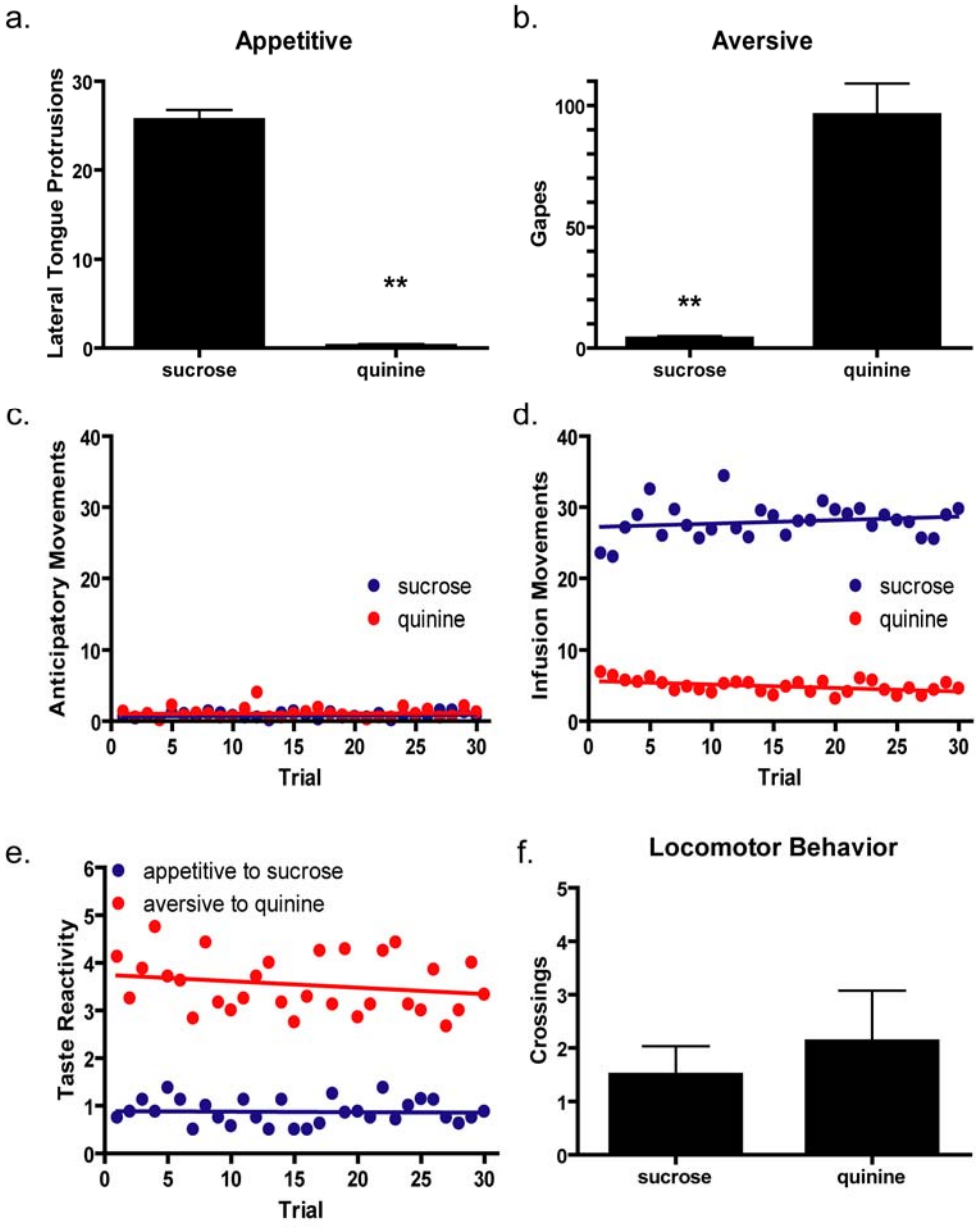
Supplementary Figures



Supplementary Figure 1. Behavioral responses to intra-oral infusions of sucrose and quinine. **a**. Intra-oral infusions of a 0.3M sucrose solution evoked significantly more appetitive oro-facial responses than a 0.001M quinine solution [$t(7)=17.795$, $p<.05$]. **b**. Intra-oral infusions of a 0.001M quinine solution evoked significantly more aversive oro-facial responses than a 0.3M sucrose solution [$t(7)= -6.546$, $p<.05$]. **c**. Rats show no behavioral evidence of predicting intra-oral infusions. For each taste solution, the number of oro-facial responses (licks+lateral tongue protrusions+gapes) was calculated for the 6s preceding each intra-oral solution of the 30 trial block. The slope of the regression line for oro-facial responses across trials was not significantly different from 0 for either sucrose or quinine sessions. In addition, the y-intercepts of the regression lines for sucrose and quinine were not significantly different. The data indicate that rats could not predict the timing of intra-oral infusions of either sucrose or quinine. **d**. Rats

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show no behavioral evidence of learning (e.g. habituation). For each taste solution, the number of oro-facial responses (licks+lateral tongue protrusions+gapes) was calculated for the 6s after infusion onset