (meal definition) × 3 (restriction: ad libitum vs. 1 or 7 days of restricted feeding) repeated-measures ANOVA, and post hoc within-subject Newman-Keul's tests were used to interpret significant effects. The focus of the present analyses was on the effects of meal definition; detailed analyses and interpretations 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

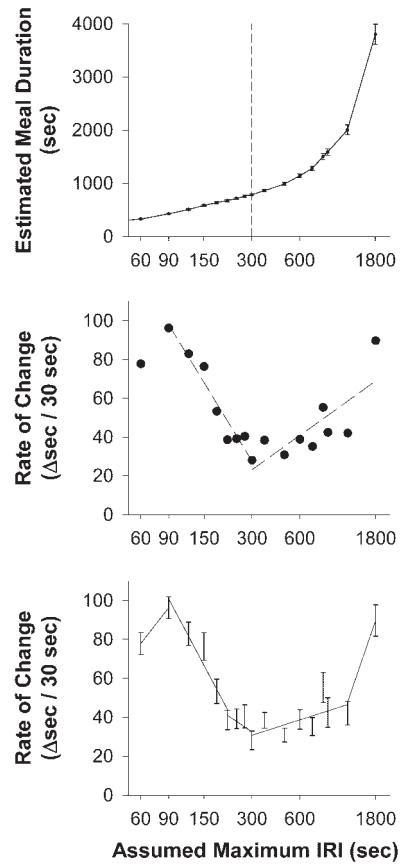

Fig. 1. Zero- (*top*) and first-order (*middle* and *bottom*) functions of estimated average meal duration for rats as a function of the maximum interresponse interval (IRI) between nosepoke responses for food or water considered to continue a meal. The first-order function, which depicts local rates of change in the slope of the zero-order function, has an absolute minimum at an IRI of 300 s (IRI-300; dashed line). Two-segment linear regression of mean values (*middle*) 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 (*bottom*) provided adequate fit of individual values [$F(5,1694) = 43.24$, $P < 1 \times 10^{-14}$, $r^2 = 0.49$; linear generalized cross-validation (GCV) = 3,516]. 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 SE values for observed results. Data were obtained from 100 12-h 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 recommended for similar applications (62).

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 (Fig. 1, *top*). Linear regression revealed that the resulting first-order scatterplot (Fig. 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 210–720 showed a relative stability in the rate of change, with an absolute minimum at 300 s. 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 four intervals: IRI 90, 210, 300, and 1,200 s [Fig. 1, *bottom*; basis functions (BF): BF1 = max(0,IRI-300), BF2 = max(0,IRI-300), 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 s 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 ~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 definition. On average, rats consumed nine or ten 13-min 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 (SDs comprising 36–45% of means) than in eating-related measures (SDs 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 s; for food, 9.2 s) and the smallest observed average between-meal interval (34.1 min).

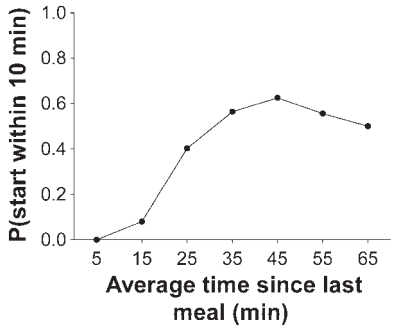

Fig. 2. Average probability (*P*) of initiating a meal within the next 10 min as a function of the average time since completion of the prior meal. Data were obtained from 100 12-h nocturnal nosepoke sessions of 25 mature, male Wistar rats with day as the unit of analysis.

Table 1. *Selected characteristics of nocturnal meal structure in nondeprived Wistar rats*

Measure	Mean	Range Min–Max	SD	SE
No. of meals	9.4	4–15	2.4	0.2
Average intermeal interval, min	65.5	34.1–218.7	26.7	2.7
Satiety ratio, min/g food eaten	28.6	18.8–117.1	10.6	1.1
Food-to-water ratio, g/ml	1.2	0.5–3.7	0.6	0.1
Average meal size				
Food, g	2.3	1.1–4.8	0.7	0.1
Water, ml	2.3	0.7–6.6	1.1	0.1
Average meal duration, min	13.1	6.3–25.1	3.6	0.4
Food	5.4	2.2–10.8	1.6	0.2
Water	5.3	0.9–12.5	2.3	0.2
Average within-meal rate of intake				
Food, g/min	0.4	0.3–0.7	0.1	0.01
Water, ml/min	0.5	0.2–1.0	0.2	0.02

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% (mean ± SE: 57 ± 5%) of the variance in subjects' average scores across 3 wk 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-wk 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).[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-to-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, as 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 summary, 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-to-water ratio for fluid and the satiety ratio for food.

[1] Supplemental data for this article may be found at http://ajpregu.physiology.org/cgi/content/full/00175.2004/DC1.

Table 2. *Factor loadings of meal-related feeding and drinking parameters in rats*

Measure	*Factor 1:* "Meal Patterning"	*Factor 2:* "Prandial Thirst"	*Factor 3:* "Satiety Ratio"	*Factor 4:* "Drinking Rate"	*Factor 5:* "Eating Rate"
No. of meals	−0.90				
Intermeal interval	0.75		−0.51		
Satiety ratio			−0.91		
Food-to-water ratio		−0.89			
Food					
Total responses			0.90		
Total duration			0.53		−0.82
Responses per meal	0.95				
Duration per meal	0.88				
Response rate					0.97
Water					
Total responses		0.91			
Total duration		0.55		−0.75	
Responses per meal	0.51	0.83			
Duration per meal		0.56		−0.71	
Response rate				0.93	
Variance explained, %	26.2	22.1	17.7	13.8	13.5

Note: Blank entries represent loadings of <0.40.

