Voluntary Consumption of NaCl, KCl, CaCl$_2$, and NH$_4$Cl Solutions by 28 Mouse Strains

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Abstract

Male mice from 28 inbred strains (129P3/J, A/J, AKR/J, BALB/cByJ, BUB/BnJ, C3H/HeJ, C57BL/6J, C57L/J, CAST/Ei, CBA/J, CE/J, DBA/2J, FVB/NJ, I/LnJ, KK/H1J, LP/J, NOD/LtJ, NZB/B1NJ, P/J, PL/J, RBF/DnJ, RF/J, RIIIS/J, SEA/GnJ, SJL/J, SM/J, SPRET/Ei, and SWR/J) were tested with NaCl (75–450 mM), KCl (30–300 mM), CaCl$_2$ (3–100 mM), and NH$_4$Cl (10–300 mM) solutions using two-bottle preference tests with water as the second choice. For each mineral, there was a wide range of strain variation in solution intakes and preferences. This variation had a substantial genetic component as assessed using heritability estimates. In most cases, the strain means were continuously distributed; however, strains with deviating high or low intakes or preferences were also observed. The associations among the responses to different minerals were only modest, suggesting distinct genetic controls of sodium, potassium, calcium, and ammonium consumption. These results provide a valuable resource for investigators who wish to identify genes involved in the regulation of mineral consumption and balance.

Keywords

Mice; genetics; appetite; preference; salt; sodium; potassium; calcium

INTRODUCTION

The consumption of inappropriately high or low amounts of several minerals can exacerbate many chronic diseases, including hypertension and osteoporosis (e.g., reviewed in [Beauchamp and Engelman, 1991;Tordoff, 2001]). However, despite impressive progress toward characterization of the genetic bases of some of these diseases, there has been little concerted effort to understand the genetic controls of mineral intake. The comparison of animals from inbred strains is an initial step towards identification of genetic factors controlling mineral intake. In previous studies, strain differences in spontaneous intake of NaCl solutions have been noted. For example, rat strains can be characterized by high NaCl intakes (SHR), moderate NaCl intakes (e.g., Dahl-R, Sprague-Dawley, Dark Agouti, WKY, GHR, NT), or low NaCl intakes (e.g., Fisher-344, Dahl-S, Brattleboro) (Wolf *et al.,* 1965;Catalanotto *et al.,* 1972;Fregly, 1975;DiNicolantonio *et al.,* 1983;Midkiff and Bernstein, 1983;Ferrell and Gray, 1985;Midkiff *et al.,* 1985;Ely *et al.,* 1987;Bachmanov, 1989,1993;Yongue and Myers, 1989;Rowland and Fregly, 1990;Bernstein *et al.,* 1991). NaCl preferences have also been characterized in 12 mouse strains: 129, A2G, BALB/c, BPH/2, BPL/1, BPN/3, C3H/He, C57BL/6, CBA, DBA/2, NZB/B1NJ, and TO (Ninomiya *et al.,* 1989;Lush, 1991;Beauchamp and Fisher, 1993;Gannon and Contreras, 1993;Bachmanov *et al.,* 1998a,1998b). However, each of these previous studies included five or fewer strains and involved different methods,

so it is impossible to compare all the strains directly. Nevertheless, there was a considerable range of responses among the strains tested, including strong preferences for hypotonic and isotonic NaCl (BPN/3, BPL/1 and TO [Lush, 1991;Bachmanov *et al.,* 1998a]), high intakes of hypertonic NaCl (NZB/B1NJ [Bachmanov *et al.,* 1998b]), and strong avoidance of all concentrations of NaCl (CBA [Bachmanov *et al.,* 1998b]).

In contrast to this work on strain differences in ingestion of NaCl solutions, there is little parallel work with other minerals. Rats of the SHR strain ingest more calcium and potassium solutions or calcium-containing diet than do rats of the WKY strain (Ferrell and Dreith, 1986;Bachmanov, 1989,1993;Tordoff, 1992a). Mice of the BPL/1 strain drink less $CaCl_2$ and more of some concentrations of KCl than do mice of the BPN/3 or BPH/2 strains (Bachmanov *et al.,* 1998a). We are unaware of any published work on strain differences in intake of other minerals.

To provide a more comprehensive examination of strain differences in mineral intake, we used 48-h two-bottle choice tests to assess the voluntary intake of and preference for, various mineral chlorides by 28 inbred strains of mice. We concentrated on four mineral chlorides: NaCl, KCl, $CaCl_2$, and NH_4Cl for several reasons. Each cation is regulated by independent homeostatic systems, and at least for sodium, potassium, and calcium, is the target of a specific appetite (e.g., Eckert, 1938;Scott *et al.,* 1950;Milner and Zucker, 1965;Adam and Dawborn, 1972;Lobaugh *et al.,* 1981;Denton, 1984;Rowland and Fregly, 1988;Tordoff *et al.,* 1998;Tordoff, 2001). At the same time, there is much interplay among the control mechanisms for consumption of each mineral. Physiological examples of this include the regulation of aldosterone by both potassium excess and sodium deficiency, the linked excretion of sodium and calcium, and co-transport involving Na^+/Ca^{2+}, Na^+/K^+, and Na^+/H^+-ATPases. Behavioral examples include the increase in NaCl intake observed during calcium or potassium deficiency (e.g., Zucker, 1965;Adam and Dawborn, 1972;Tordoff, 1992b,1996). There are also similarities among NaCl, KCl, $CaCl_2$, and NH_4Cl in taste. For example, high concentrations of all four mineral salts have a salty taste component, and all except NaCl are rated as bitter [at least by humans (McBurney and Shick, 1971;Schiffman and Erickson, 1971)]. By comparing the voluntary intake of different minerals by many strains of mice, we hoped to gain insight into the relationships among the factors controlling consumption of these salts.

METHODS

Procedure

Mice, diet, housing conditions, and construction of drinking tubes are described in an accompanying paper (Bachmanov *et al.,* 2002). Tests of mineral intake began 3 days after the end of food and water intake tests (Bachmanov *et al.,* 2002). The mice received four series of two-bottle choice tests. These involved ascending concentrations of NaCl (75, 150, 300, 450 mM), KCl (10, 30, 100, 200, 300 mM), $CaCl_2$ (3, 10, 30, 100 mM), and NH_4Cl (10, 30, 100, 300 mM). The concentrations used were chosen based on previous work (Bachmanov *et al.,* 1996,1998a,1998b) and pilot studies so as to span the range from indifference to strong avoidance by most strains of mice. Each concentration was tested for 48 h, with the positions of the tubes containing taste solution and water switched after 24 h to control for side preferences. There were no breaks between testing different concentrations of the same compound, but between testing different compounds, the mice received deionized water in both drinking tubes for at least three days. Body weight was measured (to the nearest 0.1 g) at the beginning of each series of taste solutions, and at the end of the experiment.

