A System for Studying the Microstructure of Ingestive Behavior in Mice

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GANNON, K. S., J. C. SMITH, R. HENDERSON AND P. HENDRICK. A system for studying the microstructure of ingestive behavior in mice. PHYSIOL BEHAV 51(3) 515–521, 1992. — A system for long-term monitoring of mouse eating and drinking behavior with 6-s resolution is described. The apparatus utilizes infrared beams to monitor activity at a single feeding port and electronic contact detector circuitry to record individual licks at two drinking ports. Eight SWR/J mice were monitored using this system and data from the last day of testing with food and water were analyzed. Mice ate an average of 4.54 ± 0.40 g of powdered food and drank an average of 5.81 ± 0.65 ml of water. Mice had an average of 36.25 ± 3.96 food bouts and 32.25 ± 7.56 water bouts lasting 3.00 ± 0.48 and 0.88 ± 0.35 min, respectively. Information regarding the temporal association between eating and drinking and the circadian patterns of ingestion was provided, utilizing customized software that augments the system. Important implications for the use of mice in ingestion pattern research are discussed.

Rodents Ingestion Feeding patterns Drinking patterns

RAT ingestive behavior has been described in great detail utilizing microcomputer systems capable of long-term data collection and sophisticated analyses (6,19–21). As a result, much is known about how rats ingest various substances such as NaCl (2) and sucrose (20), and what experimental manipulations serve to modify patterns of ingestion (18,19). Long-term pattern analyses of feeding and drinking have provided valuable insight into the regulatory processes (or the breakdown thereof) operating in experimental subjects. More reliable inferences about taste and other ingestion-related phenomena result from studying detailed patterns of ingestion in contrast to studying only total consumption of substances over 24-h or 48-h periods. The primary focus of past research efforts, however, has been on the rat. Relatively little effort has been devoted to acquiring long-term, high resolution patterns of food and fluid ingestion for mice.

Several reasons may be offered for the neglect of mice in the domain of ingestion-pattern research. First, it may be assumed that mice are miniature rats and behave accordingly, although it is not known whether results from rat studies generalize well to this species. Second, a prevalent belief may be that rats make better subjects (i.e., are larger and less active) than mice when implementing various experimental methodologies. Third, the psychobiology of feeding and taste research has been dominated by studies using rats and there may exist a reluctance to switch from this traditional and successfully utilized subject. Routine incorporation of mice into feeding and taste research, however, can be potentially advantageous.

Aside from the interspecific comparative information that could arise from studying mice, there are certain advantages that mice, as experimental subjects, offer relative to rats. Many of the rats used in studies of ingestive behavior, even uniformly albino stocks (e.g., Sprague Dawley), are outbred, resulting in a lack of precise control of genetic variables. Although some genetically uniform rat stocks exist, they are not typically used and far more mouse strains are available with much better genetic characterization (13). Various inbred strains or mutant stocks of mice exist which serve as models for specific abnormal or pathological states such as alcoholism (17), diabetes (16), obesity (8), and anorexia (14).

In the realm of taste genetics, inbred strains of mice are routinely screened to assess preference or avoidance of various test substances based on two-bottle, daily consumption measures (1,7,9–11,15). The use of genetically identical mice has proven fruitful in that dramatic monogenic influences on the taste system have been reported (3,12,22,23). To further characterize strain differences in ingestive behavior and taste preference, more precise behavioral methods of testing mice need to be devised that go beyond gross measures of intake. Overall consumption measures provide only a superficial glimpse into the complexities of taste and ingestion.

To enable the study of mouse ingestive behavior in greater detail, an automated system was designed that continuously monitored activity at one feeding port and two drinking ports while allowing mice to live undisturbed in their cages. The cages were designed to accommodate the animal’s small size, prevent the mice from residing in the food chambers, and virtually eliminate food spillage. Feeding and drinking behaviors were quantified using criteria presented below which accounted for the bulk of ingestive activity. To demonstrate the utility of this sys-

1 Requests for reprints should be addressed to James C. Smith.
term, SWR/J mice were monitored with access to food and water. It was thought that SWR/J mice would be appropriate test subjects due to their characteristic small size and hyperactivity relative to other mouse strains.