for each intra-oral solution of the 30 trial block. The slope of the regression line for oro-facial responses across trials was not significantly different from 0 for either sucrose or quinine sessions. However, the y-intercept of the regression line for sucrose was significantly higher than that for quinine ($p<0.0001$) indicating more oro-facial responses were evoked by sucrose than by quinine. **e**. Hedonic reactivity to sucrose and quinine does not change over the course of behavioral session. For sucrose, the number of appetitive (lateral tongue protrusions) oro-facial responses was calculated for the 6s after infusion onset for each intra-oral solution of the 30 trial block. For quinine, the number of aversive (gapes) oro-facial responses was calculated for the 6s after infusion onset for each intra-oral solution of the 30 trial block. The slope of the regression line for oro-facial responses across trials was not significantly different from 0 for either sucrose or quinine sessions. **f**. To determine if intra-oral infusions of sucrose and quinine differentially evoked locomotor responses, the number of quadrant crossings for the 6s after infusion onset were calculated. The number of quadrant crossings during sucrose and quinine infusions were not significantly different ($p>.05$).

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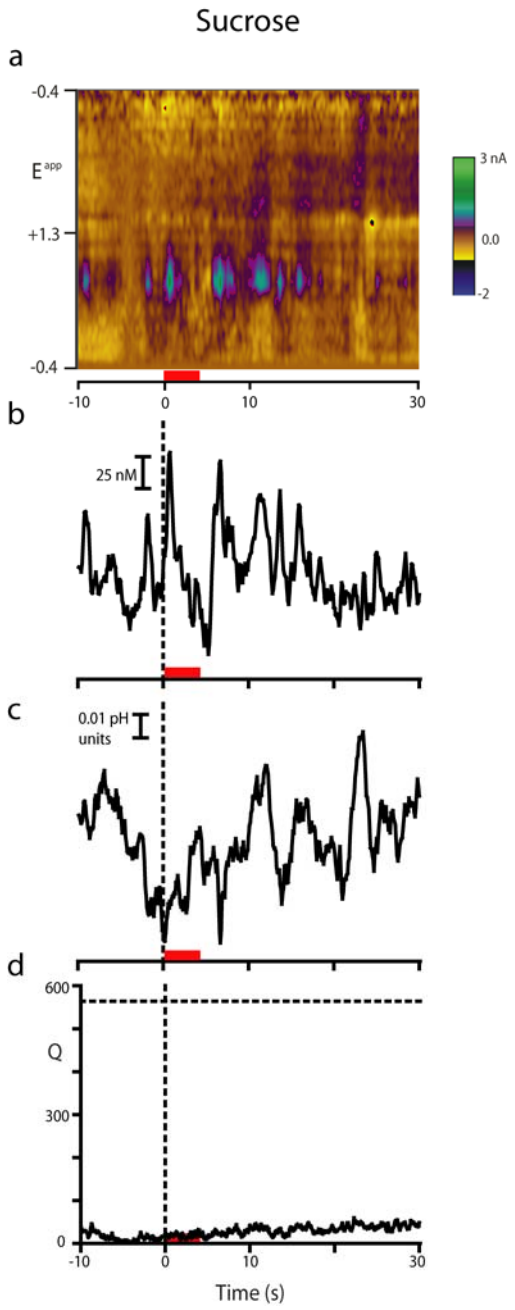

