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Cocaine decreases saccharin preference without altering sweet taste sensitivity

Jennifer K. Roebber[#], Sari Izenwasser^{#,+}, and Nirupa Chaudhari^{*,#}

[#]Graduate Program in Neurosciences, University of Miami Miller School of Medicine, Miami, FL, 33136 USA

⁺Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, Miami, FL, 33136 USA

^{*}Department of Physiology and Biophysics, University of Miami Miller School of Medicine, Miami, FL, 33136 USA

Abstract

In rodents, saccharin consumption is suppressed when the sweet taste stimulus is paired with moderate doses of cocaine. Several hypotheses have been used to explain the seemingly contradictory effect of decreased consumption of a normally preferred substance following a highly rewarding drug. A common theme across these hypotheses is that saccharin is interpreted as less rewarding after cocaine pairing. We considered the alternative possibility that suppression is caused not by a change in reward circuitry, but rather by a change in taste detection, for instance by altering the afferent taste response and decreasing sensitivity to sweet taste stimuli. To evaluate this possibility, we measured saccharin taste sensitivity of mice before and after a standard cocaine-pairing paradigm. We measured taste sensitivity using a brief-access lickometer equipped with multiple concentrations of saccharin solution and established concentration-response curves before and after saccharin-cocaine pairing. Our results indicate that the EC₅₀ for saccharin was unaltered following pairing. Instead, the avidity of licking saccharin, an indicator of motivation, was depressed. Latency to first-lick, a negative indicator of motivation, was also dramatically increased. Thus, our findings are consistent with the interpretation that saccharin-cocaine pairing results in devaluing of the sweet taste reward.

Keywords

Anticipatory contrast; conditioned stimuli; cocaine; taste sensitivity; lickometer; brief access taste test; ACE; saccharin

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Corresponding Author: Address: Nirupa Chaudhari, Ph.D., Department of Physiology and Biophysics, 1600 NW 10th Ave., Miami, FL 33136, USA, Telephone: (305) 243-3187, Fax: (305) 243-5931, NChaudhari@med.miami.edu.

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1 Introduction

Cocaine exposure and consumption of sweet tasting solutions both cause increased dopamine signaling in the nucleus accumbens, leading to pleasurable or rewarding sensations (Avena *et al.*, 2008, Mark *et al.*, 1991, Rada *et al.*, 2005). These two stimuli enhance dopamine signaling through separate mechanisms. Cocaine directly inhibits the dopamine transporter, thereby elevating synaptic dopamine levels. In contrast, sucrose elicits neural circuit activity that indirectly increases dopamine release in the nucleus accumbens (Avena *et al.*, 2008, Norgren *et al.*, 2006, Rada *et al.*, 2005). There also are notable differences in the reward value and specific pathways for sweet stimuli and cocaine. For example, dopamine firing patterns in the nucleus accumbens are usually blunted after repeated access to sweet rewards, but not cocaine (Di Chiara *et al.*, 2004, Rada *et al.*, 2005). In addition, lesion studies have shown that taste circuits in the thalamus and projections from the parabrachial nucleus are essential for sweet- but not cocaine-elicited reward (Grigson *et al.*, 2000, Norgren *et al.*, 2006, Nyland *et al.*, 2012).

It has been known for many years that rodents will avoid intake of a palatable taste cue when it is repeatedly presented prior to an injection of cocaine (Ferrari *et al.*, 1991, Glowa *et al.*, 1994, Grigson, 1997). This appears to cause a paradox where a reinforcing drug of abuse causes avoidance rather than enhanced pursuit of the conditioned stimuli. There are several behavioral hypotheses that seek to explain this observation. For instance, changes in homeostasis following drug injection may cause a 'taste shyness' around the conditioned tastant (Hunt and Amit, 1987). Alternatively, anticipation of the future high drug reward may dampen the value of the less rewarding natural stimulus (Grigson, 1997). Other hypotheses include the idea that the drug is aversive to the mice and induces conditioned taste aversion (Lin *et al.*, 2014) or conditioned disgust (Parker *et al.*, 2008).

