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Genetic control of oromotor phenotypes: A survey of licking and ingestive behaviors in highly diverse strains of mice

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Abstract

In order to examine genetic influences on fluid ingestion, 20-min intake of either water or 0.1 M sucrose was measured in a lickometer in 18 isogenic strains of mice, including 15 inbred strains and 3 F1 hybrid crosses. Intake and licking data were examined at a number of levels, including lick rate as defined by mean or median interlick interval, as well as several microstructural parameters (i.e. burst-pause structure). In general, strain variation for ingestive phenotypes were correlated across water and sucrose in all strains, indicating fundamental, rather than stimulus-specific, mechanisms of intake. Strain variation was substantial and robust, with heritabilities for phenotypes ranging from 0.22 to 0.73. For mean interlick interval (MPI; a measure of lick rate) strains varied continuously from 94.3 to 127.0 ms, a range consistent with previous studies. Furthermore, variation among strains for microstructural traits such as burst size and number suggested that strains possess different overall ingestive strategies, with some favoring more short bursts, and others favoring fewer, long bursts. Strains also varied in cumulative intake functions, exhibiting both linear and decelerated rates of intake across the session.

Keywords

taste; microstructure; cumulative intake; central pattern generator; lickometry; heritability

1. Introduction

Fluid consumption in rodents is a fundamental, readily quantifiable behavior. Common assays for this behavior range from multi-day tests of consumption amounts in the home

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cage, to recording the sequence of licks from a drinking spout. Measuring intake over a relatively long trial (e.g. 10–60 minutes) with lick-by-lick fidelity offers insights into specific ingestive processes and decisions, especially when the burst/pause structure of intake is considered (i.e., ingestive “microstructure”). For example, initial lick rate and burst size are variables that correlate with ingestive taste reactivity behaviors and unconditioned intake in brief trials, two behavioral measures thought to reflect hedonic evaluation [1, 2]. Moreover, both burst size and initial lick rate covary with sucrose concentration and can be altered by physiological states, which are thought to produce hedonic plasticity [2–5]. On the other hand, the local or primary lick rate (as defined as the average interval between licks less than 1 sec in duration) reflects the output of a central pattern generator (CPG) that is not easily modulated by extrinsic factors [6–9].

In the current study, we sought to characterize variation in ingestive phenotypes among 15 commonly used inbred strains of mice, as well as 3 F₁ hybrid crosses. Understanding endogenous traits of inbred strains and the degree of phenotypic variability is important in the development of transgenic animal models [e.g., 10]. Moreover, strain differences can serve as a starting point for linkage analysis and system genetics approaches [11]. Inbred strains chosen for this study are listed in Table 1. Most of these are considered “classical” strains [e.g., 12], and their inclusion in this study was prompted by use in previous studies characterizing significant strain variation in intake- and feeding-related behaviors [13–22]. Three strains in our panel — CAST/EiJ, WSB/EiJ, and PWK/PhJ — are wild-derived inbred strains. CAST/EiJ mice belong to the *M. m. castaneus* subspecies and all of these inbred animals descend from founders trapped in Thailand in the early 1970s [23]. PWK/PhJ is an inbred *M. m. musculus* strain that descends from mice trapped near Prague in 1972. WSB/EiJ is a pure inbred *M. m. domesticus* strain that descends from animals trapped in Maryland USA in 1976. These strains are highly useful additions to the panel in terms of their degree of genetic variability [24–26], and have also been included (as part of a larger panel) in a number of studies investigating variation in solution intake and body weight [14, 15, 27].

With respect to fluid licking and microstructure, mice of the commonly used strain C57BL/6J have been shown in several studies to possess slower lick rates (longer mean interlick intervals) to water or other tastant solutions than DBA/2, 129P3/J, or SWR/J mice [7, 28–31]. Further strain differences have been shown in other microstructural parameters including overall consumption, burst count, burst size and initial licking rate [7, 31, 32]. These four strains are included in the current study, along with other classical inbred strains (A/J, AKR/J, BALBcBy/J, C3H/HeJ, CBA/J, FVB/NJ) and the three wild-derived strains mentioned above. Several of these possess interesting phenotypes related to feeding or body physiology, such as high body-fat strains CBA/J and AKR/J [27], or C3H/HeJ and DBA/2J mice, which have a relatively poor ability to regulate caloric intake when presented with food choice [19]. We also included the strains NZW/LacJ and BTBRT⁺/t⁺J, the latter used to model autism, of interest due to its tendency to display abnormal motor stereotypies [33].

In the current study, licking microstructure was measured in response to both water and a strongly preferred concentration of sucrose (0.1 M) in thirsty mice using this diverse panel of inbred strains as well as several hybrid crosses. It is important to emphasize that although

we could make *a priori* predictions about lick rate for several strains, as mentioned above, this study provides the first look at detailed microstructure for most of these strains.

2. Materials and Methods

2.1 Animals

We collected data from a total of 160 adult mice (*Mus musculus*) and from 15 fully inbred strains and 3 F₁ crosses (mean $n = 8.8$ per type). Abbreviations used in this report, numbers of each strain and sex tested, and initial body weights are provided in Table 1. F₁ hybrids were bred from the following pairings: C57BL/6J ♀ X DBA/2J ♂ (B6D2F1); C57BL/6J ♀ X SWR/J ♂ (SWB6F1); and DBA/2J ♀ X SWR/J ♂ (SWD2F1). Mice were obtained either directly from the Jackson Laboratory (Bar Harbor, ME) and acclimated for a few weeks to the vivarium at the University of Tennessee Health Science Center (UTHSC), or were bred at UTHSC.

The average age of all mice prior to testing was 90.8 days. We tried to use matched animals by age and weight, but we occasionally tested older mice. Prior to testing, all mice were housed in plastic home cages (28 × 17.5 × 13 cm) in a temperature and humidity-controlled vivarium on a 12:12 h light-dark cycle. Food and water were available *ad lib*. Fresh bedding was provided, and water bottles were removed from the cages of singly housed mice, approximately 23 h prior to testing in a lickometer. Thereafter during the experiment, fluid was only available during daily tests, whereas food remained available in the home cage (but not the test chamber) on an *ad lib* basis. All experiments adhered to procedural guidelines approved by the UTHSC Animal Care and Use Committee.

