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Forty mouse strain survey of water and sodium intake

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Abstract

We measured voluntary water and sodium intakes of 40 inbred strains of mice. Groups of ~10 males and ~10 females from each strain received a series of 48-h tests with a choice between a bottle of water and a bottle of one of the following: water, 25, 75, and 225 mM NaCl, 25, 75, and 225 sodium lactate. Sodium solution intakes were influenced by strain, sex, anion and concentration: Nine strains drank significantly more chloride than lactate, and only one strain (I/LnJ) drank significantly more lactate than chloride. The other 30 strains drank similar volumes of chloride and lactate. Sodium intakes were higher in females than males of 8 strains and did not differ by sex in the other 32 strains. Some strains had consistently high sodium intakes and preferred all sodium solutions to water (129S1/SvImJ, MA/MyJ, NZW/LacJ and SWR/J), some showed indifference (i.e. preferences not significantly different from 50%) to all concentrations tested (A/J, C57BL/6J, FVB/NJ and SEA/GnJ), and some had consistently low sodium intakes (AKR/J, C3H/HeJ, C57BL/10J, CBA/J, DBA/2J, I/LnJ, JF1/Ms, LP/J, NON/LtJ, PERA/EiJ, PL/J, and RIIS/J). The results illustrate the diversity of voluntary sodium intake in mice and will assist in the selection of appropriate strains for focused genetic and physiological analyses.

Keywords

Inbred strains; Mouse phenome; Two-bottle choice test; Preference

Voluntary sodium intake, also called “need-free” or “spontaneous” sodium intake, usually refers to the consumption of NaCl solution by nutritionally-replete animals that have water freely available. In contrast to the many findings indicating that sodium intake due to sodium deficiency is mediated by the renin–angiotensin–aldosterone system [e.g., [14,35,36]] the physiological and neural basis of voluntary sodium intake is unclear [see [41]]. Taste acceptability is a major factor. Amiloride-sensitive sodium channels (“ENaCs”) contribute to the recognition and acceptance of sodium in rodents [e.g., [10]] but the connection between this and sodium intake has not been well characterized [reviews, [21,25]]. The failure to delineate an underlying mechanism for voluntary sodium intake is a serious shortfall because human sodium intake appears to be voluntary rather than deprivation-related [8], and excess sodium intake may have adverse health consequences, most notably, hypertension.

Studies comparing the phenotypes of inbred mouse strains can provide information about underlying genetic mechanisms. There have been several mouse strain surveys of voluntary sodium intake [1,4,6,9,18,24,28], although all but one have involved five or fewer strains, the exception being a 28-strain survey from our laboratory [1]. Comparison across studies is made difficult because there are only a few strains in common and several critical differences in methodology. Voluntary sodium consumption is influenced by a variety of environmental and experiential factors, including the diet the mice are fed [50], the duration of the test [43], the position of the drinking spouts [46], the sodium concentrations tested, and the order the

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concentrations are presented [2,4,6]. Here, we attempted to provide a rigorously controlled, comprehensive strain comparison. Whereas most previous strain surveys are limited to male mice and all are limited to tests with NaCl, we screened mice of both sexes and tested them with NaCl and another sodium salt, sodium lactate (NaLa).

There are several hundred commercially available inbred mouse strains and it is not feasible to test them all. In the following study, we tested 40 strains chosen as “priorities” of the Mouse Phenome Database Project [39]. This set encompasses most of the commonly used laboratory strains as well as less common strains that provide a wide range of genetic diversity. A strong advantage of using this set of 40 priority strains is that the data generated can be compared with other phenotypes submitted to the Database.

A critical issue with studies of ingestion involving different strains or species is the appropriate basis for comparison, particularly when there are large differences in body size. All being equal, large animals consume more than small animals. Since some strains of mice are almost three times larger than others this can become crucial for interpretation. One approach has been to employ preference scores, which are a ratio of NaCl intake to total fluid intake and thus independent of body weight. However, preference scores can be misleading when NaCl and other hypertonic solutions are available because water intake is driven by osmotic effects, not simply by relative palatability. To address this problem, we also measured water intakes of mice and compared these with body weights in order to determine what would be the most appropriate method to adjust fluid intakes for body size.

1. Methods

1.1. Mice

A total of 790 mice, comprising approximately 10 males and 10 females of the following 40 strains were tested: 129S1/SvImJ, A/J, AKR/J, BALB/cByJ, BTBR T^{+tf}/J , BUB/BnJ, C3H/HeJ, C57BL/10J, C57BL/6J, C57BLKS/J, C57BR/cdJ, C57L/J, C58/J, CAST/EiJ, CBA/J, CE/J, CZECHII/EiJ, DBA/2J, FVB/NJ, I/LnJ, JF1/Ms, KK/H1J, LP/J, MA/MyJ, MOLF/EiJ, MSM/Ms, NOD/LtJ, NON/LtJ, NZB/B1NJ, NZW/LacJ, PERA/EiJ, PL/J, PWK/PhJ, RIIS/J, SEA/GnJ, SJL/J, SM/J, SPRET/EiJ, SWR/J, and WSB/EiJ. The names of some strains have changed slightly since we conducted this experiment in 2002; here, we use the most recent ones (as of February 2007). The mice were bred by The Jackson Laboratory and shipped to our institution when ~8 week old. Because of supply problems, a few mice were slightly older or younger [see [44] for details].

While at The Jackson Laboratory, the mice were housed in same-strain, same-sex groups and fed Purina 5K52 or 5K54 chow. After they arrived at our institution, they were individually housed in plastic “tub” cages and fed AIN-76A diet. This is a semisynthetic diet containing by weight: 20% protein (casein), 65% carbohydrate (sucrose and cornstarch), 5% fat (corn oil), and 10% fiber (cellulose), minerals and vitamins. It contains 44 mmol/kg Na⁺ (~0.5% NaCl) and has an energy content of ~15.9 kJ/g [Dyets, cat. No. 100000; [15]]. When mice were not being tested, deionized water was available from an inverted 300-mL glass bottle with a rubber stopper and stainless steel drinking spout. Cages were changed every 4 d (between choice tests). The vivarium was maintained at 23 °C on a 12:12-h light/dark cycle with lights off at 7 pm. Male and female mice were housed in the same vivarium on separate racks. A detailed description of mouse husbandry, housing conditions, and other procedures is available on-line [42,44]. Abbreviated details are given below.