Experiment 2

Objective 1: estimation of alternative drinking-naive and drinking-implicit threshold meal intervals. To compare the validity of other methods used to estimate the meal threshold criterion, drinking-naive meal breakpoints were identified using prevailing approaches: log-survivorship and frequency histogram analysis. Figure 3 depicts the aggregate frequency histogram of all interfeeding intervals (n = 6,361) from 12 nocturnal feeding sessions of six rats (*bottom*) and the corresponding log-survivorship breakpoint analysis (*top*). An inverse y-weighted double exponential function fit the interval attrition scatterplot ($y = 0.9693e^{-0.2295x} + 0.0307\,e^{-0.0003977x}$; $r^2 = 0.96$) and indicated an intermeal breakpoint of 18.6 s between feeding events (see Fig. 3, *top*). However, the aggregate frequency histogram (Fig. 3, *bottom*) clearly showed that many intervals beyond the purported meal threshold (e.g., 18–47 s) were continuous with the large distribution of presumed intrameal 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., intrameal) distribution. Therefore, we also identified an intermeal breakpoint of 120 s 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 that 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 interfeeding intervals under the assumption of a two-process, drinking-naive model (i.e., within-meal vs. between-meal interfeeding intervals) (Fig. 3, *bottom*). Although a formal maximum likelihood two-model function could have been fit to this distribution (e.g., Ref. 65), all published studies in rodents have adopted a subjective approach (see Ref. 19), which we therefore adopted for comparative interest. Potential arguments could be made for a subjective breakpoint at 60 s or anywhere along a trough from 96 to 317 s (Fig. 3, *bottom*). Smoothing the function by aggregating data into larger bins suggested an arbitrary breakpoint between 76 and 317 s (see Fig. 4, *top*). Thus the subjective log-survivorship-based estimate of 120 s was also an acceptable estimate for the subjective, two-process histogram approach.

As a third general approach, we estimated a meal threshold criterion under a three-process model composed of two within-meal processes (i.e., intrameal sustained feeding and intrameal pauses to drink) and one between-meal process (i.e., meal initiation) (65). A practical motivation for this was to determine whether a breakpoint based only on interfeeding intervals that implicitly recognized intrameal 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*) intrameal, interfeeding intervals that did not contain drinking; *2*) intrameal, interfeeding intervals that contained drinking; and *3*) intermeal feeding intervals as defined by the drinking-explicit definition (see Fig. 4). Peak probability functions were individually fit to each of these histograms (see MATERIALS AND METHODS for details). Excellent fits were observed for each distribution, supporting the proposed classification of interfeeding intervals (r^2 values = 0.9999997, 0.95, and 0.98 for intrameal feeding intervals without drinking, intrameal intervals with drinking, and intermeal intervals, respectively; see Fig. 4). The combined mixed distribution model also fit the aggregate frequency histogram well, further supporting the three-process model [$r^2 = 0.999993$; $F(1,13) = 386{,}229$; Fig. 4, *top left*]. Under this model, the intersection of the intermediate and slowest functions (Fig. 4, *bottom left* and *bottom right*, respectively) represents the threshold meal criterion that minimizes the expected number of misassigned events (65) and was interpolated to be an interfeeding interval of 1,065 s (17.7 min). As a second empirical criterion under the three-process model, the interfeeding interval that minimized the number of observed (as opposed to expected) interfeeding intervals, which were classified differently from the drinking-explicit definition, was