2.2 Apparatus

Licking tests were conducted in a Davis MS-160 computer-controlled lickometer (DiLog Instruments, Inc., Tallahassee FL) designed by James C. Smith and Ross Henderson [34]. In the MS-160, water-restricted mice were placed in an opaque test cage (30 × 14.5 × 16 cm) with a stainless-steel mesh floor, and access to a stainless steel drinking tube (orifice diameter = 3 mm) containing 18 MΩ deionized water (days 1–2) or 0.1 M sucrose (mixed in deionized water, Sigma, St. Louis, MO; day 3) via a small opening at the front of the chamber. A test period began when a shutter opened to allow access to the drinking tube, and the mouse made contact with the tube. The test period ended after 20 minutes when the shutter closed. Lick contact with the spout completed an imperceptible (< 50 nA) circuit that allowed the onset time of each lick (ms resolution) to be recorded to a computer file.

2.3 Procedure

All mice were tested in the MS-160 for three consecutive days, with one 20-minute session per day. Mice were weighed just prior to testing each day. On the first two days the mice were presented with water and on the final day with an appetitive stimulus, sucrose, at a concentration (0.1 M) that is readily consumed by mice [35]. This relatively short test cycle was chosen to minimize potential effects of prolonged water restriction, and for comparability with our previous report on water licking in B6 and D2 mice [7]. If a mouse only licked a few times or not at all during the first day of testing, it was re-tested on the

same day, after the other mice had been tested. Of the 160 mice tested in this experiment, 5 (2.8%) licked less than 40 times at either opportunity on day 1; all of these performed well above this level on day 2, and their data from days 2 and 3 were used in the analysis.

In this study all data were collected over a 19-month period; due to the inevitable difficulties of obtaining all strains it was not practical to interleave all 17 genotypes during test weeks. Overall, an average of 7.6 ± 3.5 mice (mean $\pm$ SD) were tested in any one week. However, efforts were made to minimize the potential that our phenotyping outcomes were not exposed to undue technical or environmental drift. When possible, individuals belonging to a single strain were tested in two batches, on two different weeks. Exactly half of the mice were tested during the spring-summer months, half during fall-winter. A single individual tested all but four of the mice. Within each strain, mice were randomly assigned to one of two highly similar lickometers, with 79 mice tested on rig “A” and 81 on rig “B”.

2.4 Data Analysis

2.4.1 Interlick intervals (ILI)—Data files consisting of a serial listing of interlick intervals (ILI, measured to the nearest ms) were analyzed using custom software (Visual Basic 2008, written by S.J.S.). ILI less than 40 ms (1.3% of all recorded ILI) were considered physiologically implausible as ILI and more likely the result of brief loss of contact with the spout during an ongoing lick. ILI of this duration were added to the following ILI.

2.4.2 Mean primary ILI (MPI) and median ILI—During “meals”, mice consume fluid from a spout discontinuously – runs of licks (called *bursts*) separated by *pauses* of various durations [36, 37]. Within bursts, licking is fairly regular, with the length of ILI varying across strain and species [7, 8, 30, 32]. To capture this “fastest lick rate”, we measured the *mean primary ILI* (MPI) as the mean of all ILI less than 160 ms [7, 8]. This cutoff value of 160 ms was chosen as capturing most or all licks during an uninterrupted run while avoiding the inclusion of ILI at twice that periodicity (which can occur if the tongue is extended but fails to make contact with the spout during a licking cycle). This cutoff works well for a variety of mouse strains, but may not be appropriate for slower-licking strains. We therefore also assessed the criterion-free measure of *median ILI*. This measure should be robust so long as >50% of all ILI in a session represent an uninterrupted run of licking.

2.4.3 Lick efficiency—We also calculated *lick efficiency*, which is a measure of the proportion of session ILI briefer than 160 ms (a value chosen to be consistent with the MPI measure and for the same reasons). Lick efficiency provides a measure of how continuous or discontinuous a mouse’s ingestive behavior is [31].

2.4.4 Bursts and pauses—Consistent with much of the microstructure literature, we defined a burst as 3 or more licks with ILI less than 1 s [7, 8, 30, 32, 38–40]. With this criterion, the *number of bursts* and the *average burst size* (measured as licks per burst) could be calculated for each mouse. ILI intervals greater than 1 s can therefore be considered *interburst intervals*. We also examined pauses of intermediate length – pauses less than 1 s but greater than 160 ms represent very brief breaks in licking behavior that may reflect taste

reactivity behaviors [41, 42] or missed licks (tongue protrusions failing to contact the drinking spout).

2.4.5 Ingestive style index—It has been noted that some mouse strains tend to engage in frequent, short bursts of licking whereas others tend to engage in few, longer bursts of licking [30]. We developed an *ingestive style index* for our dataset as follows. As noted above, we calculated an average burst size and an average number of bursts for each of the 18 strains. An all-strain average was calculated from these values. For each individual mouse, we calculated a percentage score relative to the all-strain average for burst size and burst number. From these percentages, the ingestive style index for each mouse was computed as:

$$ISI = \log_{10} (\% \text{ of all- strain burst size} / \% \text{ of all- strain burst number})$$

An ISI of 0 is indicative of a mouse whose burst size and burst number are proportional to the all-strain averages. A positive ISI indicates a preference for long but infrequent bursts, whereas a negative ISI indicates a preference for short, frequent bursts (relative to the all-strain average). ISI was calculated separately for the water and sucrose sessions.