It was not practical to test simultaneously the 790 mice involved in this experiment. Instead, they were tested in five squads of 188, 170, 135, 186, and 111 mice, conducted over a 12-month period in October 2001–2002. The primary consideration for which mice were tested

in which squad was their availability, so the more common strains were tested in the earlier replications and the rarer and more difficult to breed strains were tested later. Details of which mice were tested in which replication are provided on-line [42,44]. We did not attempt to control for seasonal effects.

1.2. Procedures

After 6–10 d to habituate to laboratory conditions, all mice received a series of 48-h two-bottle choice tests. The mice first received a test with a choice between water versus water, then a series of six tests with calcium solutions, and then a series of six tests with sodium solutions. The current paper is based on the two-bottle choice tests that were conducted with water and sodium solutions. The results with calcium solutions are described elsewhere [48]. The specific concentrations tested here were (in the order they were tested): 25, 75, and 225 mM NaCl, and 25, 75, and 225 mM NaLa. These concentrations were chosen based on previous work in mice [1] with the aim of spanning the range from indifference to strong avoidance. The order of tests (i.e., in ascending concentration for each sodium salt) was chosen so as to be consistent with the majority of previous studies, to simplify testing procedures, and to reduce any potential carry-over effects [6].

For the “water versus water” test, the mice received two tubes of deionized water. For the other tests, the mice received a choice between water and the compound listed. The NaCl was purchased from Sigma Chemical Corp. and the NaLa from Fisher Scientific. All solutions were made with deionized water.

The drinking tubes used for the choice tests are described elsewhere [5,42]. Each consisted of a 25-mL plastic serological pipette with 0.2-mL gradations and a 63.5-mm long stainless steel straight spout. Each spout had a tip with a 3.175-mm diameter hole from which a mouse could lick fluid. Drinking tubes were always presented in pairs, with one containing deionized water and the other taste solution (or, for one test, deionized water in both tubes). The drinking tube spouts extended ~25 mm into the mouse cage and their tips were ~15 mm apart. Each test lasted 48 h. The position of the fluid meniscus was read to the nearest 0.2 mL against a graduated scale at the start and end of the test in order to determine fluid intake. At the midpoint of the test, at 24 h, the position of the two drinking tubes was switched in order to control for the tendency of some strains to drink from the tube on their left [3,46]. Earlier research using identical procedures has shown that fluid spillage and evaporation by C57BL/6J and 129S1/SvImJ mice contribute < 0.5 mL over 48 h to fluid loss [45,47], and so we did not correct for this. For these two strains, the majority of fluid loss occurred during insertion and removal of the drinking tubes from the cage, but it is possible that other strains spill more fluid, so this may be a potential source of error. Occasionally, data were lost due to drinking tube leakage or other technical problems. In these cases, the mice were retested at the end of the test series.

Body weight was measured (to the nearest 0.1 g) at the beginning and end of the test series.

2. Data analysis

2.1. Measures of consumption

Intakes during 48-h tests were divided by two to provide average daily intakes. To determine the measure of intake that was most independent of body size, we compared mean body weights of each strain and sex with corresponding total water intakes collected during the test with two bottles of water. The correlations were moderate but significant (male, $r=0.40$; female, $r=0.48$; Fig. 1). Intakes adjusted for body weight (i.e., mL/kg) were also significantly correlated with body weight but in the opposite direction [male, $r=-0.21$, female, $r=-0.19$; see [3] for a similar result]. However, the relationship was eliminated when water intakes were expressed in relation to body surface area, as first suggested by Richter and Barelare [[33]; i.e., mL/BW^{0.667}; male,

$r=0.03$, female, $r=0.07$; see Fig. 1]. Consequently, we adjusted all fluid intakes for body weight to the power of $2/3$.

Nearly all mice gained weight during the 26-day test series (Table 1). To account for this as precisely as possible, we assumed weights increased linearly across the 26 d and used the appropriate intermediate value between the start and end weights to adjust intakes for each test. The following formula was used, where solution intake is in milliliters, BW is body weight in kilograms, and Test day is the day the test started in the 26-day series.

$$\text{Adjusted solution intake} = \frac{\text{Measured solution intake}}{\left(BW_{\text{start}} + \frac{\text{Test day}(BW_{\text{end}} - BW_{\text{start}})}{26} \right)^{0.667}}$$

A second issue is whether solution consumption is better expressed as intake or as a preference score; that is, as a percentage of total intake [solution intake/(solution intake+water intake) $\times 100$]. To determine how well the two measures of ingestion agreed, we calculated Pearson correlation coefficients between adjusted intakes and preferences based on the 80 means (40 strains $\times$ 2 sexes) for each of the 6 tests with sodium solutions. The resulting correlations were only moderate (r 's=0.51–0.66; Table 2). Preference scores are non-normally distributed at the extremes. In one series of analyses we attempted to eliminate this using an arcsine-root transformation but this only marginally increased correlations (data not shown). We considered the correlations between adjusted intakes and preferences too low to assume that both were measures of the same underlying phenotype. Consequently, we used both adjusted intakes and preference ratios in subsequent analyses.

2.1.1. Statistical analyses—Total water intakes and body weights were first analyzed using omnibus analyses of variance (ANOVAs) with factors of Strain and Sex. Body-weight adjusted sodium intakes and preferences were analyzed using similar ANOVAs with additional factors of Concentration and Anion (chloride or lactate).

Several of these analyses produced complex interactions so more focused analyses were conducted. To determine differences *between* strains, we conducted post hoc analyses of the omnibus ANOVAs using Tukey's tests to reveal "homogenous" groups with statistically similar means [see [1,3] for examples of this approach]. However, for some measures in this experiment there were 15 or more overlapping homogenous groups that, in addition to being hard to describe, were of little value for subsequent interpretation. To provide a more concise and simple identification of outlying strains, we calculated 95% confidence intervals based on the mean of the 40-strain means (i.e., overall mean $\pm 1.96 \times$ standard error of the means), and used these as a criterion to parse strains into high, medium, or low phenotypes.