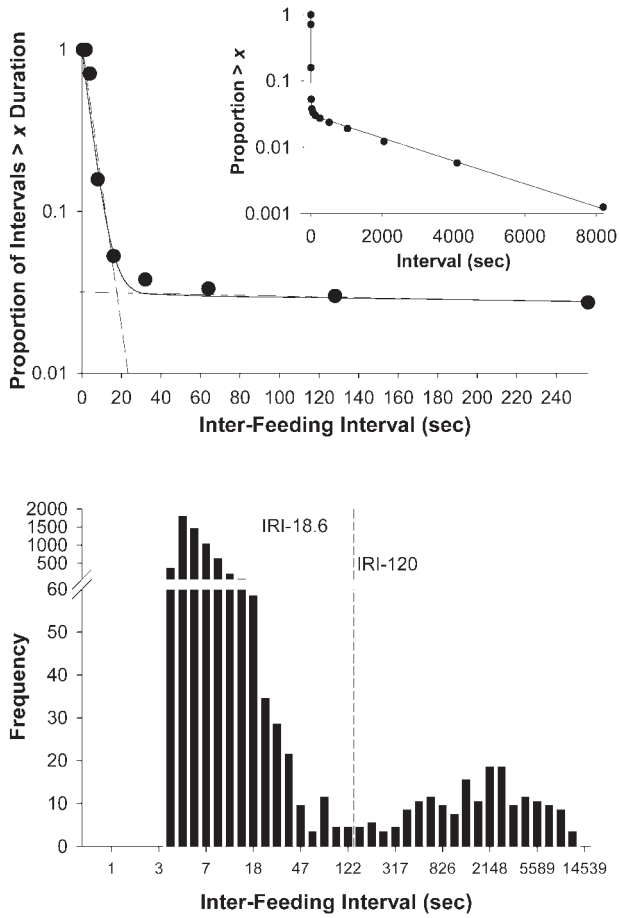

Fig. 3. Comparison of log-survivorship analysis of interfeeding intervals (*top*) with the log-normal frequency histogram of interfeeding intervals (*bottom*). Calculated log-survivorship analysis suggested a breakpoint of 18.6 s between interfeeding intervals, represented as the intersection of the extensions of the purported fast intrameal (more vertical) and slow intermeal (more horizontal) feeding functions illustrated at *top*. This breakpoint appears to interrupt a coherent distribution of interfeeding intervals (long dashed line in *bottom*). An alternative 2-process, drinking-naive breakpoint of ~120 s (short dashed line in *bottom*) was suggested both by selecting an interfeeding 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 intrameal and intermeal feeding intervals (*bottom*). Data represent all (n = 6,361) nocturnal interfeeding intervals from 12 nocturnal test sessions of 6 male Wistar rats. *Bottom*: time is depicted on a logarithmic scale, as recommended (62).

identified. This breakpoint (826–852 s) only classified six intervals (0.09%) differently from the drinking-explicit definition, in each case merging estimated meals, and was treated as 840 s (14 min).

Objective 2: description of prandial drinking pauses. Under the drinking-explicit definition, each of the six rats exhibited prandial drinking pauses during their nocturnal meals (mean + SE: 4.7 + 1.0 pauses/night). Individual pauses averaged slightly longer than 5 min (5.7 + 0.6; range: 0.4–12.7 min) and contained ~2 ml of water intake (1.9 + 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 s (range: 7–269 s) from the prior feeding response.

Objective 3: effect of meal definition on the estimated postmeal interval. Figure 5 shows the effects of the meal definition used on the estimated distribution of postmeal intervals. Figure 5, *left*, depicts postmeal intervals on a linear time scale as has been conventionally done. All breakpoints suggested a highly positively skewed, non-Gaussian distribution for postmeal intervals. Logarithmic transformation of time (Fig. 5, *right*) revealed a homogenous, unimodal distribution of postmeal intervals for the drinking-explicit breakpoint (IRI-300 food or water), indicating that postmeal 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-naive log-survivorship and frequency histogram analysis (IRI-19 or IRI-120 food) resulted in heterogeneous, nonunimodal log-transformed distributions. Interfeeding intervals of <7.5- to 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 Fig. 5).

Objective 4: effect of meal definition on the estimated instantaneous probability of initiating a meal. Figure 6 shows the effects 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 (Fig. 6, *left*, 0–16 min) and long (Fig. 6, *right*, 0–4.5 h) postmeal time scales. As shown in Fig. 6, none of the observed meal initiation functions fits the basic assumption of log-survivorship analysis (Fig. 6, *top*, dashed lines), which is that the meal initiation rate (b) was constant regardless 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 postmeal intervals and a higher probability of resuming feeding following longer postmeal 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 after meal completion. Rather, at brief postmeal 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 postmeal intervals is reflected in the bimodal distribution of postmeal intervals in Fig. 5 and supports the hypothesis that the definitions systematically (but inadvertently) split meals.

On long time scales (Fig. 6, *right*), the three-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 postmeal intervals (Fig. 6, *left*), the drinking-implicit definitions yielded smaller starting probabilities than the drinking-explicit definition. The hypoth-