2.4.6 Inferential statistics—Many of the microstructural variables listed above were assessed for strain differences using inferential statistics. With few exceptions, variability of these variables across strains was non-homogenous (Levene's test), precluding parametric analysis. Standard data transforms (e.g., log transform) usually improved, but did not eliminate, non-homogeneity. Therefore we took a standardized approach of using the nonparametric Kruskal-Wallis H test to examine differences across the 15 inbred and 3 hybrid strains on microstructural variables of interest.

2.4.7 Cumulative intake—Finally, we examined cumulative intake functions. The number of licks generated during each minute of the 20-min session was calculated for each mouse and converted to proportions to allow averaging across individuals in a strain. This generated a function for each strain in which the proportion of total licks in a session began at 0 (at time 0) and rose to 1.0 (by time 20 minutes). We noted that in some strains, this function was fairly linear, whereas in most strains, the rate of licking was high early in the session and decelerated as the session progressed.

2.4.8 Heritability and genomic analyses—Heritability (h^2) was estimated from within- and between-strain variances for microstructural phenotypes (includes those described above for water and for sucrose, e.g. water MPI and sucrose MPI are 2 separate phenotypes) using body weight, sex, logarithm of age, and testing rig as controlled cofactors in a linear model. We used all inbred strains ($n = 15$) and the F1 hybrids. The sum of squares attributable to strain was divided by the total sum of squares to yield an estimate of h^2 . Delete-one jackknife resampling was used to reduce bias and estimate error terms for h^2 for all traits as described in Williams and colleagues [24].

The data were entered into GeneNetwork (GN, www.genenetwork.org) into the Mouse Diversity Panel data set as Trait IDs 49910 to 49933 (Table 2). Corresponding data for some phenotypes are also available at this site for the BXD strains of mice [8]. GN automatically compares phenotypes against a panel of ~10,000 informative markers for all strains. The results of this rough genome mapping should only be used with caution. Given the modest sample size ($n = 14$ strains with 8 to 10 replicates/strain; BTBR and the hybrid strains omitted) and given the complex genetic structure of our specific diversity cohort, it is not possible to map traits using a genome-wide strategy [43]. Correlations (Spearman Rank) for each trait were also calculated at GeneNetwork with other published inbred strain phenotypes.

3. Results

3.1 Interlick intervals

Strains varied in their rate of licking within bursts (Figure 1), with MPI significantly varying as a function of *Strain* to both water ($\chi^2(17) = 128.4$, $p < 0.0005$) and sucrose ($\chi^2(17) = 113.3$, $p < 0.0005$). SWR, D2, and the SWD2F1 hybrids had the fastest within burst lick rate (94.3 – 98.2 ms), whereas A, PWK, and CAST mice had the slowest (119.2 – 127.0 ms). The mean for all 18 strains was 109.4 ± 2.2 ms. There was a high correlation between MPI measured during the water session and the sucrose session ($r = 0.954$, $p = 1 \times 10^{-9}$), with only the WSB strain shifting its rank ordering more than 2 between the two conditions (from 9th fastest to 14th fastest). This strain, along with the A, NZW, CAST, and PWK strains, had unusually high median ILI (149.3 – 163.4 ms to water; the next slowest strain was FVB, 134.4 ms). For these 5 strains, the 160 ms cutoff for MPI may make these strains appear to lick faster than in actuality. Careful examination of the ILI histograms for each mouse in these strains suggests that these mice were less reliable in licking behavior – that is, ILI distributions for individual mice of the A, NZW, CAST, PWK, and WSB strains often appeared broad and multimodal, in contrast to ILI distributions of the remaining strains that were typically tight (most ILI clustered within 20 ms of the MPI) and strongly unimodal.

3.2 Lick efficiency

Another way to measure lick reliability is the lick efficiency metric; there were profound strain differences in lick efficiency for water ($\chi^2(17) = 111.7$, $p < 0.0005$) and sucrose ($\chi^2(17) = 92.3$, $p < 0.0005$). As seen in Figure 2 (white bars), the 5 strains identified as having an unusually high median ILI (A, PWK, NZW, CAST, and WSB) also had the lowest lick efficiency proportion of session ILI less than 160 ms in length in the water session (0.744 – 0.778). The BTBR, SWR, and AKR strains had the highest lick efficiency (0.953 – 0.963); the mean for all 18 strains was 0.866 ± 0.018 . Lick efficiency during the sucrose session was highly correlated with lick efficiency during the water session ($r = 0.892$, $p = 1 \times 10^{-6}$). Rank ordering and mean lick efficiency (0.865 ± 0.020) was generally similar during the sucrose sessions except that the PWK strain was notably more efficient and the B6 strain notably less efficient in the sucrose vs. water session. In Figure 2, mouse strains are rank-ordered by MPI during the water session inverse correlation (water session: $r = -0.536$, $p = 0.022$; sucrose session: $r = -0.558$, $p = 0.016$). Interestingly, there was a significant correlation between percent body weight loss (test day relative to pre-water

restriction weight) in the water session ($r = -0.543$, $p = 0.020$) and a similar-sized correlation during the sucrose session that was not statistically significant ($r = -0.425$, $p = 0.079$). Thus, in strains where mice lost a greater percentage of body weight, lick efficiency was lower.

The majority of ILI that were not less than 160 ms, particularly for inefficient strains, were nonetheless short (161–1000 ms; gray bars, Figure 2) and did not terminate a burst of licking by our criterion. Pauses briefer than 1 s make it likely that the mice were still attending to the spout during these shorter pauses.

3.3 Total licks and fluid consumption

There was a large range of total licks to water ($\chi^2(17) = 80.4$, $p < 0.0005$) and sucrose ($\chi^2(17) = 65.4$, $p < 0.0005$) during the 20-minute intake sessions (Figure 3; note that for this and subsequent figures mouse strains are arranged by increasing total licks to facilitate comparisons across meal pattern variables). For water, the A strain averaged the fewest number of licks (495.2) and BTBR mice the most (1471.8); the 18-strain average was 825.9 ± 61.6 licks. For sucrose, the lowest intake was in D2 mice (940.2 licks) and the highest was in CAST mice (2150.8 licks); the 18-strain average was 1454.9 ± 82.2 licks. Total licks in these two sessions were highly correlated across strains ($r = 0.914$, $p = 1 \times 10^{-7}$). This strong correlation is presumably caused by the similar water-deprivation state of the mice during both sessions.