METHOD

Subjects

Eight young adult (mean age = 82.5 days) male SWR/J mice bred at Florida State University were tested in the apparatus for a 2-week period. Animals were weaned at 20 days of age and housed with male littermates until testing, when they were housed individually in cages described below. A 12-h light: 12-h dark cycle (lights on at 0800 h) was maintained throughout rearing and testing. Mice had free access to pelleted Purina Rodent Chow (5001) and tap water prior to testing and powdered Purina Rodent Chow (5012) and distilled water during the test period.

Apparatus Design

Murine living quarters consisted of eight wire-bottomed, stainless steel Hoeltge cages (10 × 24 × 13 cm) that were arranged in two rows on a PVC frame (Fig. 1). Cages were modified so that a feeding station was attached to the front and two drinking cylinders with acorn stoppers and stainless steel sipper tubes could be secured to the rear of each cage. A box mounted on one end of the frame housed the electronic detection circuitry.

Food ingestion monitor. Each feeding station consisted of a stainless steel compartment that held a cylindrical food jar (height = 37 mm; inside diameter = 26 mm) containing powdered food. The food jar was recessed beneath a horizontal plate, and access to the food was through a 16-mm hole cut in the plate. The top of the food jar was approximately level with the floor of the cage. A concave insert was placed inside the food chamber to prevent the mice from gaining bodily entry into the space above the food jar. Interfaced between the food chamber and the floor of the cage was a 1-cm high partition that prevented food from being scattered backward into the cage. The design of the food chamber eliminated the problems of food spillage and excreta-contaminated food. An infrared light source (Honeywell SE 1450-003L) and a phototransistor (Honeywell SD 1440-003L) were positioned approximately 3.2 cm apart across the mouth of each food jar. In order to eat, an animal would insert its head through
the restricted opening into the food jar, thereby interrupting an infrared light beam. The time spent with the mouse’s head in the food jar was assumed to be closely related to the actual time spent eating.

Beam interruptions at the food jar were monitored by electronic circuitry (Fig. 2). The circuit not only converted beam breaks into signals suitable for the computer, but monitored the intensity of each infrared beam positioned across a food jar. Partial obstruction of an infrared beam would turn on a corresponding light emitting diode (LED) with an intensity proportional to the degree of obstruction. Given this feature, the system could be checked before each experimental session for blocked beams or failed components.

The phototransistor’s collector was connected to the non-inverting input of an RCA CA3140 operational amplifier that was biased at +6 V. The output of the operational amplifier was connected to the base of a 2N2222 transistor through the beam intensity-monitoring LED. In this way, the LED indicated the condition of the infrared beam [i.e., if the beam was fully on (no obstruction) the LED was off; if the beam was partially obstructed, the LED was partially on]. This transistor’s collector was tied to an I/O board in the computer. Upon obstruction of the photobeam, the output of the detector circuit would switch from +5 V to 0 V (ground).

Fluid ingestion monitor. Attached to the back of each cage were two Plexiglas blocks. Each block held an inverted graduated, 25-ml glass cylinder fitted with a neoprene stopper through which a bent, stainless steel sipper tube was inserted. Clips positioned at the top of each block secured the drinking cylinders. Different sized drinking cylinders could be used by simply changing the size of the clips. Sipper tubes were inserted through angular holes drilled near the bottom of the Plexiglas block. Stainless steel collars were clamped on the sipper tubes so that, when placed in the Plexiglas block, the tips of all sipper tubes were a uniform distance from the back of the cage. Access to each sipper tube was restricted by a stainless steel plate with a 3 × 28 mm vertical slot cut in it. To drink, an animal would insert its tongue through the slot and lick the recessed end of the sipper tube. This configuration prevented inadvertent or extraneous contact with the tube.

Individual licks on sipper tubes were monitored with an electrical leakage-compensating contact detector (Fig. 3) capable of discriminating licks with onsets 50 ms apart. With this system, a low potential was applied between an electrically isolated lick tube and the metal floor of the cage. Upon contacting the tube, the mouse would complete the circuit resulting in an extremely small current (0.1 microamp) flow that was detected by the circuit. To determine if this current level had any effect on drinking behavior, eight mice were given simultaneous access to two water tubes, one with the electrical contact circuit intact and the other without. Two-tailed t-tests indicated that water consumption did not differ between the two drinking tubes across two consecutive days [on both days, t(7) < 0.21, p > 0.05].

