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Pontine and Thalamic Influences on Fluid Rewards: I. Operant Responding for Sucrose and Corn Oil

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Abstract

The reward strength of orosensory sucrose and corn oil was measured using fixed and progressive ratio operant schedules. Because the orosensory effects of the stimuli were of interest, Experiment 1 compared operant responses for sucrose in sham and real feeding rats. The results demonstrated that rats would work for sucrose solutions without the accompanying postingestive effects. Furthermore, the break points for high concentrations of sucrose (1.0 M or 2.0 M) were significantly higher in sham feeding rats than in real feeding controls. Experiment 2 investigated the role of the parabrachial nucleus (PBN) and of the thalamic orosensory area (TOA) in sucrose and corn oil reward. During free access, rats with PBN lesions (PBNx) licked significantly less sucrose solution than their controls, but both groups ingested a similar volume of corn oil emulsion. When an operant was imposed, these same PBNx rats failed to respond for sucrose and continued only modestly for corn oil. In contrast, the TOA lesioned rats (TOAx) showed no impairment in responding for sucrose or corn oil during either the free access or operant sessions. Furthermore, rats with TOA lesions demonstrated significantly higher break points for sucrose than did their controls. Together, the data imply that the PBN but not the TOA is critical for the perception of, or responding to the reward value of sucrose and corn oil.

Keywords

parabrachial nucleus; progressive ratio; reward; sham feeding; thalamic orosensory area

1. Introduction

Sugars and fats are inherently rewarding to both rodents and humans. Prewaning and adult rats show concentration dependent intake of sucrose and corn oil in both brief access [1, 2] and sham feeding [3, 4] conditions. In the sham feeding paradigm, ingested fluid is drained out through an implanted gastric fistula and thus excludes the postingestive effects of the stimulus. These results suggest that, even without postingestive feedback, orosensory stimulation with sucrose or corn oil is rewarding. Nevertheless, whether the same sensory pathways are responsible for orosensory detection of the two stimuli is not clear. Sensory coding of sucrose requires the gustatory system [5]. The central neural pathways for sensory coding of corn oil, on the other hand, have not been determined.

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Although the gustatory system may be involved in their detection, free fatty acid solutions and corn oil emulsions do not necessarily provide the same orosensory cues. Free fatty acids stimulate taste receptor cells through delayed rectifying potassium channels [6]. Furthermore, in rats, lingual lipase can hydrolyze triglycerides to free fatty acids within 1-5 seconds [7]. Finally, a fatty acid transporter, CD36, has been discovered in taste cells [8]. These data suggest that fats or oils are digested into free fatty acids, and that those free fatty acids activate the gustatory system through taste receptors. Despite these data, other biological and behavioral data are at odds with this scenario. First, lingual lipase is secreted from the von Ebner's gland in different species, but lipase activity does not occur in the oral cavity of every species [6, 9]. Second, although chorda tympani (CT) transection impairs discrimination of free fatty acids, rats with these nerve cuts are still able to acquire a conditioned taste aversion (CTA) to corn oil [10, 11]. Third, adding a lipase inhibitor to a corn oil emulsion does not change the preference for the emulsion [7]. Finally, after CTA training, rats showed stronger aversion to corn oil when the CS was a corn oil/sucrose mixture but to sucrose when it was a linoleic acid/sucrose mixture [12].

These results indicate that the orosensory cues produced by corn oil/sucrose or linoleic/sucrose mixtures are different, and thus imply that corn oil emulsions and fatty acid solutions have different orosensory features. As described, corn oil is unlikely to affect the gustatory system via digestion into free fatty acids. Other characteristics of corn oil, such as smell and texture may contribute to these differences. Several studies have shown that the olfactory system is not necessary for conditioning using oils as the CS. Anosmic rats were able to show preference for oils and to use corn oil as a cue for conditioned preference or aversion learning [13-15]. Based on these data and other studies [16], we hypothesized that the trigeminal system is important in the sensory detection and coding for corn oil.

The general hypothesis under test is that sucrose and corn oil are processed through different central pathways. Specifically, we propose that sensory processing of sucrose depends on the gustatory system and sensory processing of corn oil depends on the intraoral trigeminal somatosensory system. In this case, lesions of nuclei along one of the two pathways should disrupt behaviors that are related to the sensory and reward processing of one but not the other stimulus. Accordingly, we tested the hypothesis by studying the effects of central gustatory and trigeminal lesions on three sensory/reward-related behavioral tasks using sucrose and corn oil as the stimuli.

This first manuscript in the series used operant tasks to measure the apparent reward strength of sucrose and corn oil and to assess the role of the gustatory parabrachial nucleus (PBN) and the thalamic orosensory area (TOA) in these effects. The second manuscript used conditioned taste aversion (CTA) to determine whether an intact PBN or TOA was necessary for this paradigm when sucrose or corn oil was the conditioned stimulus (CS) in sham feeding rats. Finally, the third manuscript used the anticipatory contrast paradigm and sham drinking rats to examine whether an intact PBN or TOA was required to compare the relative rewarding properties of different concentrations of sucrose or corn oil.

For the data summarized here, the rats were trained to perform on fixed ratio (FR) and progressive ratio (PR) schedules as a measure of the reward strength of sucrose and corn oil. Operant responses for food reward previously have been measured in real feeding rats [17, 18]. Thus, Experiment 1 used an open gastric fistula to test whether rats would respond for orosensory reward without significant intestinal or nutritional feedback. Experiment 2 tested the overall hypothesis using rats with lesions of either the parabrachial nucleus (PBNx) or the thalamic orosensory area (TOAx). This study is the first to compare the effects of PBN and TOA lesions on FR and PR responding using both the real and the sham feeding paradigm. Preliminary reports of the results have been presented at the annual meeting of the

Society for the Study of Ingestive behavior in 2008 [19] and the annual meeting of the Society for Neuroscience in Washington D.C. in 2008 [20].

2. Experiment 1: Operant Responding in Sham and Real Feeding Rats

Animals consume more sucrose when postingestive feedback is limited or excluded. The intake functions for sucrose solutions vary with the testing methods. For rats in 30-min, real feeding tests, sucrose intake was an inverted-U function of concentration [21]. In brief access [22-24] or sham feeding tests [21, 25], however, sucrose intake increased monotonically as the concentration increased. Moreover, sham feeding rats maintained a significantly higher rate of licking than real feeding animals throughout the test period [26]. The major factor that accounted for differences in the intake functions was postingestive negative feedback. Brief access and sham feeding reduce or prevent accumulation in the gastrointestinal tract and the subsequent induction of satiety. The fact that sham feeding rats exhibit concentration dependent intake of sucrose implies reward in the absence of postingestive effects. Indeed sham and real intake of sucrose increases the release of DA in the NAc [27-30]. Because accumbens DA levels track reward value [31], these results further support the idea that orosensory stimulation without postingestive outcome can produce rewarding effects. Thus sham feeding provides a good model for studying the rewarding properties of orosensory stimulation. Based on the effects of sham-feeding, we hypothesized that the absence of satiety signals not only maintains, but actually increases the reward value of sucrose.

Operant tasks, including FR and PR schedules, have been used as a measure the reward strength of natural and non-natural stimuli. Among other uses, the FR task is used to screen the reward properties of addictive drugs [32, 33]. The PR schedule was first introduced as a method to measure reward strength by Hodos in 1961 [34]. With a PR schedule, the effort required to receive a fixed reward increases progressively throughout testing. Reward strength often is identified by break point, the highest ratio completed within a given test session. Subsequently, many studies have applied these operant tasks to measure the strength of natural [18, 35-38] and non-natural rewards [39-41]. Typically break points increase with the concentration [17, 18, 35] or the volume [37, 42] of a normally preferred fluid. Studies testing the reward strength of sucrose with real feeding have shown similar results [17, 18, 35]. Interestingly, while operant tasks have been used to assess reward strength in real feeding rats, they have not been applied similarly during sham feeding. Therefore, to prepare to test our hypothesis regarding the neural circuitry mediating the orosensory reward, we first used an FR10 and a PR5 to assess the reward strength of a range of concentrations of sucrose in rats with an open gastric fistula.

