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Effects of lesions of the bed nucleus of the stria terminalis, lateral hypothalamus, or insular cortex on conditioned taste aversion and conditioned odor aversion.

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Abstract

The effects of permanent forebrain lesions on conditioned taste aversions (CTAs) and conditioned odor aversions (COAs) were examined in 3 experiments. In Experiment 1, lesions of the bed nucleus of the stria terminalis had no influence on CTA or COA acquisition. Although lesions of the lateral hypothalamus induced severe hypodipsia in Experiment 2, they did not prevent the acquisition of CTAs or COAs. Finally, in Experiment 3, lesions of the insular cortex retarded CTA acquisition but had no influence on COA acquisition. The implications of these findings are discussed with regard to the forebrain influence on parabrachial nucleus function during CTA acquisition.

If an animal survives the ingestion of a toxic food, it will avoid eating that food on subsequent encounters (Garcia & Ervin, 1968; Revusky & Garcia, 1970). In this way, conditioned taste aversions (CTAs) protect against the repeated self-administration of poisonous edibles. For experimental purposes, in the laboratory, the toxic food can be separated into the critical constituent elements: the taste (conditioned stimulus [CS]) and the aversive postingestive consequences (unconditioned stimulus [US]). Although a great deal has been learned about the behavioral parameters of CTA (see Barker, Best, & Domjan, 1977; Braveman & Bronstein, 1985; Milgram, Krames, & Alloway, 1977), the underlying neural substrates still remain something of a mystery. Indeed, after more than 30 years of study, the component parts of the central CTA system have not been identified. Nonetheless, there is a consensus that the parabrachial nucleus (PBN) is critically involved in the associative mechanism that links the taste CS with the illness US (for a review, see Reilly, 1999). Thus, rats with lesions of the PBN continue to ingest undiminished amounts of the CS even after multiple taste-illness pairings (Grigson, Reilly, Shimura, & Norgren, 1998; Reilly, Grigson, & Norgren, 1993). However, the demonstration that rats with precollicular decerebrations (which disconnect the brainstem from the forebrain while presumably leaving the PBN intact) also are incapable of acquiring CTAs (Grill & Norgren, 1978) reveals that the PBN is necessary, but not sufficient, for CTA acquisition. It would seem, then, that interplay between the PBN and forebrain is required for CTA acquisition. However, which forebrain structures are essential?

CTAs are readily acquired even following as few as one CS-US pairing and when several hours intervene between the taste experience and the subsequent illness (e.g., Garcia, Ervin, & Koelling, 1966; Revusky & Garcia, 1970; Smith & Roll, 1967). The alacrity with which an aversion can be acquired belies the fact that CTA is a complicated, multistage phenomenon (e.g., Reilly & Bornovalova, 2005). Given its complex nature, it is perhaps unsurprising that most brain structures that have been examined have yielded conflicting CTA results. For example, some studies have found CTA deficits consequent to lesions of the insular cortex (IC; Bermudez-Rattoni & McGaugh, 1991; Braun, Slick, & Lorden, 1972; Dunn & Everitt, 1988; Nerad, Ramirez-Amaya, Ormsby, & Bermudez-Rattoni, 1996; Schafe & Bernstein, 1998), but others have found little or no disruption (Kiefer & Braun, 1977; Mackey, Keller, & van der Kooy, 1986; Roldan & Bures, 1994; Yamamoto, Fujimoto, Shimura, & Sakai, 1995). Similarly, lesions of the lateral hypothalamus (LH) have been reported to impair (Caulliez, Meile, & Nicolaidis, 1996; Roth, Schwartz, & Teitelbaum, 1973; Ruch, Grigson, & Norgren, 1997; Schwartz & Teitelbaum, 1974; Touzani & Sclafani, 2002) or have no influence upon (Roldan & Bures, 1994; Touzani & Sclafani, 2001; Yamamoto et al., 1995) CTA acquisition.