The input stage of the contact detector was an RCA CA3140 FET input operational amplifier. The input bias was set to approximately 0.6 V with a 1N4148 diode. The compensating

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**FIG. 2.** Schematic diagram of an infrared photobeam detector.
feedback circuitry, in conjunction with the input bias, was configured so that the input stage was a high impedance current source supplying approximately 0.1 microamp regardless of the mouse's body resistance. If leakage current developed due to contaminated lick tube isolators, the circuit would respond to hold the input to 0.6 V and still trigger with a 0.1 microamp input current. If the steady state leakage current reached 0.25 microamp, an onboard LED would begin to glow with its intensity indicating the amount of leakage. As with the onboard food LED's, continuously glowing solution LEDs would indicate a problem in the system.

The output stage utilized a 2N2222 transistor and operated in a manner similar to that of the infrared photobeam circuit used to monitor entry into the food jars. The output stage switched between +5 V and 0 V (ground) in response to each lick on the drinking tube.

Data collection system. The +5 V to 0 V outputs of the contact and beam-break circuits drove a Metabyte PIO-12 I/O board installed in a Zenith computer (model Z80A16L-52) with a memory capacity of approximately 500 Kbytes. The system utilized a battery backup unit (Tripple Mfg. Co., model BC-200) that served to protect the computer and electronic circuits from power line failure and voltage fluctuations. In the event of power failure, the system could remain operable for approximately 30 min.

A 24-h timer was used to control the light cycle in the experimental room. Room illumination was monitored by a cadmium sulfide photocell and was connected to an unused status bit of the computer's serial port.

A data collection program written in C monitored electrical contact and beam break occurrences in sequential 6-s time periods referred to as bins. During an experiment, an ongoing tabulation of the number of licks on each sipper tube and seconds spent eating from the start of the experimental session was displayed on the computer screen. The system could be programmed in 1-h increments to collect data for up to 24 h. Typically, the system collected data for 23 h with one hour each day devoted to measuring food and fluid consumption, replenishing substances, and performing general animal and equipment maintenance. A 23-h session would result in 13 800 bins generated for each of 24 incoming channels (three ingestion ports for each of the eight cages). During an experimental session, the data were stored in system memory (RAM) requiring 336 Kbytes. At the end of a session, which occurred either under program control or via manual termination, the data were transferred to a 5¼" floppy diskette under an operator-specified file name providing permanent data storage for future analyses.

BOUT CRITERIA

A convenient way to quantify the ingestive data arose from observations that mice, like rats, eat and drink in discrete episodes or bouts. A set of bout criteria was established in order to define operationally this episodic ingestive behavior as accurately as possible. A drinking bout was initiated by three licks on a drinking spout and was terminated after 50 consecutive bins (300 s) elapsed with no licking activity. Only those bouts containing at least 30 licks were counted. An eating bout was initiated by 3 s with the mouse's head in the food jar (as indicated by obstruction of the beam) and terminated following 50 consecutive bins without feeding activity. All bouts containing less than 10 s of eating were eliminated from further consideration.

Bout-criteria parameters were extensively manipulated in an effort to select those which accounted for the greatest proportion of eating and drinking behavior while eliminating any short-duration investigative behaviors. The above-mentioned criteria accounted for approximately 97.4% of total feeding activity and 99.6% of total drinking behavior (for the last day of testing). Following data collection, bout parameters could be altered for subsequent analyses without affecting the raw data.
DEPENDENT VARIABLES

A computer program written in C processed the raw data disk and generated a printed table containing the ingestive data for individual subjects. For each animal and each ingested substance, the total number of licks (or total time spent eating) was provided, as well as the amount of ingestive activity accounted for by the specified bout criteria. As shown in Table 1, each individual bout was listed along with the time bins in which it occurred, the number of licks (or seconds) each bout contained, and the duration of the subsequent interbout interval (IBI). Correlations between bout length and subsequent IBI duration were also generated for each animal.

A file, automatically created on the data disk, contained the following summary information for each subject and each substance: number of day bouts, night bouts, and total bouts; means for day bout length, night bout length, total bout length, day licks/bout, night licks/bout, total licks/bout, day IBI, night IBI, and total IBI; total number of licks and finally, an eating efficiency score (for food only). Eating efficiency scores reflected the average proportion of time during the feeding bouts in which the infrared beam over the food jar was obstructed by the animal's head.

Food consumption measures were obtained by manually weighing food jars before and after each daily experimental session. The ingested volume of solution was determined directly from the graduated cylinders by the experimenter.