To determine effects *within* each strain, a series of 40 mixed-design ANOVAs involving Sex, Anion and Concentration were conducted for each dependent variable. In essence, for these analyses the results from each strain were treated as if they had been generated in separate, independent experiments. Tukey's post hoc tests were used to determine whether differences existed between individual pairs of means. Additional analyses of water intake and total fluid intake during taste solution tests were also conducted but revealed little of interest, so are not described here. For all tests, the criterion for significance was $p < 0.01$.

3. Results

3.1. Body weight

Table 1 shows body weights of male and female mice from each strain at the start of the series of two-bottle choice tests. For most strains, males weighed more than females (the exceptions were AKR/J, CE/J, CZECHII/EiJ, KK/H1J, RIIS/J and SPRET/EiJ). Based on the mean body

weights for their sex, 19 male and 16 female groups weighed above the 95th confidence interval of the overall strain mean, and 11 male and 13 female groups weighed below the 95th confidence interval of the overall strain mean. Weight gain during two-bottle choice tests was also strain- and sex-related (Table 1). At the end of testing, the pattern of strain differences had changed little although at this time only three strains had no significant sex difference: CE/J, KK/H1J, and RIIS/J strains [data not shown; see also [32]].

Correlations between body weight and unadjusted sodium intake ranged from $r=0.28$ to 0.43 , depending on the concentration and salt tested (Table 2). Adjusting for body weight^{0.667} successfully removed these correlations (r 's= -0.07 to 0.11). Correlations between body weight and sodium preference were also low (r 's= -0.08 to 0.19).

3.2. Water intake

There was a Strain $\times$ Sex interaction affecting water intake, $F(39,709)=3.22$, $p < 0.0001$. However, the only sex differences found significant by post hoc comparisons were for the C57BR/cdJ strain (males, 60 ± 4 mL/kg^{0.667}; females, 98 ± 6 mL/kg^{0.667}) and NZW/LacJ strain (males, 130 ± 15 mL/kg^{0.667}, females, 93 ± 7 mL/kg^{0.667}); there were no differences in water intake between the sexes for the other 38 strains. Seventeen strains had water intakes below, and 10 strains had water intakes above the 95% confidence intervals (Fig. 2). The C58/J and NZW/LacJ strains had notably higher water intakes than did all the other strains.

3.3. Comparison of sodium chloride and lactate intake

Based on the results of the omnibus ANOVA, the overall mean intake of NaCl (37.7 ± 0.8 mL/kg^{0.667}) was significantly higher than the overall mean intake of NaLa [33.6 ± 0.7 mL/kg^{0.667}; $F(1,709)=73.2$, $p < 0.00001$]. Initial inspection of the results suggested that strains with high intakes of a particular concentration of NaCl also had high intakes of the same concentration of NaLa. To investigate this relationship in detail, we compared intakes of each strain and sex in scatter plots. Correlation coefficients for individual concentrations are given in Table 2. The overall linear regression equation between all three NaCl and NaLa intakes was, NaLa intake= $0.79 \times$ NaCl intake $+6.0$ ($r=0.87$, $n=240$). The positive constant might reflect nonlinearity of the relationship between chloride and lactate intake or simply slightly higher intakes of all fluids in tests with lactate than tests with chloride.

Although there were strong correlations between chloride and lactate intakes there were also sufficient numbers of outliers that we considered it inappropriate to simply ignore the anion when describing results. As a compromise between full description and thrifty use of journal space, we show the results with individual anions when making within-strain comparisons but use averages of the chloride and lactate intakes when making between-strain comparisons.

3.4. Within-strain comparisons of sodium intake

Below are summarized results of ANOVAs based on sodium intakes of each strain analyzed individually. Fig. 3 shows means and standard errors for each sex, anion, and concentration tested.

3.4.1. Sex $\times$ Anion $\times$ Concentration—There were no significant three-way interactions for any of the 40 ANOVAs conducted.

3.4.2. Sex $\times$ Anion—There were no interactions between Sex and Anion.

3.4.3. Anion $\times$ Concentration—Eight strains showed an interaction between Anion and Concentration. Mice from the AKR/J, C57BL/10J, C57BR/cdJ, I/LnJ, KK/H1J, and MSM/MsJ strains drank more 225 mM NaLa than 225 mM NaCl but drank either similar volumes

of lower concentrations of NaCl and NaLa or more NaCl than NaLa. The NZW/LacJ strain drank similar volumes of 225 mM NaCl and 225 mM NaLa but drank less 25 and 75 mM NaLa than the corresponding concentrations of NaCl. The SWR/J strain drank similar volumes of 25 mM NaCl and NaLa but significantly less 75 and 225 mM NaLa than corresponding concentrations of NaCl.

3.4.4. Sex × Concentration—The only interaction between Sex × Concentration was observed for the BALB/cByJ strain. Whereas males of this strain drank similar volumes of all three sodium concentrations tested, females drank more 225 mM NaCl than 25 mM NaCl, with the 75 mM NaCl intakes of females being intermediate between these two means.

3.4.5. Sex—Females of 8 strains drank significantly more sodium than did males: AKR/J, BALB/cByJ, BUB/BnJ, C57BL/10J, C57BR/cdJ, LP/J, MA/MyJ, and NZB/B1NJ. There were no examples of males that drank significantly more sodium than same-strain females.

3.4.6. Anion—Nine strains drank significantly more chloride than lactate (C57BLKS/J, CAST/EiJ, FVB/NJ, JF1/Ms, MOLF/EiJ, NON/LtJ, NZW/LacJ, SJL/J, SWR/J). Only the I/LnJ strain drank significantly more lactate than chloride. The other 30 strains did not differ in anion intake.

3.4.7. Concentration—There were effects of concentration on sodium intake in all strains except the 129S1/SvImJ, A/J, RIIS/J, SPRET/EiJ, and WSB/EiJ.

3.5. Sodium preference, avoidance and indifference

Table 3 presents sodium preference scores for each mouse strain. A preference score of 50% indicates that a mouse either cannot detect or is indifferent to a taste solution. To determine whether each strain responded to taste solutions with anything other than indifference, mean strain preferences (for both sexes combined) were compared to 50% using one-sample *t*-tests (Table 3).

