ALN® magazine

Measuring Food and Water Intake in Rats and Mice

Eating and drinking support life and are compelled and controlled by positive drives of hunger and thirst and negative restraint of satiety.

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Eating and drinking are episodic, and organized into meals with bouts of specific start times, durations, and amounts. All systems, signals, and switches (physiologic, metabolic, neurologic, anatomic, endocrine, behavioral, etc.) must influence eating and drinking bout by bout. So too, food and water intake should be observed and recorded bout by bout.

Why measure food and water intake?

All animals sense hunger and thirst and satisfy these by eating and drinking. If they do not; they die. Uncovering and understanding the systems for compelling and controlling what and when animals eat and drink has interested biologists for centuries. Some consider this the "holy grail" of biology.

We biologists are lucky; we experience what we study. We have an insider's view. We know the tug of war of hunger and satiety. We can know a lot about eating just by thinking about our own hunger and satiety. We become hungry, we eat; we become satisfied, we stop eating; time passes and we become hungry again and we eat again and so it goes. Some of us live to eat, others eat to live; regardless, it's vitally important to all of us. It's also subtly important to all of us. Everything changes with eating; hormones, metabolites, body temperature, blood pressure, body composition, obesity, glucose tolerance, neural activities, fertility, health, disease, longevity. You name it; you measure it; eating changes it.

All *in vivo* lab animal experimentation would be improved by paying more attention to food and water intake. In this article, we'll review concepts and methodologies for measuring food and water intake of mice and rats.

Concepts and Definitions in Food and Water Intake

Elliot Stellar[1] championed the simple, powerful model that feeding behavior relies on the ongoing interaction between mechanisms that drive eating (hunger) and those that restrain it (satiety). These mechanisms operate to control short term eating behaviors and long term control of body weight and composition. Gerard Smith[2] observed that "after Richter's classic [1922] experiments in rats it became clear that [food] intake in all mammals under a variety of conditions is *episodic*, not continuous." Smith has championed the concept that the *meal* is the functional unit of eating, and that total food intake and its control should be considered in the context of individual meals and the sum of these meals.

Actually, meals are not the smallest unit of ingestive behavior. *Bouts* are the smallest unit of consumption. A bout is an *episode* of uninterrupted feeding or drinking, having *a start time, duration,* and *amount consumed.* A bout can be as small as one nibble, one bite of food or one sip or lick of liquid, or may be comprised of a series of these in quick succession. After a period of not eating or drinking the bout ends; the time between bouts is the *Inter-Bout-Interval (IBI).*

Meals are comprised of the sum of bouts of feeding and/or drinking that are close to each other through time. The amount of time that passes before a new bout is considered to be part of a different meal is called the *Inter-Meal-Interval (IMI)*. The IMI can be considered as simply a long IBI.

Water intake is under separate but analogous control. Sensing thirst and controlling drinking is complex, and is largely regulated by the interaction of positive and negative feedback to maintain body fluid homeostasis. Eating and drinking often but not always occur close together in time. Food and water intake can be measured *periodically* or *episodically*.

Periodic food and water weighings are measured over a set unit of time (minutes, hours, days) defined by the researcher (not the animal). From these weighings, the amount of food or water consumed during the period is calculated by difference. The period's intake is the sum of the period's bouts, but no information is gathered about meals or their component bouts during the period.

Episodic food and water intake is the automatic recording of the animal's individual eating and drinking episode bouts. Each eating or drinking episode is recorded as a unique bout vector record including the bout start time, duration, and amount. From bout vectors, meal records can be constructed. Since animals eat and drink episodically, episodic data represents the animal's *native ingestive behavior.*

Methods for Measuring Food and Water Intake

Periodic Measurement

Food intake can be measured *periodically manually.* A known amount of a diet is given to the animal. After a defined time elapses (set number of hours, days, etc.), the food is reweighed and the amount consumed is calculated by difference. Again, feeding episodes, bouts, and meals cannot be distinguished by these periodic manual weighings.

These methods are time consuming. They cannot be done 24/7. People collecting the periodic data can affect the animals' behavior. The act of moving the animal and its food to weigh it can interrupt or start meals, affecting food and water intake, both negatively and positively. Paradoxically, the more manual measures taken in a day, the less likely that the data is true to the animal's native ingestive behavior. Instruments have been developed to automate this process, saving labor and limiting disruptive human contact with feeding animals.

With the introduction of the electronic "Mettler" balance in 1973, it became possible to measure food and water intake *periodically automatically.* Powdered food in a cup is placed on a Mettler-type balance and offered to animals and scheduled periodic food and water weighings are recorded automatically. This decreases human interference with behavior, and also allows undisturbed food and water weighings across light and dark periods, 24/7. Much can and has been learned about total, periodic food and water intake using systems based on this operating principle.

