Automated High Throughput Generation of Concentration-Response Functions for Bitter Taste in Human Subjects

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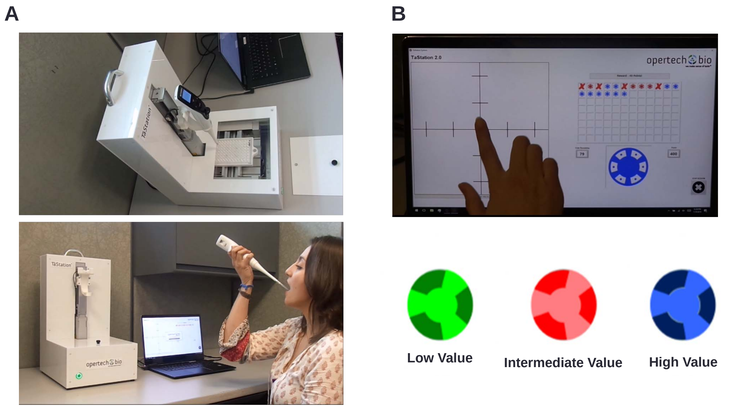
ABSTRACT

Bitter taste in humans is thought to be mediated by a group of approximately 30 GPCRs expressed in type II taste cells. The molecular interactions between bitter tastant and cognate receptor should fundamentally be equivalent to those observed for any other ligand-receptor pair. Bitter taste responses, then, 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 the predominance low-throughput methodologies that rely upon large numbers of subjects subjectively evaluating a few samples at a time. The ability to accurately measure taste responses across multiple trials is a necessity for an efficient concentration-response analysis. In this regard, bitter tastants present a further challenge due to their tendency to impart a temporally prolonged taste stimulus that can carry an impact across trials. We have developed a rapid throughput technology and methodology for human taste measurement, called the TaStation™, with the capacity for establishing robust concentration-response functions for bitter taste within single 45-minute test sessions. Samples of tastant solutions (control standards of sucrose, NaCl, citric acid, quinine and water—representative stimuli for the basic tastes or sweet, salty, sour, and bitter, and neutral, respectively—and novel bitter tastant "test articles") are distributed in a 96-well plate, which is placed on an x-y motion table. At the start of a trial the plate is moved to align a randomly selected well directly beneath an automated pipette mounted on a Z-axis gantry. The pipette is lowered into the well, withdraws 200 ul of solution, and then is presented to a subject seated before a touch-sensitive display. When prompted by a command that appears on the display, the subject removes the pipette from the gantry and self-administers the 200 ul of taste stimulus. After tasting, the subject then is prompted to respond by touching the display. Underlying a visual field on the display is a Cartesian grid in which specific sets of coordinates have been designated to be associated with each of the control standards. Subjects have previously learned in training sessions the locations of these targets through trial-and-error. Correct responses on control standard trials are rewarded by the appearance of virtual poker chips carrying point values, and incorrect responses are penalized by reductions in the point tally. On trials in which test articles (novel bitter tastants) are presented, responses made anywhere on the display are rewarded. Using this approach we rapidly established robust concentrations-response functions for 4 bitter tastants—denatonium, quinine, salicin, and caffeine—in a cohort of 5 adult subjects. Data were plotted as distance of each subject's response from the x-y coordinates assigned to the quinine standard and averaged across all 5 subjects. A nonlinear regression applied to the resulting data points indicated a vast range of potencies among these bitter tastants, with EC50 values of 78 nM, 56 uM, 640 uM, 26 mM, for denatonium, quinine, salicin, and caffeine respectivley. No loss of performance accuracy was detected across trials the 96 trials that might have been expected from cross-trial carry over effects of lingering bitter stimulus. Our results demonstrate the effectiveness of conducting a concentration-response analysis for bitter taste using the TaStation™.