As oftentimes noted in the literature (e.g., Bures, Bermudez-Rattoni, & Yamamoto, 1998; Gallo, Ballesteros, Molero, & Moron, 1999), two other factors also contribute to the rather confusing CTA-brain lesion literature. First, variations in the size and extent of the lesions, as well as the nature of the induction method (i.e., selective or nonselective lesions, permanent or temporary lesions), undoubtedly contribute to between-studies differences in behavioral results. Second, the nature of the CS and US, as well as a lack of procedural consistency, also clouds analysis of brain-behavior experiments. In the latter category, Reilly and Bornovalova (2005) identified a number of experimental design problems that confound interpretation of data obtained from lesion studies

of CTA. Four of these issues merit consideration because they are evident in the literature relevant to the present study. First, the inadvertent practice of preexposing the subjects to the taste stimulus that will later be used as a CS is problematic because CTAs are more readily acquired by novel taste stimuli than familiar (i.e., preexposed) taste stimuli, an effect termed *latent inhibition* (see Lubow, 1989, for a review). When this occurs, it is not possible to determine whether any lesion-induced deficit is due to a disruption of CTA or latent inhibition. Second, possibly because CTAs can be acquired following one CS-US pairing by neurologically intact rats, it is a common practice in the CTA-brain lesion literature also to use only a single acquisition trial. This design choice may not allow an accurate determination of whether the lesions attenuate or prevent CTA acquisition, an interpretational issue that is particularly problematic following inadvertent preexposure to the CS, or if the lesion disrupts the initial neophobic response to the taste stimulus prior to its pairing with the US. In this latter case, if a lesion disrupts the perception of novelty, then CTA may be retarded because of a latent inhibition-like effect. Third, many studies use two-bottle (CS vs. water) tests to assay the acquired aversion. This method may not be entirely appropriate because, regardless of the strength of the aversion, the rat can avoid the CS by drinking the water. In so doing, the strength of the aversion will be amplified, and genuinely weak CTAs would appear strong. Indeed, this practice has the potential to obscure a lesion-induced disruption of CTA. The problem may be avoided by using a one-bottle (i.e., CS-only) test procedure because it is more sensitive to the detection of between-groups differences in the strength of an aversion (Batsell & Best, 1993), as may occur when examining the influence of brain manipulations on CTA acquisition. Finally, another common practice involves determining the strength of a CTA by examining CS intake in extinction, using repeated CS-only test trials. This practice is based on the (typically unstated) assumption that extinction involves unlearning of the previously acquired association. A great deal of empirical evidence indicates that this assumption is not valid and that extinction actually involves the acquisition of new knowledge (likely an inhibitory association) that competes with the original association to govern the level of manifest responding during CS-only test trials (e.g., Calton, Mitchell, & Schachtman, 1996; Rosas & Bouton, 1997). Thus, a lesion-induced performance difference revealed during postacquisition CS-only test trials cannot unambiguously be attributed to a disruption of taste-illness learning; it may indicate a disruption of the inhibitory learning that occurs in extinction. The experiments described in the present article were designed to avoid these design and interpretational issues that exist in much of the extant CTA literature, thereby making the results obtained more reliable and definitive regarding the influence of these brain areas on CTA learning.

Because CTA is a taste-guided behavior, our approach to the selection of appropriate target structures was based on the projection sites of the PBN within the central gustatory system. Ascending gustatory information from the mouth synapses in the nucleus of the solitary tract before projecting to the PBN (Norgren & Leonard, 1971). A bifurcating pathway sends axons from the PBN along two routes to the forebrain. Axons of the thalamocortical or dorsal pathway synapse in the gustatory thalamus (GT; parvicellular region of the ventral posteromedial nucleus; Halsell, 1992) en route to the IC (Kosar, Grill, & Norgren, 1986). The second, ventral pathway involves projections, which in all cases are reciprocal, to a number of forebrain structures, including the central nucleus of the amygdala (CNA; Karimnamazi & Travers, 1998), the basolateral amygdala (BLA; Norgren, 1976), the LH (Bester, Besson, & Bernard, 1997), and the bed nucleus of the stria

terminalis (BNST; Alden, Besson, & Bernard, 1994). There are, moreover, connections between the GT and CNA (Nakashima et al., 2000), CNA and BLA (Pitkanen, Savander, & Le Doux, 1997), CNA and IC (Ottersen, 1982), BLA and IC (Krettek & Price, 1977), and PBN and IC (Saper, 1982). The fibers of the PBN-IC pathway pass through but do not synapse in the CNA (Frey, Morris, & Petrides, 1997).