Data Analysis

Intakes from each day of each of the 2-day choice tests were averaged to provide measures of daily water intake and solution intake. Body weight values used in analyses were based on the average body weights collected before and after each test. Total fluid intake was calculated as the sum of water intake and solution intake. Solution preference scores were calculated as the ratio of average daily solution intake to average daily total fluid intake ($\times 100$).

Eighty of the 10,948 measurements made were lost due to technical errors (e.g., spilled or leaking drinking tubes; error rate $< 0.75\%$). The data lost appeared to be distributed randomly among the strains. The data set contained several measures for each of four taste solutions in a total of 17 concentrations. With the large number of potential comparisons involved, there is a strong possibility of Type I errors occurring. To ameliorate this, we set the criterion for significance at $P < 0.01$ for all statistical tests. However, exact probability values are given throughout the text to allow investigators who prefer other criteria for significance to apply them.

Three inbred strains included mice with different genotypes because these strains are bred using a forced heterozygosity procedure. These were 129P3/J (Tyr^c/Tyr^c, albino coat color phenotype, n = 10; and Tyr^{c-ch}/Tyr^c, light chinchilla coat color phenotype, n = 2), SM/J (A^w/a, white-bellied agouti coat color phenotype, n = 7; and a/a, black coat color phenotype, n = 5) and SEA/GnJ ($Bmp5^{se}/Bmp5^{se}$, short ears phenotype, n = 6; and $Bmp5^{se}/+$, normal ears phenotype, n = 6). The only significant difference detected was related to 10 mM KCl intakes by the two SM/J genotypes. However, this was probably due to chance because 10 mM KCl was neither preferred nor avoided by most of the strains (see Results). We also noticed that one NOD/LtJ mouse lost 2.8 g (9.5% of initial body weight) during the NH_4Cl test period and drank progressively larger volumes of fluid (during tests with 100 and 300 mM NH_4Cl, total intake of this mouse was 19–20 ml/day), which is typical of the diabetes that develops in this strain. However, the other NOD/LtJ mice did not show weight loss or high fluid intakes (relative to other strains), and NH_4Cl intake and preference of the diabetic mouse were within the range displayed by other mice from this strain, so we did not exclude its data. Therefore, data for individual types of mice of the same strain were pooled together for subsequent analyses.

Two-way ANOVAs were used to assess the effects of strain (between-subjects), concentration (within-subjects), and their interaction on each of the five dependent variables (solution intake per mouse, solution intake per unit of body weight, water intake per mouse, total fluid intake per mouse, and solution preference) for each of the four taste solutions. This was followed up by planned comparisons, consisting of one-way ANOVAs for each concentration separately. Tukey's Honestly Significant Difference post hoc tests from the one-way ANOVAs were used to identify differences between pairs of means and ascertain homogeneous groups.

Heritability estimates and genetic correlations were calculated as described in the accompanying paper (Bachmanov *et al.,* 2002). Cluster analysis was conducted using Statistica software (StatSoft, Tulsa, OK). The correlation matrix was used as input, and the correlations were transformed to dissimilarities ($1 - r$), which were used as distance measures. Single linkage was used as the amalgamation rule.

RESULTS

Relationship of Mineral Solution Intake and Preference to Water Intake and Body Weight

Fluid intake often depends on body size [see detailed discussion in (Bachmanov *et al.,* 1998b,2001,2002)]. A positive correlation was found between water intake and body weight in the same 28 strains (Bachmanov *et al.,* 2002). To examine the possibility that body size might contribute to differences in solution intake or preference, we calculated correlation

coefficients of body weight or water intake (given as the only liquid) with solution intakes or preferences (Table I). Significant correlations involved intakes of the weakest NaCl and KCl solutions only. These dilute mineral solutions were neutral to many strains (see results below), and therefore their consumption is proportional to total fluid intake, which depends on body size. For intakes of most solutions, and for all solution preference scores, the distributions of strain means were unrelated to body weight or water intake. We therefore concluded that for the 28 strains tested here, mineral solution intakes and preferences are generally independent of body size and customary fluid intake (represented by intakes of water available as the only fluid), and so do not require adjustments for these variables. Thus, we report unadjusted daily solution intakes per mouse and preference scores.

Strain Differences in Mineral Solution Intakes and Preferences

For each of the four minerals, significant effects of strain, concentration, and their interaction on each of the five dependent variables (solution intake per mouse, solution intake per unit of body weight, water intake per mouse, total fluid intake per mouse, and solution preference) were detected using two-way ANOVAs (all P's < 0.0000001). In the subsequent planned comparison one-way ANOVAs for each solution concentration, there were highly significant differences between the 28 strains in both intake and preference of every taste solution, with the exception of preference for 30 mM KCl solution (Table II). The concentration-response functions for each taste solution are shown in Figures 1, 2, 3, 4, and distribution patterns are presented in Figure 5.

For all minerals, mouse strains displayed a wide range of intakes and preference scores. As indicated by significant interactions between the effects of strain and concentration in the ANOVAs, patterns of strain distributions were affected by solution concentration.

NaCl—Most mouse strains were indifferent to, or moderately preferred, 75 and 150 mM NaCl, and they avoided 300 and 450 mM NaCl. Across all NaCl concentrations tested, the strongest avoidance and the lowest intakes were found in CBA/J, C3H/HeJ, and (except for 75 mM NaCl) AKR/J mice. For weaker (75 and 150 mM) NaCl solutions, CAST/Ei mice showed the strongest preferences (close to 90%). Intakes of these solutions were the highest in BUB/BnJ mice, which probably was affected by the high water consumption of this strain (Bachmanov *et al.,* 2002). The highest preference scores and intakes of 300 mM NaCl were found in NZB/ BINJ mice.

