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RAPID COMMUNICATION

High Lick Rate Is Maintained Throughout Spontaneous Liquid Meals in Freely Feeding Rats

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RUSHING, P. A., T. A. HOUPT, R. P. HENDERSON AND J. GIBBS. *High lick rate is maintained throughout spontaneous liquid meals in freely feeding rats.* PHYSIOL. BEHAV. **62**(5) 1185–1188, 1997.—To investigate the microstructure of spontaneous meals in freely feeding rats, 16 adult male Sprague Dawley rats were housed individually in custom-designed lickometer cages and maintained on a milk diet. Licks were recorded over 23 h at millisecond accuracy via a computer-controlled lickometer. Analysis of lick data revealed an average of about 12 discrete meals/day occurring mainly during the dark phase. The most striking feature of both dark and light meals was the maintenance of a high initial rate of licking until an abrupt decline at the end of the meal. This pattern of licking is very different from the exponential decay of lick rate reported in scheduled test meals of palatable solutions. Thus, the microstructure of licking for meals is affected in an apparently fundamental way by whether a meal is scheduled or spontaneous, suggesting a basic difference in the underlying physiologic controls. © 1997 Elsevier Science Inc.

Ingestion   Lickometer   Meal patterns   Microstructure

THE analysis of the microstructure of licking with millisecond resolution reveals the final motor output of the central neural network that controls ingestion of liquid diets. Although much is known about the microstructure of scheduled test meals in deprived animals (2), the microstructural pattern of licking in spontaneous meals has not been reported. In the present study, we describe the microstructure of licking during spontaneous meals of milk over a 23-h period in freely feeding rats. Our results show that the pattern of licking during spontaneous meals is very different from that reported in scheduled meals, suggesting a basic difference in the underlying physiologic controls.

METHODS

Subjects and Housing

Sixteen adult male Sprague Dawley rats (Charles River, Willmington, MA) were individually housed in open-topped, polycarbonate lickometer cages with stainless steel mesh floors. The dimensions and floor plan of a cage are shown in Fig. 1. One feature of the cage is a stainless steel niche that provides a semi enclosed area where rats rest and sleep. The rationale for the niche comes from a previous report (10) suggesting that when a niche is provided in the test cage, meals are more discrete.

The rats were maintained on a reverse 12-h light-dark cycle

(lights off, 1300 h). A milk diet was continuously available except for a short period from 1000 to 1100 h when daily maintenance was performed. The diet consisted of one 396-g can of sweetened condensed milk, two cans of distilled water, and 3 mL of vitamins (Poly Vi Sol, Mead Johnson, Evansville, IN). The caloric density of this diet is approximately 1.3 kcal/mL. Water was freely available at all times. In addition, a sound conditioner (Marsona, model 1250, Wilmington, NC) was used to mask extraneous noise.

Procedure

Animals were first adapted to eating the milk diet and living in the lickometer cages for 2 weeks to ensure that intake had stabilized. Data were then collected continuously during the 23 h after daily maintenance. Animals were undisturbed during this 23-h period of data collection.

Individual licks were recorded with a computer-controlled, eight-channel electronic lickometer and custom-designed software for data collection (DiLog Instruments, Tallahassee, FL). The data collection program timestamps licks at 0.001 s resolution in binary code for an entire 23-h session in separate ".RIF" files for each animal. In addition, ancillary ".CSV" files are created containing information including the start and
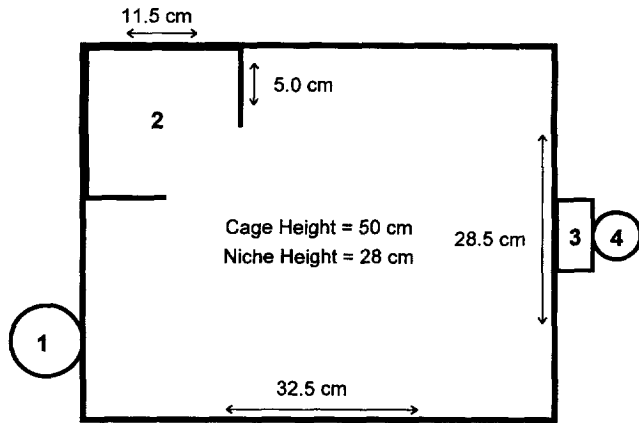
FIG. 1. Floor plan showing the dimensions of the lickometer cage as viewed from above. The components indicated are (1) water bottle, (2) niche, (3) lickometer block and (4) lick tube containing the milk diet.

