

C57BL/6J and DBA/2J mice vary in lick rate and ingestive microstructure

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Fluid licking in mice is an example of a rhythmic behavior thought to be under the control of a central pattern generator. Inbred strains of mice have been shown to differ in mean or modal interlick interval (ILI) duration, suggesting a genetic-based variation. We investigated water licking in the commonly used inbred strains C57BL/6J (B6) and DBA/2J (D2), using a commercially available contact lickometer. Results from 20-min test sessions indicated that D2 mice lick at a faster rate than B6 mice (10.6 licks/s vs. 8.5 licks/s), based on analysis of the distribution of short-duration ILIs (50–160 ms). This strain difference was independent of sex, extent of water deprivation or total number of licks. D2 mice also displayed a faster lick rate when the strains were tested with a series of brief (5 s) trials. However, when ingestion over the entire 20-min session was analyzed, it was evident that D2 mice had an overall slower rate of ingestion than B6 mice. This was because of the tendency for D2 mice to have more very long pauses (>30 s) between sequences of licking bursts. Overall, it appeared that D2 mice licked more efficiently, ingesting more rapidly during excursions to the spout that were fewer and farther between.

Keywords: Inbred strain, ingestion, licking, mouse, pattern generator

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Fluid licking is a highly stereotyped behavior in rats, mice and many other mammals that involves the rhythmic co-ordination of muscle groups involved in tongue protrusion and retraction, jaw opening and closing and swallowing. The co-ordination of these oromotor movements is thought to be under the control of central pattern generators (CPGs), motor 'programs' extant among premotor neuron networks that

send rhythmic inputs to motor neuron pools in cranial nerve nuclei V, VII and XII (for reviews see Nakamura & Katakura 1995; Travers *et al.* 1997). Current evidence suggests a substrate for rhythmic licking organized among premotor neurons in the medullary reticular formation (RF). Premotor neurons associated with intrinsic and extrinsic tongue muscles are located in a number of medullary and pontine RF cell groups (Travers & Rinaman 2002; Travers *et al.* 2005). Neurons rhythmically active during licking are found in both the parvocellular and intermediate zones of the RF (e.g. Travers *et al.* 1997), and reversible lesion studies in awake rat preparations suggest a necessary role for the rostralateral medullary RF (Chen & Travers 2003). However, the specific identity and physiological properties of the neurons and networks that underlie the CPG for licking are unknown.

The species of choice for investigating the physiological and anatomical substrates of licking has been the rat (Weijnen 1998) but the study of strains of mice with different lick or ingestion rates holds substantial promise for genetic approaches to the study of oromotor CPGs (e.g. Okayasu *et al.* 2003; Tomiyama *et al.* 2004). Horowitz *et al.* (1977) examined *ad lib* fluid licking over a series of 20-h periods in undrugged C57BL/6 (B6) and DBA/2 (D2) mice, and their F₁ progeny, using an infrared-beam lickometer. Local lick rate, as defined by interlick intervals (ILIs) <390 ms differed substantially between strains; B6 mice exhibited a slower lick rate (mean ILI ≈ 130 ms) than D2 mice (mean ILI ≈ 97 ms), and F₁ mice expressed an intermediate rate (mean ILI ≈ 109 ms). These data indicated robust and genetically influenced differences in lick rate, and that the strain difference was stable over time and in response to different stimuli.

Other strain differences in licking have been reported: Smith *et al.* (2001) showed a significant strain lick-rate difference between water-deprived inbred mice, with SWR/J mice possessing a shorter modal ILI (faster lick rate; 109 ms) than AKR/J mice (129 ms) in a short (30 s) trial. More recently, Dotson & Spector (2005) assessed lick rate in four strains of mice (B6, D2, 129P3 and SWR) in a commercially available lickometer (Davis MS160). These strains differed significantly in terms of mean ILI when the analysis was limited to ILIs 50–200 ms, with D2 and SWR mice licking significantly faster than the other strains.

In order to provide a broader characterization of inbred strain differences in lick rate and the robustness of their generalizability, we examined licking in water-restricted B6 and D2 mice across several temporal and situational contexts. We utilized a licking microstructure analysis to evaluate how licking in these strains was organized across various time frames, ranging locally from one lick cycle to the next, to more broadly across bursts of licking as well as over an entire ingestion bout. Treatments that influence meal size tend

more often to influence the size and number of bursts of licking rather than the rate of licking intrinsic to bursts (Davis 1996; Spector *et al.* 1998). Therefore, understanding principal strain differences in the organization of licking in both local lick rate and at the level of bursts and pauses will provide a better platform to genetically evaluate the operating characteristics of food intake mechanisms in a variety of genetic models of obesity.

Methods

Animals

Data were collected from adult male and female mice (*Mus musculus*) from inbred strains B6 ($n = 37$) and D2 ($n = 32$). Mice were obtained directly from the Jackson Laboratory (Bar Harbor, ME, USA), or they were the direct offspring of such mice. The average age of mice prior to testing was ~13 weeks; mice were generally age and weight matched between strains. The mean pretest body weight was 20.8 g for B6 females ($n = 14$) and 23.8 g ($n = 23$) for B6 males. The mean pretest body weight was 20.8 g for D2 females ($n = 15$) and 25.9 g for D2 males ($n = 17$).