Mettler balance based systems are difficult and tedious to run. The scale must be placed under or beside specialized cages fitted with holes in the bottom or tunnels to access the food in the cup on the scale. Because the food cup is on the scale, food is accessed from above. The rat can paw through the food, removing it from the cup but not eating it. They can and do sleep, urinate, defecate in their food. Hygienic issues aside, fouling their food also renders weighings of food less accurate. These systems are not based on home cages. Animals are not left in these cages for extended studies instead they are transferred back and forth from home cages. This too may disturb behaviors. Since mice eat much less and are generally much more active than rats, these problems are exaggerated with mice and powder food.

A variation of the Mettler-based system allows hanging food and water from a custom built balance. This improvement allows pelleted foods and water to be dangled from the top and into the cage. This allows for feeding of pelleted foods and eliminates fouling of food from urine and feces. In these systems, crumbles of food or drips of water can fall into the cage and bedding. This loss in weight due to spillage can not be distinguished from eating and drinking. This is especially problematic when studying mice that only eat 2 to 5 gm/day.

Even with flawless weighings, the periodic weighing of food and water has limited value simply because the data is periodic. The scheduled time weight measurements do not record exact start and stop time of intake, nor can they be used to determine rate of eating in bouts or changes in rate of eating within a meal. Predetermined, periodic weighings do not establish eating and drinking start and stop times positively in time. Meal patterns emerge, but are pixilated to the preset weighing periods. *IntraMeal* bout dynamics can not be measured with periodic weighings. To study intake at the bout level with firmly established bout start and stop time, *a trip switch* must be added to periodic weighing devices. Several trip switch-based devices exist.

Trip-Switch Devices

Beam break systems detect and record the timing of light beam break near food and water, but they do not record quantity of food consumed. Beam breaks near food and water are the trip-switch. Animals can break the beam by being near the food yet not eating, and the beams can be broken by shifting of bedding and other objects in the cage. Beam break systems have been used very fruitfully in assessing general activity types and durations, but it is not possible to reliably separate feeding activity from other activities, or associate beam break timing information with the amount consumed. Similarly, *visual systems* for remote viewing and recording of animal movements in a cage (as useful as they are in activity behavior research) do not positively record eating and do not associate the timing of activity near food with the amounts consumed.

Lick meters record every time an animal completes an electric circuit from a water bottle sipper through its tongue, body, and feet to the floor of the cage. This detection of licking is the trip-switch. Lick Meters are used very fruitfully mapping neurophysiologic mechanisms of controlling the starting, stopping, and persistence of licking, and thus presumably drinking and eating combined. A measure of the intensity and timing of interest in a fluid can be assessed. Nutrients and some, but not all, foods can be suspended in water and tested in lick meters. Since foods must be added to water, lick meters cannot separate eating from drinking behavior. Lick meters are not used to record intakes over long periods. Also, the amount of fluid removed by each lick is not consistent, so it is only possible to estimate the quantity of intake. Lick meters can establish the start and stop of drinking/eating bouts in time but do not establish the amount of a bout.

Tablet meters dispense defined weight food tablets or fluid volumes. Tablets are dispensed in response to animals' bar pressing (a learned behavior) or as a replacement of a tablet removed by the animal as detected by beam breaking. Likewise, fixed volumes of water can be delivered for drinking. The bar press or beam break are the "on" trip-switch. Tablet meters do not have an "off" trip-switch. Tablet feeders are used fruitfully in deprivation-based, motivation and reward psychology experimentation. Tablet feeders have also been used for food intake studies. Tablets are commercially available in different sizes – 20 or 45 mg are common sizes. The tablets are real food and tablets of varying composition are available, though very high fat tablets for studying obesity are not. Tablet feeders are mounted in custom cages. Animals are placed in them for the limited study time and then returned to their home cage. Tablets feeders can fail by becoming jammed and it is possible to activate and count a pellet delivered but have no pellet be delivered. So, it is difficult to validate the actual number of tablets delivered and then eaten. Tablets can be removed and hoarded and not eaten. The precise start and finish time of eating cannot be determined. The trip-switch in tablet meters is the call to dispense tablets. There is no way to verify that it is actually eaten and if eaten, when the eating ended. The trip-switch only records the start time of bouts. The stop time and dura-