3.6. Between-strain comparisons of sodium intake

Fig. 4, Fig. 5, and Fig. 6 rank the 40 strains according to daily intakes of each concentration of NaCl and NaLa, and show the relationship between sodium intake and preference. There were four strains that had mean intakes above the 95% confidence interval for all three concentrations of sodium: 129S1/SvImJ, MA/MyJ, NZW/LacJ and SWR/J. Six others had mean intakes above the 95% confidence interval for two of the three concentrations: BTBR *T⁺tf/J*, C57BLKS/J, C57BR/cdJ, C58/J, KK/H1J and NOD/LtJ. There were 12 strains that had mean intakes below the 95% confidence interval for all three concentrations of sodium: AKR/J, C3H/HeJ, C57BL/10J, CBA/J, DBA/2J, I/LnJ, JF1/Ms, LP/J, NON/LtJ, PERA/EiJ, PL/J, and RIIS/J. The CZECHII/EiJ, MOLF/EiJ, SM/J, and WSB/EiJ had mean sodium intakes below the 95% confidence interval for two of the three sodium concentrations. The NZB/B1NJ and PWK/PhJ strains had 25 mM sodium intakes below the lower confidence interval, 75 mM sodium intakes within the confidence interval, and 225 mM sodium intakes above the higher confidence interval. The A/J, C57BL/6J, FVB/NJ and SEA/GnJ strains had all three sodium intakes within the confidence interval.

3.7. Heritability

Heritability was calculated from the ratio of $SS_{\text{among strains}}/SS_{\text{total}}$ where *SS* is the sum of squares obtained in a one-way ANOVA based on data from each sex of each strain, or both sexes of each strain [9]. Heritability coefficients of sodium intake and preference ranged from 0.47 to 0.76, depending on the particular sex, concentration and anion being tested (Table 4).

In most cases, sodium preference scores had slightly higher heritability coefficients than did sodium intakes.

4. Discussion

The results describe the voluntary water and sodium consumption of 40 strains of mice. Although there have been previous strain surveys of water and sodium intake, including one of 28 strains [1], this study is not only larger but more comprehensive in that it includes mice of both sexes, tests with two sodium salts, and evaluates the impact of various methods of assessing sodium solution consumption. The most important conclusion to be drawn is that all five factors – Strain, Sex, Anion, Concentration and the measure of consumption – influence the ranking of strains, and the manner in which each factor interacts with the others is not always simple. There are few generalities and nearly always exceptions to the “typical” patterns of water and sodium intake in mice.

4.1. Methods to compare intake among strains

An issue that has considerable effect on interpretation is the appropriate unit of measurement of sodium consumption. Raw intake per mouse is confounded by body weight; large mice generally drink more sodium but also more of everything else, so this is not an informative measure to use when comparing across strains. Dividing raw intake by body weight reduces the problem to some extent but overcorrects so that intake is negatively related to body weight [see Fig. 1 and Ref. [3]]. We found that adjusting fluid intakes by dividing them by body weight to the power of two-thirds provided a measure that was independent of body weight. This adjustment may be more than a useful mathematical construct. Allometric functions with similar power exponents link body weight to metabolic rate and to body surface area, both of which are determinants of fluid loss, and thus likely to be reflected in intakes [see [27,33]].

The most common method to eliminate bias related to body size is to express intake as a preference score, which is a ratio of taste solution intake to total fluid intake. Since preference scores are ratios of intakes, they are generally believed to be independent of body size, and we demonstrate that this is the case here (Table 2). However, a concern is that intakes of the two available fluids are not independent, making interpretation difficult. This is particularly true for tests involving high concentrations of sodium because these stimulate thirst. Rodents titrate their water and sodium intakes in a manner that dilutes concentrated sodium to near isotonicity in the gut [e.g., [37,38]]. Although in this experiment, adjusted sodium intakes and sodium preference scores were moderately congruent (r^2 's=0.51–0.62) there were many exceptions. For example, the C58/J strain had the 2nd highest intakes of 25 mM NaLa but ranked 35th in 25 mM NaLa preference. Conversely, the RIIS/J strain ranked 34th in 25 mM NaLa intake but 9th in preference. It can therefore be misleading to use preference scores as the sole measure of sodium consumption to compare across strains. Ultimately, the most appropriate measure of consumption will depend upon the questions being asked.

A second technical issue highlighted by the results of this study is how to categorize mice into homogenous groups and consequently identify outlying strains. Probably the most statistically defensible approach is to look for differences between each pair of strains during post hoc analyses. We have used this approach previously in studies involving fewer strains [e.g., [1, 3,4,6]]. However, with the 40 strains and two sexes involved here, the number of comparisons to be made becomes enormous. It generally resulted in a dozen or more overlapping homogenous groups for each sex and phenotype. It is dubious whether this information is useful, except as a statistical demonstration that the distribution is more-or-less continuous, and thus likely due to a complex (polygenic) trait. Instead, here we simply split strains into three categories; those with a phenotype above, within, or below the 95% confidence intervals for the overall mean of all strains. This approach has the advantage of being easy to display

but it could also be misleading because the confidence intervals are based on a normal distribution, which was not present for the strains tested here. The tails of the distribution, which in a normally distributed sample would be expected to contain just 5% (i.e., 2) of the strains, usually contained most of them. Simply because a strain was above the 95% confidence interval did not imply that it was an outlier. We suspect that defining outliers can best be done either by targeted comparisons of selected strain pairs or simply by ranking the strain means.

4.2. Sodium concentration–intake functions

A surprising result was that sodium concentration had little effect on the volume of sodium ingested by the majority of strains. There could be several reasons for this. First, some strains drank very little of all three concentrations of sodium (e.g., CBA/J, PERA/EiJ). It may be that intakes of these strains were maximally depressed with even the lowest (i.e., 25 mM) sodium solutions tested. Conversely, the NZW/LacJ and MA/MyJ female mice drank so much sodium at all concentrations there may have been a ceiling on intake. Second, some strains were indifferent to sodium solutions (e.g., NON/LtJ) raising the possibility that they may not be able to taste them. Third, the concentrations of sodium tested may not have been optimal to elicit concentration–response functions. In outbred rats, the highest sodium intakes and preferences occur at ~150 mM sodium and it may be that if a similar peak is present for some strains of mice, it would be straddled by the closest concentrations tested here (75 or 225 mM). Fourth, unlike some of the previous strain surveys, the mice tested here were naive [except for prior tests with calcium solutions [48]] and were given sodium solutions in ascending order. Both prior experience and the order of solution presentation can influence sodium intakes [4,6,7]. Fifth, the mice in this experiment were fed a semisynthetic diet rather than the more typical cereal-based chow used in previous studies. Sodium preferences are higher in mice fed semisynthetic diet than chow [50], largely because water intakes are lower. The level of sodium or other ingredients in the semisynthetic diet might skew concentration–intake and preference functions.