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 lickometer tests, whereas food remained available in the home cage (but not the test chamber) on an *ad lib* basis.

Apparatus

Licking tests were conducted in a Davis MS-160 computer-controlled lickometer (DiLog Instruments, Inc., Tallahassee, FL, USA). 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 deionized water (18 MΩ) 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 min when the shutter closed. Lick contact with the spout completed an imperceptible (<50 nA) circuit that allowed the time of each lick to be recorded to a computer file.

Lick testing

All mice were tested in the MS-160 for two consecutive days. If a mouse only licked a few times or not at all during the first day of

testing, it was retested on the same day, after the other mice had been tested. A few mice ($n = 3$) licked less than 50 times at either opportunity on day 1, and were subsequently removed from the experiment. A subset of mice ($n = 23$) was tested for two additional days (total 4 days), while the remaining mice ($n = 39$) were given a different task on the third day: These mice were tested in the MS160 rig with a series of brief (5 s) trials, where they could initiate up to 16 trials with a single lick to one of four bottles containing distilled water. Each bottle was presented once, in random order, in a block of four trials. This was repeated for a total of 16 trials (four blocks).

Tongue measurement

A subset of mice (10 B6, 10 D2) of both sexes was allowed to recover in their home cages with *ad lib* food and water for 1–2 weeks. Mice were weighed and euthanized; the tongue was excised at the level of the trachea and placed on a glass slide after rinsing with deionized water. Two measurements utilizing common tongue landmarks were made under a dissecting microscope using a millimeter ruler: apex to anterior border of median eminence, and apex to anterior border of the vallate papilla. The tongue was then sectioned at the anterior border of the median eminence, and weighed on an analytical balance.

Analysis

All lick and microstructure data were analyzed using custom software written by S.J.S, J.P.B. or D.H. Interlick interval (ILI) frequency histograms were constructed for each individual mouse, and measures of ILI duration were calculated, including mean ILI (20–500 ms), mean primary ILI (MPI; mean of 50–160 ms) and peak or modal ILI (<500 ms). Data were also analyzed using microstructural analysis. A burst was defined as a run of licks bounded by ILIs 1000 ms or greater, and intervals between bursts (>1000 ms) were defined as pauses. Fluid consumption during 20-min sessions was measured by weighing drinking tubes before and after the session; dividing this number by the number of licks provided an estimate of the mean volume per lick.

Statistical analyses were performed using a general linear model [factorial or repeated-measures analysis of variance (ANOVA)] with categorical factors for strain and sex. Significant group differences were analyzed with *post hoc* tests (Bonferroni corrected).

Results

Licking and ILI distributions for B6 and D2 mice

Water-deprived licking was measured in the Davis MS-160 for individual B6 and D2 mice. Data combined across days 1 and 2 for 37 B6 and 32 D2 mice are reported in Table 1.

Table 1: Average (\pm standard error of mean) measures of ILI duration among B6 and D2 male and female mice

Strain	Sex	n	Licks/20 min	Av. mean ILI 20–500 ms	Av. peak ILI < 500 ms	Av. MPI, 50–160 ms
B6	♀	14	506.4 \pm 20.7	140.2 \pm 1.6	123.9 \pm 1.2	119.1 \pm 0.7
	♂	23	646.8 [†] \pm 29.5	135.5 \pm 1.9	124.0 \pm 1.2	118.8 \pm 1.0
	Both	37	593.7* \pm 22.8	137.3* \pm 1.4	123.9* \pm 0.8	118.9* \pm 0.7
D2	♀	15	436.8 \pm 38.4	120.2 \pm 4.5	95.3 \pm 1.9	95.2 \pm 1.3
	♂	17	465.3 \pm 42.0	118.9 \pm 2.4	95.3 \pm 1.7	95.1 \pm 1.1
	Both	32	452.0 \pm 28.3	119.5 \pm 2.4	95.3 \pm 1.2	95.2 \pm 0.8

*B6 vs. D2, $P < 0.001$.

[†]B6 ♀ vs. ♂, $P < 0.05$.

Values (average of day 1 and day 2 data) are given for 37 B6 and 32 D2 mice of both sexes. Measures of ILI duration include average mean ILI (from 20 to 500 ms), average peak ILI (mode of distribution <500 ms) and average MPI (mean of distribution from 50 to 160 ms).

On average, mice from these two strains licked 528 times during the 20-min test session, with a range of 117–1263 on day 1, and 239–1279 on day 2. Lick counts averaged over 2 days differed between strains, and sex: significant effects were found for strain ($F_{1,65} = 13.1$; $P < 0.001$) and sex ($F_{1,65} = 5.9$; $P < 0.05$). The strain \times sex interaction was not significant. B6 male mice licked significantly more during the test session than did B6 females, or male or female D2 mice (Bonferroni, $P < 0.05$). This increase in consummatory behavior in B6 males was not associated with body weight on the test date, whether it was expressed as the raw value or as a percentage of baseline undeprived weight. For example, following 24 h of water deprivation and just prior to testing on day 1, B6 males had a lower mean body weight (21.2 g) than D2 males (22.9 g) but they licked on average 28% more. During the second test session, all mice generally took more licks than on trial 1, likely because of increased thirst but also because of familiarity with the test chamber (repeated measures, $F_{1,67} = 76.9$; $P < 0.00001$).