2.1. Materials and Methods

2.1.1 Subjects—The subjects were 16 male Sprague-Dawley rats (Charles River, Wilmington, MA) with implanted gastric fistulas and bilateral guide cannulas both of which had been used in a previous microdialysis experiment. During this prior experiment, the rats were divided into 2 groups and exposed to 0.3M sucrose solution while sham or real feeding for 20 min. In the current experiment these rats were assigned according to their prior experience, i.e. those that had sham fed began the operant task also sham feeding and vice versa. The rats weighed 350-600g at the beginning of this experiment. They were individually housed on a 12:12-h light-dark cycle with ad libitum access to tap water and standard laboratory diet [Rodent diet (W) 8604; Harlan Teklad, Madison, WI]. Once the experiment began, the rats were maintained on a 15 h food deprivation regimen. Water was removed one h before the start of the test period and returned one h after its conclusion. The operant sessions occurred between 8:00 to 10:30 AM daily. Normal powdered chow was

available between 12:00 and 5:00 PM. Body weight was monitored daily to ensure it did not fall below 85% of their free-feeding weight.

2.1.2. Apparatus—The rats were tested in 12 identical operant chambers measuring L30.5 × W24.0 × H29.0 cm. Details of this chamber have been described elsewhere [43]. In brief, each chamber was equipped with a house light, a white noise generator, and 3 sipper tubes that could be programmed to advance and retract depending on the testing schedule. These sipper tubes entered the chamber through 1.3-cm holes spaced horizontally on one wall of the chamber. Licking was monitored using a triple lickometer circuit. Each test chamber was located in a sound attenuating cubicle that was fitted with a ventilation fan. The operant tasks and on-line data collection were managed by a PC computer via an interface (MedPC; Med Associates Inc, St. Albans, VT).

2.1.3. Experimental Design—Responses to 5 different concentrations of sapid sucrose (0.03M, 0.1M, 0.3M, 1.0M, and 2.0M) were measured in a once daily FR10 or PR5 session, one concentration per day in ascending order. Eight rats (G1, sham-real) were tested while sham feeding on an FR10 and the first PR5 schedule, and then while real feeding on a second PR5 schedule. Another 8 rats (G2, real-sham) had the order reversed, real feeding during the FR10 and the first PR5, then sham feeding on the second PR5. The three retractable fluid spouts were, from left to right, sucrose, operant (empty), and inactive (empty). Contacting the inactive spout had no programmed consequences. The FR10 schedule required the rat to touch the center active spout 10 times to obtain 10-sec access to the left spout that contained one of the sucrose solutions. These sessions were 30 min long. The PR 5 also began with a 10-lick contingency but the access requirement then increased by 5 contacts after each successful 10-sec trial. The PR5 sessions ended when 10 min elapsed without the rat meeting the current PR requirement.

2.1.4. Procedure—Before each rat was placed in an operant chamber, its stomach was flushed with lukewarm water. For sham feeding, a flexible tube was screwed into the gastric fistula and passed through a slot in the wire mesh floor to drain solutions to a waste pan. For real feeding, a flexible tube sealed with silicone rubber adhesive (white RTV 12, GE Silicone) was screwed into the fistula to prevent drainage. Once a sham feeding rat finished its daily session, the volume of the solution in the waste pan was measured and the rat's stomach was flushed again with lukewarm water. For all rats the gastric fistula was closed with its screw before the rats were returned to their home cages. The sham or real intakes of the solutions were measured for each rat at the end of its daily session.

During habituation, the rats were placed in the chamber for 15 minutes with the house light and white noise on. After 3 days of habituation, operant responding for 5 concentrations of sucrose began. For both the real and sham conditions, each concentration was presented twice for the FR10 schedule and three times for the PR5 schedule. All procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine.

2.1.5. Statistical Analysis—One rat in G1 had inappropriate drainage and one rat in G2 died during the experiment. Data from these two rats were excluded. The data from both groups (each n=7) consisted of the means of intake, spout contacts, and break points averaged across 2 FR10 or 3 PR5 sessions for each stimulus. These data were analyzed separately for the two schedules using a 5 × 2 ANOVA varying sucrose concentration (0.03M – 2.0M) and feeding condition (real vs. sham). Post hoc Newman-Keuls tests were conducted when appropriate.

2.2. Results

Sham Feeding increased FR10 responses to 1.0 and 2.0M sucrose—At higher concentrations, the sham feeding rats (G1) made more operant spout contacts on the FR10 schedule of reinforcement than the real feeding group (G2; Fig. 1A). The response curve for G1 increased as a function of sucrose concentration with the maximums at 1.0M and 2.0M. In contrast, although the operant contacts for G2 rats peaked at 0.3M, across the 5 concentrations none of the differences was significant. A two-way ANOVA (stimulus $\times$ group) showed there was a group [F(1, 12)=5.27, $p < 0.05$], concentration [F(4, 48)=16.41, $p < 0.0001$], and group $\times$ concentration interaction effect [F(4, 48)=6.08, $p < 0.003$]. Post hoc tests on the significant group $\times$ concentration interaction revealed that operant spout contacts were significantly higher in G1 than in G2 at 1.0M (post hoc $p < 0.0003$) and 2.0M (post hoc $p < 0.0002$) sucrose. The intake curves for both groups were essentially identical to their operant response curves (Fig. 1B). The sham feeding intakes were significantly higher than the real feeding intakes at 1.0M (post hoc $p < 0.0002$) and 2.0M (post hoc $p < 0.0002$). There was again a group [F(1, 12)=5.94, $P < 0.04$], concentration [F(4, 48)=17.30, $P < 0.0001$], and group $\times$ concentration interaction effect [F(4, 48)=6.33, $P < 0.0004$]. The number of contacts made on the sucrose spout mirrored the spout contacts made on the operant spout (Fig. 1A) and the actual intake of sucrose (Fig. 1B; sucrose contact data not shown).

Sham feeding increased PR5 responses at high concentrations—The PR5 tests compared how hard the two groups of rats would work for sucrose during real and sham feeding. Group 1 responded for sucrose during sham feeding in the first PR5 test and during real feeding in the second PR5 test; G2 had the feeding experience reversed. The conclusions from operant spout contact, break point, intake, and sucrose spout licking data are in close parallel. Therefore, the data presented here include only the contacts made on the operant spout (Fig. 2A) and break points (Fig. 2B). For both G1 (Fig. 2A, left) and G2 (Fig. 2A, right), operant responding was a monotonic function of sucrose concentration during sham feeding and an inverted-U function across the same stimuli during real feeding. A two-way ANOVA (feeding $\times$ stimulus) revealed a significant main effect of concentration [G1, F(4, 48)=16.28, $P < 0.0001$; G2, F(4, 48)=3.97, $P < 0.008$] and a significant feeding $\times$ concentration interaction [G1, F(4, 48)=9.59, $P < 0.0001$; G2, F(4, 48)=8.16, $P < 0.0001$] for both groups. A significant main effect of feeding condition (sham vs. real) occurred in G1 [F(1, 12)=11.31, $P < 0.006$] but not in G2 [F(1, 12)=1.19, $P = 0.30$]. Although the response curves were similar for the two groups, more significant differences appeared between real and sham feeding in G1 where sham feeding preceded real feeding than in G2 where sham feeding followed real feeding. Operant spout contacts were significantly higher at 1.0M and 2.0M (both post hoc $p < 0.0002$) during sham feeding than during real feeding in G1, but similar significance occurred only at 2.0M (post hoc $p < 0.0002$) in G2. These PR data are consistent with the FR10 data showing that rats responded more for high concentrations of sucrose during sham feeding than during real feeding, though the effect was slightly reduced in magnitude if real feeding experience preceded sham feeding.

