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Pontine and Thalamic Influences on Fluid Rewards: II. Sucrose and Corn Oil Conditioned Aversions

Nu-Chu Liang, Patricia S. Grigson, and Ralph Norgren

Department of Neural and Behavioral Sciences, College of Medicine, The Pennsylvania State University, Hershey, PA 17033, USA

Abstract

In this study conditioned aversions were produced in sham feeding rats to limit postingestive feedback from the oral stimulus. All control rats learned an aversion to either 100% corn oil or 0.3M sucrose when ingestion of these stimuli was followed by an injection of lithium chloride (LiCl). Rats with lesions of the ventroposteromedial thalamus also learned to avoid either corn oil or sucrose. After 3 trials, rats with damage to the parabrachial nuclei (PBN) learned to avoid 100% corn oil, but failed to do so when the stimulus was 0.3M sucrose. These results support our hypothesis that the PBN is necessary to appropriately respond to a taste, but not an oil cue as a function of experience (i.e., pairings with LiCl). The results also are consistent with our results from operant tasks demonstrating that the trigeminal thalamus, the ventroposteromedial nucleus, is not required for responding to the rewarding properties of sucrose, oil, or for modifying the response to these stimuli as a function of experience.

Keywords

parabrachial nucleus; conditioned taste aversion; ventroposteromedial nucleus; sham feeding; sucrose; corn oil

1. Introduction

A conditioned taste aversion (CTA) usually involves the ingestion of a novel fluid stimulus followed by the application of a second stimulus that causes malaise in humans. In our laboratory (and many others) this second stimulus typically is an intraperitoneal (ip) injection of LiCl. After one such pairing, animals usually learn to avoid the oral stimulus when next presented. In this classical conditioning paradigm, the novel oral stimulus is the conditioned stimulus (CS) and the LiCl is the unconditioned stimulus (US) [1]. Conditioned taste aversion is a robust phenomenon. It can be acquired after one pairing of the CS with the US, and the association does not require close temporal contiguity between the stimuli [2, 3]. The conditioned aversion paradigm provides a model for investigating reward pathways activated by orosensory stimuli. That is, following acquisition of a CTA, the hedonic properties of a stimulus can switch from positive to negative [4–6], and the

Corresponding Author: Nu-Chu Liang Department of Psychiatry and Behavioral Sciences School of Medicine The Johns Hopkins University Ross Building Room 621 720 Rutland Avenue Baltimore, MD 21205 Phone: (410) 955-2996 Fax: (410) 502-3769 nliang2@jhmi.edu.

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dopamine response to the normally preferred taste cue is reversed in the nucleus accumbens [7].

In the first article in this series, we found that the parabrachial nucleus (PBN) is critical for responding to the rewarding properties of sucrose, and to a lesser extent, oil. This disruption in behavior is not due to a failure to detect the stimuli or to associate an operant response with the reward. Instead, the lackluster behavior appears to be due to a motivational deficit in that the rats will not voluntarily consume sucrose or perform an operant to obtain it. They will consume oil, but will work only desultorily to obtain it. In contrast to the PBN the ventroposteromedial nucleus of the thalamus (the thalamic orosensory area, TOA) does not contribute to the generation of these motivated behaviors and, in fact, may inhibit them.

In this second article in the series we use sham feeding and a CTA task to test the hypothesis that the PBN processes neural activity from orosensory sucrose and the trigeminal thalamus processes similar activity from oral corn oil. Previous studies have demonstrated that the PBN is required for the acquisition of a CTA when the CS is a taste stimulus [6, 8–10]. Nevertheless, rats with PBN lesions can learn a CTA when the CS is a trigeminal stimulus e.g. capsaicin [11]. Given that PBN lesions block CTA learning in real feeding rats [6, 12], we predicted that sham feeding rats with PBN lesions would continue to show this deficit. On the other hand, PBN lesions should not block a CTA to a sham fed corn oil CS because real feeding rats with PBN damage can learn to avoid this normally preferred fluid following pairings with LiCl-induced malaise, i.e. there is no deficit [13]. In contrast, bilateral lesions of the TOA were expected to have no effect on a CTA to sucrose, but were expected to block a learned aversion to the corn oil CS if the TOA mediates the establishment of an oil-LiCl association.