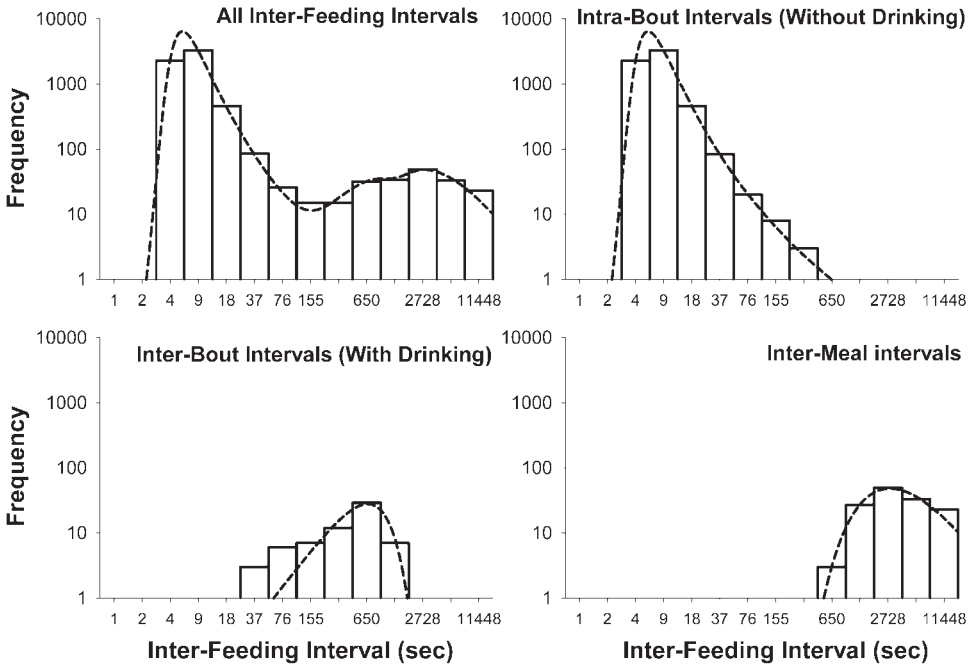

Fig. 4. Identity of interfeeding intervals under the 3-process, drinking-explicit meal definition. Under the drinking-explicit meal definition, aggregate distribution of feeding intervals (*top left*) resolves to 2 forms of intrameal intervals (*top right* and *bottom left*) and 1 type of intermeal interval (*bottom right*). Intrameal intervals purportedly represent bouts of sustained feeding (*top right*, intrabout intervals without drinking) and prandial drinking pauses (*bottom left*, interbout intervals with drinking). From these distributions, drinking-implicit meal IRI breakpoints were calculated by minimizing the number of observed (frequency histogram bars; IRI-840) or modeled (dashed lines; IRI-1065) interfeeding intervals that were assigned differently from the drinking-implicit definition with respect to being intrameal vs. intermeal intervals. A 2-process, drinking-naive analyses of all interfeeding intervals would suggest a much shorter breakpoint (*top left*; ~IRI-120) than the 3-process, drinking-informed analyses. Data represent all (n = 6,361) nocturnal interfeeding intervals from 12 nocturnal test sessions of 6 male Wistar rats. Time (x-axis) is depicted on a logarithmic scale, as recommended (62). Frequency (y-axis) is presented on a logarithmic scale to allow continuous display of relative interval frequencies.

esized underestimation of early starting probabilities is reflected in the truncated distribution of postmeal intervals in Fig. 5 and is hypothesized to reflect that the drinking-implicit definitions systematically overlooked the briefest postmeal 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 to 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 Fig. 7, subjects reliably ate larger meals when they had not eaten for longer periods of time (a preprandial correlation) and went longer without eating after 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 that 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 values = 0.83 and 0.94, respectively, under the drinking-explicit definition).

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 after 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-naive, two-process meal definitions (0/10 and 1/10 for the IRI-19 and IRI-120 food definitions, respectively). Under the drinking-naive 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 after estimated meal termination as judged by each definition. Meal definition significantly influenced the frequency with which eating [definition: $F(4,36) = 17.55$; definition × time: $F(56,504) = 1.85$, $P < 0.001$], active behaviors [$F(4,36) = 5.78$; $F(56,504) = 3.13$, $P < 0.001$], drinking [$F(56,504) = 2.26$, $P < 0.001$], and rest [$F(4,36) = 30.43$; $F(56,504) = 5.56$, $P < 0.001$] were observed after estimated meal termination. Under the drinking-inclusive definitions, primarily active and

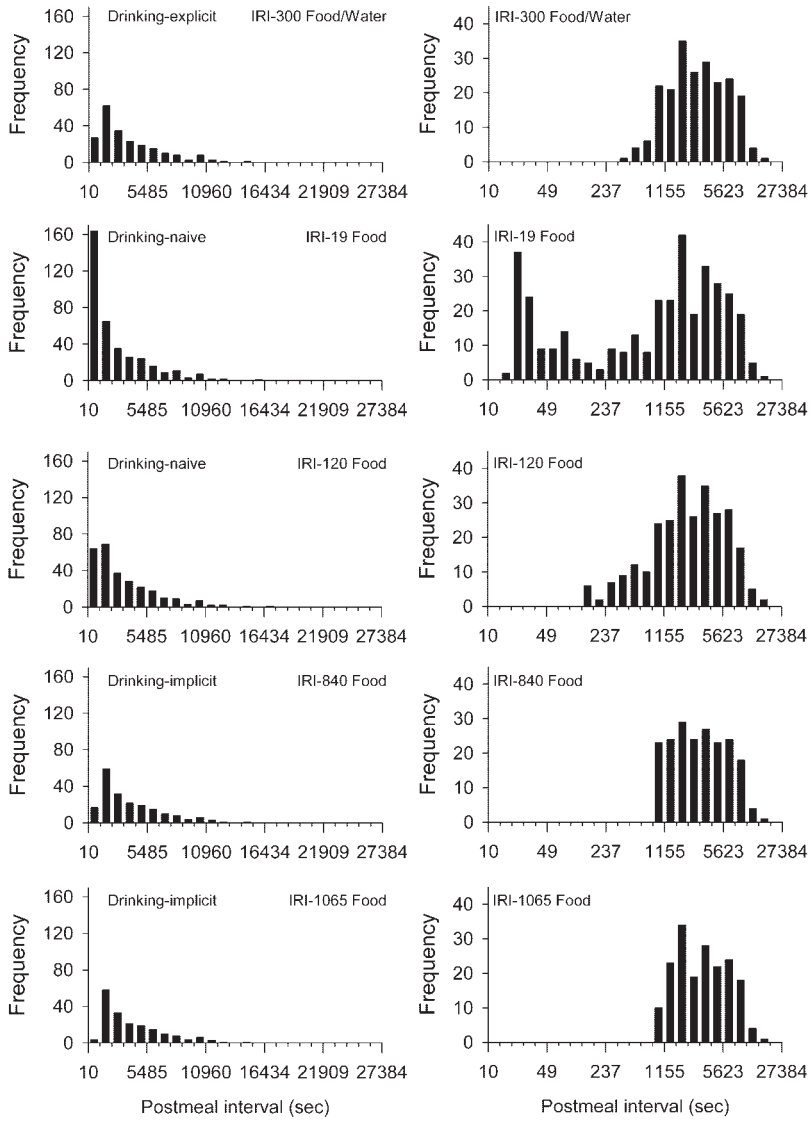

Fig. 5. Frequency histogram of postmeal intervals on linear (*left*) or logarithmic (*right*) time scales under candidate meal definitions. Postmeal intervals are unimodal, homogeneous, and log-normally distributed under a meal definition that explicitly accounts for prandial drinking (*top*; IRI-300 food or water). Drinking-implicit definitions produce similar log-normal distributions, but these are truncated at the left tail [2 *right bottom* panels (IRI-840, IRI-1065 food)], suggesting that the briefest postmeal intervals were omitted. Drinking-naive definitions produce much more heterogeneous and even bimodal postmeal distributions [2 *right middle* panels (IRI-19, IRI-120 food)], suggesting that meals were split. Data represent 24 nocturnal test sessions of 6 male Wistar rats.

drinking behaviors initially followed meal completion but subsequently diminished until resting predominated after 6 min. Under the drinking-naive 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-naive 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 × 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 Fig. 9, the drinking-explicit meal definition (IRI-300 food or water) led to the perception that food restriction selectively increased meal size and duration (Fig. 9, *B* and *C*) and tended to slow the average sustained rate of eating (Fig. 9*D*) without altering meal frequency (Fig. 9*A*).