KCl—Most mouse strains were indifferent to 10–100 mM KCl, and they avoided 200 and 300 mM KCl. AKR/J mice had notably stronger avoidance and lower intake of 100 mM KCl compared with the rest of the strains. C57BL/6J mice had the highest preference scores for 100–300 mM KCl and the highest intakes of 300 mM KCl. For weaker KCl solutions, intakes by SM/J mice were similar to, or higher than, those of the C57BL/6J mice, but these high solution intakes by the SM/J mice are probably a consequence of their high customary fluid intakes (Bachmanov *et al.,* 2002).

CaCl$_2$—The 3 to 30 mM CaCl$_2$ solutions were either neutral or aversive to mice. All strains exhibited avoidance of the strongest (100 mM) CaCl$_2$ solution. Except for 30 mM CaCl$_2$, the strain distributions were homogeneous. Preferences for 30 mM CaCl$_2$ appeared to be dichotomous, with one group of strains being relatively indifferent (preference scores ranging from 37% to 57%), and the other group avoiding this solution (preference scores 4–26%; Fig. 5). 129P3/J mice displayed the weakest avoidance and the highest intakes of 100 mM CaCl$_2$.

NH$_4$Cl—The 10 to 100 mM NH$_4$Cl solutions were either neutral, or aversive to mice. All strains exhibited avoidance of the strongest (300 mM) NH$_4$Cl solution. SPRET/Ei mice had

the lowest preference scores and intakes for 10–100 NH_4Cl. NZB/B1NJ and C57L/J mice had the weakest avoidance and the highest intakes of 300 mM NH_4Cl.

Heritability

Estimates of heritability were calculated based on the relationship of variation among the strains (representing the genetic component) to the variation within the strains (representing the environmental component; see Fig. 6). For each of the four minerals, heritabilities depended on concentration, with responses to more concentrated solutions being more strongly influenced by genetic factors. The highest levels of heritability were similar for all four minerals (~0.5 to 0.7), indicating that at least half of the variation in mineral consumption of this mouse population is genetically determined. At lower concentrations, estimates of heritability were higher for solution intakes than for preference scores. This was probably because when there is no strong preference or avoidance, solution intake is proportional to customary fluid consumption, and thus genetic differences in water intake contribute to solution intake, but they do not affect preference scores. This explanation is also consistent with the correlations between water intake and intakes (but not preferences) of weaker mineral solutions (Table I).

Interrelationships among Mineral Solution Intakes and Preferences

In most cases, there were positive and significant correlations between intakes and preferences of the four or five concentrations of each mineral solution (Table III). In general, intake and preference of the closest concentrations of the same mineral were the most strongly correlated; as the difference between concentrations increased, the correlations became weaker. The weakest correlations were observed among KCl solutions (Table IIIb). This was probably due to the indifference of mice to the two weakest KCl concentrations, so that strain distributions for these solutions were more-or-less random for preferences and depended on customary fluid consumption for intakes and thus did not correlate with the preference or aversion responses to higher KCl concentrations. This is consistent with the low heritabilities of the dilute KCl solution intakes (Fig. 6) and with significant correlations between intake of these solutions and water intake (in the water-only test; Table I).

For analysis of interrelationships among responses to different minerals, we used only preference scores because intakes may show relationships due to non-specific factors (e.g., customary fluid intake or body weight), whereas preference scores are independent of these factors and better represent responses specific to particular mineral solutions. We also excluded from the analysis 10 and 30 mM KCl solutions that evoked responses with low heritability (< 0.2). Because these solutions did not evoke obvious preference or aversion, the differences among strain means are probably randomly distributed and by chance may show a false positive relationship with another stimulus. The results of cluster analysis based on correlations among all the selected preference scores are presented in Figure 7. Consistent with the correlations among different concentrations of the same mineral, in most cases, the four or five concentrations of a mineral were clustered together (with the exception of 300 mM NH_4Cl, which was more closely related with NaCl solutions than with the other NH_4Cl concentrations). Each mineral could be divided into two subgroups of weaker and stronger concentrations: 75–150 mM and 300–450 mM for NaCl, 100 and 200–300 mM for KCl, 3–30 and 100 mM for $CaCl_2$, and 10–100 and 300 mM for NH_4Cl. This suggests that strain distributions for the lower and higher concentrations of the same mineral may be affected by different genetic factors.

Responses to different minerals displayed only modest similarities, with preferences for $CaCl_2$ and NH_4Cl (10–100 mM) being the most closely related. The lack of close correspondence among preferences for different minerals is consistent with the responses of individual strains. For example, C3H/HeJ mice had low NaCl and KCl preferences, and high $CaCl_2$ preferences; CE/J mice had high NaCl and low $CaCl_2$ preferences; BUB/BnJ mice had

high NaCl and $CaCl_2$, and low KCl preferences; SJL/J mice had high NaCl and low NH_4Cl preferences.

Multidimensional scaling yielded results similar to the cluster analysis (data not shown). The correlation matrix of all 123 measures collected for the 17 taste solutions tested is available on our website (Tordoff and Bachmanov).

DISCUSSION

This study compared the voluntary consumption of four mineral salt solutions (chlorides of sodium, potassium, calcium, and ammonium) by male mice from 28 strains. For each mineral solution, there was a wide range of intakes and preferences among the strains. This variation had a substantial genetic component. For most of the measures collected, the majority of strain means were continuously distributed but strains with deviating high or low intakes or preferences were also observed.

The results of this study are consistent with previously reported strain differences in NaCl consumption (Ninomiya *et al.,* 1989;Lush, 1991;Bachmanov *et al.,* 1998b;Kotlus and Blizard, 1998), with the exception that here we found smaller differences between 129P3/J and C57BL/6J strains compared with previous reports (Lush, 1991;Beauchamp and Fisher, 1993;Gannon and Contreras, 1993;Bachmanov *et al.,* 1998b). This discrepancy could be due to the different substrains used (Tordoff, unpublished data) or differences in testing procedures that could affect NaCl preference (e.g., Beauchamp and Fisher, 1993;Bachmanov *et al.,* 1998b,1999).

The continuous patterns of strain distributions for consumption of all four minerals are consistent with multigenic controls of these traits, which would be expected of a complex behavior such as mineral solution intake. Several physiological mechanisms that can mediate strain differences in voluntary NaCl consumption have been suggested, including differences in gustatory neural responses to sodium and their sensitivity to amiloride (Ninomiya *et al.,* 1989,1996;Bernstein *et al.,* 1991;Gannon and Contreras, 1993), sodium metabolism (Bachmanov *et al.,* 1997), or the rennin-angiotensin system (Bachmanov *et al.,* 1994). The other three minerals, KCl, $CaCl_2$, and NH_4Cl, also evoke taste sensations, and thus genetic differences in taste sensitivity could affect solution preference. Consumption of sodium, potassium, and calcium is regulated by specific appetites tied to homeostatic mechanisms, and thus genetic differences in these mechanisms may mediate the variation in voluntary consumption. Future genetic analyses of mineral consumption will unveil these mechanisms.