We have recently completed a study of the influence of CNA and BLA lesions on the acquisition of CTA (St. Andre & Reilly, 2006). The present study focuses on three other structures that are recipients of ascending taste projections from the PBN: BNST, LH, and IC. To ensure comparison among the studies, a standardized behavioral procedure was employed that utilized saccharin as the CS and toxicosis induced by lithium chloride (LiCl) as the US, in a procedure that involved two or three conditioning trials and a single CS-only test trial.

As suggested by the data from decerebrate rats, an intact PBN is interacting with one or more forebrain structures during CTA acquisition. If this analysis is correct, then it should be possible to lesion at least one forebrain structure and obtain CTA deficits that are as profound as those seen following PBN lesions. The goal of the present study was to determine whether the BNST (Experiment 1), LH (Experiment 2), or IC (Experiment 3) is interacting with the PBN during CTA acquisition.

Lesions of the medial, gustatory region of the PBN also prevent the development of conditioned odor aversions (COAs; Grigson, Reilly, Scalera, & Norgren, 1998). To obtain a more complete understanding of the relationship between the PBN and the BNST, LH, and IC, we examined whether lesions of these forebrain structures influence the acquisition of COAs as well as CTAs. Although there appears to be no direct pathway from the olfactory bulb to the PBN (Shipley, Ennis, & Puche, 2004), some neurons in the PBN are responsive to both gustatory and olfactory stimuli (DiLorenzo & Garcia, 1985). As noted above, the BNST, LH, and IC maintain reciprocal connections with the PBN. So, in addition to their known direct connections with the olfactory system (Shipley et al., 2004), our selected forebrain structures may receive olfactory information indirectly via the PBN. It is also possible that lesions of the BNST, LH, or IC could interrupt the processing of US-related information. This type of deficit has been demonstrated with lesions of the lateral PBN (e.g., Reilly & Trifunovic, 2000a, 2000b) and the area postrema (e.g., Berger, Wise, & Stein, 1973; Coil & Norgren, 1981; Ritter, McGlone, & Kelley, 1980). If BNST, LH, or IC lesions disrupt both CTA and COA, then one possible explanation would be a deficiency in the lesioned rats' ability to experience visceral feedback, as seen with lesions of the lateral PBN and area postrema. On the other hand, a finding of impaired CTA and normal COA in lesioned rats would seem to rule out an interpretation in terms of a disruption of ascending viscerosensory information. Thus, investigating the effects of brain lesions on both CTA and COA afforded greater explanatory power than examining CTA in isolation.

Experiment 1: Bed Nucleus of the Stria Terminalis

Animals consume less of a particular food item when it is novel compared with when it is familiar. This innate fear of novel edibles is called *gustatory neophobia* (Barnett, 1963; Corey, 1978; Domjan, 1977), which protects against the ingestion of large amounts of a potentially toxic new

food. However, the absence of aversive gastrointestinal consequences leads to an attenuation of the initial neophobic reaction manifested as an increase in consumption until, after repeated encounters, intake asymptote is reached for the now familiar and safe food. On the other hand, as noted above, if animals become ill after eating a novel food, they rapidly develop an aversion and avoid eating that food on future encounters. Thus, any CTA experiment minimally involves an interaction between the innate fear triggered by the detection of novelty and the development of an acquired aversion based on the knowledge that the food caused the later illness.

The BNST has been implicated in both unconditioned fear and anxiety responses (e.g., Gewirtz, McNish, & Davis, 1998; Schulkin, Morgan, & Rosen, 2005; Walker, Toufexis, & Davis, 2003). Thus, there is some reason to expect that BNST-lesioned (BNSTX) rats may be impaired in the expression of neophobia as well, perhaps, as the development of CTAs. To investigate these possibilities required a design involving multiple conditioning trials to quantify the magnitude of any obtained deficit, an analysis not feasible in one-trial conditioning procedures. Although gustatory projections from the PBN are known to terminate in the BNST, we were not sure whether ascending olfactory or viscerosensory axons pass through as well as synapse in the nucleus. Accordingly, we decided to use electrolytic lesions in the present experiment. The absence of lesion-induced deficits would provide definitive evidence that the BNST has no role in neophobia, CTA, COA, or viscerosensory processing. On the other hand, if behavioral deficits were obtained, additional research would be undertaken using neurotoxic lesions to determine the underlying neural substrate (intrinsic neurons or fibers of passage).