An example of the pattern of licking across the 20-min test period is shown in Fig. 1. This individual B6 mouse licked a total of 561 times on day 1, and these licks were organized into a small number of discrete bursts of drinking behavior, interrupted by pauses (Fig. 1a). The temporal patterning of individual licks is shown in Fig. 1b; the delays between the licks (ILIs) are typically less than 500 ms in duration within any burst of licking behavior. A frequency distribution of ILIs < 500 ms (5 ms bins) was constructed for this mouse (Fig. 1c); the mode of this distribution was the bin 126–130 ms. A smaller cluster of ILIs had a peak of 251–255 ms – approximately double the value of the overall mode – suggesting instances where the mouse ‘missed’ a lick in the sequence, or where the mouse maintained contact on the sipper tube between two consecutive licks. A much smaller secondary ‘harmonic’ is barely discernible at 376–380 ms. Most of the ILIs (>95%) for any individual occur in the first cluster.

B6 and D2 mice differed in their mean ILI distribution (Fig. 2a), with the primary and secondary peaks for D2 mice

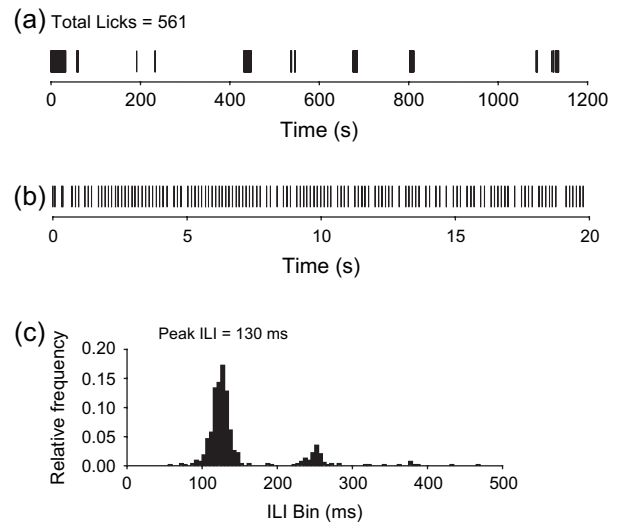


Figure 1: The pattern of licks in a 20-min test session for an individual B6 mouse. (a) This mouse licked 561 times during the session and these licks were organized into a small number of bouts of drinking behavior. (b) Expanded view of the first 20 s of licking shows temporal pattern of individual licks. (c) A frequency distribution of ILIs < 500 ms (5 ms bins) shows a primary distribution with a mode of 130 ms; second and third peaks are approximately double and triple the modal value of the primary distribution.

shifted toward a lower value, indicating that D2 mice in general possessed ILIs of shorter duration than did B6 mice. This robust strain difference was confirmed by analysis of several different average measures of ILI length: differences in mean value were found among B6 and D2 mice on either day 1 or day 2 for mean ILI (20–500 ms), modal or peak ILI (5 ms bins) and MPI, which is the average ILI of the first cluster (50–160 ms) (Table 1). For all three of these measures

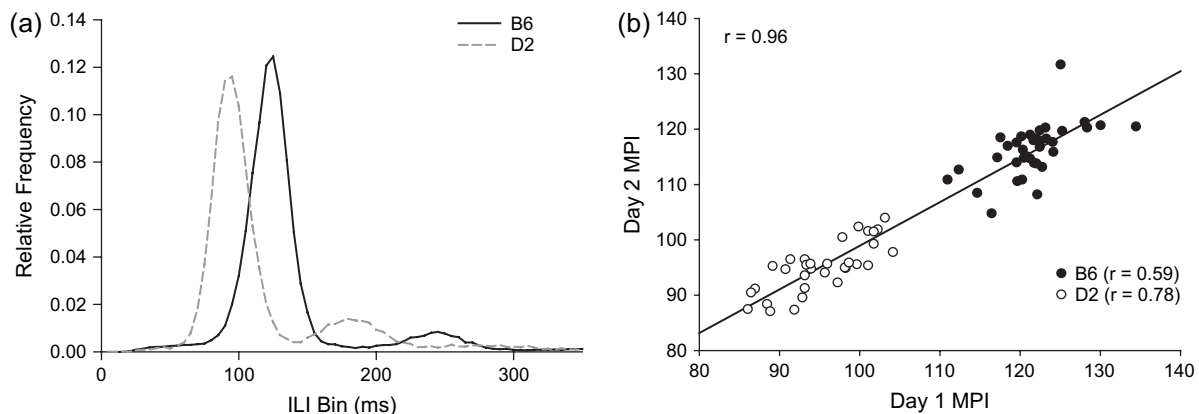


Figure 2: (a) Mean ILI distributions for B6 ($n = 37$) and D2 ($n = 32$) mice, averaged across two consecutive test sessions. The primary and secondary peaks for D2 mice are left-shifted, reflecting a shorter duration ILI. (b) Scatter plot of individual scores (MPI) for B6 (filled circles) and D2 mice (open circles) plotted as a function of test day. Little overlap occurs between individuals of either strain and the scores for each day are highly correlated with strain ($r = 0.59$ for B6 and 0.78 for D2; $P < 0.05$).