4.3. Sex differences in sodium consumption

Extensive work with outbred rats indicates that females drink more sodium than males and that reproductive steroids have both organizational and activational effects on sodium consumption [e.g., [12,17,22]]. We were therefore surprised to find that only 8 of the 40 strains tested here showed significant sex differences in sodium intake, and none showed significant sex differences in sodium preference. Consistent with other literature, if a sex difference was present it was due to higher sodium intakes of females than males. It has been suggested that the higher sodium intakes of females reflect the demands of reproduction [see [34,36]] but this argument appears weak given that most strains did not differ in sodium intake by sex. It would be interesting to know if there are differences in the regulation of estrogen or other reproductive steroids between the 8 strains showing sex differences in sodium intake and the 32 that do not.

We did not attempt to control for estrus cycle-related influences on sodium consumption in the present work. However, these did not appear to add much variation to sodium intakes because males and females had similar within-strain variability. It is possible the females cycled together because they were housed together on the same racks, which may allow pheromonal synchronization of reproductive state. It would take considerable effort to investigate this.

4.4. Role of the anion in determining sodium consumption

Human psychophysics and rodent gustatory electrophysiology suggest that the anion accompanying sodium has little effect on taste quality but the larger the anion, the less intense the sodium [16,19,23,26]. Overall, our results are consistent with this: Mice drank ~11% less NaLa than the equivalent concentration of NaCl. The correlations between mean strain NaCl and NaLa intakes were very high ($r=0.90, 0.96, 0.86$ for 25, 75, and 225 mM sodium,

respectively). Indeed, it is doubtful that a test–retest correlation of the same compound would be any higher. This argues that the response to sodium is the predominant factor and that the anion makes little contribution to this phenotype. Correlations involving preferences for NaCl and NaLa were also high, but not quite as high as those for intakes ($r=0.80, 0.89, 0.84$ for 25, 75, and 225 mM sodium, respectively). It is noteworthy that although intakes of NaCl were higher than intakes of NaLa there were no corresponding differences in preference scores for NaCl and NaLa (for example, the overall mean preference for 75 mM NaCl was $61.7 \pm 0.7\%$ and for 75 mM NaLa it was $61.1 \pm 0.8\%$). This was because lower NaLa intakes were accompanied by lower water intakes.

The above discussion refers to all the mice combined. There were several examples of individual strains that responded differently to the chloride and lactate. Nine strains drank significantly more chloride than lactate but only one, the I/LnJ strain, drank significantly more lactate than chloride. The general tendency for higher chloride than lactate intakes is not simple; the same mice tested with calcium salts drank more calcium lactate than CaCl_2 [48].

4.5. Potential mechanisms underlying strain differences in voluntary sodium consumption

The mice involved in this study included strains from distant genealogical backgrounds that were likely to encompass wide genetic diversity. This was reflected in the wide range of responses to sodium. We attempted several comparisons to determine whether sodium intake was related to a strain's genetic origins. For example, we compared strains derived from Swiss, C57, Castle or "wild" stock [see [40] for strains belonging to each of these families]. We also compared sodium intakes of various strains that were genetically similar based on seven strain families identified by SNP parsimony analyses [30]. However, there were no reliable differences among these genealogical branches. This argues that differences in sodium consumption were unlikely to have been due to a few polymorphisms carried in the early divergence of these strain families. Instead, the variability could be due to multiple polymorphisms in founder wild populations that were randomly fixed in different inbred strains during inbreeding, or to many relatively recent mutations.

Two recent approaches toward gene identification deserve consideration in the context of the present results. First, there have been reports that genome-wide haplotype analysis of inbred strains can be used to identify quantitative trait loci [e.g., [20,30,31]]. However, more recent research has shown these approaches are underpowered, and are prone to false positives [11, 29,49]. We suspect that inbred strain haplotype analysis will be useful for assessing the contribution of candidate genes once these have been identified but *de novo* gene identification will require even more than 40 strains to be useful. Second, an ambitious effort to produce > 1000 advanced recombinant inbred strains has begun [13,51]. This Collaborative Cross is based on 8 founder strains of which 7 were tested here (129S1/SvImJ, A/J, C57BL/6J, NOD/LtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ, but not NZO/HILtJ). Unfortunately, the 7 founder strains we tested had only a modest range of sodium intakes [e.g., for 75 mM NaCl, lowest strain mean (WSB/EiJ) = $34 \pm 7 \text{ mL/kg}^{0.667}/\text{d}$; highest strain mean (129S1/SvImJ) = $53 \pm 6 \text{ mL/kg}^{0.667}/\text{d}$]. Thus the Collaborative Cross will not be ideal for discovering genes related to sodium intake, although it remains possible that some of the advanced recombinant inbred strains may have recombined alleles of the founders in a way that will introduce greater phenotypical variation.

4.6. Covariates of sodium intake

Associations between the pattern of results found with various strain sodium intakes and other phenotypes can provide clues about specificity and common genetic mechanisms underlying the phenotypes. Some caution must be used with this because although in general, intakes of the two salts and three concentrations of sodium were closely associated (Table 2), there were exceptions, and the chances of finding spurious correlations are high with at least 36 ways of

summarizing “sodium intake” (i.e., 2 anions $\times$ 3 concentrations $\times$ males, females or both sexes $\times$ intakes or preferences). Nevertheless, some general observations appear worthwhile. First, there were fairly strong correlations between body-weight adjusted intakes of sodium and water (r 's=0.47–0.79; see Table 2). This indicates that there are shared genetic mechanisms underlying fluid intake that are not simply due to body size. It also suggests that the measure of sodium consumption used here is not specific. Perhaps a more specific measure could be produced by regressing sodium intake against water intake. We note that the correlation between sodium and water intake is not simply a general proclivity to consume all fluids because correlations between consumption of sodium and calcium [described elsewhere [48]] were much weaker (r =0.09 to 0.45, average of the 36 correlations, r =0.25). Correlations between sodium consumption and blood pH, ionized calcium, total calcium, and various measures of body composition were all low (r 's=–0.19 to 0.16). Correlations of sodium intake with bone mineral density and bone mineral content [described in [48]] were slightly higher (r 's=0.17 to 0.31).