Strains also vary in body weight ($\chi^2(17) = 96.9$, $p < 0.0005$), which did not correlate with total licks but did correlate with fluid consumption (water session: $r = 0.535$, $p = 0.02$; sucrose session: $r = 0.531$, $p = 0.02$). Total licks correlated with water consumption ($r = 0.590$, $p = 0.01$; for sucrose consumption, the correlation was not significant, $r = 0.425$, $p = 0.08$).

Although it is difficult to measure fluid consumption accurately in our testing conditions, the correlations suggest that larger mice may obtain more fluid per lick rather than generate more licks per session. We computed *volume per lick* by dividing consumption (in μl) by total licks. WSB and CAST mice were outliers, averaging 0.6 or fewer μl per lick to both water and sucrose (the next lowest was 0.88 μl per lick in CBA mice licking water; the mean across all 18 strains was 1.08 ± 0.08 per water lick and 1.10 ± 0.07 μl per sucrose lick.) On the other end, SWB6F1 and B6D2F1 mice had high volumes per lick for both water and sucrose (e.g., greater than 1.42 μl per lick). Volume per water lick ($r = 0.555$, $p = 0.02$) and sucrose lick ($r = 0.599$, $p = 0.009$) significantly correlated with initial body weight. Strains significantly varied in volume per water lick ($\chi^2(17) = 77.6$, $p < 0.0005$) and volume per sucrose lick ($\chi^2(17) = 84.0$, $p < 0.0005$).

3.4 Microstructural analysis: burst number and size

Mice displayed different behavioral strategies in fluid consumption. In principle, the total licks of a session should be the product of the number of bursts and the average size of bursts of licking (this is true on an individual basis and approximately true when examining strain means). A mouse with a large intake might initiate more bursts of licking or it might maintain longer bursts of licking (or some combination). Variations might indicate

differences across strains in the neural control of burst initiation (burst number) or in the neural control of burst termination (burst size). Strains significantly varied in the size of water ($\chi^2(17) = 86.9$, $p < 0.0005$) and sucrose bursts ($\chi^2(17) = 107.6$, $p < 0.0005$; Figure 4A) and well as the number of bursts initiated to water ($\chi^2(17) = 61.8$, $p < 0.0005$) and sucrose ($\chi^2(17) = 78.1$, $p < 0.0005$; Figure 4B). Interestingly, across strains, the average size of bursts (Figure 4A) correlated with total licks for both water ($r = 0.695$, $p = 0.0014$) and sucrose ($r = 0.700$, $p = 0.0011$), whereas the average number of bursts (Figure 4B) did not correlate with total licks. (In Figure 4, strains are in ascending order of total licks to water to facilitate this comparison.) During the water session, burst number ranged from as few as 9.1 (SWR) to as many as 23.5 (BALB) with a mean of 16.2 ± 0.96 , and burst size ranged from 34.77 licks (FVB) to 110.85 licks (CAST) with a mean of 57.8 ± 5.70 . During the sucrose session, burst number ranged from 14.0 (129 strain) to 47.3 (B6) with a mean of 28.5 ± 2.20 , and burst size ranged from 28.5 licks per burst (B6) to 134.9 licks per burst (BTBR) with a mean of 62.7 ± 7.4 . In general, there was an inverse correlation between burst number and burst size across strain (water: $r = -0.50$, $p = 0.04$; sucrose: $r = -0.75$, $p = 0.00033$): some mice took few, but long bursts and others took many, but smaller bursts. These patterns were consistent across the two stimuli. The number of bursts for water and sucrose were correlated across strain ($r = 0.52$, $p = 0.027$) as were the size of bursts ($r = 0.88$, $p = 1.5 \times 10^{-6}$).

Despite the fact that all strains licked more to sucrose than water over the entire session (c.f., Figure 3), several strains averaged longer bursts for water than sucrose (Figure 4A: A, AKR, B6, B6D2F1, C3, CAST, D2, FVB, and SWB6F1). We investigated the possibility that this result might be an artefact of the phenomenon that burst size tends to decrease as a session progresses [39, 44]. Bursts in the sucrose session were serially-ordered for each mouse, and average burst size was computed over the same number of bursts produced by the mouse in the water session (the *water-matched burst size*). For example, a mouse taking 12 bursts in the water session and 26 bursts in the sucrose session had a water-matched burst size computed over the first 12 bursts in the sucrose session. Using this measure, burst size was higher in sucrose sessions than water sessions for all strains except the FVB (Figure 4C). Therefore, the lower burst size exhibited by 9 strains to sucrose (Figure 4A) is likely an artefact of increased meal duration. Indeed, the longest burst of the session tends to be the first. When the longest single burst per mouse is compared, nearly all strains generated their longest burst to sucrose rather than water (Figure 4D).

As noted above, the mouse strains exhibit a range of ingestive strategies, with some consuming fluid in large, infrequent bursts and others consuming fluid in many, shorter bursts. We quantified these strategies with the ISI measure (see Method), which simplified identification of these behavioral styles independent of the total number of licks generated (Figure 5). There were pronounced strain differences in ISI during both the water session ($\chi^2(17) = 71.6$, $p < 0.0005$) and the sucrose session ($\chi^2(17) = 99.4$, $p < 0.0005$). For example, SWR, CAST, and 129 mice had a positive ISI for both the water and sucrose sessions (few, long bursts), whereas FVB and BALB mice had a negative ISI for both sessions (many, short bursts). Other strains exhibited an average strategy, such as the C3, D2, and CBA mice. There was a positive correlation between water and sucrose ISI across strain ($r = 0.772$, $p = 0.0018$), suggesting that these styles are independent of taste stimulus. Strains

falling near the long diagonal of Figure 5 show very similar behavior for both water and sucrose, whereas deviation from the long diagonal indicates that a style was more pronounced with sucrose (usually) than water. The A strain, for example, favors a style of many small bursts, but this style is more evident when licking sucrose than water. Similarly, the BTBR strain favors fewer, longer bursts, which is again more evident when sucrose is the stimulus than when water is the stimulus.