2. Materials and methods

2.1. Subjects

The subjects were 30 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 275–300g at the beginning of this study. They were individually housed in hanging wire mesh cages on a 12:12-h light-dark cycle with ad libitum tap water and standard laboratory pellets [Rodent diet (W) 2018; Harlan Teklad, Madison, WI]. Once the experiment began, the rats were maintained on an 18 hr food and water deprivation regimen with distilled water (dH₂O) and the same powdered chow [Rodent diet (W) 2018C; Harlan Teklad, Madison, WI] available from noon to 4PM daily.

2.2. Surgery

The rats were divided into those with bilateral PBN lesions (PBNx, n=10), PBN surgical controls (Con PBN, n=5), TOA lesions (TOAx, n=10), and TOA surgical controls (Con TOA, n=5). The lesions were performed first and, after recovery, the gastric fistulae were implanted. The lesion and fistula implant procedures were identical to those described in the previous paper [14]. All rats had at least one month of recovery before behavioral testing commenced.

2.3. Training Protocol

Four days before baseline measures began, the rats were transferred to stainless steel, wire mesh training cages (11" long × 9" wide × 7" high) that have a slot down the center of the floor to accommodate the gastric cannula drain. During this period, the rats were acclimated to consuming powdered chow and to licking dH₂O from a spout on the cage front. Water and food were removed the afternoon before training began and, from then on, the rats were maintained on 18 hr food and water restriction. Every morning they received fluid for 15

min with the gastric fistula opened, and dH₂O and powdered chow for 4 hr in the afternoon with the gastric fistula closed. The CTA measures included baseline water intake, a series of conditioning trials, and one-bottle and two-bottle tests. The rats were first trained, tested, and extinguished for CTA using a 100% corn oil CS (Mazola, ACH Food Companies, INC., Memphis, TN). They were then returned to free drinking and feeding for two weeks. Thereafter, for all rats, the food and water restriction regimen was re-imposed for a second LiCl-induced CTA using a 0.3M sucrose CS (Fisher Chemicals, Fairlawn, NJ). Every 100 ml corn oil contained 0.75 ml Tween-80 (Sigma-Aldrich, St. Louis, MO) to maintain its fluidity in the stomach.

Each morning, the seal of each gastric fistula was removed and the stomach was flushed with lukewarm water until the effluent was clear. A flexible tube was then screwed into the gastric fistula and passed through the slot to drain the solutions. Fifteen minutes later, the fluid (dH₂O or the CS) was presented in an inverted Nalgene graduated cylinder with a silicone stopper and a stainless steel spout affixed to the front of the cage. Once the rat finished its daily session, its stomach was flushed again with lukewarm water, and the gastric fistula closed with its screw. The fluid recovered from the gut was measured. To ensure proper sham feeding, this volume had to equal or exceed the amount ingested.

Baseline water intake normally stabilized within 7 days. Once it did, CTA acquisition began. On the first day of the cycle, the rats were presented with the CS for 15 min and, after a 15 min interval, they were injected with 0.15M LiCl (US, 1.33ml/100g body weight. i.p.). On the following two days, they received water in the morning without injections. There were a total of three such CS-US acquisition cycles. Subsequently, using the same 3-day cycle, they had a 15 min 1-bottle CS test without an injection of the US, and then a 2-bottle test with the CS and dH₂O simultaneously available for 15 minutes. The location of the bottles on the cage was counterbalanced left and right across tests. Once again, LiCl was not injected.

Extinction trials followed the 2-bottle test. An extinction sequence consisted of two 1-bottle tests followed by one 2-bottle test, with two water days elapsing between each. There were three such extinction cycles following the corn oil CTA, but there was only one following the sucrose CTA. During the first extinction cycle, a mineral oil emulsion was used instead of corn oil for the first 1-bottle test. This was to test whether the rats would generalize the oil aversion. Again, for the 2-bottle test, the location of the water and the CS solution was counterbalanced left and right. Because the gastric cannulas began to fail for some rats, only one sucrose extinction cycle was completed.