Another possibility is that there may be an afferent, taste-driven explanation for the decreased consumption. For example, decreased ability to taste the cue (saccharin) may result in decreased consumption following cocaine exposure and conditioning. Such a mechanism may be quite independent of hedonic value and motivation. It is known that cocaine inhibits several transporter proteins, thus blocking transport, not only of dopamine, but also of the other monoamines, serotonin and norepinephrine (Amara and Sonders, 1998, Mateo *et al.*, 2004). Peripheral taste cells employ both serotonin and norepinephrine as neurotransmitters (Huang *et al.*, 2007, Huang *et al.*, 2005) and the transporters for norepinephrine and serotonin are present and functional on cells of taste buds (Chaudhari and Roper, 2010, Dvoryanchikov *et al.*, 2007). It also has been shown that knockout of both dopamine and serotonin transporters are required to abolish cocaine place preference, even though dopamine remains the primary neurotransmitter underlying the rewarding effects of cocaine (Sora *et al.*, 2001). Similarly, mice with genetic deletion of transporters for serotonin or norepinephrine show altered responses to taste-selective behavior (Jones *et al.*, 2010). Cocaine also has been shown to alter many aspects of appetite and ingestion (Cui and Lutter, 2013, Vicentic and Jones, 2007). Cocaine dependence in humans also causes profound changes in diet, specifically by increasing consumption of fats and carbohydrates, but decreasing sugar intake (Ersche *et al.*, 2013).

If cocaine acutely alters signaling in taste buds, repeated exposures may well result in chronically altered gene expression. Cocaine has been shown to lead to long-term transcriptional alterations through CREB expression (Xu *et al.*, 2013) and chromatin remodeling (Taniguchi *et al.*, 2012). Cocaine could alter taste sensitivity by modifying gene expression in existing and particularly in newly formed taste bud cells, which are replaced every few days (Beidler and Smallman, 1965). In support of this, the frame for cocaine-induced anticipatory contrast is similar to that of taste bud cell turnover. Thus, we considered whether long-term exposure to cocaine might change chemosensory detection and neural signals from the taste periphery.

The goal of the present study was to apply a model commonly used in taste studies but not commonly used in drug studies to measure rodent taste sensitivity before and after saccharin is paired with cocaine to determine if the pairing paradigm causes taste sensitivity to be dampened. We used a brief-access lickometer assay, as it is commonly used to identify taste quality and to separate orosensory-mediated responses from higher order responses (Loney *et al.*, 2012, Mantella and Youngentob, 2014, McCaughey and Glendinning, 2013). This assay has been used to quantify differences of taste sensitivity in mice with mutations in taste-related genes (Tordoff and Ellis, 2013, Treesukosol *et al.*, 2009) and to compare orosensory ability and drug preference for both nicotine (Glatt *et al.*, 2009) and alcohol (Brasser *et al.*, 2012). Brief-access lickometry has been optimized to elicit concentration-dependent licking for different taste stimuli and to effectively separate mice with altered taste function (Glendinning *et al.*, 2002, Sinclair *et al.*, 2014). A brief-access lickometer assay generates a concentration-response curve of the acceptance of a taste stimulus. Importantly, voluntary consumption, preference in a 2-bottle assay, and brief-access lick-rate all are dependent on similar concentrations of the stimulus and display parallel responses (Inoue *et al.*, 2007). A substantial difference between the brief-access lickometer assay and the commonly used two-bottle preference test is that licking responses reflect taste-dependent acceptance or rejection that is minimally influenced by post-ingestive effects. Thus, we used a Davis rig lickometer to determine if detection of a sweet taste stimulus is altered when mice are exposed to a standard saccharin-cocaine pairing paradigm. We administered this brief-access test both before and after cocaine exposure (pre-test vs. post-test, respectively).

2 Materials and methods

2.1 Animals

Sixteen male C57BL/6 mice between 2–4 months of age were individually housed on a 12-hour light/dark cycle with food and water available. All mice were handled according the 2010 NIH guide for the Care and Use of Laboratory Animals^{8th ed.} and all procedures were approved by the University of Miami Institutional Animal Care and Use Committee (IACUC). Male mice were exclusively used to match conditions used in previous studies.

2.2 Drugs and Dosage

Cocaine was obtained from NIDA (Rockville, MD, USA) and was dissolved in 0.9% NaCl. Cocaine (30mg/kg) was injected i.p. for experimental animals while an equal volume

(0.01ml/g) of normal saline was injected for controls. Dosage and procedures for pairing saccharin and cocaine in C57BL/6J mice were followed exactly as described in Freet *et al.* (2013). Saccharin solutions were made by dissolving saccharin sodium salt hydrate (Sigma #S1002) in deionized autoclaved water.