3.5 Cumulative intake

We also examined the possibility of strain differences as the meal progressed. As noted above, most mice engaged in their longest burst of licking in the very first burst, and, in fact, generally consumed 50% of their total session licks within the first 2–5 minutes of the 20 minute session (Figure 6, white bars). Cumulative intake functions varied across strains, with some strains drinking in a decelerated fashion and others in a more linear fashion (Figure 7 shows the cumulative intake functions during the sucrose session for the three most and least linear drinkers). In general, variability within strains was low, particularly during the sucrose session, and pattern of intake during the water and sucrose session was similar (with a couple of exceptions).

3.6 Heritability and Genomic Analysis

Estimates of h^2 values were calculated for a large subset of microstructural phenotypes (22; includes behaviors measured with either water and sucrose; note that we did not estimate h^2 for either the initial body weight or the water-matched sucrose burst size) using a linear model applied to each phenotype, or trait. Strain effects for each trait were significant ($p < 0.0002$). We also investigated body weight, sex, age, and testing rig as cofactors. Significant effects ($p < 0.01$) of all cofactors were detected at a 0.11 rate across all analyses, with the most common being effects of age (4/22 traits or testing rig (also 4/22 traits). However, these effects appeared to be randomly distributed across traits rather than predictive of variation in specific aspects of microstructure.

Estimates of h^2 for each trait ranged from approximately 0.22 to 0.73 (Figure 8). By including the cofactors listed above we are eliminating some genetic variance from our estimates of h^2 ; these estimates will therefore be biased toward lower values. Jackknife re-sampling for all h^2 estimates yielded error values (SD) for each h^2 mean ranging from 0.019 – 0.045. When comparing the same microstructural measure between water and sucrose, h^2 was found to be higher for water a majority of times.

As mentioned above, GeneNetwork automatically conducts marker regression with entered phenotypes. Significant associations ($p < 0.05$) were found for 12/23 traits, but are not discussed in detail here, due to the unsuitability of this dataset (too few strains) for genome-wide mapping. However, we have previously used the same methods and traits to phenotype 66 BXD RI strains, derived from B6 $\times$ D2 crosses (Boughter et al., 2012). This makes it feasible to use the new inbred strain data for discrete analysis of loci securely mapped in the BXD cohort. For example, BXD Trait ID 12297 (MPI for water over 20 min) corresponds precisely to MDP Trait ID 49919 (also MPI for water over 20 min) in the present study. This trait in the BXDs maps with a highly significant likelihood ratio statistic (LRS) of 24.9 ($p <$

0.001) near the candidate gene *Atp1a2* (Na⁺/K⁺ ATPase subunit alpha-2) on Chr 1. Using the current strain (MPD) data (i.e. Fig.1), we revisited this locus in GeneNetwork in an attempt to refine the BXD-generated map. This attempt failed completely: No significant ($p > 0.05$) associations were found between MPI and genotype on Chr 1. However, this negative result is useful because it suggests that the sequence variants that are associated with the difference in ILI length between D2 and B6 mice may not be shared with other common strains of mice.

Correlation analysis with published phenotypes revealed interesting relationships between other studies and our own. Pre-test body weight (GN 49910) was significantly correlated with many tissue and whole-body mass phenotypes, including 27 types of body weight phenotypes originating from 9 different studies (number of overlapping strains ranged from 8–13; $r_s = 0.75 - 0.98$; $p_s < 0.009$). This robust concordance across different studies and labs yields confidence in the potential of this database-driven approach for uncovering relationships between phenotypes that may offer mechanistic insight. On the other hand, for our ingestive phenotypes, there were fewer studies in the database with which to compare. Water consumption (GN 49911) in our 20-minute test was correlated (10 strains compared; $r_s = 0.78$, $p < 0.006$) with total 3-day water intake [45]. However, sucrose consumption (GN 49912) was correlated (12 strains compared; $r_s = 0.65$, $p < 0.02$) with intake of 75 mM NaCl in two-bottle choice tests [13]; this concentration of NaCl is appetitive (preferred to water) to many strains.

4. Conclusion

The current study represents the first attempt to quantify the microstructural pattern of intake across a large array of mouse strains. The intake sessions were simple: water-restricted mice were offered a single bottle for 20 minutes of water (first test) or 0.1 M sucrose (second test) following an earlier training day identical to the first test session. In addition to measuring the amount consumed, we assessed variation in ingestive behavior at a number of levels: The rate of licking in uninterrupted volleys of licks (MPI and median ILI, thought to reflect the properties of the CPG governing licking), the length of short and long pauses within and between bursts (lick efficiency), the number and length of bursts of licking behavior (burst number, thought to reflect controls of burst initiation along with burst size, thought to reflect controls of burst termination), and the change in the rate of licking over the course of a session (cumulative intake). We also compared strains in terms of relative preference for many short bursts or few long bursts during the course of a session (quantified by the ISI). This experimental strategy identified a number of interesting differences across strains – in local lick rate, in ingestive style, and in cumulative licking across the session, each of which is ripe for further mechanistic analyses.