Supplementary Figure 2. Dopamine release events during a representative sucrose infusion trial. **a**. Voltammetric recording during the 10s before, 4s of, and 26s after an intra-oral infusion of 0.3M sucrose. Time is the abscissa, the electrode potential is the ordinate and current changes are encoded in color. Several dopamine (the green/purple oxidation features (~0.6V) and the yellow reduction features (~-0.2V on the negative going scan)) release events are observed throughout the recording. PCR reveals rapid, transient fluctuations in dopamine concentration (**b**) and pH (**c**) over the 40s trial. **d**. The residual (Q) associated with the collected data is well below the 95% confidence limit indicated by the dashed horizontal line. In all figures, the red bar denotes the duration of the intra-oral infusion.

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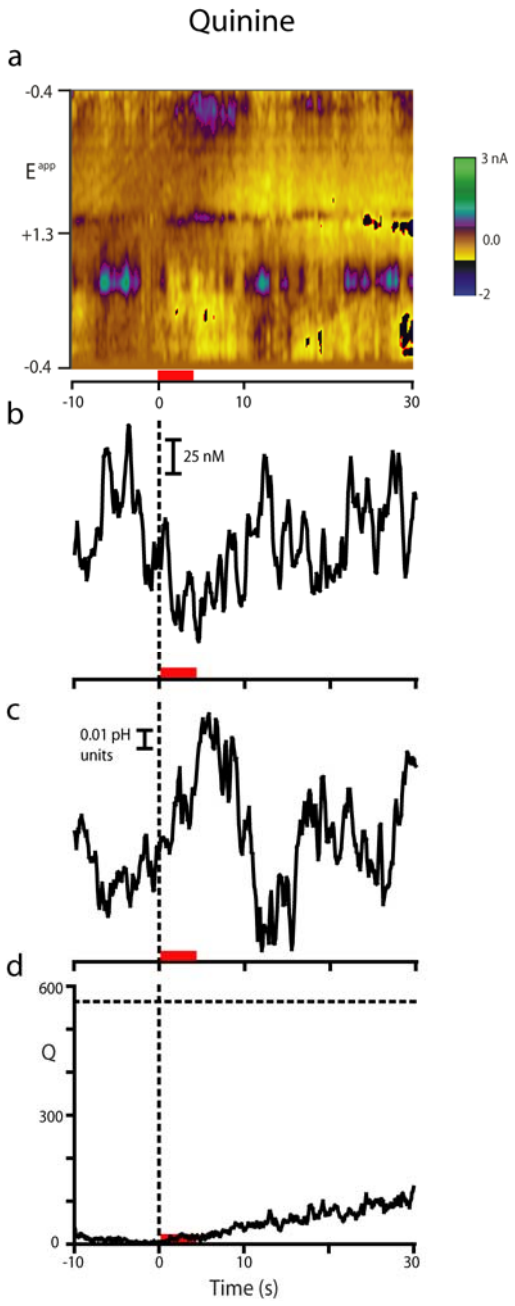

Supplementary Figure 3. Dopamine release events during a representative quinine infusion trial. **a**. Voltammetric recording during the 10s before, 4s of and 26s after an intra-oral infusion of 0.001M quinine. Time is the abscissa, the electrode potential is the ordinate and current changes are encoded in color. While dopamine (the green/purple oxidation features (~0.6V) and the yellow reduction features (~-0.2V on the negative going scan)) release events are observed throughout the recording, there is an absence of dopamine release events during the intra-oral infusion. PCR reveals rapid, transient fluctuations in dopamine concentration (**b**) and pH (**c**) over the 40s trial. **d**. The residual (Q) associated with the collected data is well below the 95% confidence limit indicated by the dashed horizontal line. In all figures, the red bar denotes the duration of the intra-oral infusion.