The response function for break points was similar to that of the operant spout contacts (Fig. 2B). A two-way ANOVA (feeding $\times$ stimulus) revealed a significant main effect of concentration [G1, F(4, 48)=40.6, $P < 0.0001$; G2, F(4, 48)=5.72, $P < 0.0008$] and a significant feeding $\times$ concentration interaction [G1, F(4, 48)=14.25, $P < 0.0001$; G2, F(4, 48)=8.42, $P < 0.0001$] for both groups. A significant main effect of feeding condition (sham vs. real) occurred in G1 [F(1, 12)=12.18, $P < 0.005$] but not in G2 [F(1, 12)=1.29, $P = 0.28$]. Again, more significant differences appeared between real and sham feeding in G1 where sham feeding preceded real feeding than in G2 where sham feeding followed real feeding. In G1, break points were significantly higher at 1.0M and 2.0M (both post hoc $p < 0.0002$) during sham feeding than during real feeding. Similar significance occurred only at 2.0M (post hoc

$p < 0.0002$) in G2. Furthermore, during sham feeding, break points for both 1.0M and 2.0M were significantly higher than the other 3 lower concentrations of sucrose in G1 (post hoc p values between < 0.05 and < 0.0002). In G2 these differences reached significance only for 2.0M compared with 0.03M ($p < 0.001$) and 0.1M ($p < 0.009$). Thus, the slope of the response curve for G2 (real-**sham**) was flatter than that for G1 (**sham**-real) during sham feeding. This is consistent with a two-way ANOVA (group $\times$ stimulus) comparison demonstrating that break points at 1.0M and 2.0M sucrose for G1 were significantly higher than those exhibited by rats in G2 [group $\times$ concentration interaction, $F(4, 48) = 8.35$, $p < 0.0001$; post hoc $p < 0.002$]. These results demonstrated that prior experience with nutritional feedback dampened the reward value of high concentrations sucrose during sham feeding,

The two groups also showed differences in break point during real feeding. A two-way ANOVA (group $\times$ stimulus) revealed a significant main effect of group $F(1,12) = 5.34$, $P < 0.04$, concentration [$F(4,48) = 14.10$, $P < 0.0001$], and the interaction [$F(4,48) = 4.26$, $P < 0.005$]. Group 1 (**sham**-**real**) made fewer operant responses for low concentrations of sucrose than did G2 (**real**-**sham**; N-K 0.1M, $p < 0.02$; 0.03M, $p = 0.051$). Thus, previous sham feeding experience (G1) reduced their operant responding for lower concentration of sucrose during real feeding – perhaps by making them more sensitive to postingestive feedback.

Overall, rats worked harder on the progressive ratio schedule to receive high concentrations of sucrose during sham feeding than during real feeding. The difference was greater when the rats were tested with sham feeding first and then with real feeding than when tested in the reverse order. During sham intake, the break points for G1 rats were 53 and 100 licks higher for 1.0 and 2.0 M sucrose, respectively, than when they real fed. For G2 rats the same differences were only 20 and 56 licks, respectively [Wilcoxon matched pairs test: 1.0M (G1 vs. G2): 53.1 ± 19.5 vs. 20.2 ± 9.9 , $z = 3.12$, $p < 0.002$; 2.0M (G1 vs. G2): 100.7 ± 15.5 vs. 56.2 ± 13.6 , $z = 1.98$, $p < 0.05$].

2.3. Discussion

Using both between and within group designs, this experiment revealed that an orosensory cue without postingestive feedback can support operant behavior. Further, during sham feeding, the intake and reward value of sucrose increases monotonically with concentration. These results support our hypothesis that the absence of satiety signals not only maintains, but actually increases the perceived reward value of sucrose. During an FR10, sham feeding rats responded more for 1.0 and 2.0M sucrose than did real feeding rats. The PR5 experiments demonstrated that, regardless of testing order (e.g. sham-real or real-sham), the break points for concentrated sucrose were significantly higher during sham feeding than real feeding. With real feeding, the inverted-U response function of the FR10 is consistent with previous studies using sucrose [17, 18, 35]. The purpose of imposing a PR schedule was to acquire a break point for different concentrations of sucrose as an index of reward value. The results revealed a positive correlation between break point and sucrose concentration, which is also consistent with previous studies [17, 18, 35, 42] and with the idea that the perceived value of these solutions is increased during sham feeding.

The operant response curves for the same stimulus differ depending on parametric manipulations. Thus, the measured reward value of sucrose solutions is context dependent. In the current experiment, context included test session length, operant schedule, sham or real feeding, and the order in which the rats experienced the feeding condition. Another manipulation is the deprivation state of the animal. When real feeding rats were water, but not food deprived, they failed to demonstrate a concentration-dependent increase in break point for sucrose [17, 36]. In these studies, the caloric effects of the stimuli may have been discounted because the animals were motivated by dehydration rather than by energy deficit. When the animals are motivated for energy, as in the present study, satiety signals affect the

operant response function across stimulus concentrations. This is consistent with studies demonstrating that break points for sucrose increase or decrease depending on the levels of orexigenic or anorexigenic peptide in the brain [44-48]. These data confirm that the perceived reward value of a stimulus is context dependent. Thus, when break point serves as the index, the measured reward value is influenced not only by the concentration of the tastant, but also by the need state of the rat.

The effects of real or sham feeding on operant responding also depended on stimulus concentration. In previous intake tests, compared with real feeding, sham feeding rats ingested significantly more sucrose down to as low as 0.1M [21, 49]. In the present study, with an FR10 imposed, operant responding was significantly greater only for the highest concentrations during sham vs. real feeding. The testing procedure could account for these differences. Early in testing, both sham and real feeding rats were learning the operant task. Because the lowest concentrations were presented first, the rats may not have been at peak performance. In addition, when the schedule switched from FR10 to PR5, the reward stimulus also switched from 2.0M to 0.03M. This substantial decrease in expected concentration may have produced a negative contrast effect in that the rats made appropriate operant responses but did not lick vigorously on the sucrose spout. For this reason, the lack of a difference in operant responding between real and sham feeding for the lower sucrose concentrations might be due to the experimental circumstances.

While sham feeding increased responding for sucrose, particularly at the higher concentrations, the magnitude of this effect was modulated by experience. Specifically, the break point curve for rats sham feeding sucrose was flattened by a history of real feeding under the same or similar circumstances (Fig. 2B, right). In previous reports, animals that had experienced real sucrose intake needed four to five daily, consecutive, sham feeding sessions to extinguish an apparent conditioned postingestive feedback [26, 50]. The current PR5 protocol contained 3 sessions for each concentration of sucrose. Thus it is possible that the slope of the G2 break point curve reflects a conditioned satiation effect from their real feeding experience. Interestingly, the inverted-U shaped curve for rats real fed sucrose also was dampened, though to a lesser degree, by a history of sham feeding experience (Fig. 2B, left). Apparently experience with sham feeding has relatively little effect on the response to subsequent postingestive feedback [49]. The current data are consistent with these prior observations. With sham and real feeding, prior experience order effects are not symmetrical. This conclusion would be bolstered with the addition of sham-sham and real-real controls.

In conclusion, the results of Experiment 1 demonstrated that rats would work for palatable fluids without their accompanying postingestive metabolic effects. This allowed us to investigate whether damage to the gustatory or trigeminal relay would affect the apparent reward value of orosensory sucrose and corn oil using the same operant schedules.

3. Experiment 2: Operant Responding in Lesioned Rats

As mentioned above, the general hypothesis in this study is that the orosensory rewarding properties of sucrose and corn oil are processed through different central pathways. The gustatory system is important in processing sucrose reward, and the intraoral trigeminal somatosensory system in corn oil reward. In rats, the PBN is an obligate relay for transmitting gustatory neural activity produced by sucrose to the central reward circuits. Lesions of the PBN preserve sensory detection of taste [51, 52] but prevent the acquisition of a LiCl-induced CTA [53-55]. Moreover, PBN lesions but not thalamic taste area damage blunt the increase of DA overflow in the NAc during sucrose ingestion [30]. These studies suggest that the PBN is important for processing sucrose reward.

