P604/320

Rapid Throughput Measurement of Sweet Taste Using Human Subjects.

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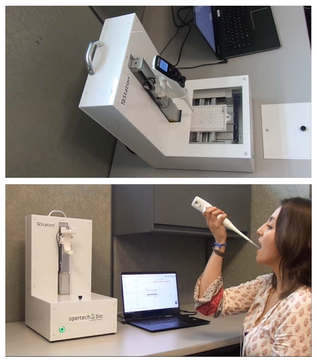
ABSTRACT

We previously reported (AChemS 2016) the development of an automated high throughput system for human taste measurement in which responses to taste stimuli are objectively measured on each of 96 trials in single 40-minute test sessions. Rapid sample delivery is achieved by an automated pipette that draws 0.2 ml of solution from a single well of a 96-well plate. In the most recent iteration of the technology an x-y motion table positions the plate beneath the pipette, which is mounted on a z-axis gantry. As before, subjects are trained by an interactive algorithm to determine whether novel tastant samples, administered from the pipette, can be distinguished from a set of control standards. Subjects are trained to associate the tastes of control standards with coordinates on a touch-sensitive display. On control standard trials subjects are rewarded by the appearance on the display of a poker chip (with remunerative value) if their response is within a specified radius of the coordinates designated for that particular control, and penalized by a point reduction/time-out if outside the radius. On trials of novel samples, all responses regardless of location are rewarded. Using this approach we established robust concentrations-response functions for the taste of 4 sweeteners in a single test of 4 subjects (2 male, 2 female). Nonlinear regression of data yielded taste EC50s of 0.17, 0.19, 0.85, and 45 mM for rebaudioside A, sucralose, acesulfame potassium, and sucrose, respectively. Sweetener potencies did not differ between male and female subjects. Response accuracy on control trials remained above 95% across the 96 trials of all tests, indicating absence of desensitization. Our results demonstrate the efficiency of the new technology for rapid characterization of sweetener taste.

AUTOMATED SYSTEM FOR HIGH THROUGHPUT MEASUREMENT OF HUMAN TASTE 1

A



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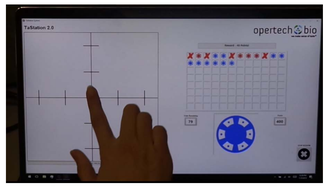
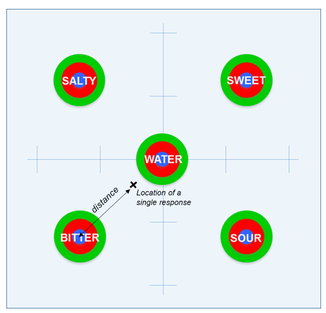