4.1 Mouse strains vary in rate of licking

The most basic unit of a liquid meal in rodents is the lick. A series of licks show remarkable rhythmicity in the interlick intervals which are typically tightly distributed around an interval close to 7 licks per second in rats and 10 licks per second in mice [2, 5–8, 32, 38, 40, 44, 46–51]. This rate of licking is relatively resistant to changes in motivational state or stimulus,

leading to speculation that the rate of licking is controlled by a CPG. We [7] and others [28, 30] previously reported that D2 mice have a faster rate of licking than B6 mice (a difference in MPI of 24 ms in our earlier work). In the current sample of 18 strains, MPI showed a similar range of values as in our study of BXD RI strains [8]. SWR mice and, not surprisingly, the SWD2F1 hybrid mice, were, along with the D2 mice, the 3 strains with an MPI less than 100 ms, substantially below the all-strain mean of 109 ms. The NZW, A, PWK, and CAST strains had the slowest rate of licking, but careful examination of the licking behavior for these 4 strains (along with the WSB strain) revealed that these mice did not show the normal rhythmicity in licking behavior. That is, rather than having most ILI tightly clustered around the MPI, distributions were unusually broad or multimodal. Whether this difference is attributable to neural differences or reflects environmental constraints of the testing environment [6, 30] awaits further study. Among the more reliably rhythmic strains, the C3, AKR, and B6 mice licked at a rate slower than the all-strain average. The other two F1 phenotypes (SWB6F1 and B6D2F1) were positioned almost exactly intermediate to the parent strain values. This intermediacy, along with strong heritability and continuous distribution of strain phenotypes, supports polygenetic control of lick rate among mice.

Reliability of licking was assessed in our data using the lick efficiency metric, which is the proportion of ILI in a session that were less than 160 ms. In our usage, “efficiency” refers to the regularity of licking and not, as in the usage of some researchers, the amount of fluid obtained per lick [52]. The threshold value of 160 ms was carefully chosen. We, and others, have noted that rodents licking a drinking spout produce most of their session ILI near the MPI value, but often show a second, far less prominent distribution of ILI near twice the MPI [7, 8, 50, 53]. The value of 160 ms was chosen to separate the primary distribution of ILI from ILI of twice this duration or greater, bearing in mind that the threshold had to be suitable for a wide variety of mouse strains showing a wide range of MPI (c.f., Figure 1).

Using this criterion, mouse strains varied from highly regular, more efficient, licking (BTBR, SWR, and AKR) to more variable, less efficient licking (WSB, NZW, A, PWK, and CAST). These tendencies were consistent for water and sucrose licking. Furthermore, for inefficient strains, the majority of ILI greater than 160 ms were short pauses less than 1 s, and thus occurred within bursts of licking. This result suggests that less-efficient strains are engaged at the drinking spout, but are either missing spout contacts despite extending the tongue or are interrupting licking for very brief behaviors such as swallowing or sniffing the spout. Future studies using simultaneous videography could help to clarify the behavioral context of such short pauses. It is intriguing that BTBR mice are among the very most efficient strains; these mice display greater frequency and less variance in repetitive grooming behaviors [33], suggesting some commonality among brain circuits for rhythmic motor behaviors.

Although body weight was not correlated with lick efficiency for either water ($r = 0.309$, $p = 0.2$) or sucrose ($r = 0.212$, $p = 0.26$), it was interesting that the three wild-derived strains (WSB, PWK, CAST) were among the inefficient group. These strains had the lowest mean body weight among the panel (Table 1), and among all strains, body weight was significantly correlated with volume per lick. Although not examined for all strains, we have

compared tongue size [for methodology, see 54] in individuals from two of the wild-derived strains (PWK and CAST) with the commonly used D2 strain. As expected, the strains with smaller body size have significantly smaller tongues (unpublished data), which may play a role in strain variation for both lick efficiency and volume per lick.

4.2 Mouse strains vary in ingestive style

When drinking a liquid meal, the next organizational level of ingestive behavior beyond the lick is the burst (sometimes called cluster or bout). We defined a burst as a run of 3 or more licks separated by pauses less than 1 s. Most studies of rodent microstructure use this criterion [7, 8, 30, 32, 38–40], or 500 ms [4, 47, 52, 55, 56], though a recent study of rat licking behavior suggests a criterion of 4 s [53]. Parametric studies indicate that all 3 suggestions are likely to produce similar conclusions [32, 38].

Our mouse strains exhibited considerable variation in the size of bursts and the number of bursts in a session (c.f., Figure 4). Intriguingly, only the size of bursts correlated with the total number of licks in the session across mouse strains. Nevertheless, the size of a meal can be controlled either by variations in burst size or in the number of bursts (or both), and the strains of mice tested in the current study seemed to exhibit these phenotypes variously. We attempted to quantify this variation by comparing individual mouse data for burst size and burst number to all-strain averages (the ISI, c.f., Figure 5), and identified some strains favoring more, short bursts (e.g., FVB, B6, BALB), and others favoring fewer, long bursts (e.g., SWR, 129, BTBR). Others were intermediate (e.g., C3, D2, CBA). These results are consistent with previous studies conducted with SWR, B6, 129, and D2 strains [7, 30, 32], and extends this style analysis to several additional mouse strains.

Because our ISI is a ratio of burst number and burst size statistics, the measure has the merit of being independent of total licks. That is, our ingestive style index allows a comparison between mice that consume little with those that consume a large amount. However, because the ingestive style index also relies on the all-strain average, it would not be possible to compare values obtained in two studies using different strain panels, particularly if all-strain averages for burst number or burst size differed across studies. Nevertheless, relative comparisons within a dataset are meaningful, and the divergent behavioral strategies of, for example, FVB and 129 mice merit further investigation.

4.3 Mouse strains vary over the course of a meal

The apex of the organization of ingestive behavior is the entire meal or session. Our mouse strains varied considerably in the total number of licks generated, and therefore also in total consumption (c.f., Figure 3) – a 3-fold difference when licking water, and a 2-fold difference when licking sucrose. This variation was not well explained by body weight. Although total licks for water and sucrose were highly correlated across strains, licking for sucrose was greater, which likely reflects both increased palatability and another day of water restriction (increasing thirst). Interestingly, there was no obvious relationship between the increase from water to sucrose session and the strains *Sac* taster status [57]. For example, The WSB strain, a *Sac*-taster, increased licking to sucrose over water in excess of most strains, but so did the A strain, a *Sac*-nontaster. The D2 and AKR strains, however, consistent with their

Sac-nontaster status, increased licking to sucrose less than most strains. Future studies should examine licking responses of nondeprived mice to better tease apart the contributions of palatability and physiological state to variations in total intake.