2.3 Water and Food deprivation

Glendinning *et al.* (2002) devised a mild water- and food-restriction paradigm that motivates mice to lick in a Davis Rig Lickometer and optimizes the concentration-dependence of the response (Glendinning *et al.*, 2002). Prior to Lickometer Training Sessions 1 and 2, mice were fully deprived of water for 23.5 hours. This familiarizes the animals to the drinking spout. For all subsequent sessions, mice were subjected to a limited water and food restriction: 1g LabDiet 5001 mouse chow plus 3ml of water for the 23.5 hours prior to testing (Glendinning *et al.*, 2002). All animals were weighed and examined daily for weight loss or behavioral changes associated with dehydration. None of the animals showed a >20% weight drop or sudden increase in lethargy during food or water restriction.

2.4 Lickometer Sessions

Each mouse was placed in a Davis MS-160 “lickometer” (DiLog Instruments Tallahassee, FL) during seven separate sessions. On the first training day, mice were water deprived for 23.5 hours prior to the experiment and placed individually in the lickometer cage for 1 h to familiarize them with the novel environment. A stationary drinking spout was present and the mouse was free to drink water *ad libitum*. Mice were given 1 hour following training to recover in their home cage with full access to food and water before being water deprived for the next session.

On Training Day 2, the mouse was placed in the lickometer cage for 30 minutes and was introduced to a non-stationary drinking tube. During this training session, a bottle of water was presented and the mouse was allowed to drink for five seconds before a shutter prevented further drinking. After a 15 second wait, a new bottle of water was presented.

Training Day 3 serves to familiarize mice with repeated presentations of varying concentrations of saccharin, representing a novel taste (Sinclair *et al.*, 2014). On Training Day 3 and on all test days, seven bottles were filled with either water or a solution of sodium saccharin (0.9mM, 2mM, 7mM, 9mM, 18mM, or 45mM corresponding to 0.018%, 0.041%, 0.18%, 0.37%, 0.92% g/ml, respectively) and were presented in computer-generated random order. Concentrations of saccharin were chosen to span the preferred concentration range in C57BL/6 mice (Bachmanov *et al.*, 2001, Fuller, 1974, Inoue *et al.*, 2007). In all tests, water and saccharin solutions were presented one at a time over a total of 30 min. or for a maximum of 49 presentations, whichever came first. Each presentation comprised of the shutter opening to reveal a drinking spout, a variable latency before the mouse began drinking, and 5 sec of drinking time. Mice were given one day of rest with full hydration after each testing session. Every mouse was tested for all concentrations of saccharin twice prior to cocaine (or saline) exposure (Pre-test 1,2) and twice post-cocaine (or saline) exposure (Post-test 1,2). The post-tests were conducted at least 27.5 hours after the last

cocaine injection. Thus, mice were never tested in the lickometer while under the influence of cocaine. The time course of experiments is outlined in Figure 1.

2.5 Data Analysis: Maximal lick rate and the Standardized Lick Ratio

For every lickometer presentation, we recorded the inter-lick interval (in msec), the total number of licks, the identity of the solution, and the latency interval before the mouse started to drink in each trial. First, we calculated the maximum lick rate for each mouse as previously reported (Glendinning *et al.*, 2002). In brief, the maximum rate for licking water was measured during Training Days 1 and 2, when water was used as a stimulus, and the mouse was maximally dehydrated. This value was used as the mouse's intrinsic maximum rate of oromotor function and motivation to drink. In the subsequent test days, mice were food and water restricted, but not deprived, and is expected to lick at saccharin solutions in a concentration-dependent fashion.

The lick rate for each mouse to a particular solution was averaged across all presentations for that concentration on that test day, and was then normalized to the individual maximum lick rate for that mouse to calculate the Standardized Lick Ratio (SLR) (Glendinning *et al.*, 2002). By normalizing mice to their own respective maximum rate, we controlled for inherent differences between oromotor function across individual mice. For example, if a mouse showed 10 licks/5sec (averaged across 7 presentations on Test Day 1) in response to 9mM saccharin and its maximal lick rate was 40 licks/5 seconds, the resulting SLR would be 0.25. Once normalized, SLR was averaged across two days of testing for each concentration and all mice in the group (e.g. all 8 control mice were averaged across both days of pre-testing) (Figure 3, data points).