The Mouse Phenome Database [39] allows comparison of phenotypes across experiments from different projects. One interesting comparison is between the 75 mM NaCl preference of male mice of 24 strains that were common to this study and our previous study of 28 strains (r =0.80). This correlation is surprisingly high considering the many differences in methodology between these studies, including factors that are known to have strain-related effects on sodium preference such as maintenance diet and the mice's previous experience with sodium and other test solutions [4,6,50]. This suggests that the strain differences observed here are relatively robust.

The Mouse Phenome Database also reveals strong correlations between sodium preference and acoustic startle response, thymus weight, the weight of the heart left ventricle, and the weight of both ventricles [39]. However, these correlations are most likely spurious. In one case, a single, extreme outlying strain accounts for the relationship; in the others a correlation is present for just one sodium concentration or just one sex. Thus, although the Mouse Phenome Database has the potential to reveal relationships among phenotypes that might illuminate mechanisms, there are no obviously informative measures for the sodium-consumption phenotype at present (February 2007).

4.7. Sodium-liking and sodium-disliking mice

One of the functions of a strain survey is to guide the choice of strains for subsequent genetic analysis. The results of this study suggest several strains that would be worthy of further attention by virtue of their extreme intakes. The MA/MyJ, NZW/LacJ and SWR/J strains had notably higher sodium intakes than most others at all three concentrations tested and they also had some of the highest sodium preference scores. The C58/J strain also had high intakes of 25 and 75 mM sodium but it fell in the middle of the pack (18th out of 40) in 225 mM sodium intakes. There was a less clear demarcation between strains with average and low sodium intakes but four strains with consistently low intakes were AKR/J, C57BL/10J, CBA/J, and PERA/EiJ. For at least some of these strains, the extreme intakes are likely to represent a specific response to sodium because the same strains did not show particularly extreme calcium intakes [see [48]]. It will require detailed physiological and genetic analyses to identify and characterize the mechanisms responsible for the differences in acceptability of sodium by these strains.

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Fig. 1.

Relationship between body weight and three measures of water intake (per mouse, per kilogram body weight (BW), or per kilogram BW to the 2/3 power). Each point represents the mean of ~ 10 male (blue) or ~ 10 female (red) mice from 40 strains.

Fig. 2.

Mean daily water intakes of 40 strains of mice. Strains are arranged in order from lowest to highest water intakes. Symbols represent average of males and females (~20 mice/strain). There were significant sex differences for only two strains, the C57BR/cdJ and NZW/LacJ. Vertical shaded bar shows 95% confidence intervals from the average of the strain means. Horizontal lines are standard errors of the mean; these are smaller than the symbols in most cases.

Fig. 3. Mean NaCl (circles) and NaLa (triangles) intakes (adjusted for body size^{0.667}) of male (blue) and female (red) mice from 40 strains. Freestanding circles are water intakes in two-bottle water versus water test. Vertical lines are standard errors of the mean; these are smaller than the symbols in some cases.

Fig. 4.

Intake and preference of 25 mM sodium solutions by 40 strains of mice (both sexes combined). Closed symbols = 25 mM NaCl, open symbols = 25 mM NaLa. Strains are arranged from lowest to highest average sodium intake. Vertical shaded bars are 95% confidence intervals from the average of the strain means of all 40 strains (both anions combined). Horizontal lines are standard errors of the mean; these are smaller than the symbols in most cases.

Fig. 5.

Intake and preference of 75 mM sodium solutions by 40 strains of mice (both sexes combined). Closed symbols = 75 mM NaCl, open symbols = 75 mM NaLa. Strains are arranged from lowest to highest average sodium intake. Vertical shaded bars are 95% confidence intervals from the average of the strain means of all 40 strains (both anions combined). Horizontal lines are standard errors of the mean; these are smaller than the symbols in most cases.

Fig. 6.

Intake and preference of 225 mM sodium solutions by 40 strains of mice (both sexes combined). Closed symbols = 225 mM NaCl, open symbols = 225 mM NaLa. Strains are arranged from lowest to highest average sodium intake. Vertical shaded bars are 95% confidence intervals from the average of the strain means of all 40 strains (both anions combined). Horizontal lines are standard errors of the mean; these are smaller than the symbols in most cases.

Table 1

Body weights and weight gain during two-bottle choice tests of 40 strains of mice

