Generating concentration-response functions for taste stimuli and identifying taste phenotypes through the TāStation®: an automated high throughput system for the measurement of human taste

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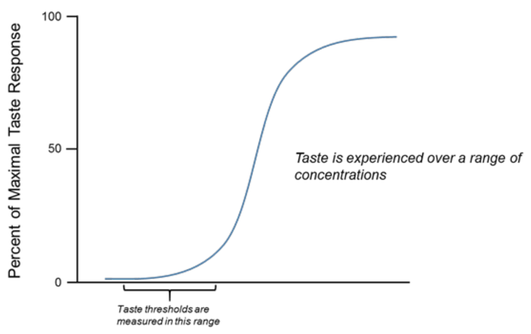
ABSTRACT

Taste is a chemosensory phenomenon mediated by receptors expressed in highly specialized cells of the tongue. As such, taste responses in principle should be amenable to the methods of pharmacology for quantitative analysis of receptor-mediated processes. Critical to any pharmacologic analysis is the establishment of robust concentration-response functions. Applying the principles of pharmacology to the study of human taste has been impeded by prevalent low-throughput methodologies that rely upon large numbers of subjects subjectively evaluating a few samples at a time. The TāStation® is a taste measurement apparatus and methodology for high throughput operant taste discrimination with human subjects. Small volume (200 ul) samples of tastant solutions are randomly selected and robotically drawn from a 96-well plate, then presented to a subject who is trained using a game-like algorithm to distinguish among taste stimuli with high acuity and consistency. Subjects learn to associate different taste standards with target coordinates through trial-and-error, rewarded for correct responses with points and penalized for incorrect responses with point reduction. Because every trial has a consequence, the TāStation® shapes subjects' behavior and performance quickly within a single test session. Using this approach we established robust concentrations-response functions for individual subjects within single test sessions. The 8x12 matrix of the 96-well plate is a practical format for arranging tastant solutions in concentration ranges with multiple replicates, and a subject can sample all 96 wells within a 40-minute test session. Thus robust concentration-response functions for any, and multiple, tastants can be generated quickly to yield precise EC50 values, quantitatively characterizing taste sensitivities for each individual subject. This allows for the identification of human taste phenotypes (non-tasters, weak, strong, etc…) for particular characteristics of a variety of tastants, such as sweeteners and bitter ingredients. Future plans to accompany this research include genotype testing to target specific taste receptors.

TASTE AS A FUNCTION OF CONCENTRATION 1

The intensity of the taste of any chemical is a function of its concentration. Although concentration-dependence can be suggested by measuring taste responses at the lowest "threshold" concentrations, only by establishing the ***concentration-response function*** can the full effect of tastants as sensory stimuli be fully appreciated and rigorously evaluated. For example, taste responses at threshold concentrations provide little, if any, insight regarding the taste sensations resulting from the high concentrations of a tastants normally encountered in food, beverages, or liquid medications.

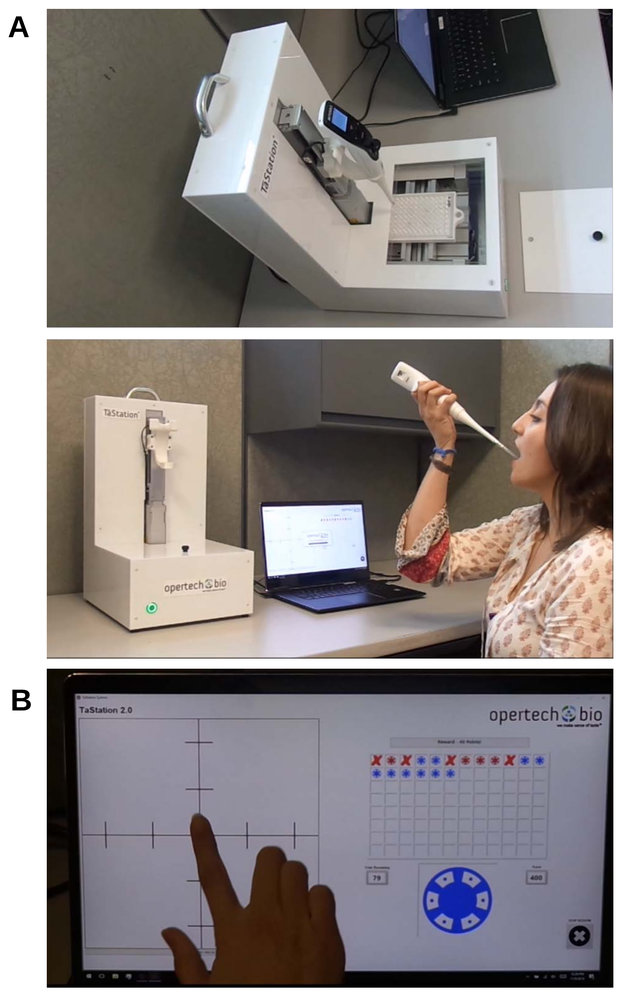
The Concentration-Response Function Provides a Thorough and Rigorous Representation of Taste