end times of the session as well as lights-on and lights-off times (via a photosensor).

Data Analysis

The lickometer data were analyzed using a version of the TongueTwister program (8) modified to accept long .RIF files and analyze meal patterns and microstructure. The modified program opens 23-h .RIF files, detects individual meals and performs analyses on those meals. The time of lights-on and lights-off is read from the accompanying .CSV file. Groups of long .RIF files can be opened automatically and simultaneously for rapid analysis.

Meals were defined as bouts of three or more consecutive licks

in bursts and clusters separated from other meals by a minimum intermeal interval (IMI) of 5 min. The 5-min intermeal interval criterion was validated by varying the minimum IMI criterion from 10 s to 60 min and calculating the mean number of meals per rat that were detected with each IMI criterion. When the minimum IMI criterion was varied from 10 s to 60 min, the mean number of meals per rat that was detected with each IMI criterion dropped from 25 at an IMI of 10 s to 7 at an IMI of 1 h (Fig. 2). A sharp discontinuity occurred at 1–2 min. At IMIs shorter than 1 min, the number of meals detected changed rapidly with changes in the IMI criterion. Varying the IMI criterion between 2 and 16 min did not change the mean number of meals detected, and increases in the IMI criterion above 16 min changed the number of meals only gradually. Thus, the IMI criterion of 5 min used in this study appears reasonable.

Burst and cluster parameters were calculated by conventional definitions (5). Bursts were defined as bouts of three or more consecutive licks with interlick intervals <0.25 s; consecutive bursts were separated by interburst intervals of 0.25–0.5 s. Clusters were defined as groups of one or more bursts; consecutive clusters were separated by intercluster intervals (ICI) of >0.5 s.

Microstructural analysis of the 23-h session was summarized for the first meal beginning after lights-off (the first dark meal), all meals that began during lights-off (dark meals) and all meals that began during lights-on (light meals). To produce the mean values for all dark meals and all light meals, the variables were first calculated for every meal of each rat, the variables were then averaged across all dark meals or all light meals of each rat, and the mean values for each rat were then averaged across all rats. The microstructural parameters calculated were meal size in licks, meal duration in minutes (from first to last lick), the mean lick rate in licks per minute during the meal, the IMI following the meal (excluding the last IMI of the dark period), the mean number and size of bursts and clusters per meal and the mean interburst intervals and ICI during the meal. Because the distribution of the
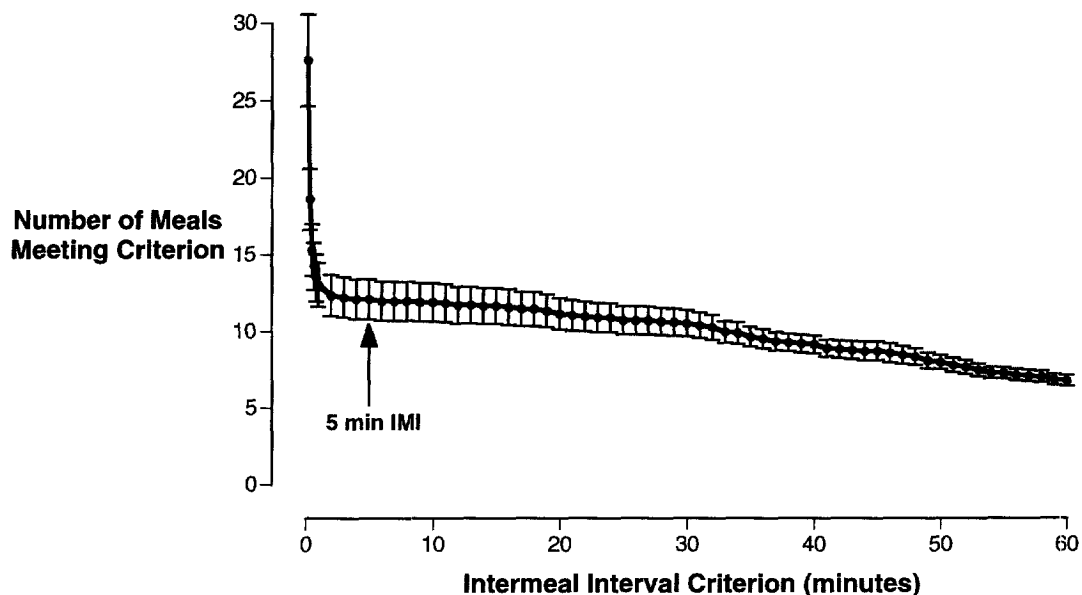