Figure 1

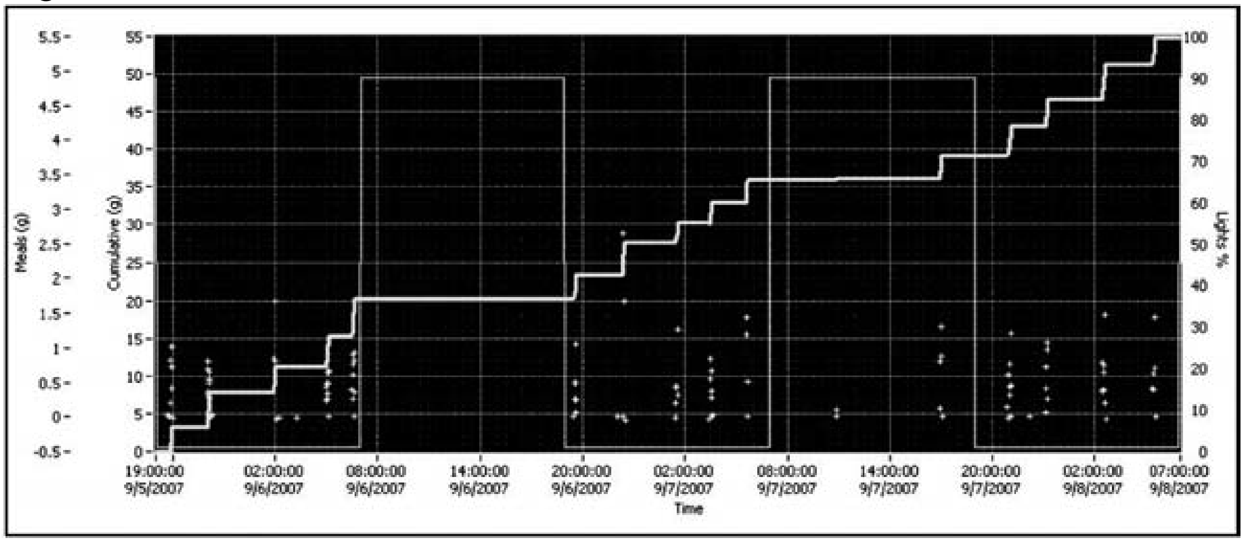

Figure 1 (Source: BioDAQ DataViewer) shows bout meter data collected from a rat over three nights and two days. The thick white trace shows accumulating food intake (y axis) over time (x axis). The thin white trace shows the lights going on and off, day and night. The white points indicate the time and amount of each of the individual bouts. Meals are defined by the IMI which is simply a long IBI. A long IBI defines the end of one meal and start of another. The 15 meals over three nights and two days are easily viewed in Figure 1.

Figure 2

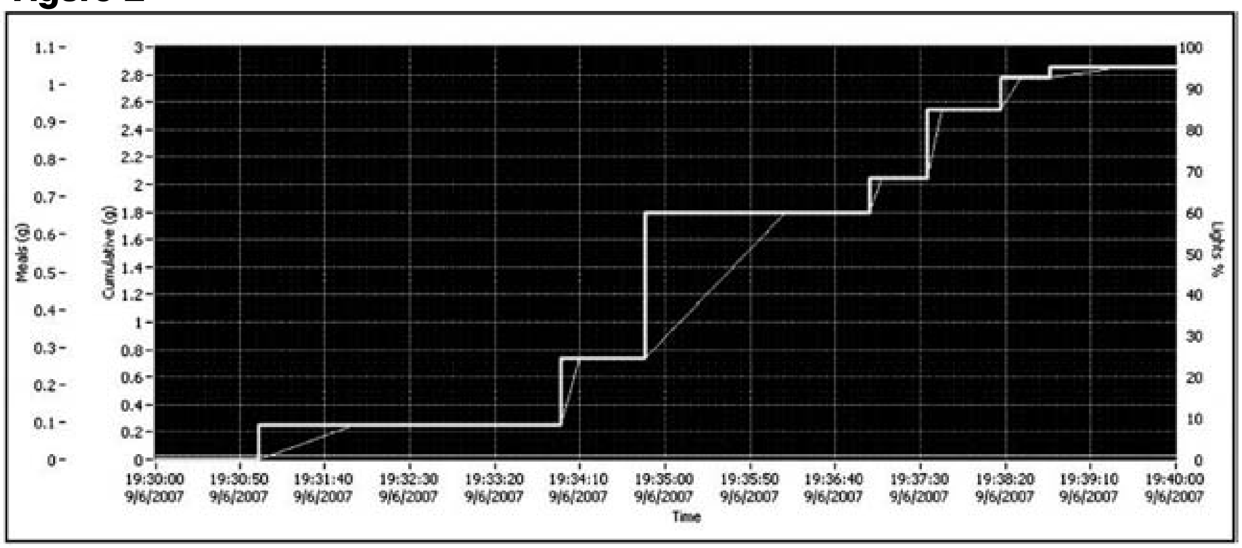

Figure 2 (Source: BioDAQ DataViewer) shows a single 2.85 gm meal that began Sept 6, 2007 at 19:31:01 and lasted 8 min and 28 sec. The vertical lines show the start time and amounts of the bouts, the diagonal lines connect the start and end time of a bout and the slopes demonstrate the rate of eating in each bout. The seven bouts and six IBI that constituted this meal are obvious.

tion cannot be determined. Also, because tablets have a fixed weight, subtle differences in bout weights cannot be assessed and bouts are pixilated by the tablet weights.