Averaged SLR values were then fit in Graphpad Prism with four-parameter dose-response curves (Figure 3, curves).

$$SLR = V_{min} + (V_{max} - V_{min}) / (1 + 10^{[(\log EC_{50} - \text{concentration}) * \text{Hillslope}]})$$

The equation above was used to model upper and lower asymptotes (V_{max} and V_{min}) and to determine the EC_{50} , defined as the concentration that evokes a response half way between V_{max} and V_{min} . In this analysis, V_{max} is the saturation or maximal lick response to saccharin. V_{min} is modeled by the equation, rather than set to zero, because the mice must lick at least once to perform a trial. The fourth parameter that the four-parameter dose response models is the Hill Slope.

Following cocaine exposure, we expected one of two changes to occur in the dose-response curve. If afferent taste sensitivity was decreased following cocaine exposure, we would expect to see a lateral shift to the right, as the mouse fails to differentiate low concentrations of saccharin from water, but can still taste very high concentrations of saccharin. This kind of behavioral output from a lickometer has been illustrated following transection of a subset of the nerves that carry taste responses (St John and Boughter, 2004). Alternatively, a change in V_{max} , signifying decreased avidity would suggest that taste sensitivity is unaltered, but that the hedonic value of the stimulus has changed (Ahmed and Koob, 1998).

2.6 Saccharin-cocaine pairing

Saccharin-cocaine or saccharin-saline pairings were carried out for 10 days as described by Freet *et al.* (2013) and diagrammed in Figure 1. Mice were placed on a water-restricted schedule where they were given access to fluids for 3 hours daily. On alternating days, mice were given either water or 7mM saccharin for 1 hour in the morning (10–11AM). Immediately following the one-hour access to saccharin, half of the mice were injected intraperitoneally (i.p.) with cocaine (30mg/kg), while the remaining mice were injected with an equal volume of saline (all injections were administered in a volume of 0.01ml/g body weight). Water was additionally provided *ad libitum* for 2 hours every afternoon (3–5PM) to prevent excessive dehydration. After the final injection, mice were given full access to food and water for 3 hours and then were subjected to the food and water restriction schedule for lickometer testing. Mice were weighed and examined prior to each session for signs of dehydration.

2.7 Statistical Analysis

Statistical analyses were conducted and graphs were produced in Graphpad Prism v5. Data are presented for seven control mice and eight cocaine-exposed mice. One of the control mice was removed before post-tests due to poor health. Data from this mouse was retained in pairing data (Figure 2). However, since the mouse did not complete concentration-response post-tests, the corresponding data were removed from all concentration-response tests (Figure 3, 4). Averaged standard lick ratios (SLRs) for all concentrations were analyzed using a 2-way ANOVA. Averaged SLRs were then fit to create nonlinear four-parameter dose response curves. These curves modeled maximal and minimal responses for liking saccharin ($V_{\max}$ and $V_{\min}$), as well as the EC_{50} and Hill Slope. To confirm curve fit trends, we analyzed individual changes in mice by running paired two-tailed Student's t-tests for the SLR of the maximum concentration tested as well as the latency to drink.

3 Results

3.1 Pre-test

Taste sensitivity for saccharin was assessed by brief access lickometer tests using a range of saccharin concentrations. We performed identical tests before and after exposure to cocaine. All lick-rate measurements were normalized to each mouse's intrinsic maximum rate of licking, eliminating much animal-to-animal variability. Saccharin concentration-lick response curves prior to cocaine exposure established baseline sensitivity. The profiles we obtained are very similar to earlier measurements on C57BL6 mice (Inoue *et al.*, 2007). We also used these curves to ensure that the experimental and control groups of mice were not significantly different at the start of the experiment. The EC_{50} and $V_{\max}$ values of each concentration-response curve were obtained following non-linear fits to the data. The EC_{50} for saccharin at the beginning of the protocol was 10.4mM for the cocaine group and 13.0mM for the control group. Before exposure, neither the EC_{50} nor the $V_{\max}$ showed significant differences across the two groups (Table 1). Additionally, the two groups of mice did not differ significantly in the time between presentation of a tube and initiation of drinking, referred to here as latency (15.01 s and 16.54 for cocaine and control groups respectively $t(13)=0.520$, $p=0.510$; two-tailed Student's t-test).