2.4. Histology

At the end of the study, the rats were euthanized with an overdose of pentobarbital sodium (150mg/kg ip), then perfused transcardially with cold heparinized 0.15M saline followed by 4% buffered paraformaldehyde at 4°C. The brains were removed and placed in paraformaldehyde. A few hours later, the brains were cryoprotected in 30% sucrose in 0.1M phosphate buffer (PB, pH 7.4) overnight also at 4°C. They were then blocked, frozen, and sectioned coronally at 50 μ m. The sections were kept in PB, then mounted and stained with cresyl violet to verify the area of lesions. The analyses were conducted on data from rats with bilateral damage of the PBN or TOA, characterized by loss of neuronal cells and increased gliosis.

2.5. Statistical analysis

The data consist of the means of the AM 15 min sham fluid intake (e.g. corn oil, sucrose, or dH₂O). The data were analyzed using mixed factorial ANOVAs followed by post hoc

Newman-Keuls tests when appropriate. Student's *t* tests for dependent samples also were conducted as appropriate.

3. Results

3.1. Histology

Five PBNx rats did not have adequate bilateral damage of the gustatory PBN, and their data were excluded. The remaining 5 PBNx rats had lesions centered in the medial PBN. Besides the medial and lateral PBN, two of the five rats had lesions that extended into the supratrigeminal area and part of the locus coeruleus (Fig. 1B). The lesions of the TOA were centered in the ventroposteromedial nucleus of the thalamus (VPM) but extended medially into the gustatory area – the parvicellular ventroposteromedial (VPMpc) nucleus (Fig. 1D).

3.2. Thalamic Orosensory Rats

Sucrose CTA—The TOAx rats and their controls responded similarly both to water and the 0.3M sucrose CS (Fig. 2A left). Sham intake of dH₂O during the last three days of baseline did not differ [$F(1, 13)=0.03$, $p=0.87$]. Compared with the last day of baseline dH₂O intake, both groups consumed significantly less 0.3M sucrose on first exposure [$F(1, 13)=10.55$, $p<0.007$]. After one sucrose-LiCl pairing, both control and TOAx rats reduced their sucrose intake further. They maintained this parallel reduction across the remaining 3 single-bottle trials [2 additional conditioning trials and one 1-bottle test, $F(1, 13)=0.94$, $p=0.35$]. In the second trial, the majority of TOAx rats avoided sucrose as did the controls. The variance on that trial arose from two of the 10 rats that consumed 4.5ml and 29ml of sucrose respectively. By trial 3 and 4, all the TOA rats completely avoided the sucrose [trial effect, $F(3, 39)=11.16$, $p<0.0001$] and the group $\times$ trial interaction was not significant [$F(3, 39)=0.35$, $p=0.79$]. Sham water intake on the 2 days between trials did not differ between groups or across days [$F(1, 13)=0.06$, $p=0.81$].

The 2-bottle test demonstrated that both groups preferred dH₂O to the sucrose CS (Student's *t*-tests, both $p<0.05$, Fig. 2B left). During the single extinction cycle, the control and the TOAx rats either avoided or barely sampled the sucrose during either the 1 or 2-bottle presentations (data not shown).

Corn Oil CTA—As with the sucrose CTA, baseline water intakes before the oil tests did not differ between the controls and TOAx rats [$F(1, 13)=0.12$, $p=0.73$, Fig. 2A right). Similarly, both groups consumed significantly less 100% corn oil on first exposure compared with the last day of baseline dH₂O [$F(1, 13)=22.77$, $p<0.0004$]. In fact, the drop in oil intake was significantly greater than in the first sucrose trial (paired *t*-test for baseline intake – 1st CS intake, $p<0.03$). After pairing with LiCl injections, corn oil intake continued to decrease across trials [$F(3, 39)=50.47$, $p<0.0001$], but neither the main effect of group [$F(1, 13)=1.81$, $p=0.20$] nor the group trial interaction [$F(3, 39)=1.50$, $p=0.22$] was significant. Again as with the sucrose trials, water intake on the intervening two days did not differ between the control and TOAx groups [$F(1, 13)=0.36$, $p=0.56$].