The summary files, created on the data disk, along with consumption data were then transferred into ASCII files for subsequent analyses using commercially-available software (Crunch Statistical Package, Version 3.0; Crunch Software Corp.).

RESULTS AND DISCUSSION

Eating and drinking behaviors were monitored for eight SWR/J mice during a 2-week period, with the data from the last day of testing presented. Consumption of food and water was found to stabilize after approximately 1 week of testing in the apparatus. Summary statistics for food- and water-ingestion variables that the system routinely quantified are provided in Table 2. SWR/J mice (n = 8), on the last day of testing, consumed an average of 4.54 g of powdered food and 5.81 ml of water. They had an average of 36.25 food bouts and 32.25 water bouts lasting approximately 3.00 min and 0.88 min, respectively. The average amount of time between bouts was 34.34 min for food and 42.40 min for water.

In order to obtain a visual representation of ingestion behavior, graphs of food and fluid ingestion as a function of time were generated. The ingestive data for a single SWR/J mouse (whose food data are shown in Table 1 and who is representative of the group) are presented in Fig. 4. Three stacked panels (corresponding to food data on the bottom and solution data on top) utilize a single X-axis that is divided into 13 800 6-s bins, spanning the 23-h testing period. The Y-axis for food has a maximum value of 6 s since this, by definition, equals one bin. The Y-axis for each solution panel (number of licks per 6-s bin) has a maximum value of 60, although this value can be altered to accommodate higher rates of licking. The dark horizontal bar

<table>
<thead>
<tr>
<th>Lights</th>
<th>Bout No.</th>
<th>Bins</th>
<th>Counts</th>
<th>IBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON (0)</td>
<td>1</td>
<td>10-15</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>576-602</td>
<td>72</td>
<td>561</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1307-1325</td>
<td>67</td>
<td>705</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2575-2598</td>
<td>98</td>
<td>1250</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3150-3157</td>
<td>33</td>
<td>552</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3827-3873</td>
<td>135</td>
<td>670</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4162-4184</td>
<td>92</td>
<td>289</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4493-4521</td>
<td>95</td>
<td>309</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4896-4942</td>
<td>97</td>
<td>375</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5069-5089</td>
<td>92</td>
<td>127</td>
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<tr>
<td>OFF (5389)</td>
<td>11</td>
<td>5515-5559</td>
<td>120</td>
<td>426</td>
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<tr>
<td></td>
<td>12</td>
<td>5724-5748</td>
<td>101</td>
<td>165</td>
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<td></td>
<td>24</td>
<td>11704-11738</td>
<td>141</td>
<td>573</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>12147-12194</td>
<td>196</td>
<td>409</td>
</tr>
<tr>
<td>ON (12598)</td>
<td>36</td>
<td>12944-12959</td>
<td>64</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>13035-13106</td>
<td>118</td>
<td>76</td>
</tr>
</tbody>
</table>

Criteria: valid bin = 3 counts; valid bout = 10 counts; valid IBI = 50 bins. Total s spent eating = 3808; total accounted for using criteria = 3739. Total s eating in daytime bouts = 984; nighttime bouts = 2755. Eating efficiency = 0.729. Total bout length, IBI correlation = -0.025; night bout length, IBI correlation = 0.279.
repsent the time during which the lights were out. As shown, the mouse's activity was not limited to the dark period. In fact, approximately one third of mouse ingestive activity, as measured here for SWR/J mice, occurred during daylight hours.

An optional feature of the program enables the expansion of any portion of the X-axis allowing for greater resolution of ingestive activity. As shown in Fig. 5, the time bins from 6900 to 8900 (taken from Fig. 4) were windowed, enabling one to see the temporal relationship between food and water ingestion. Food and water ingestion typically occurred in close association, with food bouts usually preceding water bouts.

To investigate the interplay between food and solution ingestion, a program was created which tabulated the number of times an animal went from a feeding bout to a drinking bout (or from one solution to the other) and vice versa. Since 5 min was our required amount of time for the termination of a bout, that criterion was used as the maximum cutoff for the inclusion of an ingestive switch in our analysis (i.e., only if a drinking bout occurred within 5 min of the end of a feeding bout, would it be of interest as a valid switch).