Associations among the responses to different minerals were only modest, suggesting distinct genetic controls of sodium, potassium, calcium and ammonium consumption. This is consistent with the existence of some common underlying mechanisms in responses to all these minerals, as well as the substantial role of factors that are unique to each mineral. One potential common mechanism may involve similarities in taste, as shown by similar patterns of behavioral and electrophysiological responses to NH_4Cl, KCl, and $CaCl_2$ in rodents under some conditions (e.g., Morrison, 1967;Smith *et al.,* 1983;Frank, 1991), and high neural cross-adaptation among the three minerals (Smith and Frank, 1972). Of course, the associations in long-term, two-bottle choice tests, such as those used in this experiment, could also be mediated by postingestive, motivational, and/or cognitive controls.

The weak association between the avidity for NaCl and the other three minerals argues against the hypothesis that animals use saltiness (or NaCl-like taste) to recognize and direct consumption of other minerals (Schulkin, 1991a;1991b).

NH_4Cl is commonly used as a standard stimulus for normalization of gustatory taste responses in electrophysiological experiments. Our data suggest that this practice requires caution when

comparing mouse strains, because some strains differ greatly in NH_4Cl intake and preference and thus may differ in their gustatory neural responses to NH_4Cl.

These results provide a valuable resource for investigators who wish to design mutagenesis screens or choose strains for positional cloning of underlying genes. However, at least two cautions are worth mentioning. First, many environmental factors can influence taste solution intake, and many of these probably interact with genetic factors. There may be subtle differences in laboratory environments that could produce large differences in mouse behavior (e.g., Crabbe *et al.,* 1999). Second, given the enormous number of comparisons conducted, it is likely that some of the differences found here are due to chance. To some extent, this is ameliorated by the generally similar results found with different concentrations of the same taste solution but, even so, there is still the possibility that some significant differences may be erroneous. It will require replication of critical comparisons under other laboratory conditions to eliminate these possibilities.

Two-bottle preference tests are an easy, efficient, and reliable method to measure voluntary taste solution consumption by mice. Strain differences in mineral consumption can reflect variations in taste perception or in underlying physiological or pathological processes. The data presented here provide information necessary to further the goal of identification of these mechanisms.