Compared with sucrose, fewer studies have been conducted on the sensory detection and coding of corn oil, particularly with respect to how it reaches the central reward systems. Our hypothesis for corn oil reward is based on the anatomy of the intraoral trigeminal somatosensory system. At the PBN, the central gustatory system splits into a thalamocortical pathway and a ventral or limbic projection [5, 56, 57]. Unlike taste, the thalamocortical system is the only substantial trigeminal projection to the forebrain [58]. Thus, we hypothesized that the orosensory part of the trigeminal thalamic relay, the ventroposteromedial thalamic nucleus (VPM), was required for processing oral corn oil reward. If the PBN is important for oral sucrose reward and the VPM is important for oral corn oil reward, then lesions of the PBN should decrease the apparent reward value of sucrose but not corn oil and damage to the VPM should have the converse effect. Experiment 1 demonstrated that rats would perform operant responses for sucrose reward when the postingestive effects of the stimuli are excluded. The current experiment compared the effects of the PBN and VPM lesions on FR and PR responding using both real and sham feeding.

Based on anatomical and electrophysiological evidence, taste, tongue thermal, and tongue tactile neurons are arranged medial to lateral in the VPM thalamus [58-60]. Therefore, the thalamic lesions in this experiment and the following studies were centered 0.5mm lateral to taste responses in order to include both the taste and intraoral trigeminal relays. This area is referred to here as the thalamic orosensory area (TOA) and the resulting lesions abbreviated as TOAx.

3.1. Materials and Methods

3.1.1 Subjects—The subjects were 30 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 295-335g at the beginning of this study. Their housing and maintenance conditions were identical to those in Experiment 1.

3.1.2. Surgery—The rats were divided into groups with bilateral PBN lesions (PBNx, n=10), PBN surgical controls (Con PBN, n=5), bilateral TOA lesions (TOAx, n=10), and TOA surgical controls (Con TOA, n=5). Before surgery, each rat was treated with atropine sulfate (0.15 mg/kg ip; Gensia Sicor Pharmaceuticals, Irvine, CA) and, 20 min later, anesthetized with pentobarbital sodium (50 mg/kg ip; Nembutal; Abbott Laboratories, North Chicago, IL). Once anesthetized, the rat was mounted in a stereotaxic instrument and a midline incision made to expose the dorsal surface of the skull. Bilateral holes (2.0-2.5 mm diameter) were burred in the interparietal bone for the PBN rats or in the parietal bones for the TOA animals.

Taste neurons in the PBN or the medial TOA were located by recording multiunit activity through a glass-insulated tungsten electrode ($Z = 0.5\text{--}2.0\text{ M}\Omega$ at 1Hz) while stimulating the anterior tongue with 0.3M NaCl. The search stimulus was NaCl rather than sucrose because, under anesthesia, the former produces a more robust response than the latter. In rats, at least, the neurons that respond best to one or the other stimulus are interdigitated in the nucleus of the solitary tract, the only central taste relay where the issue has been investigated [61]. For the PBN, the electrode was oriented 20° off vertical tip rostral, but it was vertical for recording in the thalamus. The coordinates for the PBN were, from β , AP: -12.0 ± 1.0 mm, ML: $\pm 1.8 \pm 0.4$ mm, DV: -7.5 ± 0.5 mm; in the thalamus AP: -3.6 ± 0.6 mm, ML: ± 1.0 to ± 1.4 mm, DV: -7.0 ± 0.5 mm.

Once the taste area was located, the electrode was replaced with a Hamilton 1.0 μ l syringe fitted with a glass pipette on the tip and filled with ibotenic acid (20 μ g/ μ l; Sigma, St Louis, MO). The pipette was then lowered into the brain at the appropriate coordinates. For PBNx rats, taste activity could be recorded through the Hamilton injector to confirm the location.

For the TOAx rats, the injector was centered 0.5mm lateral to where taste activity was located. With the pipette tip at the desired depth, the ibotenic acid (0.1 – 0.15µl /side for PBN; 0.17 – 0.2µl /side for TOA) was delivered by pressure over 15 min. Afterward the syringe remained in place for an additional 10 to 15 min. The same procedure was then repeated on the contralateral side. For the surgical controls, the skull was opened and a recording electrode was lowered several times to just above the PBN or the TOA. At the end of each cranial surgery, the skull openings were filled with Gelfoam (Upjohn, Kalamazoo, MI) and the skin was closed with wound clips. The rat was allowed to recover until its body weight reached pre-surgical level, usually more than one week.

Subsequently all rats were anesthetized again for the gastric fistula surgery for sham feeding. Details for the design and implantation of the gastric fistulas (Plouse Machine Shop, Harrisburg, PA) are described elsewhere [62, 63]. At the end of each surgery, the rats were given gentamicin (4mg ip; Abbott Laboratories, North Chicago, IL), and an anti-inflammatory (Meloxicam, 1 mg/kg sc; Metacam, Boehringer Ingelheim Vetmedica, St. Joseph, MO) every 12 hrs for as long as needed. The rats had at least one month to recover prior to the start of experimental testing.

3.1.3. Apparatus—The apparatus was the same as that used in Experiment 1 except for two details. The walls of the operant chambers were aluminum instead of Plexiglas panels. The placement of the reward, active (operant), and inactive spout was right to left, the reverse of in Experiment 1.

3.1.4. Test Stimuli—The oral stimuli consisted of 0.03M, 0.3M, and 2.0M sucrose and 2.5%, 25%, and 100% corn oil emulsions. Sucrose solutions were made with distilled water. Corn oil emulsions were blended with distilled water and Tween 80 for 8 minutes (Sigma-Aldrich, St. Louis, MO; 0.75ml Tween 80 was added to every 100ml of corn oil and water mixture).

3.1.5. Procedure—The general sequence was the following: Rats were habituated to the test chamber, they were tested on an FR10, and then on the two, sequential PR5 schedules for both sucrose and corn oil. During habituation, the FR10, and the first PR5 the rats sham fed. During the second PR5, the rats were real feeding, i. e. the gastric cannula was closed. Before each rat was placed in an operant chamber, its stomach was flushed with lukewarm water as described in Experiment 1. During habituation, the sham feeding rats had 15 min free access to sucrose solutions or corn oil emulsions. The house light and white noise were on, and the operant and stimulus spouts were both advanced. Sucrose or corn oil was presented from the future stimulus or operant spout on alternative days. There were 3 such days with sucrose (0.3M, 0.3M, 0.03M) and, again after the sucrose operant tasks, 2 days with 25% corn oil.

During the operant tests, sucrose came before corn oil, both in ascending order of concentration. The FR10 schedules were repeated twice under sham feeding condition and the PR5 tests were repeated three times for each concentration under both sham and then real feeding conditions (details of the daily schedule are shown in Table 1). After completing the last PR5 for corn oil while real feeding, all rats were tested again with 2 sessions of FR10 using 0.3M sucrose while sham feeding. All of the procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine.

3.1.6. Histology—At the end of the experiment, the rats were sacrificed with an overdose of pentobarbital sodium (150 mg/kg ip), then perfused transcardially with cold heparinized 0.9% saline solution followed by 4% buffered paraformaldehyde at 4° C. The brains were

removed to paraformaldehyde and, a few hours later, cryoprotected in 30% sucrose in 0.1M phosphate buffer (PB, pH 7.4) overnight, also at 4°C. Then they were blocked, frozen, and sectioned coronally at 50 µm. The sections were kept in PB, then mounted, and stained with cresyl violet to verify the lesions. Only rats showing bilateral damage of the PBN or TOA, characterized by loss of neurons and increased gliosis were included in the data analysis.