on either test day there were significant effects of strain ($F_{S_{1,65}} = 44.8\text{--}486.1$; $P_s < 0.00001$) but not sex. An examination of individual MPI scores among these mice from day 1 to day 2 (Fig. 2b) showed little overlap between individuals of either strain, and confirmed that ILIs were relatively stable and highly correlated ($r = 0.96$) from test session to test session. It was also evident from these data that B6 mice tended to have a longer mean MPI on day 1 (121.6 ms) than on day 2 (116.2 ms), whereas the D2 mice were stable from day 1 (95.4 ms) to day 2 (95.0 ms). A repeated-measures ANOVA confirmed that this effect was significant (strain \times concentration interaction; $F_{1,67} = 31.3$; $P < 0.0001$). Testing over four consecutive sessions in some mice showed that after this initial drop, the ILI phenotype in B6 mice was stable and D2 mice did not vary at all (e.g. Fig. 6).

Ingestive microstructure

To provide a better understanding of ingestion over longer time frames, we used a licking microstructure analysis to characterize licking in B6 and D2 mice over 20-min tests (same mice and lick data as in Fig. 2). Files were analyzed offline for several measures of licking microstructure. Results for each measure were averaged across the 2 days for each mouse and then compared using between-subjects t -tests. For this analysis, a burst was defined as a run of licks bounded by ILIs 1000 ms or greater. B6 mice tended overall to display more bursts of licking in the 20-min session than did D2 mice (Fig. 3a; effect of strain, $F_{1,65} = 25.6$; $P < 0.00001$). For number of bursts, a significant strain \times sex interaction was found, with significant strain differences between males ($P < 0.05$) but not females. As expected from the overall ILI distributions (see Fig. 2a), D2 mice of either sex licked much faster within bursts than did B6 mice (Fig. 3b) when licks in

the primary distribution (ILIs < 160 ms) were assessed (effect of strain, $F_{1,65} = 431.9$; $P < 0.00001$). Within bursts, D2 females licked at a rate of 10.5/s, and males 10.6/s. B6 females and males both licked a rate of 8.5/s. Note that expanding the upper limit of ILIs used for analysis to 1000 ms results in a narrowing of the lick-rate difference between B6 and D2 mice. As the time frame was expanded even further, the remaining rate differences were lost (Fig. 3d). These strains did not express significantly different mean burst sizes (i.e. licks per burst), but, given such, D2 mice did not express a significantly shorter mean burst duration (Fig. 3c). Therefore, the faster lick rate of D2 mice was at least partially offset by a commensurate increase in the proportions of ILIs in the first and second harmonic periods for this strain.

This trend continued as the time frame of analysis was expanded further. Although D2 mice expressed a faster lick rate in the primary ILI distribution, they paradoxically appeared to express a slower rate of ingestion when analyzed in longer time frames (Fig. 4). The rate of licking was significantly slower for D2 mice in the first minute of the meal (Fig. 4a; effect of strain, $F_{1,65} = 20.9$; $P < 0.0001$), and it was slowed even further when the average rate of ingestion (i.e. licks per minute) across the 20-min period was compared with B6 mice (Fig. 4b; effect of strain, $F_{1,65} = 6.4$; $P < 0.05$). Furthermore, faster intraburst licking in the D2 mice might be expected to produce smaller lick volumes but we actually found that on average D2 mice consumed significantly more fluid per lick (Fig. 4c; effect of strain, $F_{1,64} = 4.1$; $P < 0.05$).

The overall slower ingestion rate of D2 mice appeared to be because of the tendency for D2 mice to have a greater number of longer pauses between bursts of licking. Pause distributions for these strains are displayed in Fig. 5. D2 males and females displayed a significantly greater mean pause duration (more than twofold) than did B6 mice of either