Results of quantifying the change from eating to drinking and vice versa for the last day are as follows: SWR/J mice had an average of 36.25 food bouts, 30.75 of which were ultimately followed by a water bout. Of these changes from food to water, 83% occurred within 5 min of the food bout (i.e., were valid switches). The mice had an average of 32.25 water bouts, 30.38 of which were followed by a food bout. Of these, 25% occurred within 5 min of the water bout. Here, the typical pattern was to alternate from one ingested substance to the other. These data suggest that an eating bout tended to be followed closely (within 5 min) by a water bout. Water ingestion, however, often was not followed closely by eating.

To assess differential activity across the 23-h period, ingestive activity could be tabulated according to specified time intervals. For a single mouse, Fig. 6 shows cumulative licks on the water spout and time spent eating across the 23-h testing period. As shown, food and water ingestion functions are virtually identical with steeper slopes occurring during the night period (represented by a line beneath the X-axis tick labels).

**Table 2**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Food</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bout number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td>13.38 ± 2.77</td>
<td>11.63 ± 3.62</td>
</tr>
<tr>
<td>Nighttime</td>
<td>22.88 ± 3.52</td>
<td>20.63 ± 6.02</td>
</tr>
<tr>
<td>Bout length (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td>3.04 ± 0.85</td>
<td>0.94 ± 0.46</td>
</tr>
<tr>
<td>Nighttime</td>
<td>2.90 ± 0.49</td>
<td>0.83 ± 0.45</td>
</tr>
<tr>
<td>Time spent eating (s) or number of licks per Day bout</td>
<td>89.84 ± 27.87</td>
<td>143.98 ± 28.15</td>
</tr>
<tr>
<td>Night bout</td>
<td>102.30 ± 13.76</td>
<td>172.79 ± 46.08</td>
</tr>
<tr>
<td>IBI (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td>46.02 ± 9.11</td>
<td>58.09 ± 18.43</td>
</tr>
<tr>
<td>Nighttime</td>
<td>28.56 ± 5.50</td>
<td>35.43 ± 11.13</td>
</tr>
<tr>
<td>Eating efficiency</td>
<td>0.72 ± 0.05</td>
<td>—</td>
</tr>
<tr>
<td>Consumption</td>
<td>4.54 ± 0.40 g</td>
<td>5.81 ± 0.65 ml</td>
</tr>
</tbody>
</table>

n = 8. Mean ± SD.

**Fig. 4** Water (upper panel) and food (lower panel) ingestive activity is shown for a representative male SWR/J mouse across a 23-h period. The dark horizontal bars indicate the 12-h period in which the lights were off.

**Fig. 5** A 3.33-h segment (bins 6900–8900) taken from Fig. 5 is expanded to illustrate the temporal relationship between eating and drinking.

**Fig. 6** Number of licks (solid line) and seconds spent feeding (dashed line) are cumulated for a single SWR/J mouse across a 23-h testing period. The night period is indicated by the line beneath the X-axis tick labels.
CONCLUSION

The pattern analysis approach as applied to ingestion research has proven to be quite powerful. This approach allows one to accurately study the interplay between food and solution ingestion, assess the time course of experimental manipulations affecting ingestion, investigate the circadian rhythms of ingestive behavior, and determine the effects of pharmacological agents on ingestion. Mice have not traditionally been subjects of this type of research and, consequently, their ingestion patterns have not been as thoroughly studied as those of rats. Until now, proven methodologies for simultaneously recording mouse eating and drinking behavior in great detail have not been available. The apparatus presented here was designed specifically to monitor these behaviors in mice while eliminating food scattering and contamination. Furthermore, our quantification of ingestive activity into discrete ingestive bouts using specific criteria was shown to account for the vast proportion of eating and drinking activity. Following data acquisition, unique software programs enabled the expedient summarization of data and provided information regarding circadian patterns of ingestion and the temporal association of eating and drinking.

Utilizing mice in ingestion research could add new insights into the area of ingestion through the comparative information that results as well as through the attainment of genetic control, the existence of useful mutations, and the availability of subjects with reproducible, repeatable phenotypes. In our laboratory, preliminary investigation into mouse ingestive patterns has implicated a genetic influence on consumatory behavior (4).

Applying ingestion pattern methodology to mouse taste psychophysics that uses overall consumption measures could reap many benefits as well. Detailed patterns of sucrose octaacetate (SOA) drinking in SOA-taster and nontaster mice supported and extended findings based on two-bottle preference testing (5). Information regarding how and when mice are ingesting various substances can aid in separating consumption mediated by taste from consumption driven by postigestional or other factors.

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