3.1.7. Statistical Analysis—The total number of PBNx rats contributing to this study was 9 because one rat died prior to testing. Two other PBNx rats died during the operant tasks with corn oil. Thus, the subject number of the PBNx group for the sucrose data was 9 and for the corn oil data, 7. One TOAx rat had only an ipsilateral lesion and another had its gastric fistula fail during corn oil experiments. Finally, three TOAx rats failed to learn any operant responding for FR10 sucrose sessions. The data from these animals were excluded from the analysis. Therefore, the subject number of the TOAx group for the sucrose data was 6 (free access and FR10) or 8 (PR5) and for the corn oil data was 8. The data consisted of the means of intake, spout contacts, and break points during 3 PR5 (sham and then real feeding) sessions for each stimulus. These data were analyzed by two-way ANOVAs with repeated measures followed by post hoc Newman-Keuls tests when justified.

3.2. Results

Lesions—The gustatory PBN is located in the areas dorsal and ventral of the brachium, and lateral to the mesencephalic trigeminal nucleus (Fig. 3A). Of the 9 PBNx rats, two had large lesions that included the entire gustatory PBN, the locus coeruleus, and the supratrigeminal area (Fig 3B). The remaining 7 rats had damage centered in the PBN, but the lesions were not quite symmetrical. Five had lesions extending into the locus coeruleus on one side, and 3 of these rats had minor sparing of the PBN laterally on the contralateral side. The TOA lesions included the entire area that responds to taste, tongue thermal, and tongue tactile stimuli (Fig. 3C). Four of these rats also had damage across the midline (Fig. 3D).

Free access licking

Parabrachial rats: Before an operant was imposed, both 0.3M sucrose and 25% corn oil were presented in two 15-min free access sessions, and 0.03M sucrose was presented once (see Table 1). Rats with bilateral PBN lesions licked significantly less sucrose than did their surgical controls for both presentations of the 0.3M concentration. [$F(1, 12)=15.70$, $p<0.002$; Fig 4A]. They also licked less 0.03M sucrose than did the controls, but the difference was not significant (post hoc, $p=0.07$). Although the PBNx rats ingested less sucrose than the controls, the number of licks emitted was concentration dependent in both groups [$F(2, 24)=22.49$, $P<0.0001$]. With the 25% corn oil emulsion no significant difference in licking occurred between the PBNx and Con PBN rats [group, $F(1, 10)=0.86$, $p=0.38$; interaction, $F<1$, $p=0.99$]. Both groups increased licking of 25% corn oil in the 2nd session [$F(1, 10)=14.06$, $p<0.004$; (Con PBN vs. PBNx), 1st session, 993.6 ± 422.8 vs. 1496.1 ± 232.8 ; 2nd session, 2165.4 ± 637.9 vs. 2657 ± 450.1]. This difference probably reflected an error because, during the first oil exposure, the emulsion was improperly prepared. As a result, the emulsion delivered to all four groups would have varied during the 15-min access trials, beginning weaker in the first and getting stronger in the second. Regardless, the results indicate that lesions of the PBN decreased free ingestion of sucrose but spared intake of corn oil.

Thalamic orosensory rats: As shown in Fig. 4B, in contrast to the PBN groups, the TOAx rats licked both sucrose and corn oil no differently than their surgical controls [sucrose: group, $F<1$, $P=0.77$, group $\times$ stimulus interaction, $F<1$, $p=0.46$; corn oil: group, $F(1, 11)=3.0$, $p=0.11$, group $\times$ stimulus interaction, $F<1$, $p=0.35$]. Both TOAx and Con TOA rats

showed concentration dependent intake of sucrose [$F(2, 18)=6.4$, $p<0.008$] and, as mentioned above, they also increased oil licking in the second session compared with the first [$F(1, 11)=27.6$, $p<0.0001$; (Con TOA vs. TOAx), 1st session, 619.4 ± 158.9 vs. 1410.4 ± 202.9 ; 2nd session, 1006.9 ± 282.7 vs. 2160.8 ± 260.9].

FR10 performance

Parabrachial rats: Compared with the surgical controls, during the FR10 the PBNx rats not only failed to work for sucrose, but also displayed no concentration dependent operant responses [Fig. 5A, left; group, $F(1, 12)=15.65$, $p<0.002$; concentration, $F(2, 24)=19.97$, $p<0.0001$; interaction, $F(2, 24)=19.58$, $p<0.0001$]. The PBNx rats made near zero responses regardless of the concentration of sucrose. During the corn oil trials, the PBNx rats did respond, but still significantly less than the Con PBN rats [Fig. 5A, right; group, $F(1, 10)=37.66$, $p<0.0002$]. The PBNx rats showed concentration dependent responses for corn oil as did the control rats [concentration, $F(2, 20)=20.35$, $p<0.0001$; group $\times$ concentration interaction, $F(2, 20)=5.05$, $p<0.02$]. The responses of the PBNx rats for 100% corn oil were marginally more than for the 2.5% emulsion [2.5%, 126 ± 40.2 vs. 100%, 274.7 ± 90 ; post hoc, $p=0.06$]. Thus, the data supported the hypothesis that PBN lesions would disrupt operant responding for sucrose, but only partially validated the contrasting hypothesis that the same lesions would spare operant responding for corn oil. The PBNx rats responded to corn oil, but clearly at reduced levels.

Thalamic orosensory rats: Consistent with the results of free access trials, TOA lesions produced no deficits in operant responding for either sucrose or corn oil (Fig. 5B). Indeed, the TOAx rats tended to respond more for both sucrose and corn oil than the Con TOA group. These differences, however, were not significant [(sucrose - $F(1, 9)=1.12$, $p=0.32$; oil - $F(1, 11)=1.81$, $p=0.21$; group $\times$ concentration interactions: sucrose - $F(2, 18)=1.51$; oil - $p=0.25$ vs. $F<1$, $p=0.47$]. Nevertheless, both TOA groups responded for sucrose and corn oil as a function of concentration [sucrose, $F(2, 18)=10.47$, $p<0.001$; corn oil, $F(2, 22)=28.37$, $p<0.0001$]. These results failed to support the hypothesis that lesions of the TOA would disrupt operant responding for corn oil.

PR5 performance

Parabrachial rats: The results for the PR5 trials were similar to those for FR10. The PBNx rats showed severe PR5 performance deficits during either sham or real feeding (Fig. 6, A and B respectively). During sham feeding, the PBNx rats had significantly lower break points for both sucrose [$F(1, 12)=48.73$, $p<0.0001$] and corn oil [$F(1, 10)=24.12$, $p<0.0007$] than the Con PBN group. Consistent with Experiment 1, the break points for the Con PBN rats were a monotonic function of concentration during sham feeding [$F(2, 24)=38.71$, $p<0.0001$]. The PBNx rats, on the other hand, responded equally to all concentrations of sucrose [group $\times$ concentration interaction, $F(2, 24)=36.94$, $p<0.0001$; post hoc, Con PBN, $p<0.0002$; PBNx, $p>0.8$]. For corn oil sham feeding, the Con PBN break points increased as a function of concentration, but not linearly as with sucrose [$F(2, 20)=29.40$, $p<0.0002$]. For the PBNx rats, the break points for corn oil were higher than for sucrose but did not change across concentrations [group $\times$ concentration interaction, $F(2, 20)=11.46$, $p<0.0005$; post hoc, $p>0.4$].