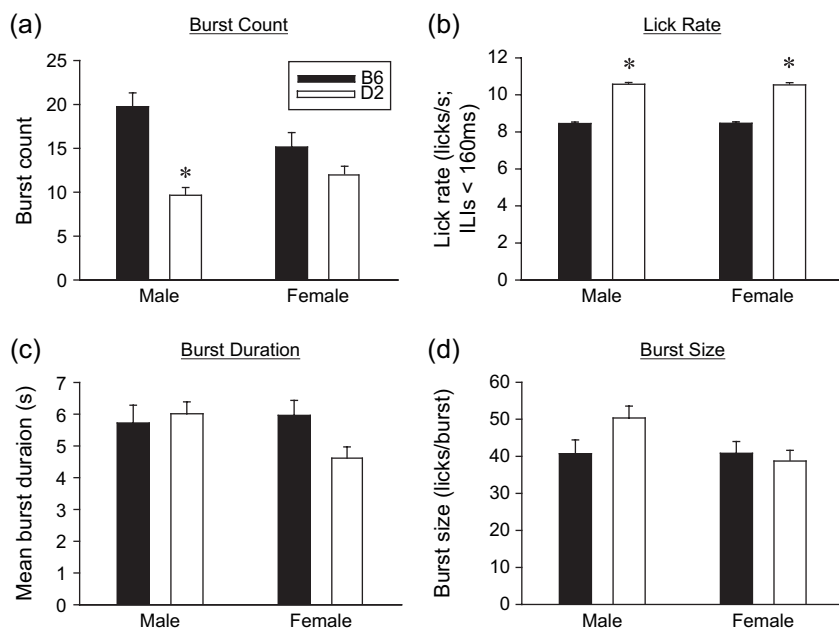


Figure 3: Measures of licking bursts among B6 (black bars; $n = 37$) and D2 mice (open bars; $n = 32$), sorted by sex. Asterisks denote significant effects of strain for mice of each sex (*post hoc* tests, $P < 0.05$). (a) B6 mice displayed more bursts than D2 mice; a significant difference was found among males but not females. (b) D2 mice of either sex licked faster within bursts than B6 mice. Differences were not found for burst duration (c) or size (licks/burst) (d).

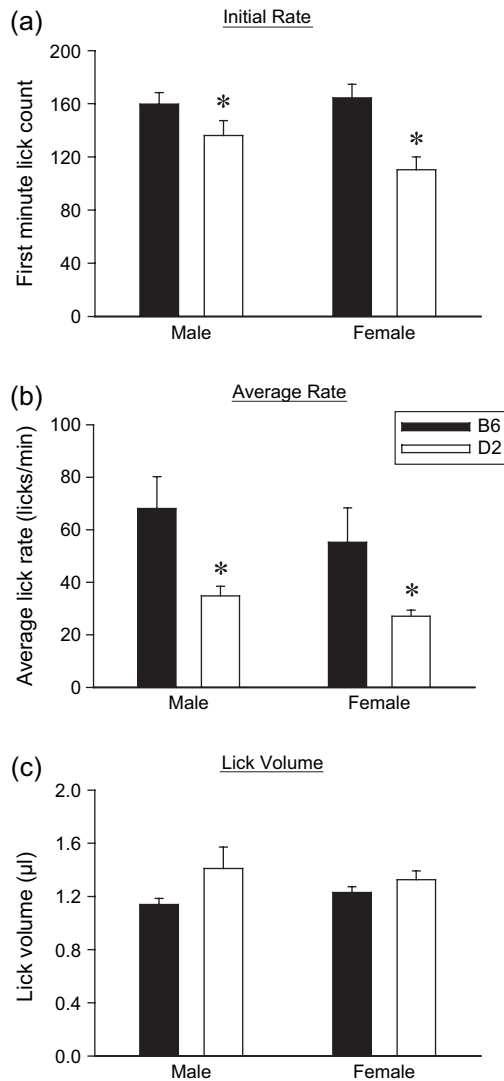


Figure 4: Measures of lick rate among B6 (black bars) and D2 mice (open bars), sorted by sex. Asterisks denote significant effects of strain for mice of each sex (*post hoc* tests, $P < 0.05$). (a) Within the first minute of licking, B6 mice of either sex licked more than D2 mice. (b) Across the whole 20-min test session, B6 mice of either sex displayed a higher lick rate per minute than D2 mice. (c) Across all mice, D2 mice consumed significantly more fluid per lick than B6 mice ($P < 0.05$) but no significant differences appeared when the mice were sorted by sex.

sex (effect of strain, $F_{1,65} = 28.3$; $P < 0.00001$). Specifically, this appears to be because of the presence of a greater number of long pauses (those >30 s; Fig. 5b).

Licking in brief trials

We examined licking behavior in 5 s trials in a subset of B6 and D2 mice ($n = 21$ B6, 18 D2; Table 2). All short-trial data were collected after two consecutive days of 20-min ses-