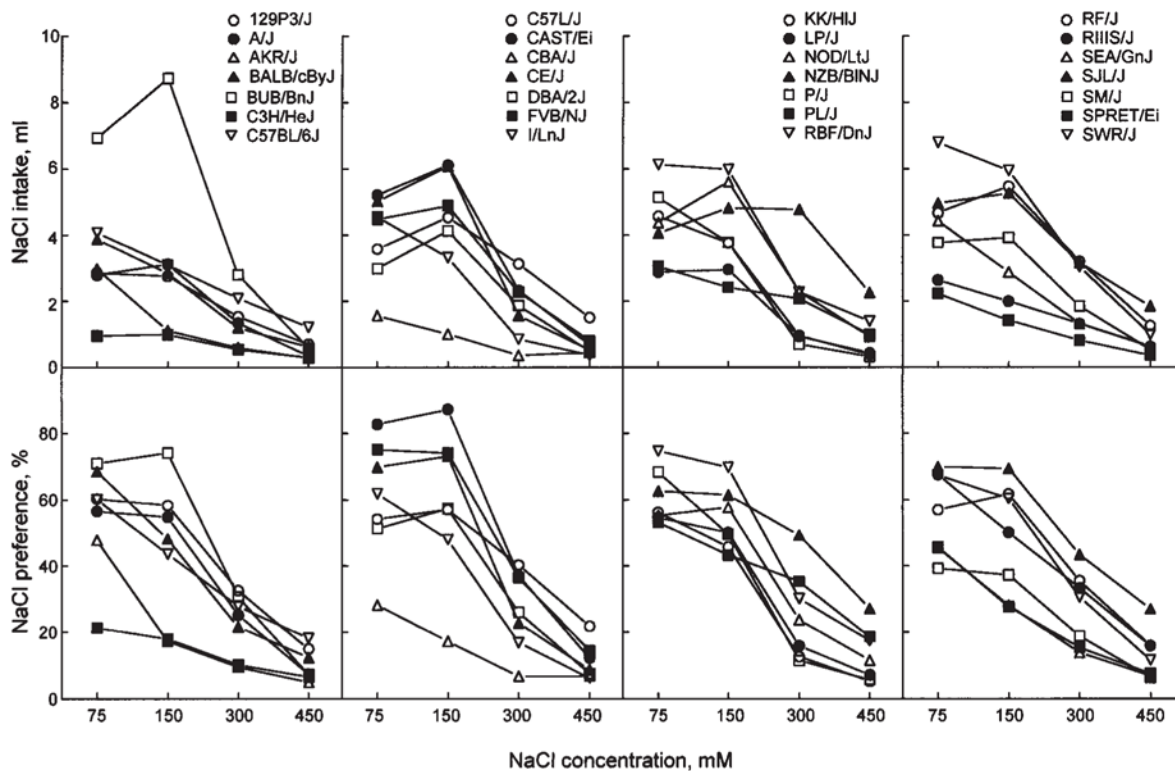# References

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Fig 1.
NaCl concentration-intake (top panels) and concentration-preference (bottom panels)
functions for 28 strains of mice. Differences between strains larger than the Tukey's critical
difference (CD) given in Table II are significant.
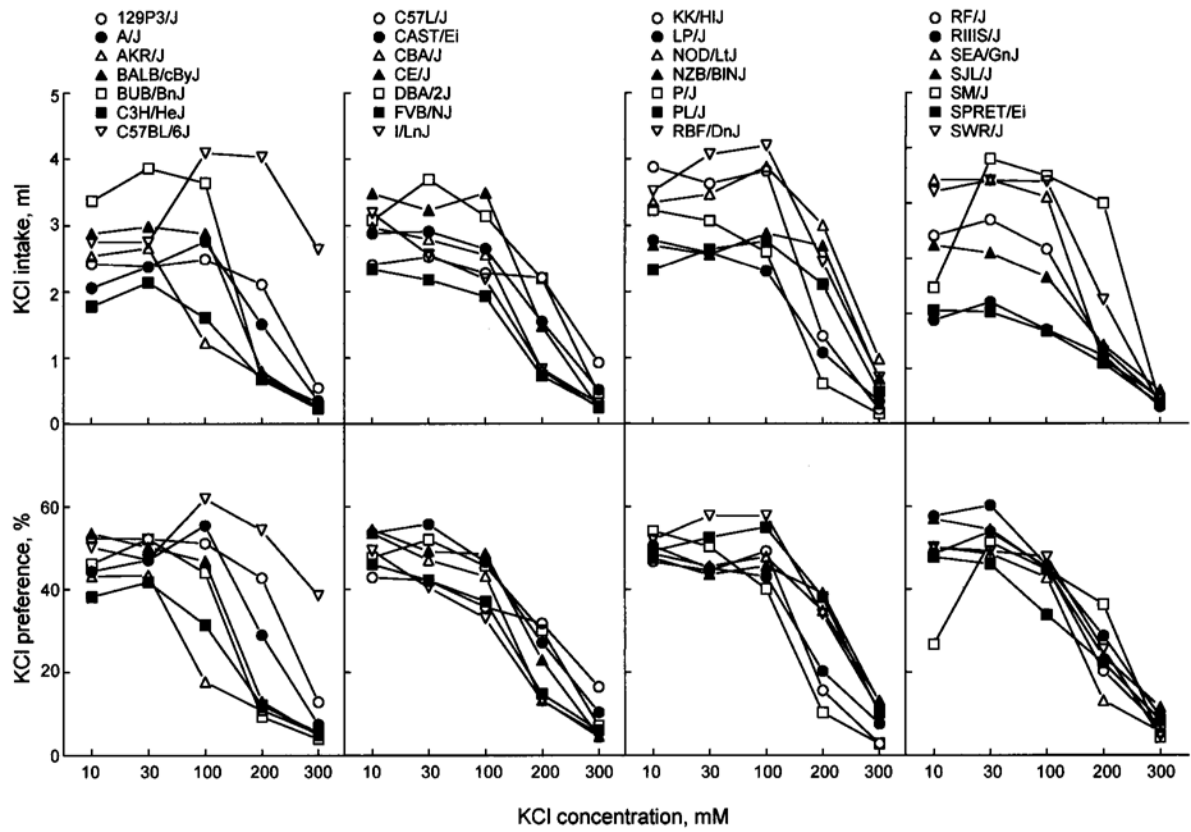
Fig 2.
KCl concentration-intake (top panels) and concentration-preference (bottom panels) functions for 28 strains of mice. Differences between strains larger than the Tukey's CD given in Table II are significant.