In the subsequent 2-bottle test, both the control and lesion groups avoided the oil CS (Fig. 2B right). The paired *t*-tests demonstrated that the TOAx rats drank significantly more dH₂O than oil ($p<0.008$) and marginally so for the controls ($p=0.055$). The water intake was not significantly greater in the controls but the *n* was only half that of the TOAx group ($t=1.42$, $p>0.19$; 5 vs. 10).

The first extinction cycle used 100% mineral oil and neither the control nor TOAx rats consumed measurable amounts (data not shown). Subsequent extinction trials used corn oil.

Except for 2 controls and 4 TOAx animals, and then only during the last cycle, the rats did not consume over 1 ml of corn oil during the 1-bottle or 2-bottle tests. Thus, neither the lesioned nor the control rats extinguished their aversions to corn oil after three extinction cycles (data not shown).

3.3. Parabrachial Rats

Sucrose CTA—During baseline training, the PBNx rats sham ingested significantly less water than the controls [$F(1, 8)=7.33$, $p<0.03$]. On the first exposure to sucrose, however, the PBNx group ingested significantly more than their controls [$F(1, 8)=13.14$, $p<0.007$; Fig. 3A left]. By the third trial, the PBNx rats ingested less sucrose than they did prior to pairing with LiCl [trial effect, $F(3, 24)=6.90$, $p<0.002$], but their intake never fell below their baseline water consumption. The sucrose intake of the controls fell to zero by trial 3 [group $\times$ trial interaction, $F(3, 24)=0.93$, $p=0.44$; post hoc, control trial 3 or 4 vs. trial 1 both $p<0.02$]. Unlike the TOA rats, on the intertrial days the PBNx group ingested significantly less dH_2O than the controls [$F(1, 8)=14.68$, $p<0.006$].

During the fourth trial (1-bottle test without a LiCl injection), all 5 PBNx rats drank sucrose (mean=10.1 ml; range 5.5–25ml), i.e. they failed to learn the CTA. During the 2-bottle test, the controls drank significantly more dH_2O than sucrose (dH_2O , 15.8 ± 4.3 ml vs. sucrose, 1.4 ± 1.2 ml; t-test, $p<0.05$). The PBNx rats drank more sucrose than water, but the preference was not significant (dH_2O , 2.9 ± 2.0 ml vs. sucrose, 9.3 ± 4.2 ml; t-test, $p=0.26$; Fig. 3B left). Under this circumstance, two of the 5 PBNx rats drank neither sucrose nor water (0 vs. 0.5ml and 1.0 vs. 1.5ml, respectively).

Corn oil CTA—Sham water intake did not differ between the controls and the PBNx rats when assessed across the three days prior to the start of oil CTA training [$F(1, 8)=1.20$, $p=0.30$]. Compared with the last water day, the control group significantly decreased their sham intake upon the first exposure to 100% corn oil (paired t-test, $p<0.002$). The PBNx rats also ingested less oil on the first exposure, but the decrease was not significant (paired t-test, $p=0.095$). After one pairing with LiCl, however, both the controls and the PBNx rats reduced their oil intake significantly (Fig. 3A right). Nevertheless, both before and after the first LiCl pairing, i.e. trials 1 & 2, the PBNx group ingested more oil than the controls [repeated measures ANOVA, group $F(1, 8)=5.60$, $p<0.05$, trial $F(3, 24)=11.23$, $p<0.0001$, and interaction $F(3, 24)=3.84$, $p<0.03$ with Post hoc Newman-Keuls tests]. This might leave the impression that the PBNx rats learned the aversion more slowly than the controls. The impression probably results from their larger initial oil intake and the floor effect encountered by the controls.