Mouse strains exhibited variations also in the rate of ingestion over the course of the session (meal progress, c.f., Figure 6 and 7). Functions for individual mice were largely consistent with the average cumulative intake functions shown, but it should be acknowledged that such averaging can alter functions somewhat. With that caveat in mind, some strains (e.g., the A, FVB, and B6) showed a largely linear rate of consumption, whereas others showed a decelerated pattern of intake (e.g., BTBR, PWK, and 129). In studies of humans consuming a solid meal, there is evidence that linear eaters have more disinhibited eating behavior than decelerated eaters, a risk factor for obesity [58]. Indeed, when linear eaters learn to eat in a decelerated fashion, the tendency to disinhibition is reduced [59]. Finding mouse models that represent these eating styles would therefore be of potential use in further understanding this phenomenon. It would therefore be of interest to compare strains identified here as exhibiting a linear or decelerated ingestive style under conditions more similar to human meals: that is, under conditions of mild food restriction.

4.4 Heritability of ingestive phenotypes

Estimates of h^2 were determined for 22 ingestive phenotypes for water and sucrose (including water and sucrose MPI, discussed above). Heritability is a useful index of the approximate degree to which variation among genotypes modulates differences in ingestive behavior. In this experiment, estimates ranged from 0.22 to 0.73. Values, however, are highly dependent on the particular environment in which animals are raised and tested. In this study all animals were studied over a period of less than two years, but due to the inevitable difficulties of obtaining all strains, it was not practical to counterbalance all 17 genotypes during testing. As mentioned earlier, using sex, age, weight, and testing rig as cofactors deflates our estimates of heritability, but they may be inflated by uncontrolled batch x strain variance.

Correlations with other published phenotypes were investigated using the current dataset at GeneNetwork. Although limited (by how many out of over ~150 possible strains can be directly compared between studies), this analysis yielded several potentially interesting associations relating to consumption. What makes these interesting is that our study measured ingestive behavior in relatively short tests (20 min) in water-restricted mice, whereas the previously published studies measured fluid intake over a much longer timeframe (days) in undeprived mice [13, 45]. This suggests that strain variation in various ingestive and microstructural phenotypes may in fact generalize to longer-term, more “normal” (i.e. in the home cage) food and fluid intake behaviors.

4.5 Future directions

The data in this report are largely exploratory, and represent the first detailed investigation of licking microstructure for the majority of strains tested. A number of interesting strain differences have been identified which could guide the choice of animal models in more mechanistic studies. For example, the control of rhythmic tongue protrusions during licking

is a function of brainstem circuits involving a CPG [60]; the pronounced differences between D2 mice and B6 mice on MPI suggest that anatomical and electrophysiological investigations of these strains might provide clues to the organization of these circuits. Highly rhythmic strains such as these might also be compared to strains exhibiting broad, multimodal patterns of interlick intervals (e.g., the WSB and CAST strains provide faster-licking and slower-licking examples).

This study also documented diverse strategies of intake by examining the size and numbers of bursts (and the summary measure, ISI). For example, B6 mice favor small but numerous bursts of licking, whereas SWR mice favor few, but longer bursts of licking (and the SWB6F1 hybrid strain is intermediate in style). Analysis of the bursting behavior of licking has long been seen as providing a window into the neurobiological controls of ingestive behavior [3, 5, 46]; comparison of strains differing noticeably in licking microstructure would be natural choices for neurobiological study of the excitatory and inhibitory inputs to the licking circuitry of the brainstem. In addition, a detailed behavioral analysis of strains differing in ingestive style would also be fruitful. For example, investigation of whether these styles are altered by physiological state (hungry vs. sated; water-deprived vs. replete), stimulus, or mode of ingestion (licking vs. lapping, drinking vs. eating) would be instructive.

Finally, this study demonstrated substantial variation in meal progress; that is, changes in intake rate over the course of the meal. For example, B6 mice in our study demonstrated a fairly linear rate of consumption, whereas 129 mice showed a decelerated rate of intake. These strains were very similar in total licks and in body weight, suggesting that the cause of these within-meal consumption rates could be related to different sensitivity to postingestive feedback or motivation. It may also be of interest to compare strains with different cumulative intake patterns in a variety of behavioral contexts as, in humans [58, 59], linear eaters have been shown to overeat in certain situations (for example, when provided with more food or when instructed to eat more rapidly). Investigation of such behaviors would be facilitated by a validated animal model of cumulative intake rates.

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Highlights

- In order to examine genetic influences on fluid ingestion, 20-min intake of either water or 0.1 M sucrose was measured in a lickometer in 18 isogenic strains of mice, including 15 inbred strains and 3 F1 hybrid crosses.
- Intake and licking data were examined at a number of levels, including lick rate as defined by mean or median interlick interval, as well as several microstructural parameters (i.e. burstpause structure).
- In general, strain variation for ingestive phenotypes were correlated across water and sucrose in all strains, indicating fundamental, rather than stimulus-specific, mechanisms of intake.
- Strain variation was substantial and robust, with heritabilities for phenotypes ranging from 0.22 to 0.73.
- For mean interlick interval (MPI; a measure of lick rate) strains varied continuously from 94.3 to 127.0 ms, a range consistent with previous studies.
- Furthermore, variation among strains for microstructural traits such as burst size and number suggested that strains possess different overall ingestive strategies, with some favoring more short bursts, and others favoring fewer, long bursts.
- Strains also varied in cumulative intake functions, exhibiting both linear and decelerated rates of intake across the session.

Figure 1.

The mean ($\pm$ standard error) of the mean primary interlick interval (MPI, defined as the average interlick interval of interlick intervals shorter than 160 ms) for 18 strains of mice licking water (filled symbols) or sucrose (open bars). Strains are arranged in rank order of MPI to water. The mean of all strains is also provided (open symbol and filled bar).