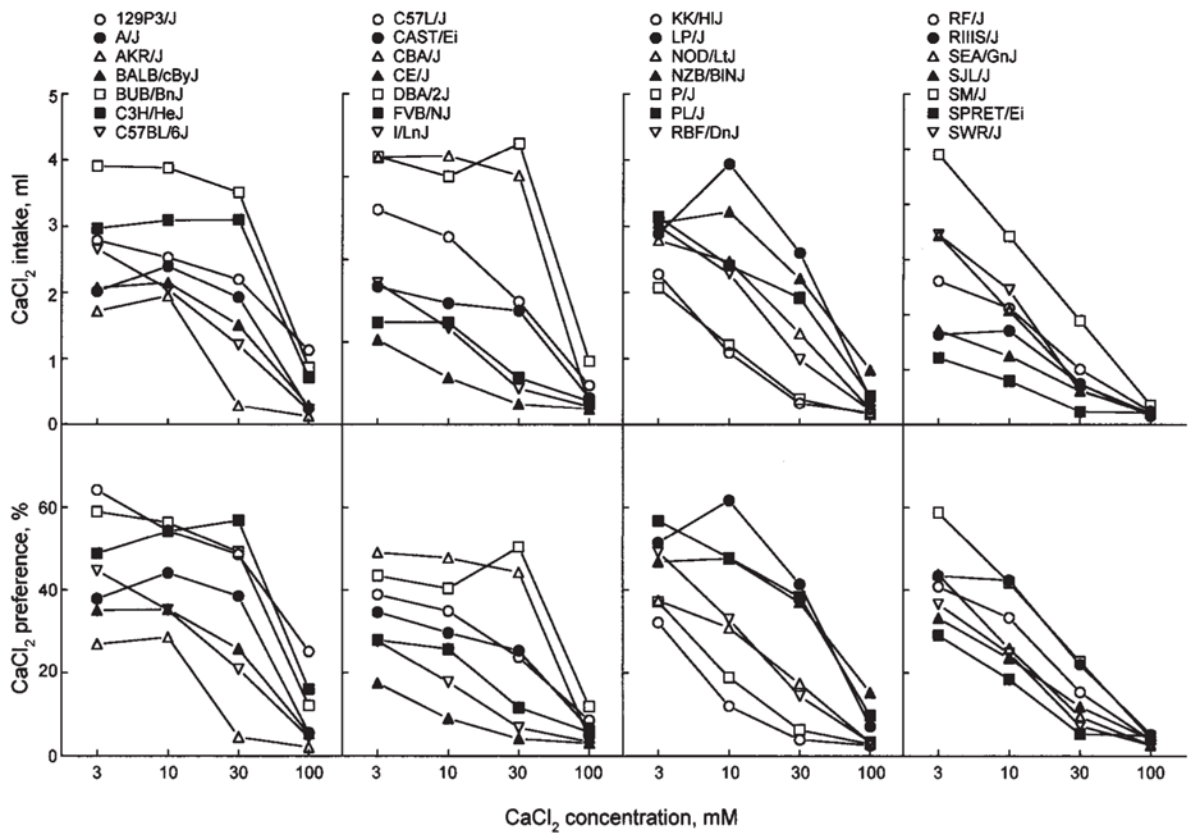
Fig 3.
CaCl$_2$ concentration-intake (top panels) and concentration-preference (bottom panels) functions for 28 strains of mice. Differences between strains larger than the Tukey's CD given in Table II are significant.
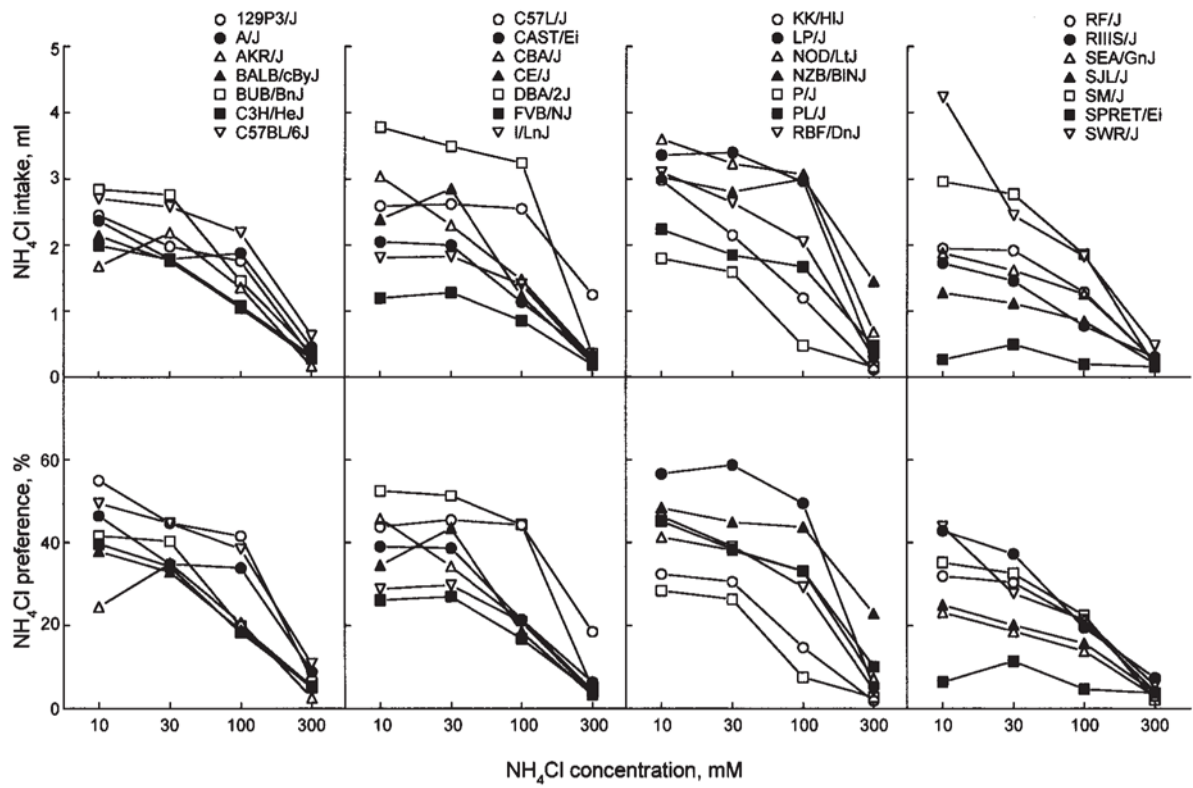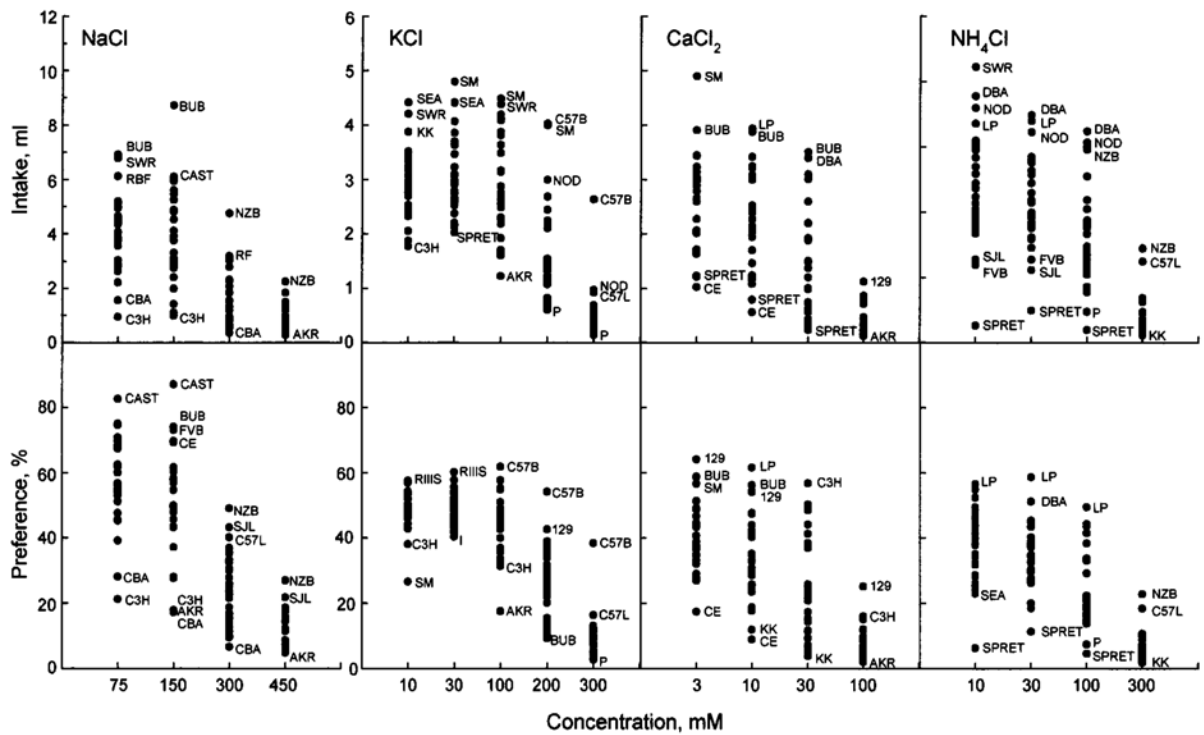
Fig 4.
NH$_4$Cl concentration-intake (top panels) and concentration-preference (bottom panels)
functions for 28 strains of mice. Differences between strains larger than the Tukey's CD given
in Table II are significant.