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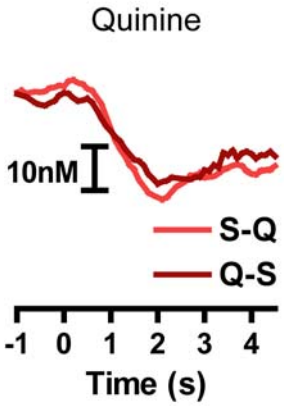

Supplementary Figure 4. The dopamine response to intra-oral infusions of quinine is not dependent on order of stimulus delivery. Intra-oral infusions of a 0.001M quinine solution evoked a similar decrease in dopamine in rats receiving a block of sucrose infusions preceding the block of quinine infusions (S-Q) and those receiving the block of quinine infusions before a block of sucrose infusions (Q-S).

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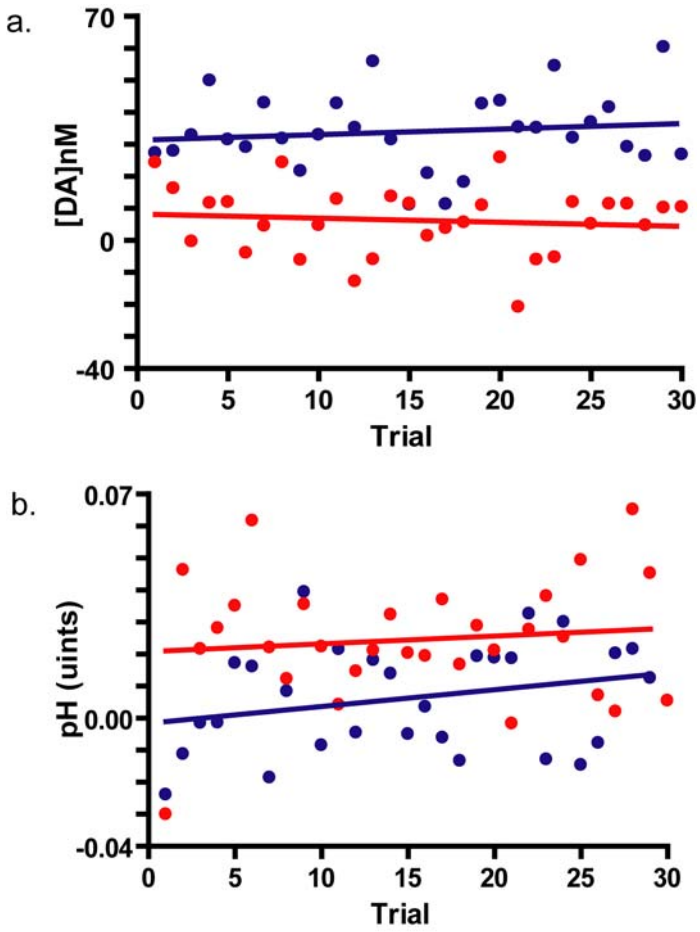

Supplementary Figure 5. Dopamine and pH responses to intra-oral infusions of sucrose and quinine do not change as a function of experience. **a**. Dopamine concentration changes were averaged for the 4.5s after infusion onset to capture the dopamine response to the infusion itself and plotted as a function of trial number. The slopes of the regression lines for sucrose (blue) and quinine (red) were not significantly different from 0. However, the y-intercepts for sucrose and quinine were significantly different indicating that sucrose evoked a significantly higher dopamine concentration than quinine across all trials ($F(1,57)=88.2$; $p<0.0001$). **b**. pH changes were averaged for the 30s after infusion onset to capture the pH response to the infusion and plotted as a function of trial number. The slopes of the regression lines for sucrose (blue) and quinine (red) were not significantly different from 0. However, the y-intercepts for sucrose and quinine were significantly different indicating that quinine evoked a significantly higher increase in pH ($F(1,273)=11.2$; $p<0.001$).