Method

Subjects

The subjects were 22 male Sprague-Dawley rats purchased from Charles River Laboratories (Wilmington, MA). The rats were individually housed in hanging stainless steel cages in a room with 12-hr light-dark cycles (lights on at 0700). All experimentation was conducted during the light phase. The rats weighed 280–320 g at the time of surgery. Food and water were available at all times, except as noted below during behavioral testing. All rats were treated in accordance with the National Institutes of Health's (1986) *Guide for the Care and Use of Laboratory Animals* and the American Psychological Association's guidelines for animal research, and the University of Illinois at Chicago Institutional Animal Care and Use Committee approved the experimental protocols.

Surgery

12 rats were given bilateral electrolytic lesions to the BNST (Group BNSTX). During surgery, the rats were anesthetized with sodium pentobarbital, and body temperature was monitored and maintained at 37 °C by a heating pad (Harvard Apparatus, Holliston, MA). The subjects were fixed into a stereotaxic apparatus (ASI, Warren, MI) using blunt ear bars, and the skull was exposed by a midline incision. After the skin and membrane were retracted, the head was leveled with respect to bregma and lambda by moving the bite bar. Trepine holes were drilled over the BNST, and a Teflon-coated tungsten electrode (AM Systems, Carlsborg, WA) was lowered to the lesion sites. On the basis of pilot surgeries, the lesion coordinates used were AP -0.4, ML \pm 1.7, DV -6.0.

Electrolytic lesions were induced by delivering a 1.2-mA DC current for 50 s from a lesion-making device (Stoelting, Wood Dale, IL). After surgery, the incisions were closed using wound clips, and the subjects' temperatures were monitored until normal and stable. Nonlesioned control rats (Group SHAM) were anesthetized and allowed to recover. Rats that received BNST lesions exhibited a transient decrease in body weight postsurgery but consumed food and liquids normally and gained weight at a typical rate.

Apparatus

All testing for each experiment was conducted in the home cages. Fluid intake was measured by attaching inverted 100-ml graduated cylinders with silicone stoppers and steel drinking tubes to the front of the cages. Fluid intake was recorded to the nearest 0.5 ml.

Procedure

In Experiment 1A, following recovery from surgery, the rats were placed on a deprivation schedule that permitted 15 min of access to water each day. This restriction schedule was selected to maintain comparability with procedures used in our PBN studies (e.g., Grigson, Reilly, Shimura, & Norgren, 1998; Reilly, Grigson, & Norgren, 1993; Reilly & Trifunovic, 2000a). When intake stabilized, the experiment began. On Day 1, all rats received 15 min of access to 0.15% (wt/vol) sodium saccharin followed, 15 min after the stimulus bottles were removed, by an intraperitoneal injection of 0.15 M LiCl (1.33 ml/100 g body weight). All rats received a second conditioning trial on Day 4 that was identical in all respects to the treatment given on Day 1. Finally, on Day 7, all rats received a 15-min test trial where the CS was provided without a subsequent US.

In Experiment 1B, after the CTA experiment concluded, the subjects were given food and water ad libitum for several days and then returned to a water-restricted schedule of 15 min per day, in preparation for the COA experiment. The COA procedure was identical to that described above for the CTA experiment, except that a 0.02% (vol/vol) solution of orange extract (Flavorganics, Newark, NJ) was used as the olfactory CS and there were three conditioning trials and one CS-only test trial.

Histology

On completion of Experiment 1B, BNSTX rats were given a lethal injection of sodium pentobarbital and perfused transcardially with physiological saline followed by 4% buffered formalin. The brains were then extracted and stored for at least 2 days in 4% buffered formalin, followed by at least 2 days in 20% (wt/vol) sucrose. Coronal sections were taken at 50 μ m using a cryostat. Sections were mounted on gelatin-coated glass slides, and the tissue was stained with cresyl violet. Reconstructions of the BNST lesions were made with the aid of a microscope (Zeiss Axioskop 40 equipped with a Q-Imaging Camera running Q-Capture software; Quantitative Imaging Corporation, Burnaby, British Columbia, Canada) on standard drawings derived from the Paxinos and Watson (2005) atlas. These drawings were then used to determine the accuracy of placement and extent of the lesions.

Results and Discussion

Anatomical

Evaluation of the histology revealed that 6 BNSTX rats received either unilateral ($n = 1$) or small lesions ($n = 5$); these subjects were dropped from the experiment. The remaining subjects had bilateral lesions damaging 75% or more of the BNST. Serial reconstructions of the smallest and largest BNST lesions are shown in Figure 1.