Figure 2.

The proportion of pauses of various lengths is shown for both the water session (A) and the sucrose session (B). The white bar indicates the proportion of interlick intervals (ILI) less than 160 ms and is equivalent to our measure of *lick efficiency*. Gray bars indicate the proportion of ILI between 161 ms and 1 s. Black bars indicate proportion of ILI greater than 1 s which also represent *interburst intervals*. (as in Figure 1); the reduction in lick efficiency as MPI increases was a significant

Figure 3.

The mean (+ or $\pm$ standard error) number of licks for 18 strains of mice licking water (filled symbols) or sucrose (open bars). Strains are arranged in rank order of total licks to water. The mean of all strains is also provided (open symbol and filled bar).

Figure 4.

The mean ($\pm$ standard error) size of bursts (A) and number of bursts (B) for 18 strains of mice licking water (filled symbols) or sucrose (open bars). The mean of all strains is also provided (open symbol and filled bar). Strains are arranged in increasing order of total number of licks during the water session (c.f., Figure 3). Despite all strains licking significantly more to sucrose than water, some strains engaged in longer bursts of licking for water than sucrose. In many cases, this may have been an artefact of some strains licking later into the session when smaller bursts are more probable. When sucrose burst number was matched to water burst number, burst size was higher to sucrose in all strains but the

FVB strain (C). Examining the largest burst (D), most strains had more sustained licking to sucrose than water.

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Figure 5.

The ingestive strategy of mice was quantified with the Ingestive Style Index (ISI, see Method) which takes into account both burst size and burst number (relative to the all-strain mean for these data). Positive ISI values indicate a preference for infrequent, longer bursts whereas negative ISI values indicate a preference for frequent, shorter bursts.

Figure 6.

The mean time during the 20 min (1200 s) session in which mice reached 50% (open bars), 75% (gray bars), and 90% (filled bars) of their lick total when ingesting water (A) or sucrose (B). Then mean of all 18 strains is also provided. Strains are rank-ordered (lowest at the top) by the NCI Index (see Method) which quantifies the extent to which cumulative intake functions deviate from linear.

Figure 7.

Cumulative intake functions for 6 selected strains that exhibited the most (FVB, A, B6) and least (BTBR, 129, PWK) linear pattern of sucrose licking. The strain mean cumulative intake function is also shown for comparison.

Figure 8. Heritability (h^2) estimates for 11 ingestive phenotypes for both water (filled bars) and sucrose (open bars). Estimates of h^2 are displayed as means ($\pm$ SD) of 18 jackknife subsamples per phenotype.

Table 1

Number of subjects in each strain and initial body weight

Abbrev.	Strain	N (♀)	Mean ($\pm$ S.E.M.) body weight (g)	
			♂	♀
129 ^I	129P3/J	10 (5)	23.19 ($\pm$ 0.28)	18.62 ($\pm$ 0.19)
A	A/J	10 (5)	22.18 ($\pm$ 0.52)	18.89 ($\pm$ 0.41)
AKR	AKR/J	10 (5)	28.50 ($\pm$ 0.36)	24.71 ($\pm$ 0.40)
B6	C57BL/6J	10 (5)	23.07 ($\pm$ 0.52)	17.62 ($\pm$ 0.24)
B6D2F1	B6xD2 F1	10 (6)	30.82 ($\pm$ 2.51)	22.47 ($\pm$ 1.39)
BALB	BALBcBy/J	8 (5)	26.12 ($\pm$ 1.58)	19.43 ($\pm$ 1.13)
BTBR	BTBR ^{tf} /J	9 (4)	31.96 ($\pm$ 0.47)	26.03 ($\pm$ 0.88)
C3	C3H/HeJ	10 (5)	24.80 ($\pm$ 0.30)	20.05 ($\pm$ 0.35)
CAST	Cast/EiJ	9 (3)	14.87 ($\pm$ 0.30)	12.10 ($\pm$ 0.12)
CBA	CBA/J	10 (5)	25.55 ($\pm$ 0.32)	20.23 ($\pm$ 0.19)
D2	DBA/2J	9 (4)	25.54 ($\pm$ 0.71)	20.65 ($\pm$ 1.40)
FVB	FVB/NJ	6 (3)	31.18 ($\pm$ 1.31)	20.99 ($\pm$ 0.50)
NZW	NZW/LacJ	6 (3)	32.79 ($\pm$ 0.77)	32.40 ($\pm$ 1.66)
PWK	PWK/PhJ	7 (5)	20.4 ($\pm$ 0.80)	14.40 ($\pm$ 0.16)
SWR	SWR/J	10 (5)	22.27 ($\pm$ 1.30)	17.60 ($\pm$ 0.20)
SWB6F1	SWxB6 F1	7 (2)	24.82 ($\pm$ 0.80)	22.45 ($\pm$ 0.55)
SWD2F1	SWxD2 F1	9 (6)	27.93 ($\pm$ 0.14)	19.91 ($\pm$ 0.80)
WSB	WSB/EiJ	10 (4)	17.02 ($\pm$ 0.55)	13.57 ($\pm$ 0.16)
Total	80	160 (80)		

^IIn some figures, this strain is referred to as S129 (strain 129).

Table 2GeneNetwork¹ Record Identification Numbers for Ingestive Traits

Trait	water session	sucrose session
Mean primary ILI (MPI)	49919	49920
Median ILI	49917	49918
Lick Efficiency	49921	49922
Total Licks	49913	49914
Consumption (ml)	49911	49912
Volume per lick (μl)	49915	49916
Burst count (per 20 min)	49923	49924
Burst size (licks)	49925	49926
Sucrose Burst size, water-matched		49927
Max burst size (licks)	49930	49931
Ingestive Style Index	49928	49929
1 st min licks	49932	49933
Initial Body Weight (gm)	49910	

¹Records located at www.genenetwork.org, Group = Mouse Diversity Panel (MDP).