FIG. 2. Mean number of meals per rat ($n = 16$) detected over a 23-h period using criteria for intermeal interval (IMI) ranging from 10 s to 60 min. Using a small IMI criterion, many meals are detected; as the IMI is increased, the number of meals meeting criterion decreases, and there is a slowing in the rate of change in the number of meals meeting criteria. The 5-min IMI criterion used in this study is indicated by the arrow.

TABLE 1

SUMMARY OF MEAL PARAMETERS OVER 23 H

	1st Dark Meal	All Dark Meals	All Light Meals
Meal size (licks)	1539 ± 141*	1433 ± 195	1255 ± 167
Meal duration (min)	5.1 ± 0.3	4.7 ± 0.6	4.4 ± 0.4
Mean lick rate (licks/min)	302 ± 19	301 ± 15	289 ± 15
Intermeal interval (min)	75 ± 8	86 ± 11	133 ± 16†
Bursts (no.)	34 ± 6	34 ± 6	36 ± 5
Burst size (licks)	80 ± 16	65 ± 11	57 ± 12
IBI (s)	0.31 ± 0.007	0.30 ± 0.004	0.32 ± 0.007
Clusters (no.)	16 ± 3	17 ± 3	15 ± 2
Cluster size (licks)	162 ± 29	132 ± 21	115 ± 20
ICI (s)	4.0 ± 0.8	4.5 ± 1.0	3.3 ± 1.0
Initial rate (licks/min)	334 ± 18	313 ± 16	298 ± 16
Decay (s^{-1})	0.005 ± 0.0006	0.005 ± 0.0006	0.005 ± 0.0005
Shape	20 ± 5	25 ± 4	31 ± 5

* Data are presented as means ± SEM.
† Statistically different from dark meals, $p < 0.05$.

ICIs has been shown to be extremely skewed, ranging from 0.5 to 100 s (5), the ICIs within meals were log-transformed before averaging. Potential differences in the parameters between dark and light meals were evaluated by t-tests ($p < 0.05$).

Weibull Function

The Weibull function was fit to the lick rate of individual meals using the Levenberg–Marqhardt nonlinear least squares method (11). The Weibull function has the form, $y = a\,e^{-(bt)^c}$, where t is the time in seconds, a is the initial lick rate at $t = 0$, b is the rate of decay (s^{-1}) and c is the shape parameter. The result, y, is the predicted lick rate at time t in seconds. The initial rate parameter has been shown to be a measure of the palatability of the test solution, while the decay parameter has been interpreted as a measure of the postingestive negative feedback that operates during a meal and ultimately produces meal termination (3,4,12). The Weibull function is distinguished from an exponential function by the presence of the shape parameter. The shape parameter can be roughly construed as the length of time before the rate of licking decreases from its initial level. Thus, the greater the shape value, the longer the initial lick rate is sustained before declining. At $c = 1$, the Weibull is a simple exponential; at very high values of c, the Weibull approximates a square wave function.