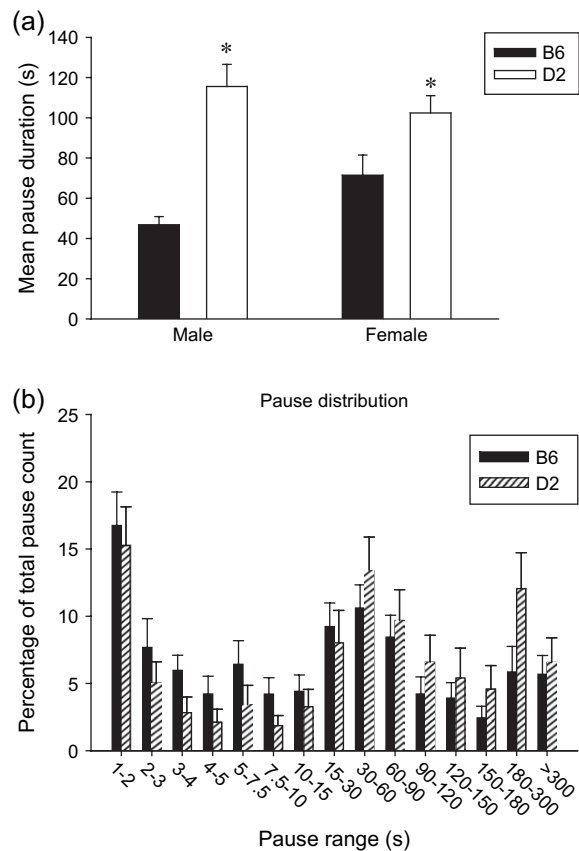


Figure 5: Mean pause (between bursts) duration and pause frequency distribution for B6 (black bars) and D2 mice (open bars). Asterisks denote significant effects of strain for mice of each sex (*post hoc* tests, $P < 0.05$). (a) B6 mice of either sex expressed a significantly shorter mean pause duration than D2 mice. (b) Pause distributions for B6 and D2 mice, collapsed across sex. D2 mice tended to take more long pauses during the trial than B6 mice.

sions; mice from either strain averaged 80% of their original body weight before this short-trial testing. B6 mice sampled over slightly more trials (mean = 15.6 of 16 possible) than did D2 mice (mean = 14.5; $F_{1,35} = 6.88$; $P < 0.05$). However, D2 mice licked a faster average rate (7.86 licks/s) over the 5-s trials than did B6 mice (6.27 licks/s; effect of strain, $F_{1,35} = 14.59$; $P < 0.001$). There were no effects of sex for either number of sampled trials or average lick rate (P s > 0.05), although a significant strain \times sex interaction for average lick rate was found ($F_{1,35} = 6.69$; $P < 0.05$), reflecting the slightly slower lick rate of D2 females (83%) as compared with D2 males (Table 2).

It was evident that mice from either strain did not lick at an optimal rate throughout a given set of 5-s trials, especially in later trials when mice often licked only a couple of times. This tendency for a slowdown in later trials was likely because of satiation of thirst (Spector & St. John 1998), and the small overall strain difference in short-trial lick rate was therefore undoubtedly affected by factors not strictly related to motor

Table 2: Short-trial licking in B6 and D2 male and female mice

Strain	Sex	<i>n</i>	Mean no. of trials completed	Average licks per s (5-s trials)	Average peak licks per s (5-s trials)
B6	♀	7	15.7 ± 0.2	6.7 ± 0.4	9.4 ± 0.2
	♂	14	15.6 ± 0.2	6.1 ± 0.2	8.5 [†] ± 0.1
	Both	21	15.6* ± 0.1	6.3* ± 0.2	8.8* ± 0.1
D2	♀	10	14.6 ± 0.5	7.2 ± 0.6	11.4 ± 0.2
	♂	8	14.5 ± 0.7	8.7 ± 0.3	11.5 ± 0.2
	Both	18	14.5 ± 0.4	7.9 ± 0.4	11.4 ± 0.2

Values are given for 21 B6 and 18 D2 mice of both sexes. Measures of short (5 s) trial licking include mean (±standard error of mean) number of sampling trials (out of 16), average licks per second per trial, and average peak licks per second per trial.

*B6 vs. D2, $P < 0.05$.

[†]B6 ♀ vs. ♂, $P < 0.05$.

ability. We therefore also assessed the short-trial lick count peak, or highest value, for each mouse. The strain difference in peak lick count was robust; B6 mice achieved a mean peak lick rate of 8.84 licks/s, whereas D2 mice achieved a peak count of 11.42 licks/s (effect of strain, $F_{1,35} = 191.17$, $P < 0.00001$). Therefore, under optimal conditions, D2 mice are capable of licking in a short 5-s trial at a far faster rate than B6 mice. There was also a significant effect of sex ($F_{1,35} = 5.01$; $P < 0.05$) and a strain × sex interaction ($F_{1,35} = 7.71$; $P < 0.01$); B6 females exhibited a higher peak count than B6 males (Table 2).

Stability of lick rate over consecutive test days

Twelve B6 and 11 D2 mice were tested in 20-min trials over a period of four consecutive days, their only opportunity for fluid consumption. Mice steadily lost body weight over this 4-day period, with weight bottoming prior to test session four at an average of 79% and 74% of original weight for B6 and D2 mice, respectively (Fig. 3a; effect of strain, $F_{1,21} = 7.19$, $P < 0.05$). B6 mice licked more over the 4-day period (effect of strain, $F_{1,18} = 5.16$, $P < 0.05$); mice of both strains increased consumption over the 4 days of testing as assessed by number of licks. Analysis of lick rate as assessed by MPI also showed a consistent and robust strain difference between B6 and D2 mice across the 4-day test period. The mean MPI value for B6 mice decreased from day 1 to day 2 but then remained static; the value for D2 mice remained fairly consistent across the 4-day period. Repeated-measures ANOVA indicated main effects of strain ($F_{1,18} = 128.3$; $P < 0.00001$) and test period ($F_{3,54} = 3.68$; $P < 0.05$), as well as a significant test period × strain interaction ($F_{3,54} = 5.91$; $P < 0.001$). Overall, these data indicate that the lick-rate strain difference is not affected by the degree of water deprivation over a short period (4 days).