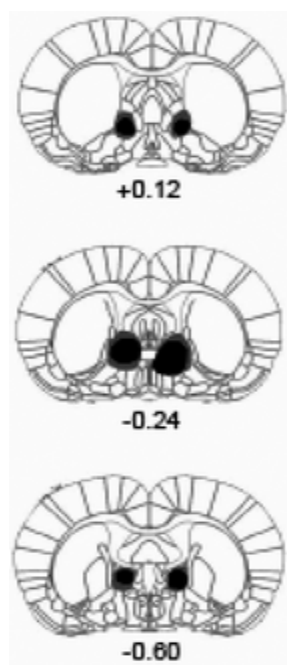


Figure 1. Serial reconstructions of the largest (gray) and smallest (black) electrolytic lesions of the bed nucleus of the stria terminalis in rats that provided behavioral data for the statistical analyses in Experiment 1. The areas of neural damage are shown at three levels (+0.12, -0.24, -0.60 mm relative to bregma) on diagrams from *The Rat Brain in Stereotaxic Coordinates* (5th ed., Figures 32, 35, & 38, respectively), by G. Paxinos and C. Watson, 2005, San Diego, CA: Academic Press. Copyright 2005 by Academic Press. Adapted with permission from Elsevier

Behavioral

In Experiment 1A, as inspection of Figure 2A shows, the BNSTX rats acquired a CTA at the same rate as the SHAM subjects. An analysis of variance (ANOVA; Statistica 6.0; StatSoft, Tulsa, OK) conducted on the data summarized in the figure found a significant main effect of trials, $F(2, 28) = 63.24$, $p < .0001$. There was, however, no main effect of lesion ($F < 1$) or Lesion \times Trials interaction ($F < 1$).

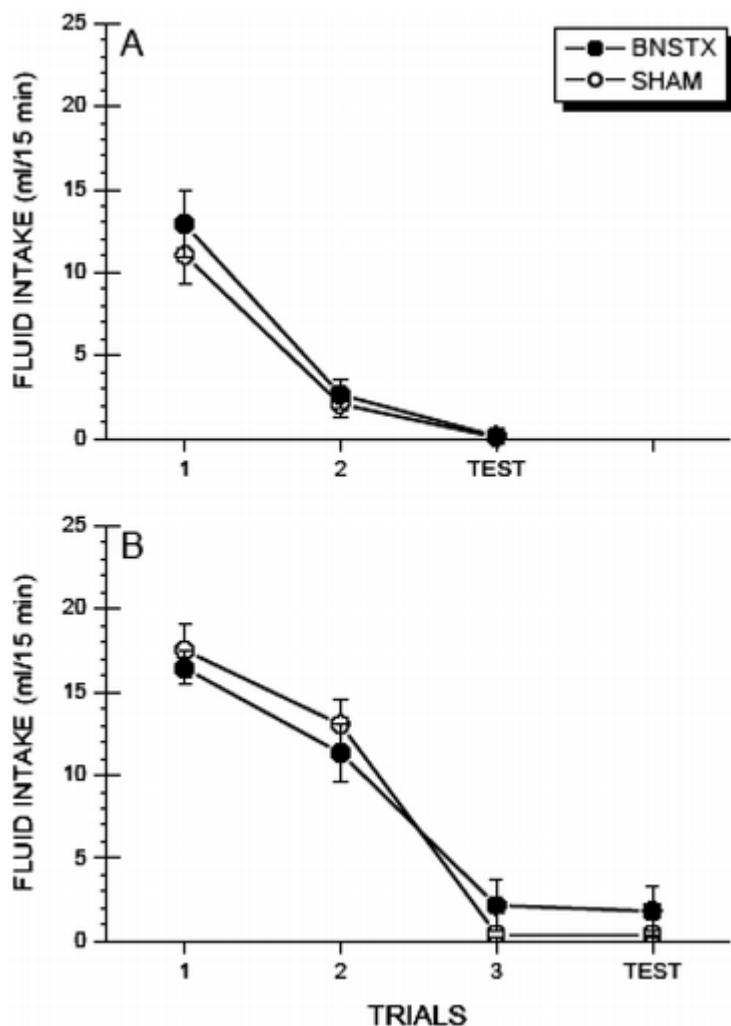


Figure 2. Mean (\pm SE) conditioned stimulus consumption for nonlesioned control (SHAM) and bed nucleus of the stria terminalis-lesioned (BNSTX) subjects during the 15-min conditioning and test trials in the conditioned taste aversion (Panel A) and conditioned odor aversion (Panel B) procedures of Experiment 1