AUTOMATED SYSTEM FOR HIGH THROUGHPUT MEASUREMENT OF HUMAN TASTE 1

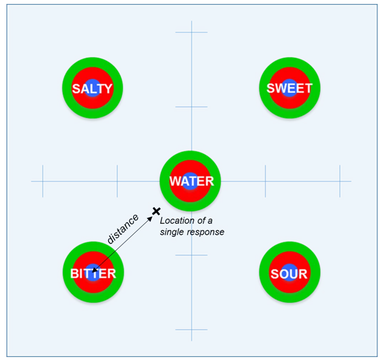


The TāStation® is a portable work station with an automated system for delivering small samples (usually 200 ul) in rapid succession to a seated subject. The subject is not given explicit instruction on how to respond, but is trained through an interactive algorithm to make responses that are dependent on his or her ability to detect and distinguish taste stimuli. The response device is a touch-sensitive monitor. Through the algorithm, which operates like a game, subjects learn to associate specific locations on the monitor with a taste stimulus. Touch responses on the screen are rewarded with an incremental point system that incentivizes both sensory acuity and rate of responding.

A) Robotic gantry lowers an automated pipette into a 96-well plate situated on an x-y motion table. 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 are prompted by the algorithm to 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.

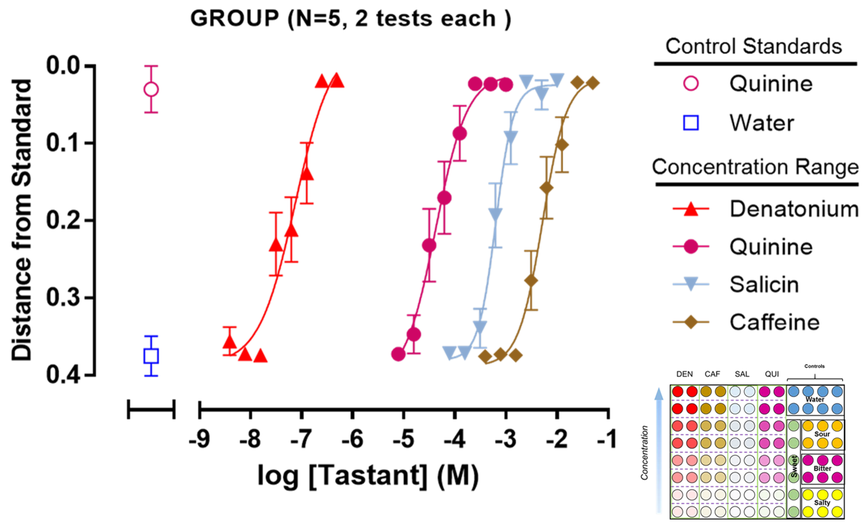
INVISIBLE TARGETS BECOME ASSOCIATED WITH TASTE STANDARDS THROUGH TRAINING 2



The tastes of basic taste stimuli and water are mapped to specific coordinates on a Cartesian grid underlying the display. Targets are invisible to the subject, who must discover their locations through trial-and-error. When subjects reach a criterion of 90% performance accuracy, they are considered "test-ready."

Concentration-response functions are generated by plotting the distance of a subject's response from the ideal coordinates of the standard taste of interest. 96 data points are recorded within a single test session (~40 minutes) for each subject.

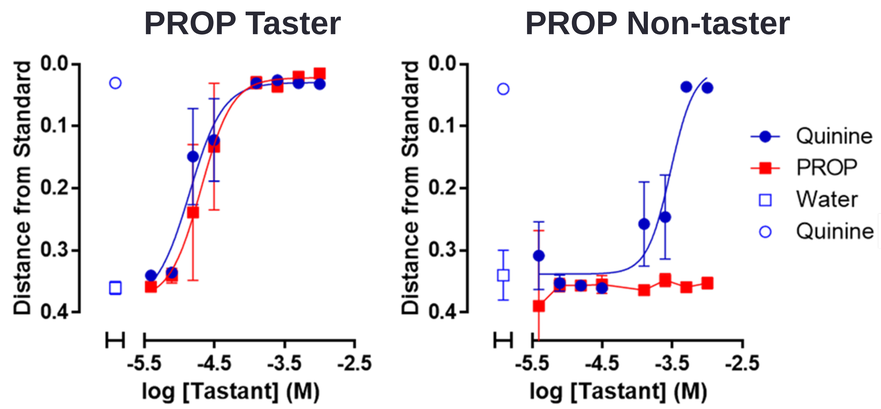
MULTIPLE CONCENTRATION-RESPONSE FUNCTIONS FOR BITTER TASTANTS ARE RAPIDLY GENERATED 3