Episodic Measurement – Bout Meter

Leveraging advances in computing, data acquisition systems, and software, and electronic strain gauge technology, a *bout meter* was patented and introduced for use in 1997. The "on and off" trip-switches in the bout meter are based on changes in the *stable weight* of the food or water.

Food and water hoppers of the bout meter system are placed on custom *strain gauge* balances and mounted on the outside of rat or mouse *home cages*. The animals access the hoppers and eat and drink from inside the cage. Food crumbs and water drips fall onto a spill tray attached to the hopper and so are not registered as loss in weight. Because the system is mounted on home cages, eating and drinking can be recorded episodically, 24/7/365 with minimal human interference.

The operating principle of the bout meter is simple; it records changes in stable weight of the food or water hoppers.

To illustrate; when rats and mice are not eating or drinking, the weight of the hoppers remains stable. The bout meter evaluates the stable weight every second but does not record anything unless eating or drinking occurs.

When the animal begins to eat or drink, it must touch, paw, bite, lick, nibble, nuzzle the food or water first. This touching disturbs the stable weight. This disturbance in stable weight is the start of bout trip- switch. The bout meter records the bout start time and start weight. As the animal continues eating or drinking (by nibbling and biting and licking, etc.) the food and water weights remain unstable. Then at some point in time the animal stops eating or drinking and the hopper weight becomes stable. This return to stable weight is the end of bout trip-switch. The bout meter evaluates the end of bout time and stable weight and calculates (by difference from stored start time and start weight) and records the bout vector as start time, duration and amount.

The bout meter continues its second by second periodic weighings while the animal continues doing things other than eating or drinking. This is the *Inter-Bout-Interval* (IBI). The IBI can be of any duration, seconds, minutes or hours. The animal determines this somehow not the investigator. Later, the animal begins eating or drinking, the stable weight goes unstable the start bout trips and a new bout recording begins. The duration of the IBI of Bout 1 is calculated as the time difference between the end of Bout 1 and the start of Bout 2. It can not be known until Bout 2 begins.

Bout meter data is shown in Figures 1 and 2 and Table 1.

Similarly, over the three nights and two days in Figure 1, the bout meter weighed the food each second, 216,000 times, and detected and recorded the start, duration, and amount of the 114 bouts the rat ate. The rat organized these 114 bouts into 15 separate meals with discrete start times, bouts, durations and amounts and the bout meter recorded them.

So, the bout meter is simple. The rat must touch the food or water to eat or drink it. This touching switches on (and holds on) the bout recording. Likewise, as the rat stops eating or drinking, it stops touching the food and the bout recording is switched off. Thus it records the native eating and drinking behaviors.

Table 1

Bout #	Start Date	Time	Duration Sec	Gm Amount	IBI Sec
1	9/6/2007	19:31:01	57	0.25	121
2	9/6/2007	19:33:59	11	0.49	38
3	9/6/2007	19:34:48	83	1.06	49
4	9/6/2007	19:37:00	7	0.25	27
5	9/6/2007	19:37:34	9	0.49	34
6	9/6/2007	19:38:17	12	0.24	17
7	9/6/2007	19:38:46	43	0.07	8702

Table 1 (Source: BioDAQ DataViewer) lists the Start Time, Duration, and Amount of these seven bouts. During this 508 second meal, the bout meter made 508 weighings and using the stable weight on and off trip switch algorithm detected, calculated, and reported these seven bout vectors.

Summary and Conclusion

Eating and drinking are compelled and controlled by positive and negative drives of hunger, thirst, and satiety. Eating and drinking is episodic, and is organized into meals with bouts of specific start times, durations, and amounts. The bout and meal amounts and starting and stopping times correspond to the summation of the internal (physiologic, metabolic, neurologic, endocrine, behavioral, etc.) systems, signals, and switches driving hunger and thirst, eating and drinking and assessing the ebb and flow of fleeting satiety. At the end of every bout, during the inter-bout-interval, these calculations resume, and "sooner or later" another bout is initiated, if "sooner" then the meal is continued if "later" then the next meal is initiated. All changes in the amounts of eating and drinking must result from changing the number of meals or the size of meals. The "holy grail" of eating and drinking is hidden in the "sooner or later" between bouts and meals. The bout meter allows us to observe and then perhaps understand, influence, and control these, if possible and if desired.

References

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