In Experiment 1B, a virtually identical pattern of results was obtained in the COA experiment (see Figure 2B). An ANOVA conducted on the COA data revealed a significant main effect of trials, $F(3, 42) = 108.73, p < .0001$, but no significant main effect of lesion ($F < 1$) and no Lesion \times Trials interaction ($F < 1$). Thus, the results of Experiment 1 are clear and unambiguous: BNST lesions have no influence on the acquisition of CTA or COA.

Experiment 2: Lateral Hypothalamus

It is well documented that LH-lesioned (LHX) rats are hypophagic and hypodipsic (for a review, see Bernardis & Bellinger, 1996). These phenomena, which are both significant and substantial, create immediate problems of interpretation in CTA and COA studies because underconsumption of the CS by LHX rats may be misinterpreted as indication of a lesion-induced disruption of perceived stimulus intensity or an enhanced neophobic reaction. This issue becomes especially problematic when only a single conditioning trial is employed. One way to address this problem

or at least to determine the occurrence and magnitude of the hypodipsia requires the inclusion of a treatment condition that involves the injection of saline rather than LiCl. In this way, for each group (SHAM and LHX), the saline-injected rats serve as a baseline against which the performance of the LiCl-injected rats can be compared.

Method

Subjects

The subjects were 30 male Sprague-Dawley rats purchased from Charles River Laboratories (Wilmington, MA). These rats were housed and maintained under the same conditions as the rats in Experiment 1.

Surgery

Eighteen rats were given bilateral *N*-methyl-D-aspartic acid (NMDA) lesions of the LH (Group LHX). The surgeries were identical to those performed in Experiment 1, except for the location and type of lesion. Trehine holes were drilled over the LH, and a glass pipette (tip diameter ~75 μ m) containing 0.15 M NMDA (Sigma Chemical, St. Louis, MO) was lowered to the lesion sites, of which there were two per hemisphere. On the basis of pilot surgeries, the lesion coordinates were AP -2.1 , ML ± 1.9 , DV -7.8 , and AP -3.6 , ML ± 1.8 , DV -8.3 . Iontophoretic infusions (Midgard Precision Current Source; Stoelting, Wood Dale, IL) were performed at these locations, each 7 min in duration driven by a -10 - μ A charge. Nonlesioned controls (Group SHAM) were either anesthetized ($n = 6$) or anesthetized and had trephine holes drilled in the same locations but did not receive lesions ($n = 6$). As expected, rats that received LH lesions exhibited a profound decrease (30–40 g) in body weight postsurgery and were hypodipsic and hypophagic during the experiments.

Procedure

In Experiment 2A, after a 1-week recovery period, all subjects were placed on a water deprivation schedule of 15 min per day. LHX subjects did not drink enough water to maintain body weight, so an additional 60 min of water was given later in the day. Because water intake remained low, food was removed from the cages during the 15-min fluid access periods to prevent eating from interfering with drinking. When water intake and body weight stabilized, the subjects were divided into four groups. Defined by lesion and US types, the groups were LHX-LiCl, LHX-saline, SHAM-LiCl, and SHAM-saline. The conditioning trials (Days 1, 4, and 7) and test (Day 10) were identical in all respects to those described in Experiment 1A, except that half of the SHAM and LHX rats were injected with LiCl; the remaining rats were injected with an equivalent volume of physiological saline.

For Experiment 2B, after Experiment 2A concluded, the US assignments were reversed. That is, the rats that received LiCl injections in Experiment 2A received saline injections in Experiment 2B, and the rats that received saline injections in Experiment 2A received LiCl injections in Experiment 2B. The COA experiment was conducted using the same parameters as the CTA study, except that a 0.02% solution of orange extract was used as the CS.