3.3 Saccharin-Cocaine pairing

We paired saccharin consumption with exposure to cocaine by first allowing mice to drink a saccharin solution (7 mM) *ad libitum* for 1 h and then injecting them i.p. with cocaine. This procedure was repeated over multiple 48-hour trials. A control group of mice was treated identically except that they were injected with saline. The volume of saccharin consumed was measured and we found that the cocaine group decreased their saccharin consumption relative to their initial rate after their second injection of cocaine (Figure 2A), consistent with previous studies (Freet *et al.*, 2013). Control mice that were injected with saline showed no decline in saccharin consumption over time (Figure 2A). The two groups of mice showed no differences in consumption of the unconditioned stimulus (water, Figure 2B), which was presented every other day in place of saccharin. Neither group showed changes in water consumption as a function of day, consistently drinking ≈ 1.7 ml of water (Figure 2B).

3.3 Post-test

Following the 10-day period of cocaine exposure, we re-assessed the sensitivity and motivation of mice to drink saccharin solutions using the same brief-access lickometer test as before. Control (saline-injected) mice displayed unaltered concentration-response curves for saccharin. For control mice, a 2-way ANOVA (drug x concentration) on SLR values of each concentration of saccharin showed that there was no significant difference between the pre- and post-tests, ($F(1) = 2.14$, $p = 0.148$). This demonstrated that the mice retain lickometer training over the 10-day interval of injections. The 2-way ANOVA further showed that there was not a significant difference $F(1) = 0.75$, $p = 0.590$ between pre- and post-tests for 7mM saccharin, the conditioning concentration used during the intervening 10-days. That is, control mice did not alter their response to the conditioning concentration of saccharin.

Mice in which saccharin was paired with cocaine showed a significant change in their dose-response curves in the post-test. A 2-way ANOVA (drug x saccharin concentration) on SLR values demonstrated that there was a highly significant difference, ($F(1) = 27.40$, $p < 0.0001$), between the pre- and post-tests. That is, dose response curves were significantly changed by cocaine pairing. Again, no interaction was found between saccharin concentration and drug ($F(5) = 1.54$, $p = 0.186$), showing that alteration of licking behavior in cocaine treated mice was generalized to all concentrations of saccharin, not only the particular concentration (7mM) that had previously been paired with cocaine. 4-parameter curve fits of lickometer data (Figure 3) showed that cocaine-exposed mice had a drop in $V_{\max}$ in the concentration-response curve for saccharin but no shift in EC_{50} (Figure 3B, Table 1). This shows that cocaine pairing caused a curve compression without any lateral shifting.

The post-exposure $V_{\max}$ for saccharin for the two groups, Control and Cocaine were 0.927 and 0.529 respectively (Table 1). These calculated values are significantly different across the groups (95% confidence interval).

To confirm that the response to the highest saccharin concentration was indeed altered for each *individual* mouse following cocaine injections, we performed within subject (paired) two-tailed Student's t-tests. The SLR for each mouse at the highest concentration (45mM) of saccharin was compared in the pre-test and post-test. In saline-injected control mice, the

SLR to 45mM saccharin was unchanged following treatment ($t(6)=0.902$, $p=0.40$). In contrast, cocaine-injected mice showed a significantly lower SLR to 45mM saccharin after the cocaine-treatment ($t(7)=2.5$, $p=0.04$). That is, while control mice licked the highest concentration of saccharin at 106% of their pre-test rate (an insignificant change), the cocaine-exposed mice dramatically decreased their licking to 69% of pre-test value.

3.4 Latency

During lickometer testing, latency is defined as the time between when a tube is presented and when the mouse starts to drink. A short latency results when the mouse initiates drinking soon after the sipper tube is presented. Since presentations are short and frequent (up to forty-nine presentations lasting no more than five seconds) and randomized, a consistently short latency indicates both a familiarity with the task and a willingness to drink low and high concentrations of saccharin. During the pre-test, the short latency is a good indication of task learning and interest in the saccharin. Following pairing of saline and saccharin, the control mice did not show a significant change in latency. In contrast, the mice in which saccharin was paired with cocaine exhibited a dramatic increase in latency (Figure 4).