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. 9*C*), underestimated the sustained eating rate (Fig. 9*D*), and did not show the restriction-induced slowing of sustained feeding seen under the drinking-explicit definition (Fig. 9*D*).

In contrast to all of the drinking-inclusive definitions, the drinking-naive definitions (IRI-19 and IRI-120 food) led to the perception that food restriction selectively increased meal frequency (see Fig. 9*A*) without altering the size or duration of

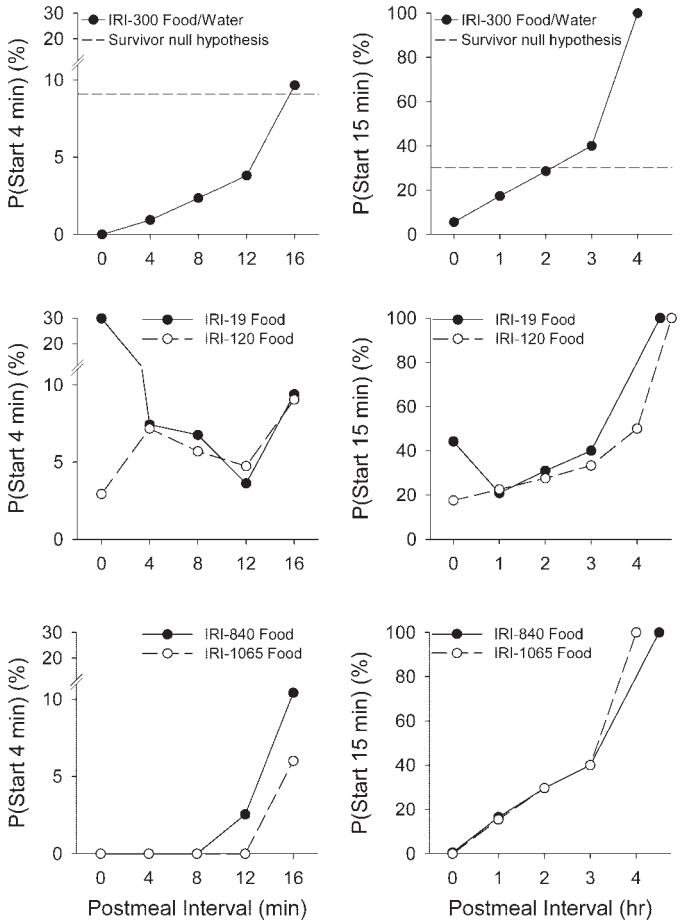

Fig. 6. Moment-to-moment probability of initiating a meal as a function of time since food was last eaten under candidate meal definitions. Panels depict the likelihood of starting a meal within a specified unit of time (4 or 15 min, see *y*-axis labels) beginning from certain postmeal time intervals (see *x*-axis). Starting probabilities are shown on short (*left*, min) and long (*right*, h) postmeal time scales. The meal definition that explicitly accounts for prandial drinking (*top*, 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 lines). Drinking-implicit meal definitions (*bottom*; IRI-840, IRI-1065 food) also yielded monotonically increasing starting probabilities but had a relative dearth of brief postmeal time scales (*bottom left*), suggesting that the briefest postmeal intervals were omitted. Drinking-naive meal definitions (*middle*; IRI-19, IRI-120 food) did not exhibit monotonically increasing starting probabilities because of an excess likelihood of resuming feeding at short postmeal time scales. Data represent 24 nocturnal test sessions of 6 male Wistar rats.

meals (Fig. 9, *B* and *C*). Across feeding conditions, the drinking-naive definitions also tended to overestimate meal frequency and underestimate meal size and duration.

To illustrate the bases for these differences, Fig. 10 depicts the event records of feeding and drinking for two representative rats under ad libitum and food-restricted conditions. As can be seen, the log-survivorship and frequency histogram-based drinking-naive definitions (IRI-19 and IRI-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. 9*A*) and meal size (Fig. 9*B*).

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. 9*C*) and the sustained local feeding rate within meals (Fig. 9*D*) that were not confounded by intrameal 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 postmeal 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-h period doubled (13.1 ± 1.2 ml) and more than tripled (22.8 ± 2.0 ml) after acute and chronic food restriction, respectively ($P < 0.00005$). 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 IRI 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, postmeal 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-naive meal definitions that were derived from log-survivorship analysis or subjective inspection of the frequency histogram of interfeeding intervals resulted in a bimodal, heterogeneous distribution of postmeal 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

Table 3. *Influence of meal definition on the estimated relations of meal size to pre- and postprandial intervals in rats*