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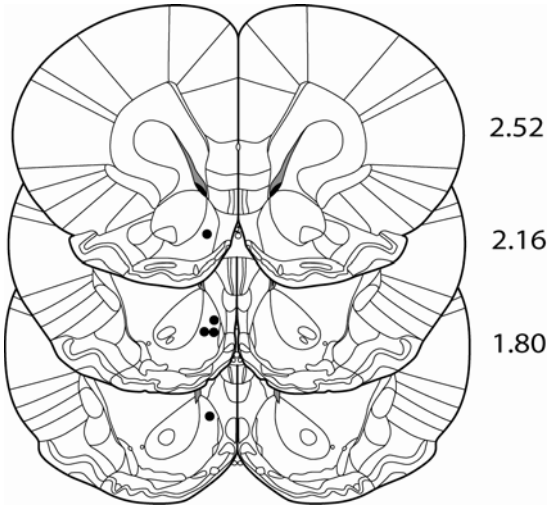

Supplementary Figure 6. Anatomical distribution of carbon-fiber electrode placements in the NAc shell. Coronal diagrams show electrode tip locations for 5 rats. Numbers to the right indicate anteroposterior coordinates rostral to bregma. Coordinates were taken from a stereotaxic atlas [4].

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Supplementary Methods

Behavioral preparation

Eight naïve male Sprague-Dawley rats (300-350g) were individually housed with ad libitum food and water with a 12/12 hr light/dark cycle (lights on at 7:00 a.m.). All experiments were conducted in the light phase between 10:00 a.m. and 6:00 p.m. All procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee. Rats were anesthetized with a ketamine (100mg/kg)-xylazine (20mg/kg) mixture and bilaterally implanted with intraoral cannulae. Each cannula consisted of an approximately 6cm length of PE-100 tubing which was phalanged at one end with a Teflon washer. The cannula was inserted just lateral to the first maxillary molar with the Teflon washer flush against the molar. The other end was exteriorized out the incision at the top of the head and held in place with a second Teflon washer. Rats were permitted at least 1 week to recover from surgery.

Behavioral measurements and procedure

One week after surgery, rats had recovered to pre-surgery body weight and behavioral measurements were made. Rats were placed in a standard operant chamber (Med Associates, St. Albans, VT). At the start of the behavioral session, white noise was administered and a house light was illuminated. After a variable inter-trial interval with an average duration of 120s (110-130s), a solenoid valve was opened and a taste solution was gravity fed through the intra-oral catheter. The solenoid remained open for 4s delivering 200µl per trial. After the solenoid closed, another inter-trial interval was randomly selected. Rats received 30 trials of one followed by 30 trials of the other taste solution. The order of presentation of 0.3M sucrose and 0.001M quinine was counterbalanced across rats. Hedonic impact was evaluated by video analysis using the taste reactivity test.

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Taste reactivity was analyzed in a frame-by-frame analysis using digital video recorded on the test day. Appetitive and aversive taste reactivity was counted for 6 sec prior to infusion onset to 6 sec after infusion onset using the technique of Grill and Norgren [1]. Mouth movements that matched the 'triangle' shape for a duration exceeding 90 ms were counted as aversive. These criteria excluded all neutral and ingestive mouth movements which were counted separately. Instances in which the tongue protruded and crossed the midline were counted as appetitive. The remaining licking behavior was counted as "neutral" licking. Counts for each animal were analyzed with a paired t-test for both appetitive taste reactivity and aversive taste reactivity. Statistical analyses of all behavioral data were performed using commercially available software (SPSS, Tulsa, OK). Locomotor behavior was analyzed in real-time video analysis using digital video recorded on the test day. The chamber was divided into 4 equal quadrants and the rat's location was noted from infusion onset for 6 sec, as above. Counts of quadrant crosses per trial for each condition were compared with a paired t-test.