Two-tailed paired Student's t-tests confirmed that mice had significantly increased latencies to licking saccharin after cocaine ($t(15) = 3.11$, $p=0.007$), whereas control mice showed no significant change in latency ($t(13)= 1.90$, $p=0.08$). That is, every cocaine-injected mouse approached the drinking spout with a greater delay after the 10-day interval of exposure to cocaine.

In a Bonferroni multiple comparison test, we confirmed that pre-test latencies across the two groups were not significantly different ($p>0.1$), nor were pre- vs. post-test latencies different for the control group ($p>0.1$). However, within the cocaine group, pre- and post-test latencies differed significantly ($p<0.001$). Finally, there were significant differences in post-test latencies ($p <0.001$) when comparing the control group to the cocaine group.

Our data demonstrated that both parameters, depression of maximum licking response and increase of latency, were altered following the saccharin-cocaine pairing. Indeed, we found a significant linear correlation ($R^2=0.62$) between the two parameters as measured for each mouse: increase in latency and depression of lick rate at 45mM saccharin (the highest concentration tested). The data suggest that both decreased avidity of licking and increased latency (i.e. decreased anticipation) result from the saccharin-cocaine pairing paradigm.

4 Discussion

4.1 Depression of response to saccharin

Previous studies have shown that after repeatedly pairing cocaine with a sweet liquid like saccharin, the consumption of saccharin is decreased (Grigson, 1997, Parker, 1995, Risinger and Boyce, 2002). This phenomenon has been interpreted a number of different ways. One hypothesis is that an anticipatory contrast effect develops where anticipation of the greater reward of cocaine decreases the normal reward value of saccharin (Grigson, 1997, Nyland *et al.*, 2012). A competing hypothesis states that a form of taste avoidance learning occurs,

where the drug-induced disruption of homeostasis causes a 'taste shyness' around saccharin when not self-administered (Hunt and Amit, 1987). There are additional aversive properties of foods and drugs (Riley, 2011), suggesting that conditioned taste aversion (Lin et al., 2014) or conditioned disgust (Parker et al., 2008) could be at play. In these cases, it is hypothesized that the nauseating effects of the drug overpower the normal reward of the drug and its associated conditioned stimulus. However, this last explanation is confounded by studies that show mechanistic differences between LiCl-induced conditioned taste aversion and the avoidance of saccharin when paired with drugs (Grigson and Freet, 2000, Grigson et al., 2000).

While a variety of behavioral models exist to explain this phenomenon, the present study aimed to answer a more simple question. Could cocaine exposure, through a standard saccharin-cocaine pairing paradigm, cause a decrease in taste sensitivity? Thus, our goal was to apply a model commonly used to quantify taste responses to the well-studied phenomenon of drug-induced suppression of saccharin preference. In brief-access lickometry, taste stimuli are presented for a short (seconds) duration. The output lick rate is not directly related to consumption in calories or volume, but instead, is a function of orosensory detection with minimal influence from post-ingestive effects (Glendinning *et al.*, 2002, Loney *et al.*, 2012, McCaughey and Glendinning, 2013).

4.2 Saccharin Concentration response Curves

By measuring brief access licking responses to multiple varying concentrations of saccharin, we have shown that mice exhibit a consistent concentration- response behavior towards saccharin. After pairing cocaine exposure with saccharin drinking, we found no significant change in EC₅₀ for saccharin. These data show that despite the potential mechanisms available for modification of taste detection, cocaine does not produce a lasting change in taste sensitivity under these conditions.

A vertical shift of the licking concentration-response curve would suggest a change in hedonic set point (Ahmed and Koob, 1998), whereas a lateral shift on the concentration axis would suggest tolerance (right shift) or sensitization (left shift) to the taste solution. Aversive stimuli such as quinine (bitter) produce a negatively oriented dose-response curve with decreasing lick rates to progressively higher stimulus concentrations (St John and Boughter, 2004). When a stimulus becomes aversive as a consequence of conditioning, the licking response is transformed from a preferred (high lick-rate) to an aversive (suppressed licking) pattern (Hashimoto and Spector, 2014). We found that after pairing saccharin with cocaine, the concentration response curve displayed a dramatic vertical depression with no lateral shift or sign-change (Figure 3B). That is, animals experienced a depression in the assigned value of the taste stimulus, without a change in EC₅₀. Because brief-access lickometer behavior is driven by taste detection, the unchanged EC₅₀ suggests cocaine exposure did not change the ability of mice to taste saccharin. Instead, our experimental data suggest a decreased palatability or preference for saccharin after this previously preferred stimulus is associated with the drug. For saccharin and other non-caloric preferred stimuli, the short-term lick-rate in the brief-access lickometer assay is taste-dependent and parallels long-term consumption (Inoue *et al.*, 2007). Thus, our findings of depressed licking are