Meal Definition	Meals	Preprandial Interval-Meal Size Relation			Meal Size-Postprandial Interval Relation			Relative Magnitude of Relations		
		Rats showing a positive relation	Weighted mean *r*	SE	Rats showing a positive relation	Weighted mean *r*	SE	Effect	*Q* value	*P* value
3-process models										
Drinking explicit										
IRI-300 food and water	215	6/6[a]	0.26[d]	0.07	6/6[a]	0.37[e]	0.07	Post = Pre	1.50	0.22 (NS)
Drinking implicit										
IRI-840 food	197	6/6[a]	0.13	0.07	6/6[a]	0.36[e]	0.07	Post > Pre	5.48	<0.05
IRI-1065 food	183	5/6	0.17[b]	0.08	5/6	0.25[c]	0.08	Post = Pre	0.60	0.60 (NS)
2-process models										
IRI-19 food	365	6/6[a]	0.50[e]	0.05	5/6	0.14[cf]	0.05	Pre > Post	28.64	<0.00001
IRI-120 food	273	6/6[a]	0.12	0.06	5/6	0.31[d]	0.06	Post > Pre	5.15	<0.05

NS, not significant; IRI, interresponse interval. See text for details. [a]$P < 0.05$ more likely than chance (sign-rank test); [b]$P < 0.05$, [c]$P < 0.005$, [d]$P < 0.0005$, [e]$P < 0.000001$ vs. 0 (*df*-weighted fixed effect meta-analysis); [f]$P < 0.01$ vs. drinking explicit definition (*df*-weighted fixed effect meta-analysis).

meal size and duration selectively. In contrast, the drinking-naive meal definitions did not consistently link feeding bouts across interposed prandial drinking bouts. Consequently, drinking-naive 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, the findings demonstrate the utility of identifying transitions in the slope of a function through first-derivative analysis, a method that can be applied to understanding 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 those 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 postmeal intervals and a higher probability of resuming feeding following longer postmeal intervals. Together with similar observations in pigs and cows (47, 65), the results support 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 postmeal intervals.

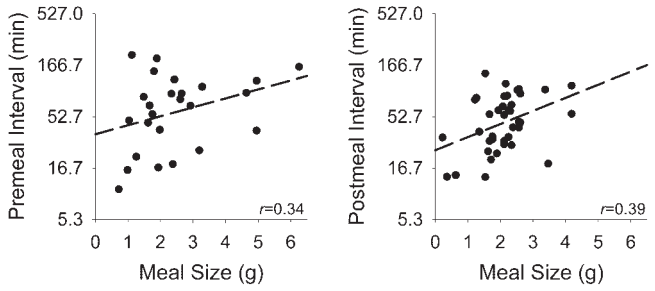

Fig. 7. Correlations between nocturnal meal size and contiguous intermeal intervals in the rat. Representative scatterplots and correlations (dashed lines) from 1 rat show comparable magnitudes of the preprandial correlation (*left*) and the postprandial correlation (*right*) during nocturnal feeding, 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. 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.

Relevance for Drinking-Naive Meal Definitions

Previous studies that derived intermeal breakpoints in the rat using log-survivorship or frequency histogram analysis have relied on interfeeding intervals without regard to drinking behavior. With the present data set, a similar two-process (drinking-naive) analysis of frequency histograms would have suggested a meal breakpoint of 1–5 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 nonunimodal distribution of postmeal 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 that extended beyond the drinking-naive breakpoint of interfeeding intervals. During pauses to drink, draughts were initiated rapidly (45 s latency) as a log-normal function of time and averaged 1.9 ml. The substantially greater influence of more frequent intrabout intervals (in the *y*-dimension) and of longer intermeal intervals (in the *x*-dimension) overwhelmed the in-

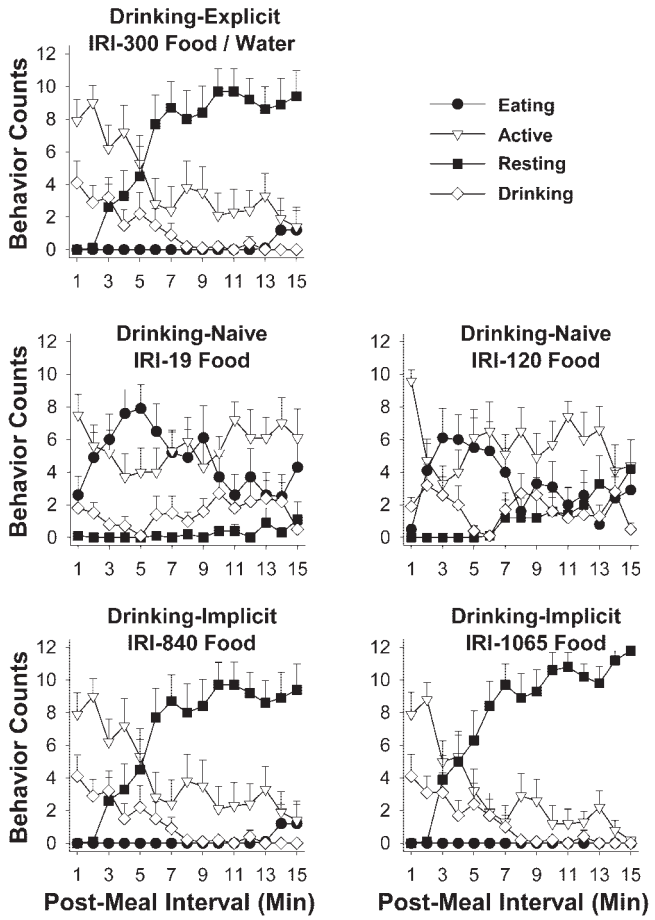

Fig. 8. Emission of a behavioral satiety sequence following estimated meal termination under candidate meal definitions. Time-sampled (5-s) counts (means ± SE) of eating, active, resting, and drinking behaviors for rats during the first full 15 min after estimated meal completion, as judged by different meal definitions, are shown. Drinking-explicit (*top left*; IRI-300 between consecutive feeding or drinking responses) and drinking-implicit meal breakpoints (*bottom*; IRI-840, IRI-1065 food) provided evidence of a postingestive behavioral satiety sequence following estimated meal termination. Rats initially showed primarily postprandial drinking and active behaviors that were supplanted by resting after 6 min. Eating remained very low. Meals defined as naive to drinking (*middle*; IRI-19, IRI-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.

termediate distribution of interbout drinking pauses and made them difficult to recognize without knowledge of drinking behavior.