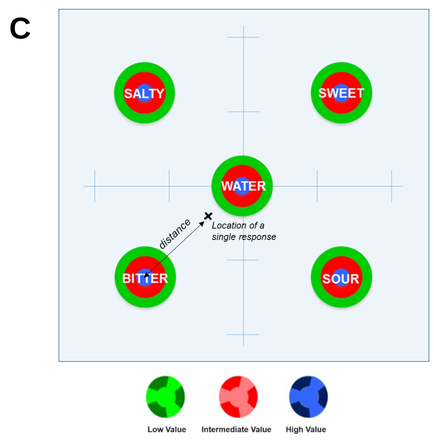


Concentration-response Functions and EC50s: The EC50, derived from a curve fit to the data by non-linear regression, represents the concentration of each tastant that elicits a half-maximal taste response and is a convenient metric for estimating placement of the concentration range in which the compound is taste-active.

Approximating the Maximal Taste Response from the Concentration-response Function: The top of the curve in the figure represents the approximate maximal taste response. The plateau of the curve is due to saturation of tastant receptors. At the lowest concentrations the tastant solution becomes difficult to distinguish from water, and therefore provides a reliable estimate of the taste threshold.

AUTOMATED SYSTEM FOR HIGH THROUGHPUT MEASUREMENT OF HUMAN TASTE 2

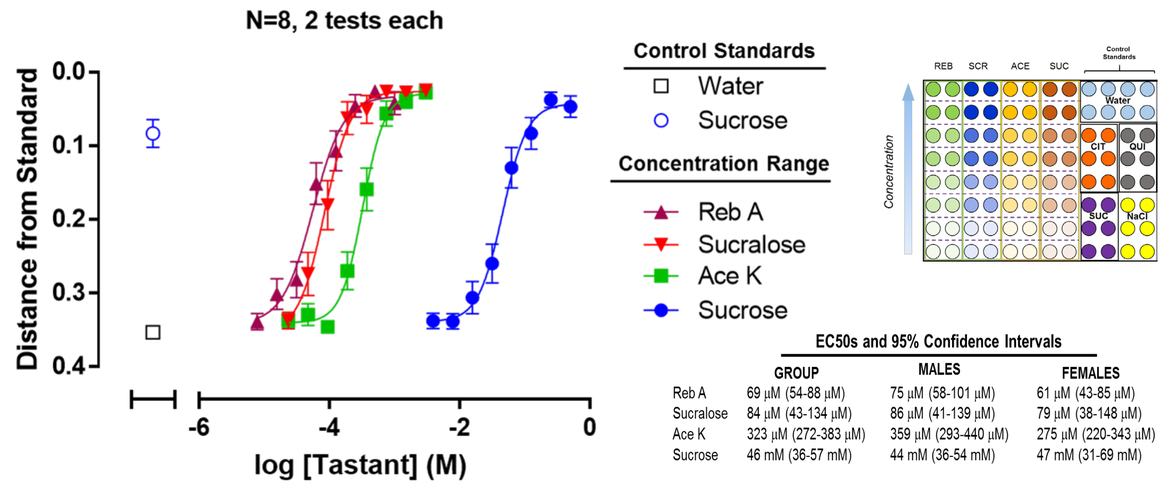




A) Robotic gantry moves an automated pipette over a 96-well plate. The pipette is lowered into a randomly selected well and withdraws a fixed volume of 200 ul. The subject is instructed by the algorithm to remove the pipette and self-administer the content of the pipette to the tongue.
B) Subjects search for poker chips buried in a visual field; the taste stimulus is a clue to their location. The subject touches the screen at a location guided by the taste of the antecedent stimulus. Response-reinforcement contingency is absolute on control trials (taste standards). On test article trials—those for novel stimuli—all responses are reinforced.
C) The distance between the coordinates of the subject's response and the ideal coordinates of the target is measured and recorded as the datum.