In the 2-bottle test, both control and lesion groups avoided the CS (Fig. 3B right). The controls sham drank significantly more dH_2O than oil (paired t-test, $p<0.008$). The same comparison for the PBNx rats was marginally significant ($p=0.055$), and most of these rats (4 of 5) completely avoided the corn oil emulsion. Further, the sham water intake was more in the controls than in the PBNx rats, but the difference was not statistically significant [controls, 17.8 ± 3.7 ml vs. PBNx, 10.2 ± 3.9 ml; t-test, $p=0.192$].

During the 1-bottle mineral oil extinction test, two PBNx rats ingested an average of 6.5ml/15min; the remaining 3 PBNx rats did not consume measurable amounts. By the last 2-bottle extinction test, which was conducted with 100% corn oil, one control preferred 100% corn oil to water (12ml vs. 1ml). The remaining rats in both groups consumed no more than 1.0 ml of corn oil during the extinction tests (data not shown).

4. Discussion

These results demonstrate that the parabrachial nucleus is required for acquisition of a learned aversion to a taste in sham feeding rats. The same animals, however, were able to learn to avoid 100% corn oil when it was paired with injections of LiCl. The results are consistent with previous observations that PBN lesions disrupted CTA in real feeding rats when the CS was a taste, but not when it was a trigeminal cue: e.g. capsaicin or 100% corn oil [11, 13]. In contrast, lesions encompassing the thalamic taste and oral trigeminal relays failed to block learned aversions to either sucrose or corn oil. This is consistent with prior studies showing that acquisition of a gustatory CTA is intact following lesions centered on the thalamic taste area. As such, the data did not support our hypothesis that thalamic lesions centered in the trigeminal relay would interfere with a learned aversion to 100% corn oil. The data are, however, in keeping with the findings in the companion manuscript [14] showing that lesions of the TOA also failed to reduce operant responding for either a sucrose or a corn oil reward.

Although a range of stimulus properties may potentially contribute to the development of a CTA, these data make it clear that the caloric value of the CS is not necessary for conditioned aversion learning. This experiment was conducted entirely using a sham feeding procedure that prevents substantial metabolic feedback. Nevertheless, all the controls and all the TOAx rats acquired conditioned taste aversions in a manner similar to real feeding animals using the same protocol. These results strengthen other experiments using non-nutritive oral stimuli such as saccharin [12] or capsaicin [11, 15] or brief intraoral infusions of the CS [6] and suggest that taste factors, alone, can support the establishment of a LiCl-induced conditioned aversion.

Lesions of the PBN but not the TOA increased the intake of both the sucrose and corn oil CS upon first exposure. Because the PBNx rats did not completely avoid corn oil until after two pairings with LiCl, they appeared to be slow to acquire the aversion. The initial intake of the corn oil, however, was more than three times greater in the PBNx rats than in the controls. Even so, the slope of decreasing intake in the two groups was near parallel indicating that learning speed was the same. When the CS was 0.3M sucrose, the same PBNx rats again sham drank more than the controls upon the first exposure but the difference was not significant. After three acquisition trials, these rats still did not avoid sucrose. Although sucrose intake decreased from its first trial maximum, it did not drop significantly below baseline water. Further, during the 2-bottle test, PBNx rats still tended to prefer sucrose.

Neophobia is defined by animals consuming only small amounts on first exposure to a novel food. Therefore, the increase in CS intake upon the first exposure by the PBNx rats could be interpreted as a lack of neophobia. Regardless, this increase in intake did not contribute to the failure of PBNx rats to acquire a CTA. Although the rats exhibited higher intake of both oil and sucrose, they learned an aversion to oil but not to sucrose. In fact, rats with PBN lesions often fail to show neophobia because their initial CS intakes are more than the intact rats [10, 11]. Furthermore, in studies when the initial intake did not differ between groups, the PBNx rats still failed to acquire a CTA while the control rats ceased intake after a single pairing with LiCl [8, 16]. As such, a general failure to exhibit taste neophobia cannot account for the failure of PBNx rats to acquire a CTA to a gustatory stimulus.