Relationship of lick rate to tongue size

Data on tongue length and weight were collected from 10 undeprived B6 (five males, five females) and 10 D2 (six males, four females) mice 1 week after testing. Mean tongue

lengths, measured either from the tip to the anterior border of the median eminence, or from the tip to anterior border of the vallate papilla, did not differ between strain ($F_{1,18} \leq 0.15$; $P > 0.7$). The average tongue weight was 46.5 mg for B6 mice, and 43.1 mg for D2 mice. Tongue weight did not differ significantly between strain, either by itself ($F_{1,18} = 3.5$; $P = 0.07$) or when expressed as a percentage of body weight ($F_{1,18} = 4.1$; $P = 0.06$). Importantly, among mice of both strains, MPI did not correlate with either measure of tongue length ($r \leq 0.14$) or tongue weight ($r = 0.46$). Collectively, these data indicate that variation in the size or shape of the tongue is not related to variation in lick rate in mice.

Discussion

By several different measures (ILI distribution, lick rate within bursts and lick rate in short trials), D2 mice possessed a significantly faster lick rate than B6 mice. This effect was independent of sex. Importantly, the strain difference in lick rate was robust over a 4-day period, and therefore independent of prolonged water deprivation, or total licks (which increased in both strains across the 4-day period). B6 mice appeared to have at least a subtle increase in lick rate resulting from experience, with significantly shorter MPI values on day 2, or days 2–4 in the extended-testing group. No such modulation was observed in D2 mice, although D2 mice licked considerably faster overall. These results confirm and extend previously published reports of lick rates, or differences in lick rate, between these strains (Dotson & Spector 2005; Glendinning *et al.* 2002; Horowitz *et al.* 1977).

What is the basis of the lick-rate difference? We hypothesize that there is a difference in the organizational properties of the oromotor CPG, although the possibility certainly exists that other factors, such as anatomical differences, play a major role. Age-matched adult B6 mice have a larger overall mandible size than D2 mice, which could in theory correspond to slower jaw movements and lick rate (Carvalho & Gerstner 2004; Lovell *et al.* 1984). However, B6 × D2 F_1 mice were found to possess a larger mandible size than either parent strain, despite possessing an intermediate lick rate (Horowitz *et al.* 1977; Lovell *et al.* 1984; unpublished data from our

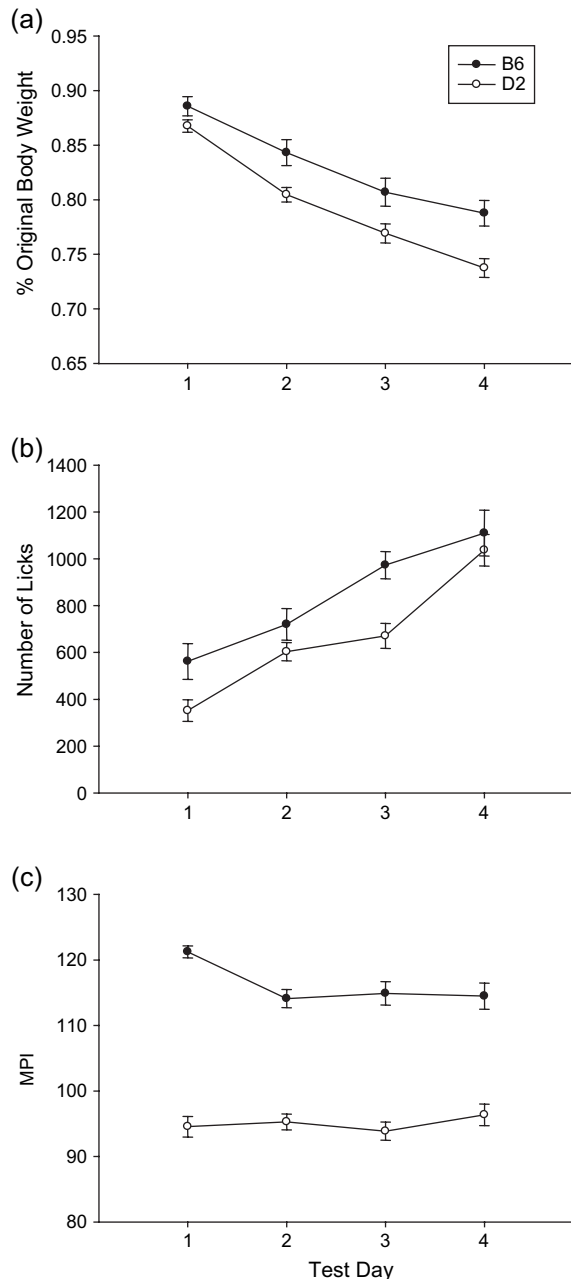


Figure 6: Summary data from four consecutive days of lick testing. These data were collected from 12 B6 (filled circles) and 11 D2 (open circles) mice. (a) There was an overall strain difference in % body weight, with B6 mice retaining a higher % ($P < 0.05$). (b) Overall, B6 mice licked more than D2 mice ($P < 0.05$). (c) There was a consistent strain difference in mean ILI from 50 to 160 ms (MPI) across the test period ($P < 0.00001$).

laboratory). We also measured tongue length, width and weight (anterior tongue portion) in 10 B6 and 10 D2 mice (both sexes) and did not find a significant correlation between any of these variables and MPI score. However, studies of skeletal muscle indicate that a subset of fast- and slow-twitch

hind limb muscles are heavier in B6 than D2 mice (Lionikas *et al.* 2003, 2005). This is a polygenic trait, and the authors observed significant effects of sex. It is possible that one or more genes influencing the weight of specific muscles such as extrinsic tongue muscles, as opposed to the tongue itself, could contribute to strain differences in lick rate.