CONCLUSION: The TāStation® was designed to have the throughput capacity for generating robust concentration-response data. The small volumes and the performance incentive help to minimize or eliminate taste desensitization, allowing a subject to effectively test many samples in a short period of time. Since so much data is generated by each subject in a test session, fewer subjects are needed for statistical power than is the case for traditional taste panels. Furthermore, the incentive structure of the game-like testing approach tends to reduce the variability of responses to replicate trials both within and across test sessions, resulting in a more reliable and repeatable dataset. The data obtained by use of the TāStation® are readily amenable to mathematical curve-fitting techniques such as non-linear regression mentioned above with all the attendant benefit for analysis of concentration-response functions for taste. Furthermore, the ability to effectively evaluate small sample sizes afforded by the TāStation® technology permits the cost-effective evaluation of ingredients that might be expensive or in short supply, such as novel natural products. The TāStation® technology also is ideal for rapid screening of ingredient libraries for taste properties of interest.

RAPID GENERATION OF CONCENTRATION-RESPONSE FUNCTIONS FOR FOUR SWEETENERS 3

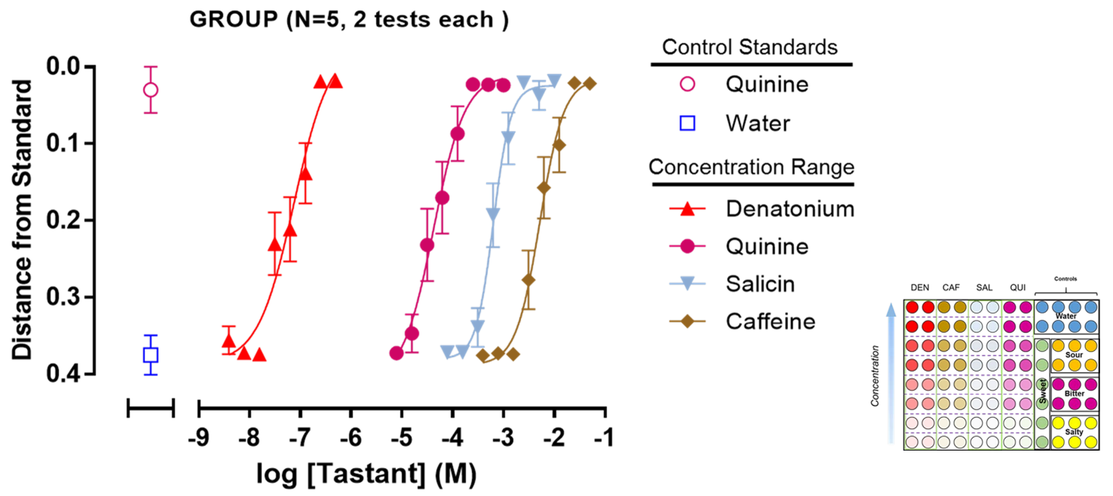


EC50s and 95% Confidence Intervals

GROUP	MALES	FEMALES
Reb A	69 µM (54-88 µM)	75 µM (58-101 µM)	61 µM (43-85 µM)
Sucralose	84 µM (43-134 µM)	86 µM (41-139 µM)	79 µM (38-148 µM)
Ace K	323 µM (272-383 µM)	359 µM (293-440 µM)	275 µM (220-343 µM)
Sucrose	46 mM (36-57 mM)	44 mM (36-54 mM)	47 mM (31-69 mM)

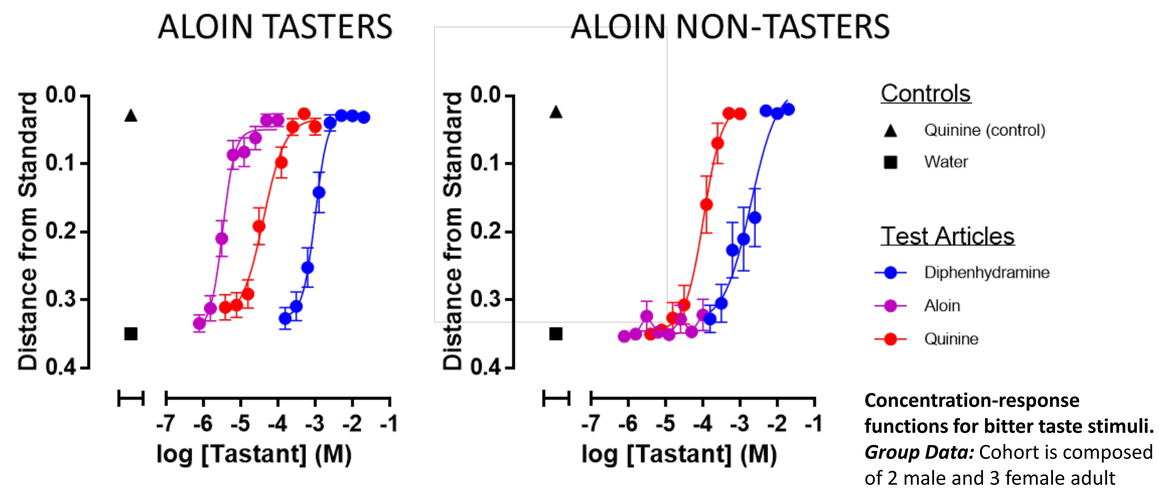
Concentration-response functions for sweet taste stimuli. A cohort of 4 male and 4 female adult subjects was trained and tested as described in Figure 2C. Data are plotted as described in the figure above except that among the control standards, only data for sucrose and water are shown. Curves were fit to the data points by non-linear regression to generate concentration-response function for each of the tastants. EC50s were derived from the curve fits.