The Weibull was fit to the lick rate in licks per minute with 1-min bin size across 30 min of all meals larger than 400 licks. Meals smaller than 400 licks were not suitable for function fitting because at 1-min resolution they were represented by only 1 or 2 points. Of the 196 meals detected during the 23-h period analyzed for 16 rats, a total of 17 meals smaller than 400 licks were excluded from the analysis. As with the other microstructural variables, the Weibull parameters for each meal were averaged across the meals of each rat, and then across all rats. Potential differences between dark and light meals were evaluated using t-tests ($p < 0.05$).

RESULTS

Using the IMI criterion of 5 min, a total of 196 meals were identified for 16 rats during the 23-h test period. These consisted of 160 dark meals and 36 light meals. The number of meals per day ranged from 5 to 20 per rat, the average number of meals per rat was 12.3 ± 1.3, with 10.0 ± 1.2 dark meals and 2.3 ± 0.3 light meals. Two rats had no meals during the lights-on period. Total average intake during the 23-h session was 85 ± 2 mL. To ensure that rats were undisturbed during testing, exact intakes (milliliters) during individual meals could not be visually recorded. Average meal intakes can be estimated, however, by multiplying the licks per milliliter calculated for the entire 23-h session by the licks per meal. From these calculations, the intake was 8.6 mL during an average dark meal and 7.5 mL during an average light meal.

As can be seen in Table 1, the meal parameters for the first meal

A

$$y = 330\,e^{-(0.0034\,t)^{37.3}}$$

B

$$y = 313\,e^{-(0.005\,t)^{25}}$$
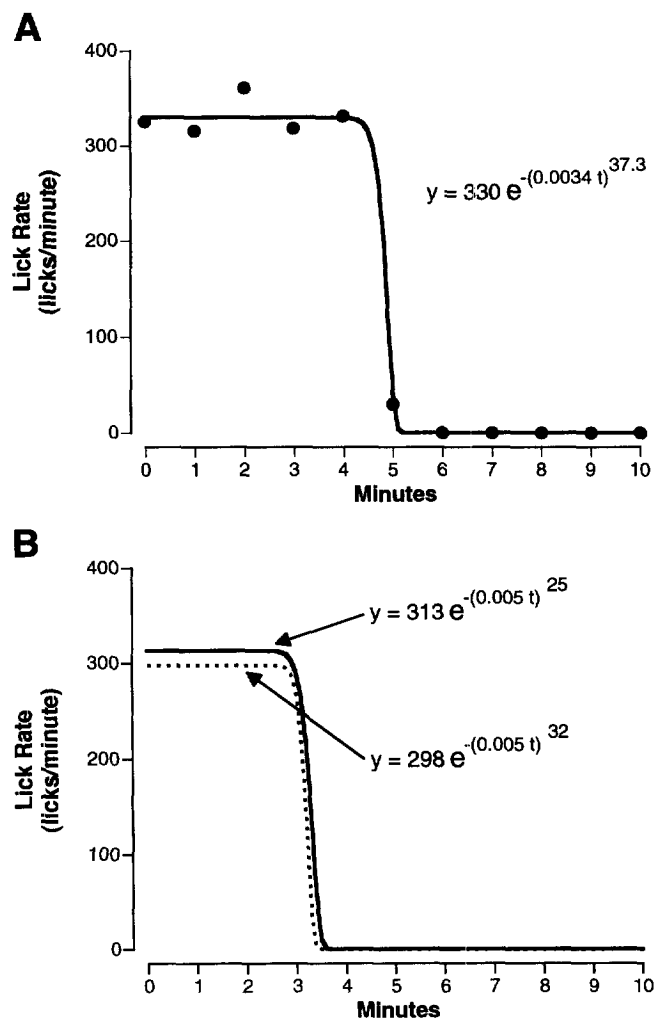
$$y = 298\,e^{-(0.005\,t)^{32}}$$