Strain	Male		Female	
	Start weight (g)	Weight gain (g/d)	Start weight (g)	Weight gain (g/d)
129S1/SvImJ	23.7 ± 0.4	0.08 ± 0.01	18.8 ± 0.2 [*]	0.07 ± 0.00
A/J	24.1 ± 0.8	0.05 ± 0.01	20.9 ± 0.9 [*]	0.02 ± 0.02
AKR/J	30.9 ± 0.7	0.13 ± 0.02	26.1 ± 1.9 [*]	0.00 ± 0.06 [*]
BALB/cByJ	27.0 ± 0.4	0.11 ± 0.01	22.1 ± 0.4 [*]	0.04 ± 0.02
BTBR <i>T⁺tf/J</i>	30.2 ± 0.5	0.14 ± 0.01	24.7 ± 0.4 [*]	0.09 ± 0.01
BUB/BnJ	30.6 ± 0.4	0.12 ± 0.01	22.8 ± 0.5 [*]	0.15 ± 0.01
C3H/HeJ	26.5 ± 0.5	0.10 ± 0.02	20.2 ± 0.6 [*]	0.09 ± 0.01
C57BL/10J	25.0 ± 0.4	0.21 ± 0.01	19.0 ± 0.3 [*]	0.12 ± 0.00
C57BL/6J	25.5 ± 0.5	0.08 ± 0.01	20.1 ± 1.0 [*]	0.04 ± 0.02
C57BLKS/J	24.0 ± 0.5	0.07 ± 0.00	18.9 ± 0.2 [*]	0.06 ± 0.00
C57BR/cdJ	25.6 ± 0.2	0.16 ± 0.02	20.1 ± 0.2 [*]	0.09 ± 0.00
C57L/J	25.3 ± 0.5	0.12 ± 0.01	20.7 ± 0.7 [*]	0.04 ± 0.02
C58/J	23.8 ± 0.8	0.04 ± 0.02	18.8 ± 0.7 [*]	0.07 ± 0.02
CAST/EiJ	13.7 ± 0.3	0.03 ± 0.01	12.1 ± 0.4	0.00 ± 0.01
CBA/J	27.9 ± 0.7	0.21 ± 0.02	21.8 ± 0.5 [*]	0.06 ± 0.02 [*]
CE/J	19.2 ± 0.9	0.05 ± 0.01	21.7 ± 1.5	0.10 ± 0.06 [*]
CZECHII/EiJ	13.4 ± 0.3	0.01 ± 0.00	10.7 ± 0.4	0.03 ± 0.01
DBA/2J	26.6 ± 0.4	0.10 ± 0.03	19.8 ± 1.0 [*]	0.07 ± 0.03
FVB/NJ	26.0 ± 0.3	0.03 ± 0.01	19.6 ± 0.7 [*]	0.00 ± 0.01
I/LnJ	24.5 ± 1.0	0.04 ± 0.01	16.9 ± 0.4 [*]	0.04 ± 0.00
JF1/Ms	19.6 ± 0.7	0.19 ± 0.01	13.6 ± 0.3 [*]	0.03 ± 0.01 [*]
KK/HIJ	34.4 ± 1.3	0.13 ± 0.02	35.2 ± 0.4	0.14 ± 0.01
LP/J	24.1 ± 0.4	0.14 ± 0.02	18.7 ± 0.4 [*]	0.03 ± 0.01
MA/MyJ	23.5 ± 0.5	0.13 ± 0.01	18.8 ± 0.3 [*]	0.08 ± 0.01
MOLF/EiJ	13.4 ± 0.3	0.03 ± 0.01	11.9 ± 0.2	0.02 ± 0.01
MSM/MsJ	12.0 ± 0.1	0.11 ± 0.03	9.5 ± 0.1	0.04 ± 0.02
NOD/LtJ	26.9 ± 0.7	0.35 ± 0.02	20.2 ± 0.7 [*]	0.22 ± 0.02
NON/LtJ	33.8 ± 1.0	0.14 ± 0.03	24.9 ± 0.5 [*]	0.15 ± 0.01
NZB/B1NJ	28.4 ± 0.7	0.12 ± 0.00	21.2 ± 0.8 [*]	0.15 ± 0.01
NZW/LacJ	27.1 ± 0.4	0.00 ± 0.02	24.5 ± 0.4	0.07 ± 0.01
PERA/EiJ	25.0 ± 0.7	0.14 ± 0.01	18.5 ± 0.6 [*]	0.02 ± 0.04
PL/J	21.8 ± 0.5	0.05 ± 0.01	16.9 ± 0.9 [*]	0.06 ± 0.01
PWK/PhJ	15.8 ± 0.3	0.17 ± 0.03	13.1 ± 0.3	0.11 ± 0.04
RIIS/J	24.5 ± 1.5	0.17 ± 0.01	20.6 ± 1.6	0.13 ± 0.01
SEA/GnJ	21.9 ± 0.6	0.07 ± 0.01	18.5 ± 0.3	0.00 ± 0.03
SJL/J	25.0 ± 0.4	0.09 ± 0.01	20.1 ± 1.0 [*]	0.06 ± 0.01
SM/J	19.5 ± 0.2	0.02 ± 0.04	15.1 ± 0.7 [*]	0.05 ± 0.00
SPRET/EiJ	16.8 ± 1.4	0.06 ± 0.00	12.5 ± 0.2	0.06 ± 0.00
SWR/J	22.7 ± 0.4	0.07 ± 0.01	15.8 ± 0.2 [*]	0.09 ± 0.01
WSB/EiJ	15.6 ± 0.4	0.08 ± 0.01	11.9 ± 0.2	0.07 ± 0.00

Values are means ± SE of ~ 10 mice per sex and strain.

* $p < 0.01$ relative to males of same strain. For starting body weights, the mice were ~ 8 week old. The 95% confidence intervals based on the average of the strain means for each sex were 21.9–25.3 g for males and 17.4–20.4 g for females. Weight gains are based on change in weight over 26 d of tests with water, calcium solutions [see [48]] and sodium solutions available.

Table 2
Correlation matrix of sodium solution intakes and preferences for 40 strains of mice

	25 mM NaCl	75 mM NaCl	225 mM NaCl	25 mM NaLa	75 mM NaLa	225 mM NaLa	BW
25 mM NaCl	0.54						
75 mM NaCl	0.94	0.85					
225 mM NaCl	0.72	0.62	0.65				
25 mM NaLa	0.90	0.82	0.66	0.80			
75 mM NaLa	0.94	0.91	0.72	0.51	0.85		
225 mM NaLa	0.65	0.80	0.75	0.92	0.55	0.62	
Water	0.79	0.73	0.47	0.69	0.77	0.77	0.04
BW	0.01	0.05	-0.07	0.02	0.11	0.48	0.10
Intakes adjusted for BW ^{0.667}							
25 mM NaCl	0.54						
75 mM NaCl	0.94	0.85					
225 mM NaCl	0.72	0.62	0.65				
25 mM NaLa	0.90	0.82	0.66	0.80			
75 mM NaLa	0.94	0.91	0.72	0.51	0.85		
225 mM NaLa	0.65	0.80	0.75	0.92	0.55	0.62	
Water	0.79	0.73	0.47	0.69	0.77	0.77	0.10
BW	0.01	0.05	-0.07	0.02	0.11	0.48	-0.08
Preferences							
25 mM NaCl							
75 mM NaCl							
225 mM NaCl							
25 mM NaLa							
75 mM NaLa							
225 mM NaLa							
Water							
BW							

Values are Pearson correlation coefficients based on 40 means from each strain (both sexes combined). The diagonal (in yellow) shows correlations between intake and preference. Correlations between intakes adjusted for body weight^{0.667} are shown on left of diagonal, correlations between preferences are shown top right. Water = total intake from both bottles in two-bottle choice of water versus water. NaLa = sodium lactate, BW = body weight. All correlations except those involving body weight are significant at $p < 0.01$ level.