Voltammetry preparation

Measurements of dopamine concentration in the nucleus accumbens in response to rewarding and aversive stimuli have been made with microdialysis techniques [2]. Most microdialysis studies, though, require very low sampling frequencies (typically, 1 sample every 10-20min). Thus, it becomes a challenge to determine whether fluctuations in dopamine are due to rewarding and aversive stimuli themselves or the cessation of stimulus exposure. Moreover, the slow sampling frequency makes for a difficult comparison with electrophysiological studies. The sampling frequency of voltammetry permitted the capture of dopamine fluctuations from baseline *during* intra-oral infusions. Naïve male Sprague-Dawley rats (n=5) were singly housed on a 12:12 light:dark schedule with water and food available *ad libitum*. All procedures were approved by the University of Illinois, Chicago Institutional Animal Care and Use Committee. To prepare rats for intra-oral infusions and voltammetric recordings, they were anesthetized with

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ketamine hydrochloride (100 mg/kg, i.m.) and xylazine hydrochloride (20 mg/kg, i.m.), implanted with bilateral intraoral catheters [3] just lateral to the first maxillary molar and were placed in a stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IL) over the NAc shell (AP: +1.7mm, ML: +0.9mm, relative to bregma, [4]) and an Ag/AgCl reference electrode placed contralateral to the guide cannula were also implanted. The NAc shell was targeted as this region of the NAc appears to be particularly important for feeding behavior [5]. Stainless steel skull screws and dental cement were used to secure all items. A bipolar stimulating electrode was then placed just dorsal to the ventral tegmental area (-5.2 AP, 1.0 ML from bregma and 7 mm ventral from the dural surface). A detachable micromanipulator containing a glass-sealed carbon-fiber electrode (75-100 μm exposed tip length, 7 μm diameter, Goodfellow, Oakdale, PA) inserted into the guide cannula, and the electrode was lowered into the nucleus accumbens core. The bipolar stimulating electrode was then lowered in 0.2mm increments until electrically evoked dopamine release was detected at the carbon-fiber electrode in response to a stimulation train (60 biphasic pulses, 60 Hz, 120 μA, 2 ms per phase). The stimulating electrode was then fixed with dental cement and the carbon-fiber electrode was removed.

Voltammetric measurements and behavioral session

Following surgery, animals were allowed one week to recover pre-surgery body weight. After recovery from surgery, voltammetric recordings were made during the behavioral session. Rats were placed in a standard operant chamber (Med Associates, St. Albans, VT) and tethered to a headstage for voltammetric recordings (for details, see [6]). A fresh carbon fiber electrode was lowered into the NAc shell. The fiber was held at -0.4 V against Ag/AgCl between scans and then driven to +1.3 V and back in a triangular fashion at 400 V/s for each voltammetric measurement [7]. The application of this triangle waveform causes oxidation and reduction of chemical species that are electroactive within this potential range, producing a change in current at the carbon-fiber. Specific analytes (including dopamine and pH) are identified by plotting

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these changes in current against the applied potential to produce a cyclic voltammogram [8]. The current arising from electrode processes (double-layer charging, etc.,) was removed by using background-subtraction. For data collected during the behavioral session, the background period (1000 ms) was taken as the minima during the 10 s before tastant administration (i.e., during the baseline period, see below). This practice does not subtract the presence of phasic dopamine release events because the background was explicitly selected for the absence of fast dopamine signals. Measurements were made every 100ms and, after driving the electrode into the NAc shell, the electrode equilibrated for 40 min before any data were collected. The position of the microelectrode was then optimized by monitoring electrically evoked (biphasic, 2 ms per phase, 24 pulses, 60 Hz, 120 μA) dopamine release. Experiments were conducted when an electrode placement yielded robust (>30:1 signal to noise) electrically evoked dopamine release. We used an additional criterion of the detection of spontaneously occurring dopamine release events as well [9-11]. Once criteria were met, the electrode was locked in place. Several stimulation trains that varied in number of pulses (1, 2, 5, 10 and 20) were administered for the generation of a training set for principal component analysis for the detection of dopamine and pH changes during the behavioral session (see below). A fluid line was then attached to the intra-oral cannula and the same behavioral session described above was started.