consistent with previously proposed hypotheses (Grigson, 1997; Hunt and Amit, 1987) that decreased saccharin consumption after cocaine exposure is caused by diminished palatability of saccharin, either through a change in relative value (anticipatory contrast) or a mild avoidance or shyness around the drug. However, in our study, mice retained increased lick rates for higher concentrations of saccharin following saccharin-cocaine pairings. Thus, our findings are inconsistent with the premise of a conditioned disgust or strong conditioned aversion (Lin et al., 2014) (Parker et al., 2008), which would result in an inverted dose-response curve, with decreased licking of increasingly concentrated saccharin.

We were mindful that chronic food restriction has been shown to dampen the contrast between more and less rewarding foods (Cottone et al., 2009). We believe this phenomenon likely did not influence behavior in our experiments. First, the paradigm we used, established by (Glendinning *et al.*, 2002) is a mild restriction for a short duration, and results in enhanced contrast between water and saccharin (Glendinning *et al.*, 2002). Second, we ensured that food restriction occurred for only 1 day immediately prior to lickometer sessions, and did not occur during the 10-day interval of cocaine exposure.

4.3 Latency

In the present study, not only did mice lick saccharin solution with less avidity after exposure to cocaine, but they also took longer to initiate drinking during each trial. The literature indicates that increased latency is not associated with unpalatability or with decreased taste sensitivity. In particular, latency increases do not accompany increasing concentrations of aversive (bitter) taste stimuli; nor are they a hallmark of decreased sensory input such as transection of taste nerves (St John and Boughter, 2004).

Latency to initiate licking has been studied in several contexts. A short latency is generally associated with oral acceptability (Mantella and Youngentob, 2014, Nyland et al., 2012), while a long latency can be indicative of failure to learn the task, or of feared or unpleasant stimuli (Haupt et al., 2007). In our experiments, the mice are unaware of the precise stimulus presented. Hence, altered latency to approach the stimulus may not reflect a specific response to the quality or concentration of saccharin.

When exposed to ethanol, mice increasingly seek out natural rewards. This is seen in lickometer studies as increased licking and decreased latency to initiate sucrose trials (Pastor et al., 2010). Cocaine treatments, on the other hand, have the opposite effect. Cocaine impairs motivation for demanding tasks. This is seen in a lowered break point in the number of lever presses to acquire a sucrose reward, as well as decreased motivation to mate with a receptive female (Barnea-Ygael et al., 2014). Considering previous literature, our observation of increased latency to lick saccharin solutions is concordant with the known effect of long-term cocaine exposure. Although our mice still found the saccharin appetitive, as evidenced by increased licking of higher concentrations, it would seem that they are less motivated to drink saccharin.

Further, our finding that the depression of lick rate is linearly correlated with increased latency adds further weight to the notion that the increase in latency is not due to gustatory interpretation of a less tasty stimulus, but rather a motivational change that counters normal

saccharin reward. We noted, however, that both parameters were more strongly influenced by cocaine pairing in some mice than in others.

Conclusions

Our findings reveal that following a standardized 10-day pairing of cocaine and saccharin, sensitivity for saccharin (measured as EC₅₀) remains unaltered. Our data indicate that despite several potential mechanisms available for taste modification, cocaine exposure does not change taste sensitivity. Rather, following saccharin-cocaine pairing, decreased licking of saccharin along with increased latency to drink suggests a decreased motivation for a previously rewarding sweet stimulus. Our findings add further support to prior interpretations that the decrease in saccharin consumption likely is due to a change in the value of saccharin as a reward.

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Highlights

- Mouse taste sensitivity was measured with a brief-access lickometer test.
- Sweet taste sensitivity was examined before and after saccharin-cocaine pairing.
- Licking responses to all concentrations of saccharin were depressed after pairing.
- The EC₅₀ for saccharin concentration-response did not shift after pairing.
- Pairing altered motivation and reward value, not taste sensitivity.