Histology

The histology was as described in Experiment 1, except that tissue was sectioned at 30 μm and stained for the specific neuronal protein NeuN (Jongen-Rêlo & Feldon, 2002; Mullen, Buck, & Smith, 1992) as follows. After mounting, a hydrophobic barrier was circumscribed around the tissue sections and allowed to dry. The sections were hydrated with phosphate-buffered saline (PBS) followed by a 60-min incubation in blocking buffer containing goat serum (Vector Laboratories, Burlingame, CA) and bovine serum albumin (Sigma Chemical, St. Louis, MO), overnight incubation in blocking buffer containing NeuN (Chemicon, Temecula, CA), 60-min incubation with blocking buffer containing biotinylated goat-anti-mouse IgG (Vector Laboratories), 60-min incubation with ABC kit (Vector Laboratories), and 15-min incubation with DAB kit (Vector Laboratories). Each incubation was PBS based and was followed by three PBS rinses. Slides were then allowed to dry, hydrated using xylene baths, and cover slipped with Permount (Fisher Scientific, Pittsburgh, PA).

Results and Discussion

Anatomical

A review of the histology revealed that 6 LHX rats received either unilateral ($n = 2$) or small lesions ($n = 4$) and therefore were dropped from the experiment. The remaining subjects had bilateral lesions damaging 60% or more of the LH. Figure 3 shows schematic representations of the smallest and largest LH lesions.



Figure 3. Serial reconstructions of the largest (gray) and smallest (black) *N*-methyl-*D*-aspartate lesions in the lateral hypothalamus of rats that provided behavioral data for the statistical analyses in Experiment 2. The areas of cell loss are shown at five levels (-1.8 , -2.4 , -3.0 , -3.6 , -4.2 mm relative to bregma) on diagrams from *The Rat Brain in Stereotaxic Coordinates* (5th ed., Figures 48, 53, 58, 63, & 68, respectively), by G. Paxinos and C. Watson, 2005, San Diego, CA: Academic Press. Copyright 2005 by Academic Press. Adapted with permission from Elsevier

Behavioral

In Experiment 2A, as expected, the LHX rats drank less fluid throughout the experiment than did the SHAM subjects. Mean ($\pm SE$) intakes during the 15-min water trial on the day before the first CTA trial were SHAM-saline = 19.7 (± 0.60) ml, SHAM-LiCl = 19.4 (± 0.82) ml, LHX-saline = 9.7 (± 0.60) ml, and LHX-LiCl = 8.8 (± 0.78) ml. A 2 (lesion: LHX, SHAM) \times 2 (US: saline, LiCl) ANOVA found a significant main effect of lesions, $F(1, 20) = 218.50$, $p < .001$, but no main effect of US (at this stage, a pseudofactor; $F < 1$) and no Lesion \times US interaction ($F < 1$).

A 2 (lesion: SHAM, LHX) \times 2 (US: saline, LiCl) \times 4 (trials) mixed design ANOVA conducted on the data summarized in Figure 4A found significant main effects of lesion, $F(1, 20) = 17.94$, $p < .001$; US, $F(1, 20) = 205.68$, $p < .0001$; and trials, $F(3, 60) = 27.95$, $p < .0001$. A significant Lesion \times US \times Trials interaction was also obtained, $F(3, 60) = 6.40$, $p < .001$. The overall difference in CS consumption negates direct comparison between the LHX and SHAM subjects. We therefore compared the saccharin intake of the saline- and LiCl-treated rats in each of the lesion groups. Post hoc analysis of the triple interaction with Fisher's least significant difference (LSD) test revealed no difference between saline- and LiCl-injected SHAM subjects on Trial 1 ($p > .30$). There were, however, significant differences between US conditions on Trials 2 and 3, as well as on the test trial ($p < .05$), for the SHAM subjects. An identical pattern of significance was obtained from the LHX rats. Thus, significant differences were found on Trial 2, Trial 3, and the test trial ($p < .05$), but not on Trial 1 ($p > .25$). Of further relevance, the SHAM-LiCl subjects consumed significantly less saccharin on the test trial than on the first CTA trial ($p < .05$). Similarly, the LHX-LiCl rats demonstrated a CTA by drinking less saccharin on the test trial than on Trial 1 ($p < .05$). We therefore conclude that, beyond the anticipated lesion-induced reduction in fluid intake, the LH has little if any role in CTA acquisition under the present conditions.