The results do not appear to be peculiar to the present methodology. Inspection of frequency histograms reported by another group (see Fig. 2 in Ref. 10) of interfeeding intervals from spontaneously feeding rats suggests a distribution of interfeeding intervals with a mode of 2 min intermediate to a "faster," much larger distribution with a mode of 9 s (presumed intrabout intervals during sustained feeding) and a "slower" distribution with a mode of 40 min (presumed between-meal intervals). The intermediate duration intervals (presumed interbout drinking pauses) are also visible as a deviation from the "vertex" of the fitted double-exponential function from 2 to 9 min (see Fig. 1 in Ref. 10). Very much like the present results, a discontinuity between intermediate and longer duration interfeeding intervals is evident as an ~30-fold increase in the frequency of interfeeding intervals from the absolute minimum at 14–23 min to a peak at 23–39 min. The time course presented in Clifton (10) corresponds well with the progressive loss of satiety in the present study that began accelerating steeply 12–16 min after meal completion (Fig. 6) with the corresponding distribution of postmeal intervals (Fig. 5).

The results collectively suggest that drinking-naive analysis of interfeeding intervals in the rat may lead to a boutlike 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 interbout and intermeal 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 interfeeding intervals that implicitly accounted for drinking were identified, as observed (14 min) or modeled

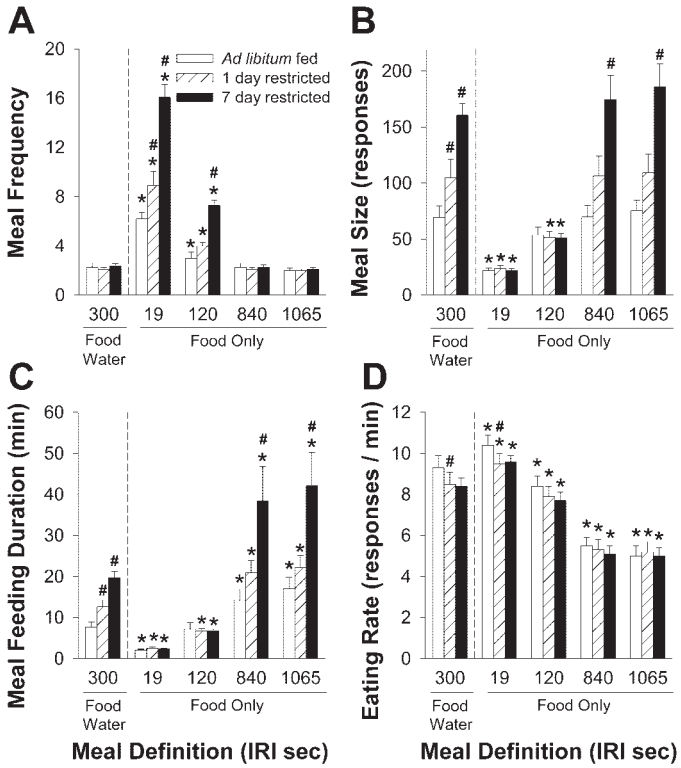

Fig. 9. Effect of meal definition on the estimated 2-h nocturnal meal pattern during ad libitum and restricted feeding. Mean (±SE) meal frequency (*A*), average meal size (*B*), average meal feeding duration (*C*), and average sustained eating rate (*D*) are shown for 11 mature, male Wistar rats fed ad libitum or under acute (1-day) or chronic (7-day) restricted (only 2-h daily access) feeding schedules. Water was available ad libitum in all conditions. Meals were defined with meal definitions that explicitly accounted for prandial drinking (IRI-300 between consecutive feeding or drinking responses), that were naive to prandial drinking (IRI-19, IRI-120 food), or that implicitly accounted for prandial drinking (IRI-840, IRI-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-Keul's tests).