MULTIPLE CONCENTRATION-RESPONSE FUNCTIONS FOR BITTER TASTANTS 4



Concentration-response functions for bitter taste stimuli. Cohort is composed of 2 male and 3 female adult subjects. Each data point in the curves was calculated as the average across 20 replicates (2 replicates per concentration x 2 tests x 5 subjects). **EC50s:** Denatonium=78 nM, Quinine=56 uM, Salicin=640 uM, Caffeine=26 mM.

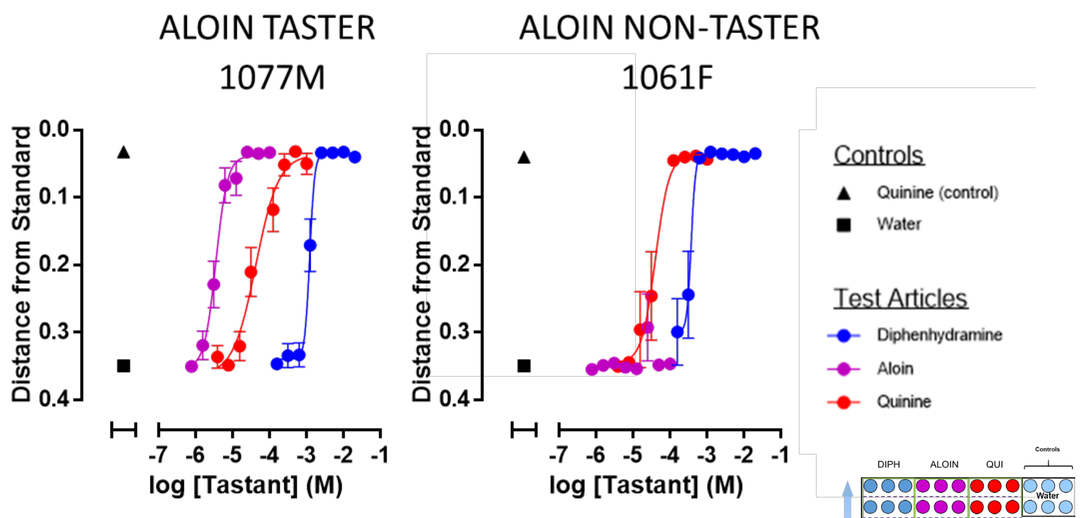
CONCENTRATION-RESPONSE FUNCTIONS FOR BITTER TASTANTS: TASTERS VS. NON-TASTERS 5



	TASTERS		NON-TASTERS	
	EC50 (mM)	95% CI	EC50 (mM)	95% CI
DIPHENHYDRAMINE	1	0.8 - 1.2	1.8	0.8 - 4.2
QUININE	0.041	0.028 - 0.064	0.1	0.07 - 0.15
ALOIN	0.003	0.0025 - 0.004	---	---

Concentration-response functions for bitter taste stimuli. ***Group Data:*** Cohort is composed of 2 male and 3 female adult subjects. Each data point in the curves was calculated as the average across 30 replicates (3 replicates per concentration x 2 tests x 5 subjects).

INDIVIDUAL SUBJECT RESULTS ARE CONSISTENT ACROSS TEST SESSIONS 6



SUBJECT 1077 M

Test Date	12/5/2017	12/12/2017	3/10/2018	3/12/2018	3/13/2018	3/14/2018
	EC50 (mM)					
DIPHENHYDRAMINE	1.2	1.4	1.2	1.3	1.3	0.71
QUININE	0.05	0.06	0.03	0.11	0.05	0.07
ALOIN	0.003	0.006	0.003	0.014	0.003	0.004

Bitter taste concentration-response functions for individual taster and non-taster subjects. Data are plotted as described above for one aloin taster and one non-taster. Each data point represents the average of 6 replicates (3 replicates x 2 tests). In the table below, EC50s are given for 6 consecutive test sessions for subject 1077M.