Unlike the PBN lesions, the TOA lesions had no effect on the acquisition of conditioned aversion to either sucrose or corn oil. Although the lesions included both the oral trigeminal and taste area of the thalamus, they failed to disrupt CTA. As mentioned, this result is consistent with several similar experiments in rats with lesions centered on the gustatory

thalamus [17–21]. These findings can be contrasted with other reports that apparently similar thalamic damage did impair acquisition of a CTA [22, 23]. These studies, however, employed only a single taste-LiCl pairing. Our experiments and Lasiter's (1985), on the other hand, used 3 CS-US pairings. After a single pairing, some rats with thalamic lesions exhibit somewhat less reduction in CS (sucrose or oil) intake than the controls, but they catch up after the second pairing. In the present case, this second trial difference was not statistically significant; in others it was [19]. Thus, when only a single CS-US pairing is employed, impaired acquisition could be reported [21].

The present thalamic lesions, however, extended into the gustatory area, but were centered on the oral trigeminal relay just lateral to taste. Thus, the behavioral data failed to support the hypothesis that the thalamic trigeminal area is necessary for learning an aversion to corn oil. This appears to contradict our assumption that the trigeminal system processes the sensory properties of corn oil needed for a CTA. It remains possible that the spinal or the principal sensory trigeminal nuclei are necessary for learned aversions to corn oil but this leaves questions as to how the sensory activity reaches the reward system from the brainstem.

The available anatomical data indicate that the trigeminal system reaches the forebrain via the thalamus and large lesions there have little if any effect on CTA regardless of the CS moiety [19, 24]. Several scenarios could explain this apparent conundrum but none is entirely satisfactory. Because the hypothesis requires that the sensory properties of oil are tactile, an obvious explanation could be that this assumption is incorrect. Olfactory sensibility is not required for oils to serve as a CS in a CTA [25]. Nevertheless, the odor of oil might substitute if other sensory information was absent. Although we did not test this possibility directly, in the first extinction trial, we did use mineral rather than corn oil. The PBNx rats avoided it, i.e. they generalized to an oil without a vapor phase and thus without an olfactory component.

If the important sensory information mediating oil recognition is neither tactile nor olfactory, then it must be gustatory. If taste is important, however, then PBN damage would have interfered with the formation of an oil CTA. In rats, the PBN is an obligate synapse for gustatory afferent activity destined for the forebrain [26–28]. Parabrachial damage blocks CTA when the CS is a taste stimulus, but not if it is primarily trigeminal, i.e. capsaicin or corn oil [11, 13].

One possibility is that the pertinent sensory activity produced by oil is trigeminal but not lemniscal. First, primary afferent axons from the lingual branch of V terminate in the rostral nucleus of the solitary tract [29]. In the vicinity of this terminal field, NST neurons respond to tactile stimulation of the intraoral cavity [30, 31]. Few of these cells project to the PBN [32] but, as with some NST taste neurons, they may terminate locally in the subjacent reticular formation [33]. Although it is not known if these neurons respond to oral oil stimulation, they represent a possible non-lemniscal route for trigeminal afferent activity to reach the forebrain.

Another possible route is the dorsal ascending secondary trigeminal tract [34]. In primates and some other species, this projection arises from the dorsomedial corner of the principal sensory trigeminal nucleus (dmPV) and ascends ipsilaterally in the central tegmental tract to the ventroposteromedial nucleus of the thalamus [35]; see Norgren & Leonard, 1973 for further references). Although this area contains neurons that respond to intraoral tactile stimulation, in rodents, this ipsilateral non-lemniscal trigeminal projection is sparse at best or absent altogether [24, 36]. Regardless, this pathway terminates in the VPM along with the

trigeminal lemniscus. Thus it also would be interrupted by thalamic lesions, leaving us back where we started without extra-thalamic oral trigeminal projections to the forebrain.

Finally, several other possible relays exist through which trigeminal afferent activity could reach the forebrain such as the posterior thalamic nuclei, the central gray, and even the hypothalamus [24, 37]. Neurons in the spinal trigeminal and paratrigeminal nuclei that project directly to the central gray or hypothalamus are normally associated with pain [24]. Trigeminal research focuses primarily on vibrissae and pain; little effort goes into the intraoral realm. The paucity of intraoral functional data frustrates evaluating the role of these extra-thalamic projections in sensing oil.