Fig 5.
Strain distribution of intakes and preferences for each concentration of four taste solutions. Where possible, outlying points have been labeled with an abbreviated strain name. Differences between strains larger than the Tukey's CD given in Table II are significant. Note that the scale for NaCl intake (top LH panel) is twice that of the other three mineral salt intakes.
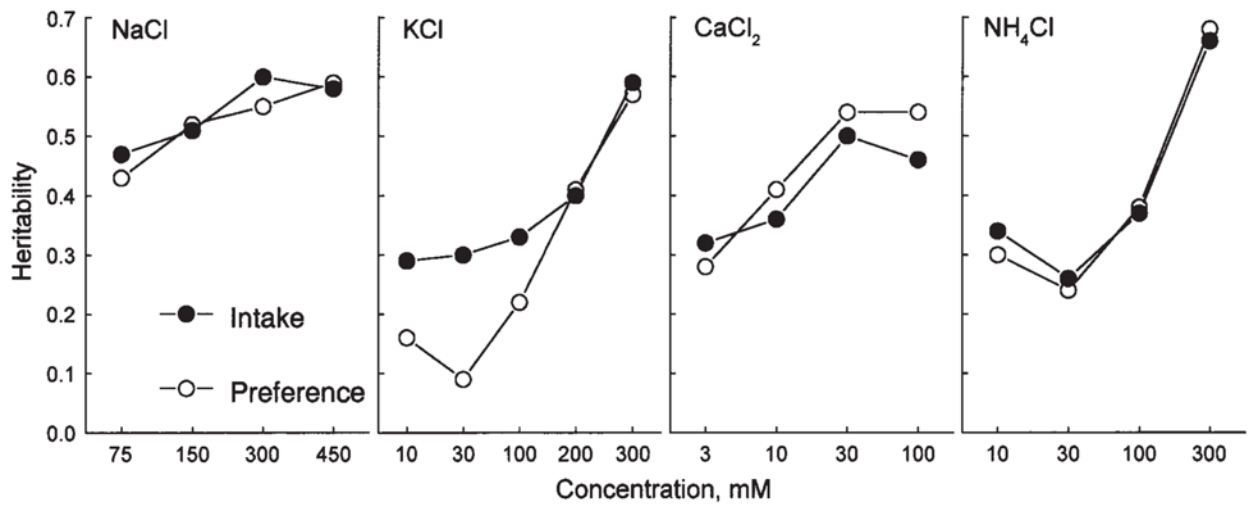
Fig 6.
Heritability estimates for intakes and preferences for each concentration of four taste solutions. Heritability was calculated as a ratio $SS_{among\ strains}/SS_{total}$ based on the sums of squares (SS) obtained in one-way ANOVAs for each concentration (Bachmanov *et al.,* 2002).
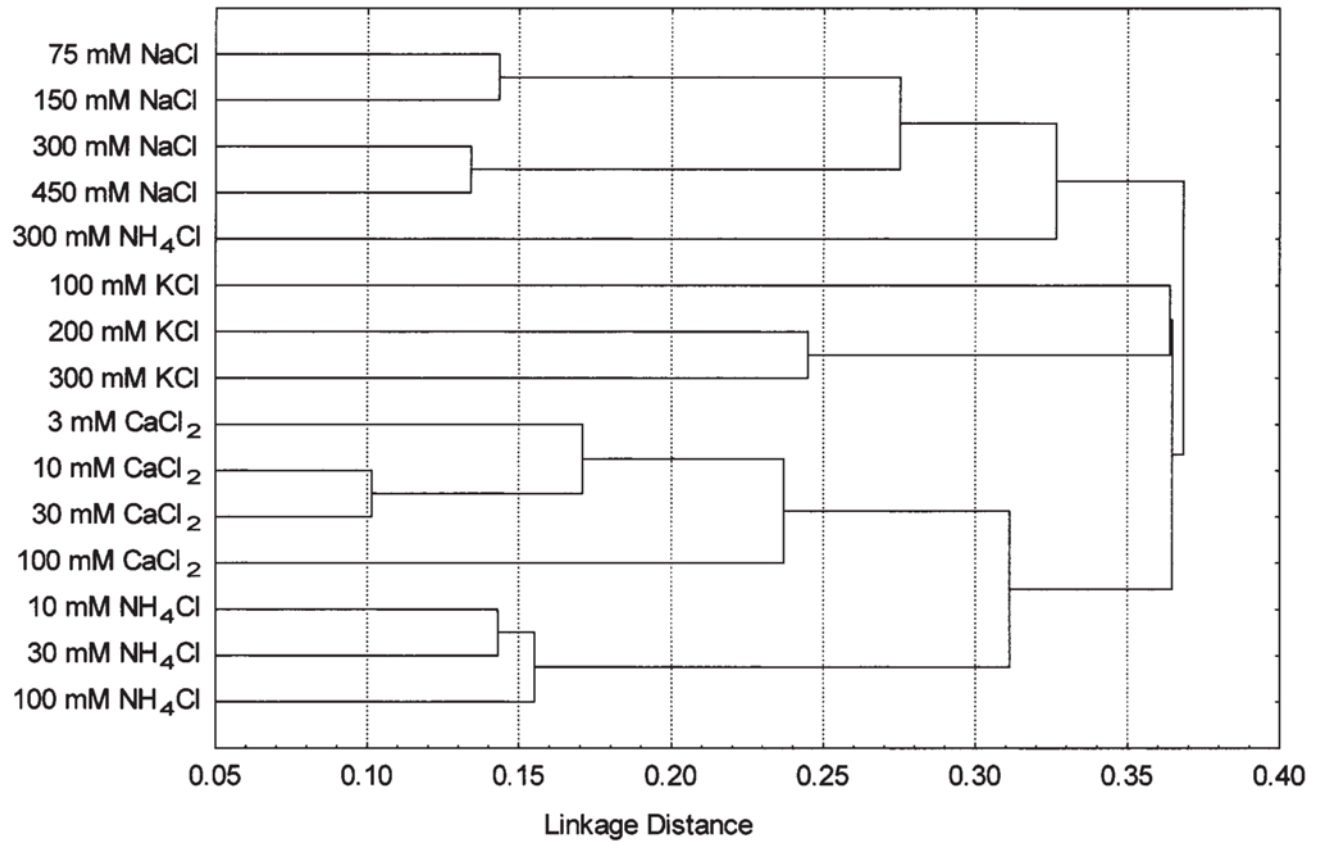
Fig 7.
Tree diagram for salt solution preferences obtained using cluster analysis.