The inflexibility of the fluid licking rate within bursts is a key consideration in understanding the genetic and neural substrates of the CPG for licking. The classic view is of an invariant rate (Stellar & Hill 1952); however, a host of environmental and physiological factors have been suggested to *subtly* modulate mean ILIs, including behavioral alertness (Vajnerova *et al.* 2003) and administration of psychoactive drugs (e.g. Das & Fowler 1995; Knowler & Ukena 1973; Vajnerova & Brozek 2002). Changes in lick rate in response to taste stimuli and tactile feedback have been reported for rats (Baird *et al.* 2005; Cone 1974; Cone *et al.* 1975; Davis & Smith 1992; Mamedov & Bures 1984). Deprivation level has also been reported to modulate mean ILI (Cone 1974; Cone *et al.* 1975), as has the type of equipment used to collect lick data (Marowitz & Halpern 1973; Weijnen 1998). In the present experiment, deprivation did not affect the strain difference. Additionally, strain values for lick rate were comparable with those collected using an optical lickometer (Horowitz *et al.* 1977).

An extremely important consideration attending all of these studies is the time frame of analysis used to evaluate ILIs. The current study shows that different analysis time frames (i.e. analyses of ILIs limited to different upper range cutoffs – 160, 500 or 1000 ms) can produce different, even opposing, conclusions about treatment/strain effects on the rate of licking. For example, as the time frame of analysis was expanded, the faster lick rate of D2 was progressively reduced until they were shown to lick slower than B6 mice when averaged over the entire test period. The aforementioned studies used different criteria for ILI analysis, calling into question whether the various conditions affected properties of the CPG controlling lick rate in the primary ILI distribution (those less than 160 ms), or ILIs of longer durations reflecting an influence on mechanisms that engage or disengage the CPG. Our findings indicate that the effects of deprivation or experience on lick rates reflecting CPG output are of considerably less influence than previously reported.

The net effect of the two phenotype differences in primary lick rate and licking microstructure is that D2 mice appear to lick in a more efficient manner overall. These differences perhaps reflect a difference in ingestion or meal-taking strategies between the two strains. This difference is clarified by analysis of the relative distributions of bursts and pauses within the ingestion period. Traditionally, the mean length of bursts is considered to be reflective of gustatory influences of the tastant as burst size increases linearly with increases in the concentrations of palatable solutions and it decreases with naturally or conditioned aversive tastants (Baird *et al.* 2005; Spector & St. John 1998; Spector *et al.* 1998). Although D2 mice expressed a faster intrinsic lick rate, the mean burst size/duration was not significantly different suggesting comparable taste reactivity across strains, although only water was used as a taste stimulus.

The principal strain differences between B6 and D2 mice in terms of burst/pause distribution were in the number of bursts expressed and the duration of intervals (pauses) between those bursts. Overall, D2 mice took significantly fewer bursts at the spout, and they expressed, on balance, proportionally more long pauses (>30 s) and proportionally fewer short pauses (<30 s) between bursts, resulting in an average pause length almost threefold longer than that for B6 mice among males (Fig. 5). This difference resulted in the overall *slower* rate of ingestion for D2 mice over the course the entire drinking period. Treatments affecting satiety-related processes have prototypical effects on the distributions of bursts and pauses. As satiety increases toward the end of meals, pauses on average tend to grow longer in duration (Davis 1996). In addition, treatments that reduce or enhance meal size tend to, respectively, decrease or increase the number of bursts in the meal (Davis & Levine 1977; Davis et al. 1994, 1995, 1997, 1998; Eisen et al. 2001; Schwartz et al. 1999). Varying the size of the sipper tube orifice also increases burst length, but not number of bursts, in mice. Either B6 or D2 mice took nearly twice as many licks in a 30-min session when presented with a 1.5 mm tube orifice than with a 2.7 mm orifice, although neither the total amount consumed nor the mean ILI were changed (Dotson & Spector 2005).

Overall, it is clear that B6 and D2 mice exhibit different and complex profiles of licking microstructure. It would be worthwhile to perform a videographic analysis to determine the nature of the other behaviors expressed by D2 mice during the longer intervals between bursts (e.g. grooming, sleep, stereotypy) to further characterize the differential portfolios of behavioral expression exhibited by these two strains. In any case, licking and ingestive phenotypes are ideal candidates for genetic analysis using derivative populations of the parent inbred strains. Moreover, it will be important and feasible in future research to determine how patterns of ingestion vary or systematically breakdown with controlled, specified mutations. Indeed, recent studies in mice and other species further support the utility of genetic approaches for dissecting the organization of CPGs and locomotor networks (Kiehn & Kullander 2004; Kullander 2005).

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