The current data eliminated the parsimonious hypothesis that prompted the experiments in the first place. We assumed that the rewarding aspects of oral oil sensibility are trigeminal and that the principal and spinal trigeminal nuclei do not have substantial direct projections to the ventral forebrain as is the case for taste. As a result, we predicted that thalamic VPM lesions would block an oil CTA but damage to the PBN would not. Our data supported the latter but not the former. This leaves open how oil becomes rewarding and underscores the need for electrophysiological data from trigeminal sensory neurons during ingestion.

The results summarized in the first paper in this series demonstrate that PBN damage reduces spontaneous intake of sucrose and interferes with operant responding for it as well. This second paper documents that the same lesions also eliminate acquiring a learned aversion to sucrose. Based on prior research, we can extend these deficits to gustatory stimuli in general. The PBN is less important for any of these tasks when corn oil, rather than sucrose, serves as the oral stimulus. Regardless of the stimulus modality, TOA damage exerted little or no influence on the same tasks. In a subsequent experiment, however, the same animals failed to demonstrate avoidance of a saccharin cue when it was paired with a drug of abuse (Nyland, Li, Liang, & Grigson, in preparation). This demonstrates that the thalamic lesions did affect at least one taste-guided behavior. In prior experiments using similar procedures, somewhat more medial thalamic damage as well as lesions of gustatory cortex disrupted suppression of a taste CS when it was paired with either a highly preferred drug or sucrose [38–40]. The third paper in this series, then, uses sham feeding of sucrose and corn oil to test whether the PBN or TOA lesion will disrupt comparison of the relative value of different stimulus concentrations in an anticipatory contrast paradigm.

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Research Highlights

- ▶ Intact rats could learn a CTA when the taste stimulus is ingested while sham feeding.
- ▶ Lesions of the PBN eliminate CTA learning to sucrose but not corn oil.
- ▶ Lesions of the TOA had no effect on CTA learning to either sucrose or corn oil.
- ▶ Pure oil orosensory may require brainstem rather than thalamic trigeminal nuclei.

Fig. 1.

Digital photomicrographs of coronal sections stained with cresyl violet, (A) PBN surgical control (B) PBN lesions (C) surgical control for TOA (D) TOA lesions. The images for the PBN used a 4 $\times$ objective; those of the TOA, 2 $\times$. The bar in (A) and (C) equals 1.0 mm. The arrows indicate the limits of gliosis after lesions. Abbreviations: BC, brachium conjunctivum; CL, central lateral nucleus; CM, central medial nucleus; MD, medial dorsal nucleus; Me5, mesencephalic trigeminal nucleus; VPM, ventroposteromedial nucleus; VPMpc, the parvicellular subdivision of VPM)

Fig. 2.

Comparisons of the CTA acquisition and test trials for the Con TOA and TOAx rats. A. Lesions of the TOA spared conditioned aversion to both sucrose (left panel) and corn oil (right panel). The data presented include the sham intake (mean $\pm$ SD) of the last day of baseline dH₂O, the CS during 3 acquisition trials, and a 1-bottle test. B. Sham intake during a 15 min, 2-bottle test for 0.3M sucrose (left) and 100% corn oil (right) compared with water. The Con TOA and TOAx rats all avoided 0.3M sucrose and 100% corn oil (* $p < 0.05$).

Fig. 3. Comparisons of the CTA acquisition and test trials for the Con PBN and PBNx rats. A. Lesions of the PBN disrupted conditioned aversion to 0.3M sucrose but not to 100% corn oil. The data presented include the sham intake (mean $\pm$ SD) of the last day of baseline dH2O, the CS during 3 acquisition trials, and a 1-bottle test. B. During a 15 min 2-bottle test the PBNx rats avoided 100% corn oil but not 0.3M sucrose while the Con PBN rats avoided both stimuli (* $p < 0.05$).