Concentration-response functions for bitter taste stimuli. ***Group Data:*** Cohort is composed of 2 male and 3 female adult subjects tested with a 96-well plate (see inset lower right) in which concentration ranges of four bitter tastants was dispensed. Data are plotted as distance of each subject's response from the x-y coordinates assigned to the quinine standard. Each data point in the curves was calculated as the average across 20 replicates (2 replicates per concentration x 2 tests x 5 subjects). The results indicate robust concentration-response data across a vast range of potencies among these bitter tastants. ***Concentration-response Functions and EC50s:*** 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.

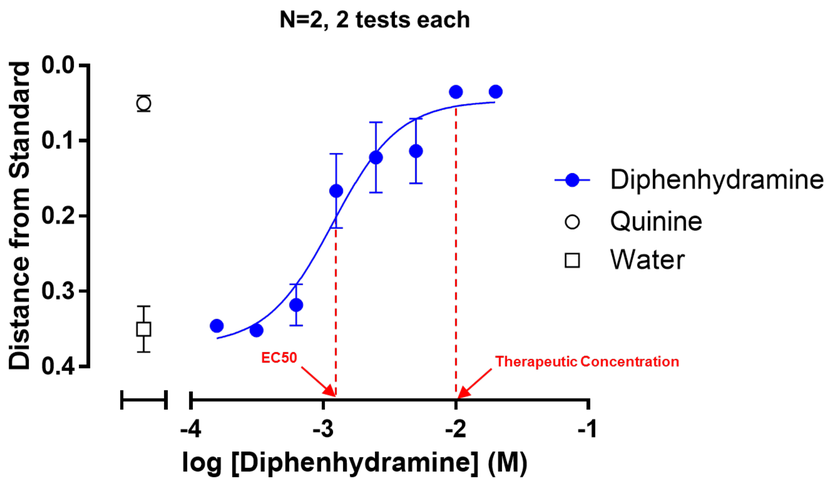
EC50s: Denatonium=78 nM, Quinine=56 uM, Salicin=640 uM, Caffeine=26 mM.

INDIVIDUAL DIFFERENCES IN CONCENTRATION-RESPONSE FUNCTIONS CAN BE QUANTIFIED 4



Concentration-response function for bitter taste of propylthiouracil (PROP) and quinine. PROP taste responsiveness was previously established for each subject by their reaction to a PROP-treated filter paper placed on the tongue. Each subject was tested twice to generate the concentration-response functions shown. Data are plotted as the distance from the ideal coordinates for the bitter standard (0.5 mM quinine). Each data point in the curves was calculated as the average across 12 replicates (3 replicates per concentration x 2 tests x 2 subjects). Error bars are SEM. Curves were fit to the data points by non-linear regression to generate concentration-response function for each of the tastants.

CONCENTRATION-DEPENDENCE OF BITTER TASTE CAN BE QUANTIFIED FOR PHARMACEUTICALS 5



DIPHENHYDRAMINE EC_{50} = 1.2 mM ($CI_{95\%}$ = 0.7 – 1.9 mM)

Total pharmaceutical exposure per test = 6 mg
Single therapeutic dose = 25 mg

Concentration-response function for bitter taste of the antihistamine diphenhydramine. *1* male and 1 female adult subjects each were tested twice to generate the concentration-response functions shown. Data are plotted as the distance from the ideal coordinates for the bitter standard (0.5 mM quinine). Each data point in the curves was calculated as the average across 12 replicates (3 replicates per concentration x 2 tests x 2 subjects). Error bars are SEM. ***Concentration-response Functions and EC50s:*** Curves were fit to the data points by non-linear regression to generate concentration-response function for each of the tastants. EC50s (and 95% CI) were derived from the curve fits.