FIG. 3. A, example of a Weibull function fit to the lick rate of a typical dark meal. Black circles indicate the lick rate in licks/min at 1-min intervals across the meal (the lick rate from 0 min to 1 min is plotted at min 0, from 1 min to 2 min is plotted at min 1, etc.). The line plots the fitted Weibull function. B, plots of the mean Weibull function fit from all dark meals (solid line) and from all light meals (dotted line). In the single meal as well as the average fit from all dark and light meals, a high lick rate is maintained throughout until an abrupt decline in the rate of licking at the end.

of the dark phase were remarkably similar to the average for all dark meals, suggesting a relative uniformity of meals throughout this period. Although light meals tended to be slightly smaller, their parameters were consistent with those of dark meals. In fact, the only statistical difference between dark and light meals was a longer average intermeal interval for light meals (Table 1); this is expected since there were fewer meals during the light phase. Also licking during both dark and light meals was consistently rapid with few interruptions, as evidenced by an average lick rate of about 300 licks/min with relatively large bursts and clusters (Table 1).

Closer examination by least squares fit of a Weibull function to the data produced an initial rate parameter of roughly 300 licks/min for both dark and light meals (Table 1). As can be seen, this is essentially the same as that for mean lick rate, indicating that this high initial rate of licking tended to be maintained throughout the meal. This is further supported by the small decay and large shape parameters, characteristics of a square wave rather than an exponential function. A graphical representation in Fig. 3 shows a Weibull fit to the lick rate of a typical dark meal for an individual rat as well as the mean fit for all dark and all light meals. The persistence of rapid licking throughout all of the meals with an abrupt decline at the end is clear, with no significant differences in the Weibull estimates between dark and light meals.

DISCUSSION

The present study provides the first detailed description of the pattern and microstructure of spontaneous meals in rats freely feeding on a milk diet. The most notable feature observed was the persistence of a high rate of licking over the course of a meal with an abrupt decline at meal termination. Thus, fitting the lick rate data to a Weibull function revealed a fit resembling a square wave rather than an exponential. This was true for meals during both the light and dark phase. Moreover, licking was not only persistent but also relatively continuous with few interruptions, as evidenced by large bursts and clusters.

This square wave pattern of lick rate observed during spontaneous meals is consistent with results from studies indicating that feeding rate does not significantly change during self-initiated meals in rats freely feeding on food pellets delivered by an eatometer device (1) or by bar-pressing (9). It contrasts dramatically, however, with the exponential decline in lick rate reported in scheduled test meals of a similar milk diet in both deprived (7) and nondeprived rats (6). Further, the maintenance of lick rate over the course of a spontaneous meal is a consistent and robust phenomenon. In ongoing experiments utilizing this spontaneous feeding paradigm, we have repeatedly observed the same distinctive pattern across a large number of rats.

There are a number of factors that might contribute to this striking difference, the most obvious being the scheduled versus spontaneous aspect of the meals. Also, rats were undisturbed during testing in our spontaneous feeding situation, while animals were removed from their respective home cages and placed into separate test chambers in the scheduled test meal studies (6,7). It is also possible that the niche available to the rats in our paradigm had some impact on meal topography. All of these potential explanations require investigation.

Experiments conducted on scheduled test meals during the last 25 years have produced a coherent model of meal control: positive feedback provided by the orosensory stimulation of a palatable diet is integrated with negative feedback arising from postingestive effects of the diet to produce an exponential decay of licking across the meal (3,12). The characteristic pattern of a high sustained rate of licking with an abrupt decline at meal termination described here during spontaneous meals, however, suggests that the underlying controls of food intake during ad lib feeding may be different from those in scheduled test meals.

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