C

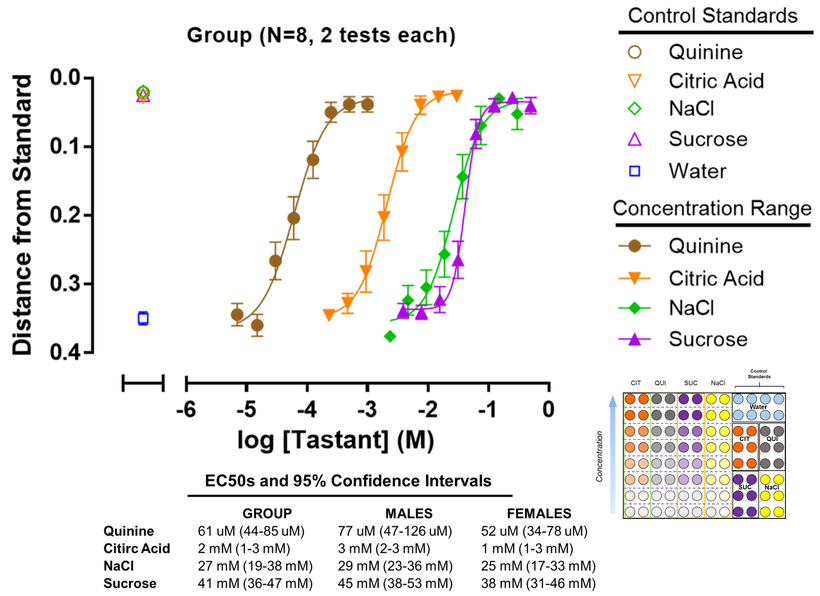


A) Robotic gantry moves an automated pipette over a 96-well plate. The pipette is lowered into a randomly selected well and withdraws a fixed volume of 200 ul. The subject is instructed by the algorithm to remove the pipette and self-administer the content of the pipette to the tongue.

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.

RAPID GENERATION OF CONCENTRATION-RESPONSE FUNCTIONS FOR BASIC TASTE STIMULI 2

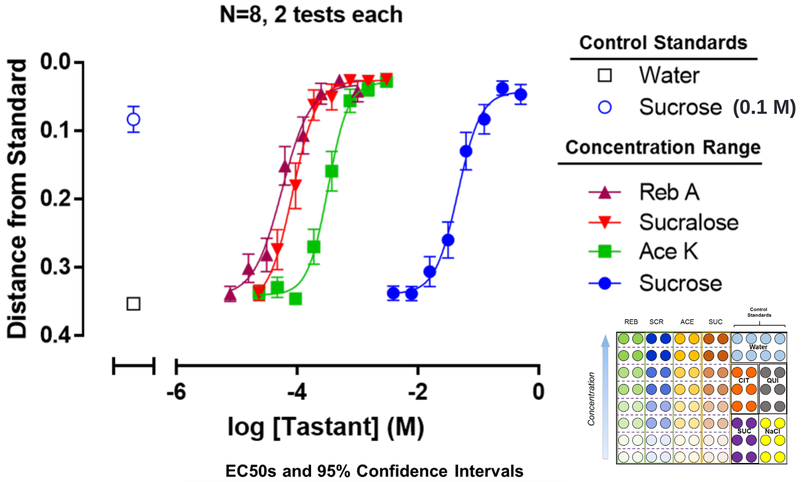


EC50s and 95% Confidence Intervals

	GROUP	MALES	FEMALES
Quinine	61 uM (44-85 uM)	77 uM (47-126 uM)	52 uM (34-78 uM)
Citirc Acid	2 mM (1-3 mM)	3 mM (2-3 mM)	1 mM (1-3 mM)
NaCl	27 mM (19-38 mM)	29 mM (23-36 mM)	25 mM (17-33 mM)
Sucrose	41 mM (36-47 mM)	45 mM (38-53 mM)	38 mM (31-46 mM)

Concentration-response functions for basic taste stimuli. Each data point in the curves was calculated as the average across 32 replicates (2 replicates per test x 2 tests x 8 subjects). Point for control standard of water was calculated as the average across 128 replicates (8 replicates x 2 tests x 8 subjects), and 96 replicates (6 replicates x 2 tests X 8 subjects) for points representing responses to the control standards of basic taste stimuli (0. 5 mM quinine, 10 mM citric acid, 100 mM NaCl, and 100 mM sucrose). Error bars are SEM. ***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 and given in the table above.