Signal identification

Analyte identification and quantification were achieved using principal component regression (PCR) analysis described in detail elsewhere [8]. Briefly, training sets were generated from background-subtracted cyclic voltammograms collected during and after electrical stimulations. Different number of pulses of stimulation were used at the same recording position (see above). The stimulations evoke an immediate increase in [DA] that rapidly returns to baseline levels followed by a longer lasting increase in pH [12-14]. At least five voltammograms were obtained for each species. The resulting current amplitude was converted to dopamine concentration or pH

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units based on calibration of the electrode in a flow injection analysis system after the *in vivo* experiment. To convert current due to oxidation of dopamine, 1μM dopamine in a buffer (pH 7.4) was used in the flow injection analysis system. To convert current due to oxidation of pH changes, the buffer was adjusted to pH 7.8. Voltammograms used in the training set were reduced by principal component analysis. Usually, only three factors were retained, which captured 99.5% of the variance in the training sets. All data presented here fit the resulting model at the 95% confidence level.

Data Analysis

Data from each trial (-10s prior to and 30s post infusion onset) was background subtracted using a 1s block at the local minima in the 10s prior to infusion onset (baseline period). For each rat, data was averaged across the 30 trials of sucrose and 30 trials of quinine infusions. The resultant current changes over time were analyzed for dopamine and pH changes using PCR. For both dopamine and pH, significant changes over time were evaluated using a two-way ANOVA with Tukey *post hoc* tests for multiple comparisons. Time was categorized into epochs: baseline (-9.9 to 0s relative to infusion onset), infusion (0.1 to 4s of infusion), post10 (4.1 to 10s relative to infusion onset), post 20 (10.1 to 20s relative to infusion onset) and post30 (20.1 to 30s relative to infusion onset).

Dopamine release events occur independent of any overt behavioral stimuli [9-11]. To determine how appetitive and aversive stimuli affected the likelihood of high concentration dopamine release events, every 100ms sample on every trial for each rat was time-stamped if its concentration was 40nM or higher. This threshold is within the range of affinities for high-affinity D1 receptors [15]. In addition, it is the average value of spontaneous dopamine release events [10]. For each solution, the probability of dopamine concentration exceeding 40nM was calculated using commercially available software (NeuroExplorer, Littleton, MA). A two-way ANOVA was

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used to identify main effects of epoch (baseline versus infusion) and solution (sucrose versus quinine). Tukey *post hoc* tests for multiple comparisons were used to identify significant differences within epoch and solution. In all cases the alpha level for significance was 0.05. Statistical comparisons were made using commercially available software (Statistica, Tulsa, OK).

Histological verification of electrode placement. Upon completion of each experiment, rats were deeply anesthetized with a ketamine/xylazine mixture (100 mg/kg and 20 mg/kg, respectively). In order to mark the placement of electrode tips, a stainless steel electrode was driven through the guide cannula to the site of the voltammetric recording and a 100 μA current was passed for 5 seconds. Transcardial perfusions were then performed using physiological saline and 10% formalin, and brains were removed. After post-fixing and freezing, 50 μm coronal brain sections were mounted and stained with cressyl violet to reveal the lesion site. The specific position of individual electrodes was assessed by visual examination of successive coronal sections. Placement of an electrode tip within the NAc shell was determined by examining the relative position of the lesion to visual landmarks (including the anterior commissure and the lateral ventricles) and anatomical organization of the NAc represented in a stereotaxic atlas [4].

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