During real feeding (Fig. 6B), the Con PBN break points were an inverted-U function of concentration but the PBNx group still displayed few if any concentration dependent responses for sucrose [group, $F(1, 12)=65.24$, $p<0.0001$; concentration, $F(2, 24)=22.74$, $p<0.0001$; interaction, $F(2, 24)=115.28$, $p<0.0001$]. In the controls but not the PBNx rats, the break points differed significantly from each other across sucrose concentrations [post hoc, Con PBN, $p<0.007$; PBNx, $p>0.3$]. Similarly, during real feeding, the PBNx rats had

significantly lower break points for corn oil than the controls [group, $F(1, 10)=17.17$, $p<0.003$; concentration, $F(2, 20)=9.84$, $p<0.002$; interaction, $F(2, 20)=5.30$, $p<0.02$]. Finally, the PBNx group failed to work harder for higher concentrations of oil [post hoc, $p>0.4$]. Thus when the work requirement increased, lesions of the PBN severely impaired operant responding for both sucrose and corn oil.

There were also break point differences during real and sham feeding for the Con PBN rats, but not for the PBNx rats (Fig. 6). At high concentrations, the Con PBN rats displayed differences in break points for either sucrose or corn oil between feeding conditions. As in Experiment 1, the break point for 2.0M sucrose was significantly higher during sham feeding than during real [feeding $\times$ concentration interaction, $F(2, 20)=5.30$, $p<0.02$; post hoc, $p<0.0002$]. The same relationship held for corn oil [$F(1, 8)=6.24$, $p<0.04$]. In contrast, the PBNx rats exhibited no differences in break points at comparable concentrations during real and sham feeding for either sucrose [$F(1, 16)=0.15$, $p=0.70$] or corn oil [$F(1, 12)=0.43$, $p=0.52$]. Thus, lesions of the PBN disrupted operant responding on a PR5 regardless of the nutritional effect of the reward.

Thalamic orosensory rats: During the PR5 tests, the Con TOA and TOAx rats both showed concentration dependent responding for both sucrose and corn oil whether sham or real feeding (Fig. 7 A and B, respectively). During sham feeding, the break points for sucrose were significantly higher in the TOAx rats than in the Con TOA rats [$F(1, 9)=11.75$, $p<0.008$]. Specifically, the break points for 0.3M (post hoc, $p<0.002$) and 2.0M (post hoc, $p<0.0004$) sucrose were significantly higher in the TOAx group than in the controls [concentration, $F(2, 18)=56.95$, $p<0.0001$; interaction, $F(2, 18)=4.19$, $p<0.04$]. In contrast, the break points for corn oil did not differ between the two groups [group, $F(1, 11)=1.41$, $p=0.26$; concentration, $F(2, 22)=23.21$, $p<0.0001$; interaction, $F<1$, $p=0.68$].

During real feeding (Fig. 7B), the TOAx rats also showed concentration dependent break points for sucrose [$F(2, 18)=26.30$, $p<0.0001$], and they were significantly higher than those of the controls [group, $F(1, 9)=5.31$, $p<0.05$; interaction, $F(2, 18)=1.11$, $p=0.35$]. The TOAx rats had significantly higher break point than the controls at 0.3M (post hoc $p<0.04$) and marginally higher at 2.0M (post hoc $p=0.06$). For corn oil, the two groups responded similarly and their break points did not differ during real feeding [group, $F(1, 11)=1.12$, $p=0.31$; concentration, $F(2, 22)=20.84$, $p<0.0001$; interaction, $F(2, 22)=1.59$, $p=0.23$]. Thus, these results failed to support the hypothesis that the TOA is necessary for corn oil but not sucrose reward responding. Lesions of the TOA did not decrease operant responses for either sucrose or corn oil. In fact, during PR5 tests the lesions appeared to enhance operant responding for sucrose but not for corn oil.

As with the PBN controls, the Con TOA rats had significantly higher break points for 2.0M sucrose during sham rather than real feeding [feeding $\times$ concentration interaction, $F(2, 16)=5.79$, $p<0.02$; post hoc, $p<0.007$]. Unlike the Con PBN rats, however, the Con TOA rats displayed no difference in break points for corn oil between real and sham feeding [$F(1, 8)=2.08$, $p=0.19$]. The TOAx group also had significantly higher break points for sucrose during sham rather than real feeding [feeding, $F(1, 10)=19.96$, $p<0.002$; concentration, $F(2, 20)=60.42$, $p<0.0001$; interaction, $F(2, 20)=13.69$, $p<0.0002$; post hoc at 2.0M, $p<0.0002$]. When responding for corn oil, the TOAx rats again had significantly higher break points during sham rather than real feeding [$F(1, 14)=15.79$, $p<0.002$]. These results indicate that, compared with their controls, the TOAx rats appeared to facilitate the differences in operant responding between real and sham feeding.

0.3M sucrose FR10 performance—After all rats had finished the second PR5 for corn oil, two additional FR10 sham feeding sessions for 0.3M sucrose were conducted (not

shown in Table 1). These sessions were added to test whether the PBNx rats would respond for sucrose after their experience with corn oil. The average operant spout contacts for these two sessions was compared with the data from the first two 0.3M sucrose FR10 sessions using dependent samples t-test. Both sets of controls increased their FR10 responses from the first to the second set of FR10 tasks, but not significantly [Con PBN (490 ± 174 vs. 747 ± 136 , $t = -2.30$, $p = 0.08$); Con TOA (280 ± 152 vs. 622 ± 124 , $t = -2.06$, $p = 0.11$)]. Both the groups with lesions, however, did perform significantly more in the second than in the first 0.3M sucrose FR10 [PBNx (29 ± 6 vs. 214 ± 46 , $t = -3.78$, $p < 0.01$); TOAx (409 ± 177 vs. 847 ± 46 , $t = -2.38$, $p < 0.05$)]. These results suggest that operant responding increases with experience.

3.3. Discussion

This study demonstrated that lesions of the PBN and TOA have differential effects on the measured reward strength of orosensory stimulation. On the one hand, the results support our hypothesis that the PBN is important for processing sucrose reward. Rats with lesions of the PBN consumed significantly less sucrose during 15-min free access. In parallel, when tested using the FR10 and PR5 schedules of reinforcement, the PBNx rats failed to work for sucrose. At the outset, however, we did not just predict that the PBN would be essential for processing sucrose reward. We also predicted that an intact PBN would not be essential for responding to an oil reward. This part of the PBN hypothesis was partially supported. When they had free access, rats with PBN lesions ingested corn oil normally. They also exhibited steady operant responding for corn oil when placed on a FR10 or a PR5 schedule of reinforcement, albeit at lower rates than their surgical controls. The hypothesis that the TOA is important for the processing of oil but not sucrose reward was not supported. Neither intake of sucrose nor oil was reduced in the TOAx rats. To the contrary, the TOAx rats tended to respond more for both sucrose and corn oil and, during the PR5 tests, they responded significantly more for sucrose than did the surgical controls. The PBN, then, is essential for consummatory and operant responding for the orosensory properties of sucrose and contributes to operant responding to the orosensory properties of corn oil. The TOA, on the other hand, is not essential for either. Indeed, when intact, it appears to exert some degree of tonic inhibition. This result refutes one arm of our hypothesis but demonstrates that the TOA lesions are effective in altering behavior guided by both oral stimuli.

The PR schedule was first designed to measure the relative strength of a reward using break point rather than the rate of response as an index [34, 37]. With a PR schedule, animals can exhibit motor impairments but still alter their break points for a reward in response to motivational variables [64]. Nevertheless, the PBN lesioned rats showed severe performance deficits in operant responding for both sucrose and corn oil. Any analysis of these deficits requires examination of the dependent variables in operant performance. These include break point, postreinforcement pause (PRP, the interval between reward termination and the ensuing operant), licks per reward trial (number of licks during each 10 sec reward trial), operant duration (time to accomplish an FR10), and the latency to reward contact. The first two variables appear to reflect motivational levels, the third, motor capability, the fourth, a combination of motivation and motor capability, and the last, associative learning.