RAPID GENERATION OF CONCENTRATION-RESPONSE FUNCTIONS FOR FOUR SWEETENERS 3

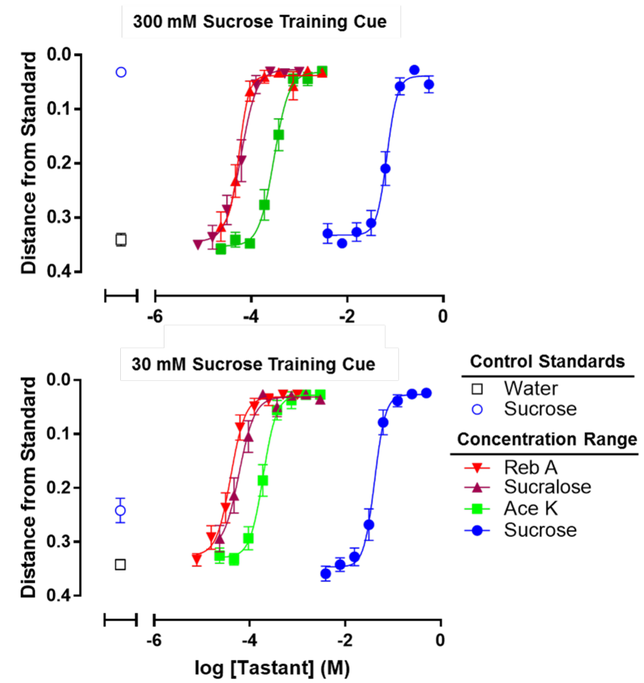


EC50s and 95% Confidence Intervals

	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)

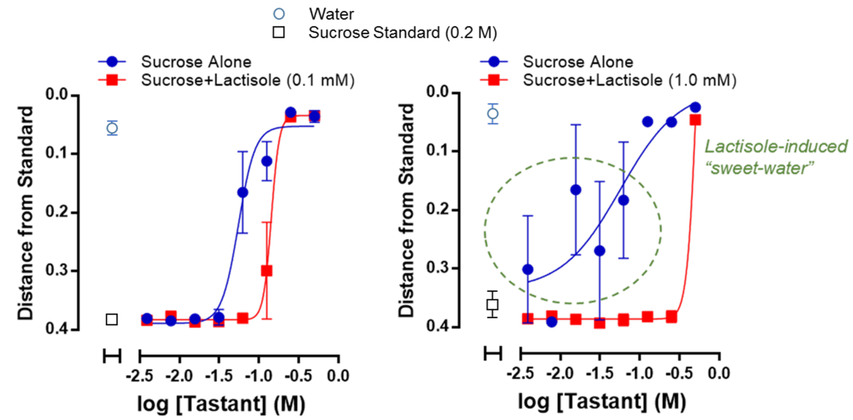
Concentration-response functions for basic taste stimuli. A cohort of 4 male and 4 female adult subjects was trained and tested as described in Figure 2. Data are plotted as described in Figure 2 except that among the control standards, only data for sucrose and water are shown.

CONCENTRATION OF SUPRATHRESHOLD TRAINING CUE DOES NOT IMPACT CONCENTRATION-RESPONSE FUNCTIONS 4



Concentration-response functions for sweeteners generated after training with 300 mM or 30 mM sucrose as the sweet discriminative cue. A cohort composed of 4 male and 4 female adult subjects was trained with either 300 mM or 30 mM sucrose as the discriminative standard cue. Otherwise all conditions are as described in Figure 3. Although all subjects achieved greater than 90% accuracy in two consecutive training sessions with 30 mM sucrose before being tested, performance accuracy on control standard trials of 30 mM sucrose was substantially impacted during the tests; in contrast, concentration-response functions for the sweeteners remained stable regardless of prior sucrose training cue concentration.

ANTAGONISM OF SUCROSE RESPONSE BY LACTISOLE IN A SINGLE SUBJECT: APPEARANCE OF "SWEET-WATER" EFFECT 5



Concentration-response functions for sucrose alone and in the presence of lactisole. A single adult male subject was trained as described in Figure 2. To each concentration of sucrose in the indicated concentration-response curve was added either 0.1 mM lactisole (left panel) or 1.0 mM lactisole (right panel). The activity of lactisole on sweet taste responses has been demonstrated to be consistent with inverse agonism (Galindo-Cuspinera et al (2006): Nature **441**, 354-357). Data shown are from the single subject tested twice. Each data point in the curves was averaged across 8 replicates (4 replicates x 2 tests).