Table I

Pearson Product-Moment Correlations of Strain Means for Water Intake (given as the only fluid available) and Body Weight with Strain Means for Taste Solution Intake and Preference (*n* = 28)

Solution and concentration (mM)	Intakes		Preferences	
	Water intake	Body weight	Water Intake	Body weight
NaCl				
75	0.55[*]	0.65[*]	0.22	0.05
150	0.42	0.49	0.19	0.06
300	0.16	0.22	−0.02	−0.11
450	0.18	0.09	−0.01	−0.18
KCl				
10	0.61[*]	0.82[*]	0.05	−0.13
30	0.56[*]	0.80[*]	0.15	0.01
100	0.38	0.67[*]	0.00	0.04
200	−0.08	0.12	−0.27	−0.20
300	−0.06	0.10	−0.19	−0.10
CaCl$_2$				
3	0.12	0.41	−0.12	−0.04
10	−0.02	0.07	−0.25	−0.31
30	−0.22	−0.14	−0.35	−0.34
100	−0.26	−0.16	−0.39	−0.36
NH$_4$Cl				
10	0.19	0.35	−0.09	−0.17
30	0.19	0.28	−0.10	−0.21
100	0.05	0.11	−0.14	−0.19
300	−0.18	−0.09	−0.31	−0.28

[*] $p < .01$.

Table II

Results of One-Way ANOVAs for Strain Differences in Taste Solution Intakes and Preferences

Test	df	Intakes		Preferences	
		F	Tukey CD (ml)	F	Tukey CD (%)
NaCl (mM)					
75	27,284	9.34	2.66	7.99	29
150	27,286	10.86	3.12	11.42	31
300	27,286	15.84	1.50	13.13	18
450	27,293	14.95	0.74	15.70	9
KCl (mM)					
10	27,293	9.42	1.72	2.03	25
30	27,290	4.58	1.97	1.07	28
100	27,288	6.40	2.14	3.08	29
200	27,292	10.56	2.08	7.49	24
300	27,292	21.79	1.52	14.63	10
CaCl₂ (mM)					
3	27,283	4.99	2.28	3.98	32
10	27,283	5.88	2.10	7.40	29
30	27,293	10.70	1.72	12.52	27
100	27,294	9.11	0.48	12.62	9
NH₄Cl (mM)					
10	27,293	3.59	1.47	4.15	31
30	27,293	5.65	2.08	4.68	30
100	27,290	10.11	1.55	8.89	25
300	27,291	9.72	1.69	10.81	20

Notes: All analyses revealed significant differences at the $p < .0001$ level except for 10 mM KCl preference ($p = .0022$) and 30 mM KCl preference ($p = .375$). *df*, degrees of freedom; Tukey CD, critical difference score for Tukey post hoc tests using $p < .01$ as the criterion for significance. Differences between means (shown in Figs. 1–5) greater than the Tukey CD are significant at the $p < .01$ level.

Table III

Table IIIa: Selected Correlations Among Intakes and Preferences of Each Concentration of NaCl

NaCl Concentration (mM)	Preference 75	150	300	450	
75	0.73*	0.86*	0.58*	0.38	75
150	0.86*	085*	0.72*	0.43	150
300	0.51*	0.68*	0.88*	0.87*	300
450	0.32	0.40	0.87*	0.97*	450
	75	150	300	450	NaCl Concentration (mM)

Intake (left axis) / Preference (right axis) / Intake (bottom axis)

b: Selected Correlations Among Intakes and Preferences of Each Concentration of KCl

KCl Concentration (mM)	Preference 10	30	100	200	300	
10	0.29	0.40*	0.29	−0.04	0.12	10
30	0.75*	0.33	0.50*	0.25	0.01	30
100	0.68*	0.85*	0.65*	0.64*	0.43*	100
200	0.01	0.35	0.60*	0.88*	0.76*	200
300	−0.02	−0.04	0.31	0.68*	0.97*	300
	10	30	100	200	300	KCl Concentration (mM)

Intake (left axis) / Preference (right axis) / Intake (bottom axis)

c: Selected Correlations Among Intakes and Preferences of Each
Concentration of CaCl$_2$

Preference

CaCl$_2$ Concentration (mM)	3	10	30	100	
3	0.79*	0.76*	0.72*	0.60*	3
10	0.81*	0.87*	0.90*	0.66*	10
30	0.61*	0.86*	0.96*	0.76*	30
100	0.42	0.57	0.73*	0.95*	100
	3	10	30	100	CaCl$_2$ Concentration (mM)

(Left axis: Intake; Right axis: Preference; Bottom axis: Intake)

d: Selected Correlations Among Intakes and Preferences of Each
Concentration of NH$_4$Cl

Preference

NH$_4$Cl Concentration (mM)	10	30	100	300	
10	0.75*	0.86*	0.82*	0.45	10
30	0.87*	0.80*	0.84*	0.42	30
100	0.78*	0.85*	0.89*	0.63*	100
300	0.35	0.38	0.63*	0.96*	300
	10	30	100	300	NH$_4$Cl Concentration (mM)

(Left axis: Intake; Right axis: Preference; Bottom axis: Intake)

c: Selected Correlations Among Intakes and Preferences of Each Concentration of $CaCl_2$

	Preference			
$CaCl_2$ Concentration (mM)	3	10	30	100
3	0.79*	0.76*	0.72*	0.60*
10	0.81*	0.87*	0.90*	0.66*
30	0.61*	0.86*	0.96*	0.76*
100	0.42	0.57	0.73*	0.95*
	3	10	30	100

(Left axis: Intake; Right axis column headers: 3, 10, 30, 100 Preference; Bottom axis: Intake; $CaCl_2$ Concentration (mM))

d: Selected Correlations Among Intakes and Preferences of Each Concentration of NH_4Cl

	Preference			
NH_4Cl Concentration (mM)	10	30	100	300
10	0.75*	0.86*	0.82*	0.45
30	0.87*	0.80*	0.84*	0.42
100	0.78*	0.85*	0.89*	0.63*
300	0.35	0.38	0.63*	0.96*
	10	30	100	300

(Left axis: Intake; Right axis values: 10, 30, 100, 300 Preference; Bottom axis: Intake; NH_4Cl Concentration (mM))

*
Notes: $p < .01$. The lower left of each table shows correlations between intakes. The upper right shows correlations between preferences. Values on the upper left-to-lower right diagonal show the correlation of intakes with preferences. The correlation matrix of all 123 measures collected for the 17 taste solutions tested is available on our website (Tordoff and Bachmanov).