During the free access trials, the PBNx rats ingested less sucrose than corn oil. In fact, under these circumstances, they licked normal amounts of 25% corn oil emulsion. During the FR10 trials, the PBNx rats failed to respond for sucrose but performed stably for corn oil though significantly less than normal. During the sucrose sessions, FR10 responses were so limited that a finer grained analysis could not be accomplished. Therefore, what follows uses only data from corn oil and the last two 0.3M sucrose FR10 sessions (conducted after completing the oil FR10 and PR5 trials). With corn oil, the PBNx rats made the same number of licks per reward (average 63-78/10sec) as their controls. This implies that the

tongue movements of PBNx rats were not impaired compared with the surgical controls. That said the time to complete an FR10 varied greatly among the PBNx rats (ranging from 1.9s to 106.7s), and some were longer than those for the control rats which ranged from 1.9s to 10.7s. Even so the two-way ANOVA comparing group and concentration revealed no significant effects of group [$F(1, 10)=2.55$, $p=0.14$], concentration [$F(2, 20)=1.23$, $p=0.31$], or group $\times$ concentration [$F(2, 20)=1.26$, $p=0.3$]. The FR10 could be accomplished with any type of contact, i.e. licks, nose pokes, or paw touches. Thus, the fact that time to complete an FR10 for corn oil did not significantly differ between groups demonstrates that the PBNx rats did not suffer from a general motor impairment. Thus neither general motor deficits nor a specific inability to lick can account for the failure of the PBNx rats to respond for sucrose.

The latency between the completion of the contingency and onset of the consummatory responses has been taken as an index of the learned association between the two events [64]. Although lesions of the PBN may not have impaired motor performance, they may have damaged the ability to associate the operant response with reward. The latency to consume sucrose varied considerably during the initial sucrose FR10 sequence. Once the oil FR10 began, however, the reward consumption latency for the PBNx rats stabilized and eventually did not differ from the controls [FR10 for 100% corn oil: Con PBN, 1.24 ± 0.04 seconds vs. PBNx, 3.86 ± 1.90 seconds; ANOVA, group effect, $F(1, 10)=2.20$, $p=0.17$]. Thus, in the FR10 series, the PBNx rats learned the contingency between the operant response and the oil reward. After the oil FR10 and PR5 trials, two additional sucrose FR10 trials were inserted to determine whether the differences in latency were stimulus specific. They were not [Con PBN, 1.19 ± 0.06 s vs. PBNx, 1.85 ± 0.26 ; t-test, $p=0.06$]; in these later two trials, the PBNx latency to lick sucrose was similar to that for oil.

The latency difference between the two sucrose FR10 sessions (one before oil testing and one after) could be explained in two ways. During the initial sucrose FR10 series, the PBNx rats might not have completely recovered from the lesions. The fact that all PBNx rats began responding after the free access to corn oil makes it unlikely that recovery of function accounts for the increase in responding during the second FR10 test. More likely, the PBN damage disrupted sucrose processing more than oil. Thus, the oil stimuli were easier to associate with an operant response. Once the operant association was in place with one orosensory reward, then the PBNx rats were able to generalize to a second, predominantly gustatory stimulus. This possibility would be supported further if the PBNx rats learned an operant immediately when corn oil was tested before sucrose i.e. reverse the order of the current study. Regardless, the existing data suggest that the failure of PBNx rats to respond for sucrose is not due to a general inability to associate an operant response with a reward.

The PBNx deficits, then, do not appear to be motor or associative. They appear to be primarily motivational. Overall, the PBNx rats showed no concentration dependent operant responses for sucrose and only weak ones for corn oil. Likewise, the break points for sucrose were close to zero and those for corn oil were low. Similar deficits occurred in the postreinforcement pause (PRP) data. In both the FR10 and PR5 sessions, the PBNx rats had long PRP compared with the controls [ANOVA: FR10 group effect, $F(1, 10)=6.67$, $p<0.03$; PR5 group effect, $F(1, 10)=4.97$, $p<0.05$]. Furthermore, the PBNx rats not only responded poorly to the operant spout but also barely touched the inactive spout. Taken together, these results suggest that lesions centered in the PBN impaired the motivation to consume sucrose and corn oil. Rats with PBN lesions consumed little sucrose during free access and would not work for sucrose at all, even when the instrumental requirement was relatively easy (e.g., FR10). With free access, these same PBNx rats consumed normal amounts of oil, but were relatively unwilling to work for this orosensory reward whether sham or real feeding.

This reduced motivation could reflect a decrease in afferent activity reaching the forebrain or a decrease in the hedonic value of the stimuli. The PBN is the second relay in the central gustatory system [57, 65]. In rodents, gustatory neurons in the PBN give rise to two axonal distributions to the forebrain. One is a more or less standard thalamocortical pathway. The other, ventral or limbic projection, reaches the central reward circuits [66, 67] perhaps by way of lateral hypothalamus, bed nucleus of stria terminalis, and the central nucleus of amygdala [5, 68]. Despite the location of this relay in the rodent taste pathway, it is unlikely that a decrease in afferent activity accounts for the apparent reduction in motivation exhibited by the PBN lesioned rats because rats with PBN lesions often have relatively normal detection thresholds [51, 52] and they will reject a gustatory cue that was preoperatively associated with LiCl-induced malaise [69]. Thus, it is possible that rats trained to perform operant tasks preoperatively would show better operant responding for both sucrose and corn oil.

A second possible explanation for the impairment of operant performance in the PBNx rats is a decrease in the apparent hedonic value of the oral stimuli. During a taste reactivity (TR) test, PBNx rats increased intake of sucrose as a function of concentration, but failed to demonstrate increases in orofacial responses [70]. Normal rats show stereotypic orofacial and body movements in response to sapid stimuli [71]. These responses have been used as an index of hedonic value [72]. Hence the inference that PBN lesions reduced the perceived reward of sucrose and so disrupted operant performance for that stimulus. In fact, lesions of the PBN blunt DA increases in the NAc during sucrose intake [30].

In contrast to PBN damage, lesions of the TOA produced significantly higher break points for sucrose. This result replicates and extends those of Reilly and Trifunovic (1999) who demonstrated that lesions centered more medially in the thalamic taste area failed to disrupt operant responding for sucrose in real feeding rats [17]. Besides adding corn oil emulsions, the current study differs from the earlier one in that a gastric fistula was in place and open, and the lesions actually increased break points for sucrose. These thalamic lesions, however, included not only the gustatory relay, but also the adjacent tongue thermal and tactile neurons. Even so, the TOA lesion failed to alter either consumption or operant responding for corn oil. Further, additional analysis indicated that during the sucrose sessions, the TOAx rats required less time to complete an FR10 and had shorter PRPs compared with controls (last two FR10 for 0.3M sucrose, Student's *t* test; PR5 of sucrose, ANOVA: group $\times$ concentration, $p < 0.05$). The shorter PRP in the TOAx rats did not reflect a general increase in activity because enhanced responding did not occur on the inactive spout. Moreover, the TOA lesions selectively enhanced overall PR responding for sucrose but not for corn oil. Thus, the TOA lesions appear to affect the processing for sucrose and corn oil reward differentially.

4. General Discussion

4.1. Comparisons between operant responding for sucrose and corn oil

These experiments confirmed that, during sham feeding, normal rats ingest sucrose as a monotonically increasing function of concentration, and an inverted-U response curve during real feeding. Experiment 2 also revealed that normal rats would work for corn oil and that these operant responses were concentration dependent. The two stimuli differ in the effect of sham feeding. The reduction in gut feedback increases operant responding for sucrose more than it does for oil. This sham feeding effect for sucrose was unchanged in rats with TOA lesions. On the other hand, the sham feeding effect for corn oil actually increased after thalamic lesions. Compared with real intake, the TOAx rats produced significantly higher break points for all 3 concentrations of corn oil during sham feeding (post hoc $p < 0.04$).