Figure 1. Experimental Timeline

C57BL6J mice were trained in the lickometer over 7 days. For each training session, water deprivation or food and water restriction began 23.5 hours prior to training. Successive training sessions introduce a new variable to the mouse each day: first, the lickometer itself; second, intermittently presented sipper tube; and third, varied saccharin concentrations. Rest days were introduced to prevent excessive dehydration and to promote consolidation of learning. Next, during the pre-test, mice were tested for their individual taste sensitivity and motivation to drink saccharin-sweetened solutions. In the following conditioning period of 10 days, mice were presented with saccharin (7mM) for one hour every other day, immediately followed by an injection of either cocaine (cocaine-injected mice) or saline (control mice). In the final post-test period, the mice were again tested for their taste sensitivity and motivation towards saccharin solutions.

Figure 2. Consumption of saccharin solution and water during the saccharin-cocaine pairing interval

Data are means $\pm$ SEM of intake volumes. **A**, Intake of 7mM saccharin solution, by mice, in the 1 hour prior to injection of saline ($\circ$) or cocaine ($\bullet$). **B**, Intake of water by the same groups of mice as in **A** and at the same time of day, but on alternating days with no injections and no access to saccharin. Saccharin and water consumption occurred on alternating days in the home cage. Cocaine-saccharin pairing occurred on days marked by ($\blacktriangle$).

Figure 3. Cocaine alters the avidity of licking saccharin solutions without changing the EC_{50}
A, Concentration-response curves for saccharin ($n=7$ mice) before (○) and after (▼) saline injections paired with saccharin. **B**, Saccharin concentration-response curves ($n=8$ mice) before and after cocaine injections paired with saccharin. Data points on graph represent means $\pm$ SEM of SLR values calculated as described (Methods). Curves represent 4-parameter nonlinear fits to the data (R^2 for pre- and post-tests in saline-injected mice, 0.998, 0.999; and in cocaine-injected mice, 0.996, 0.871 respectively). There was no significant change in calculated EC_{50} (95% CI) for either control or cocaine exposed mice. On the other hand, there was a significant change in V_{max} (95% CI) in cocaine-exposed mice, but not in saline-exposed mice compared to the respective pre-tests. We also analyzed the individual SLR data points across all saccharin concentrations by 2-way ANOVA. *** Mice exhibited significantly lower SLR values in the post-cocaine test (2-way ANOVA of saccharin concentration vs. drug, $F(1) = 27.40$, $p < 0.0001$) whereas control mice were not altered ($F(1) = 2.14$, $p = 0.148$) following injections.

Figure 4. Latency dramatically increases after saccharin is paired with cocaine injections but not after saline injections

Latency is the average time between when a saccharin solution is presented and when the mouse starts to drink. The mice undergo 10 days of saccharin-cocaine or saccharin-saline pairing between the Pre-tests and Post-tests. There is a maximum of 49 presentations of 7 concentrations of saccharin per lickometer test. Data are means $\pm$ SEM of latencies observed across two test days. *For the cocaine mice, latency during post-tests was significantly longer than during pre-tests (paired 2-tailed Student's t-tests, $t(13) = 3.1$, $p = 0.007$). Control mice did not show any difference ($t(15) = 1.9$, $p = 0.08$)

Table 1

Parameters calculated for saccharin concentration-response curves in the two groups of mice.

	Control Group (n=7)		Cocaine Group (n=8)	
	Pre-test	Post-test	Pre-test	Post-test
EC ₅₀ (mM)	13.0 ±4.16	15.0 ±2.83	10.4 ±2.97	12.6 ±4.24
V _{Max} (SLR)	0.877 ±0.165	0.927 ±0.110 ⁺	0.767 ±0.128	0.529 ±0.105 [*]

Standardized Lick Ratio (SLR) data were fit to sigmoidal concentration-response curves to derive values for EC₅₀ and V_{max} for the control (n=7) and cocaine (n=8) group. Values presented are best fit for each parameter ± 95% CI. Neither the control group nor the cocaine group showed a significant difference in the derived EC₅₀ values for their respective pre- and post-tests.

⁺ the calculated V_{max} for the post-tests was significantly different between the control and cocaine groups.

^{*} the calculated V_{max} for the cocaine group was significantly different between pre- and post-tests.

EC₅₀ values did not significantly change from pre-test to post-test within or across the groups.