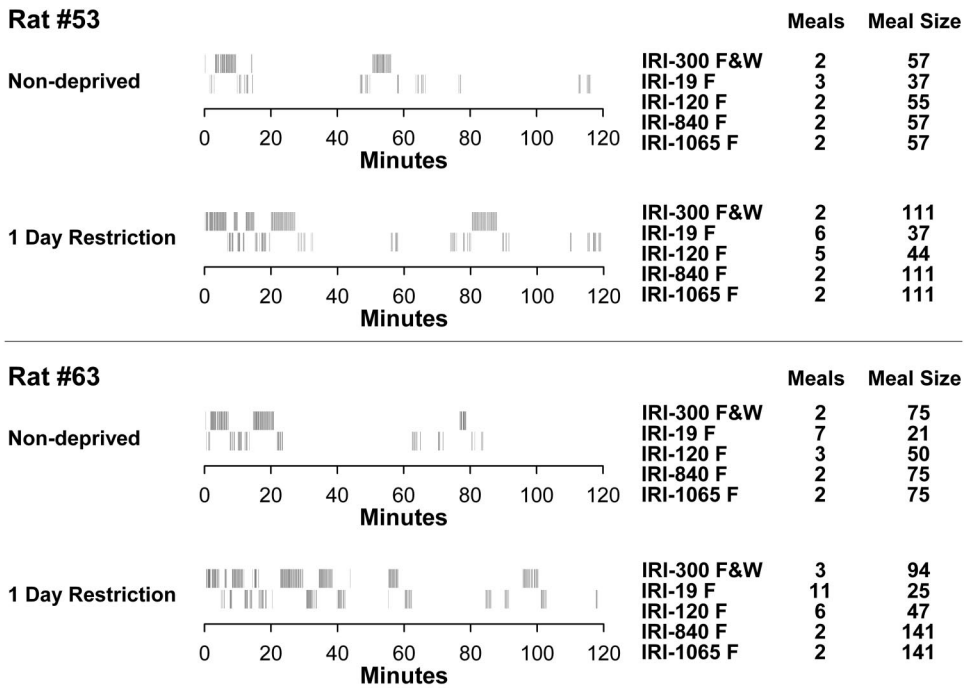

Fig. 10. Event records of feeding and drinking during 2-h nocturnal access. Typical alternating bouts of feeding (upper vertical ticks) and drinking (lower vertical ticks) in 2 representative mature, male Wistar rats under nondeprived and acutely restricted (22-h food deprivation) feeding conditions are shown. Water was available ad libitum in all conditions. Each vertical tick represents a response for food (45-mg pellet) (F) or water (100-μl aliquot) (W). Food restriction increased food intake and food-associated water intake. The drinking-naive IRI breakpoints [IRI-19 or IRI-120 s between feeding (F) responses] tended to split ingestive episodes at prandial drinking breaks. In contrast, the drinking-explicit (IRI-300 F&W) and drinking-implicit definitions (IRI-840, IR-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* and Fig. 9).

(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, although the drinking-implicit breakpoints accounted for interbout drinking, they did not differentiate a 14-min interfeeding interval bridged by drinking from a 14-min interval without drinking. As a result, nondrinking between-meal intervals were mistaken for long, intrameal drinking pauses in the present study, such that meals were merged across the briefest postmeal intervals (e.g., Figs. 5, 6, and 10). Reflecting this, the drinking-implicit definitions underestimated the magnitude of the preprandial correlation, which was not detected reliably in spontaneously feeding rats 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., Fig. 10). Consequently, drinking-implicit definitions overestimated the average duration of feeding within meals and underestimated the sustained eating rate (e.g., Fig. 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 summary, 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 (wild-type littermates of μ-opioid receptor knockout mice compared with wild-type littermates of corticotropin-releasing factor receptor-deficient mice) (unpublished observations); other methodological factors may also be relevant (e.g., strain, age, or sex of subjects, as well as 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; results from this 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 present 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-to-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 naive 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 have also 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) and postabsorptive 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 receptor antagonists into the periphery (AT_1, AT_2) or brain (AT_1) 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 nonprandial drinking occurs in both 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 Ref. 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 interfeeding intervals to derive the meal breakpoint (23), how a rat ate (i.e., meal frequency, meal 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., Ref. 64) that meal size per se is the primary determinant of daily 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 intermeal 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 premeal interval (>22 h) than in its first meal after a much briefer enforced premeal interval (30–60 min for cage cleaning) (mean ± SE) (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 midafternoon (analogous to an extended imposed premeal 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 intermeal 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 intercooling 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; Fig. 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 boutlike structure of feeding and drinking. Such lesions produce more than fourfold 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 intrabout changes in the rate of feeding. For bout analysis to be most effective, however, it would be appropriate to distinguish intrabout feeding intervals from interbout drinking pauses. In the data set of *experiment 2*, such a breakpoint was ~143 s, since not one intrabout interfeeding interval was longer than 143 s, whereas 94% of interbout drinking pauses were longer than 143 s. The similarity of this breakpoint to that obtained from subjective two-process analysis of frequency histograms (IRI-120 food) supports the assertion that the drinking-naive breakpoints were resolving bout, rather than meal, microstructure.

The present experimental methodology differed in several ways from those used in certain laboratories that employed meal pattern analysis. Relevant procedural differences that potentially could influence the distributions of intrabout, interbout, and intermeal 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 similarities of the log-survivorship breakpoint and frequency histograms of interfeeding intervals observed herein vs. 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 intrameal 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 intrameal drinking intervals to be distinguished more easily from intrabout feeding intervals in the present study but thereby also increased the potentially confounding influence of prandial drinking pauses. Conversely, a drinking-naive (or implicit) breakpoint would be more accurate if the duration of prandial drinking pauses was shorter than the duration of intermeal intervals and comparable to the duration of intrabout feeding intervals. For example, unlike what was found in the present study, Tolkamp and colleagues (61, 65) observed that the log-transformed distributions of presumed prandial pauses to drink in cows do not substantially overlap with the distributions of presumed intermeal intervals; rather, they are of similar duration to the intrabout intervals between episodes of feeding at a trough. Under such conditions, prandial drinking pauses are less likely to be confounded with intermeal intervals, and drinking-naive 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), intrabout 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 summary, a conceptually and mathematically novel basis for defining meals in rodents was validated. Unlike drinking-naive 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 of the time a rat has last eaten, log-survivorship analysis is not valid for identifying breakpoints in meals or for lick pattern analysis because it will inherently split meals. Whereas the novel method of defining meals revealed that acute and chronic food restrictions selectively increased meal size and duration, drinking-naive 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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