In normal rats during sham feeding, the break point for 2.0M sucrose was significantly higher than for 0.3M. The intake data were in parallel (2.0M vs. 0.3M: 15.1 ± 2.18 ml vs. 8.43 ± 1.48 ml, $p < 0.005$). Similar response differences did not occur between 25% and 100% corn oil. Moreover, during a PR5 with sham feeding, oil intake was an inverted-U function. Although the break points were not significantly higher, the sham intakes were significantly lower for 100% than for 25% corn oil (100% vs. 25%: 5.32 ± 0.83 ml vs. 9.22 ± 1.18 ml, $p < 0.03$). Thus, on these metrics normal rats respond differently to orosensory sucrose and corn oil. The dimensions of these differences cannot be determined from the present data because we did not match the hedonic effects of the two stimulus moieties.

4.2. Parabrachial and thalamic lesion effects

The PBNx data confirmed that this pontine relay is important in processing gustatory neural activity for the forebrain reward circuits. For corn oil, however, the parallel data are less convincing. The same PBN lesions that prevent preference and performance for sucrose have little if any effect on free intake of oil and blunt rather than eliminate operant responding for it. This implies that the PBN functions more in learning about oil reward than in processing its inherent hedonic value. This functional division may be reflected in the central anatomy of the oral trigeminal system. Primary lingual trigeminal axons synapse directly in the nucleus of the solitary tract as well as the trigeminal sensory nuclei [73-77]. Further, thermal and tactile stimulation of the oral cavity activates neurons in the gustatory NST, PBN, and the thalamus [61]. Thus, it is possible that the trigeminal sensory activity produced by oral oil stimuli has more than one pathway for reaching the limbic systems that elaborate reward.

The anatomical differences between taste and trigeminal sensibility contributed to our original hypothesis in which we predicted that the rewarding properties of sucrose would be mediated by the PBN, those for oil would depend on the thalamic sensory relay. The data do not support the strong version of the hypothesis, but they do underscore the more general point that the central gustatory system appears to handle oral sensory activity produced by sucrose and oil differently. Specifically, regardless of the measure, damage to the PBN can dramatically reduce the apparent hedonic value of oral sucrose. The same PBNx rats, however, retain their preference for corn oil but refuse to work as hard for it as their surgical controls. Thus parabrachial influence on oil reward may be evident only when pushing the motivational system by increasing the contingency from continuous reinforcement to a fixed or a progressive ratio.

If anything, the thalamic lesions present a more complicated story. Damaging the thalamic oral trigeminal relay does not blunt preference or operant responding for either sucrose or oil. In fact, after thalamic lesions, one measure of reward strength, the break point for a progressive ratio, is significantly enhanced for sucrose and to a lesser degree for corn oil. Again, this implies not sensory processing or associative impairment, but a motivational dysfunction. Specifically, the thalamus appears to have a tonic inhibitory influence on operant responding to obtain taste and even oral trigeminal stimuli (as noted, a similar trend was evident with oil). This is consistent with evidence that the thalamic circuits, including the VPM, are primarily inhibitory [78, 79].

Overall, these experiments support the hypothesis that sensory mechanisms of sucrose reward differ from those of corn oil. They also reinforce the contention that, in rats, gustatory neural activity related to reward reaches the forebrain via the ventral or limbic projections from the parabrachial nuclei. The same data are less informative about how the sensory activity produced by licking oil influences the central mechanisms of reward.

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Research Highlights

- Orosensory stimulation with sucrose or corn oil alone can support operant responding.
- Rats with PBN lesions lick oil but not sucrose at a normal rate during free access.
- PBNx rats failed to work for either sucrose or corn oil reward in operant tasks.
- TOAx rats responded either equally or more for both rewards than the controls.

Fig.1.

Sham feeding increased FR10 responses to high concentrations of sucrose. (A) Mean operant spout contacts by sham and real feeding rats over 5 concentrations of sucrose ($\pm$ SEM, average of 2 FR10 sessions). At 1.0M and 2.0M, the operant spout contacts during sham feeding were significantly higher than during real feeding. (B) Mean intake by sham and real feeding rats over 5 concentrations of sucrose ($\pm$ SEM, average of 2 FR10 sessions). At 1.0M and 2.0M, intake during sham feeding was significantly higher than during real feeding. (*: significant difference between feeding conditions)

Fig. 2.

Regardless of testing order, on a PR5 schedule operant responses and break points were significantly higher at high concentrations during sham feeding than during real feeding. (A) Mean operant spout contacts made by G1 and G2 over 5 concentrations of sucrose ($\pm$ SEM, average of 3 PR5 sessions). In Group 1, left, operant spout contacts during sham feeding were significantly higher at 1.0M and 2.0M than during real feeding. In Group 2, right, the difference was significant only at 2.0M. (B) Mean break point of G1 and G2 over 5 concentrations of sucrose ($\pm$ SEM, average of 3 PR5 sessions). In Group 1 (left), break points for 1.0M and 2.0M was significantly higher during sham feeding than during real

feeding. Such differences only occurred for 2.0M sucrose in Group 2 (right). (*: significant difference between feeding conditions)

Fig. 3. Photomicrographs of coronal sections stained with cresyl violet, (A) PBN surgical control (B) Bilateral PBN lesions (C) TOA surgical control (D) Bilateral TOA lesions. The images for the PBN are 4 $\times$, those for the TOA, 2 $\times$. The bar in A or C equals 1mm. The arrows indicate gliosis formed after lesions. Abbreviations: BC: brachium conjunctivum; CL: central lateral nucleus; CM: central medial nucleus; MD: medio dorsal nucleus; Me5: mesencephalic trigeminal nucleus; VPM: ventroposteromedial nucleus; VPMpc: the parvocellular VPM.

Fig. 4.

Effects of bilateral PBN or TOA lesions on 15 min free access to sucrose and corn oil. Mean ($\pm$ SEM) number of licks for sucrose solutions or corn oil emulsions. (A left) Con PBN rats showed concentration dependent increase in sucrose licking. PBNx rats licked significantly less 0.3M sucrose than the controls. Their licking for 0.03M sucrose approached to significantly less than controls (post hoc, $p=0.07$). (A right) Their licking of 25% corn oil equaled that of controls. (B) Both TOA groups show a trend of concentration dependent increase of sucrose licking. Free access licking did not differ between the TOAx and control groups for sucrose and corn oil (25% corn oil, $p=0.07$). (#, significant differences between concentrations. *, significant differences between the lesion and the control groups)

Fig. 5. Effects of bilateral PBN or TOA lesions on FR10 operant responses to sucrose and corn oil. Mean ($\pm$ SEM, 2 FR10 trials) operant spout contacts over 3 concentrations of sucrose (left) or corn oil (right). (A) Compared with the surgical controls, PBN damage disrupted operant responding for sucrose and reduced responding for corn oil. (B) TOAx rats and their controls did not differ on the same measures.

Fig. 6.

Effects of bilateral PBN lesions on PR5 break points for sucrose and corn oil during (A) sham and (B) real feeding. Mean ($\pm$ SEM, 3 PR5 trials) over 3 concentrations of sucrose (left) or corn oil (right). Except for sham feeding 0.03M sucrose (post hoc, $p=0.08$), the break points for the PBNx rats were significantly less than for the control rats (post hoc, $p<0.03$).

Fig. 7.

Effects of bilateral TOA lesions on PR5 break points for sucrose and corn oil during sham (A) and real (B) feeding. Mean ($\pm$ SEM, 3 PR5 trials) break point 3 concentrations of sucrose (left) or corn oil (right). The TOAx rats had significantly higher break points for 0.3M and 2.0M sucrose than the controls did during sham feeding and at 0.3M during real feeding. They did not differ in break point while responding for corn oil.

Table 1

Testing schedule.

DAY	1	2	3	4-9	10-18	19-27
Sucrose (M)	0.3		0.03	FR10	PR5	PR5
DAY	28		29	30-35	36-44	45-53
Corn Oil (%)	25		25	FR10	PR5	PR5
Feeding	Free access Sham feeding			Sham Feeding		Real Feeding