Table 3
Preferences and avoidance of NaCl and NaLa by 40 strains of mice

Strain	NaCl		NaLa	
	25 mM	75 mM	25 mM	75 mM
129S1/SvImJ	67 ± 4 [↑]	70 ± 4 [↑]	64 ± 6	71 ± 4 [↑]
A/J	53 ± 3	62 ± 3 [↑]	59 ± 3 [↑]	66 ± 4 [↑]
AKR/J	39 ± 4	55 ± 3	48 ± 3	47 ± 4
BALB/cByJ	68 ± 4 [↑]	73 ± 3 [↑]	67 ± 3 [↑]	70 ± 3 [↑]
BTBR T ^u /ffJ	67 ± 3 [↑]	75 ± 3 [↑]	64 ± 4 [↑]	75 ± 3 [↑]
BUB/BuJ	62 ± 3 [↑]	66 ± 3 [↑]	67 ± 3 [↑]	72 ± 3 [↑]
C3H/HeJ	59 ± 4	46 ± 4	54 ± 4	56 ± 6
C57BL/10J	63 ± 2 [↑]	71 ± 1 [↑]	56 ± 2	70 ± 2 [↑]
C57BL/6J	61 ± 2 [↑]	62 ± 3 [↑]	52 ± 3	58 ± 3
C57BLKS/J	62 ± 1 [↑]	69 ± 1 [↑]	60 ± 2 [↑]	69 ± 2 [↑]
C57BR/cdJ	43 ± 2 [↓]	47 ± 3	41 ± 2 [↓]	43 ± 3
C57L/J	48 ± 1	54 ± 2	48 ± 1	51 ± 2
C58/J	59 ± 7	60 ± 7	41 ± 7	55 ± 6
CAST/EiJ	67 ± 1 [↑]	72 ± 2 [↑]	64 ± 1 [↑]	70 ± 2 [↑]
CBA/J	48 ± 5	43 ± 5	53 ± 4	52 ± 5
CE/J	59 ± 4	70 ± 5 [↑]	59 ± 5	58 ± 7
CZECH/EiJ	45 ± 6	56 ± 6	32 ± 5 [↓]	50 ± 7
DBA/2J	50 ± 4	56 ± 3	41 ± 3	53 ± 4
FVB/NJ	65 ± 3 [↑]	79 ± 1 [↑]	49 ± 3	76 ± 2 [↑]
I/LnJ	51 ± 3	53 ± 3	56 ± 2	63 ± 2 [↑]
JF1/Ms	73 ± 2 [↑]	68 ± 5 [↑]	52 ± 5	62 ± 5
KK/H1J	69 ± 3 [↑]	66 ± 4 [↑]	55 ± 4	65 ± 5
LP/J	61 ± 4	57 ± 4	59 ± 5	67 ± 3 [↑]
MA/MyJ	85 ± 2 [↑]	90 ± 1 [↑]	84 ± 1 [↑]	87 ± 2 [↑]
MOLF/EiJ	54 ± 3	46 ± 2	44 ± 3	40 ± 2
MSM/MsJ	67 ± 3 [↑]	65 ± 2 [↑]	54 ± 4	63 ± 3 [↑]
NOD/LJ	52 ± 3	56 ± 5	50 ± 5	54 ± 6
NON/LJ	53 ± 3	57 ± 3	49 ± 3	50 ± 3
NZB/B1N	58 ± 1 [↑]	63 ± 1 [↑]	50 ± 2 [↑]	59 ± 1 [↑]
NZW/LacJ	76 ± 1 [↑]	82 ± 1 [↑]	65 ± 1 [↑]	77 ± 1 [↑]
PERA/EiJ	46 ± 4	36 ± 5	51 ± 6	38 ± 5
PL/J	44 ± 4	51 ± 5	37 ± 4	47 ± 5
PWK/PhJ	57 ± 5	68 ± 4 [↑]	58 ± 5	66 ± 4 [↑]
RIIS/J	61 ± 2 [↑]	62 ± 1 [↑]	61 ± 2 [↑]	70 ± 3 [↑]
SEA/GnJ	53 ± 2	59 ± 3 [↑]	54 ± 2	59 ± 2 [↑]
SIL/J	61 ± 4	67 ± 4 [↑]	59 ± 4	75 ± 3 [↑]
SM/J	49 ± 3	48 ± 4	47 ± 5	49 ± 5
SPRET/EiJ	64 ± 3	66 ± 3 [↑]	59 ± 2	67 ± 3 [↑]
SWR/J	68 ± 3 [↑]	76 ± 3 [↑]	69 ± 4 [↑]	67 ± 5 [↑]
WSB/EiJ	36 ± 4 [↓]	38 ± 3 [↓]	37 ± 4 [↓]	38 ± 4 [↓]

Notes. Values are preference scores (%; i.e., sodium solution intake/total fluid intake×100) based on intakes of male and female mice combined.

[↑] $p < 0.01$, preference score of strain significantly higher than indifference (50%).

↓
 $p < 0.01$, preference score of strain significantly lower than indifference (50%).

Table 4
Estimates of heritability for water and sodium consumption

Measure	Both sexes		Males only		Females only	
	Intake	Pref	Intake	Pref	Intake	Pref
Water	0.49	n/a	0.37	n/a	0.44	n/a
25 mM NaCl	0.67	0.71	0.61	0.64	0.57	0.66
75 mM NaCl	0.75	0.76	0.66	0.71	0.62	0.72
225 mM NaCl	0.56	0.67	0.49	0.63	0.47	0.66
25 mM NaLa	0.69	0.72	0.48	0.64	0.63	0.71
75 mM NaLa	0.64	0.56	0.58	0.50	0.61	0.58
225 mM NaLa	0.62	0.65	0.49	0.55	0.53	0.67

Notes: Heritability estimates are based on the ratio of SS_{among strains}/SS_{total}. Intake = total water intake or sodium solution intake adjusted for body weight^{0.667}, Pref = taste solution preference ratio, n/a = not applicable.