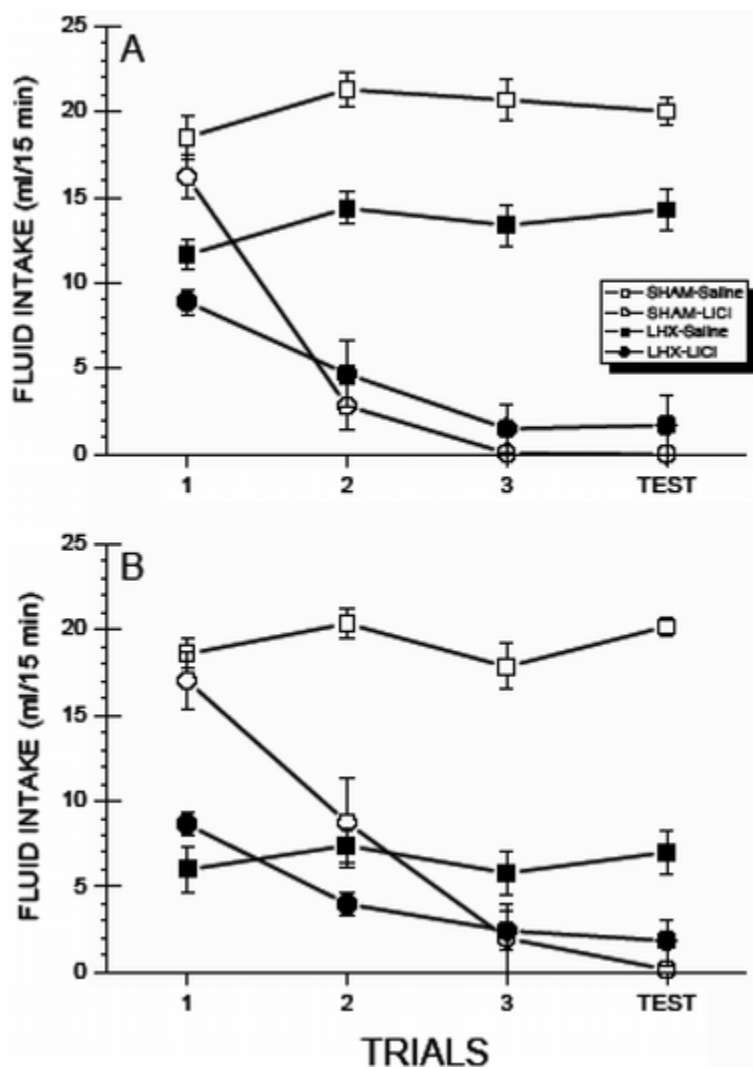


Figure 4. Mean (\pm SE) conditioned stimulus consumption for nonlesioned control (SHAM) and lateral hypothalamus-lesioned (LHX) rats during the 15-min conditioning and test trials in the conditioned taste aversion (Panel A) and conditioned odor aversion (Panel B) procedures of Experiment 2. On the conditioning trials, rats were injected with either physiological saline (saline groups) or lithium chloride (LiCl groups)

In Experiment 2B, mean (\pm SE) water intakes on the day before the first acquisition trial were SHAM-saline = 16.3 (\pm 1.98) ml, SHAM-LiCl = 16.8 (\pm 0.87) ml, LHX-saline = 6.4 (\pm 1.47) ml, and LHX-LiCl = 7.2 (\pm 1.26) ml. A 2×2 ANOVA was conducted and indicated that there was again a significant effect of lesions, $F(1, 20) = 45.27, p < .001$, but no effect of US ($F < 1$) and no Lesion \times US interaction ($F < 1$). As in Experiment 2A, the baseline water consumption of the LHX rats was significantly lower than that of the SHAM subjects. It might also be noted that both SHAM and LHX rats drank less water on the day before the COA experiment than the CTA experiment.

A three-factor ANOVA conducted on the data summarized in Figure 4B found a significant Lesion \times US \times Trials interaction, $F(3, 60) = 5.99, p < .001$. We again compared the CS intake of the saline- and LiCl-treated rats separately for the SHAM and LHX rats. Post hoc analysis (LSD test)

of the triple interaction indicated that the SHAM-saline rats differed from the SHAM-LiCl rats on each trial ($p < .001$) except for the first ($p > .50$). Surprisingly, post hoc evaluation of the data from the LHX subjects found no significant difference between saline- and LiCl-injected rats on each of the four trials (Trial 1 $p > .30$, Trial 2 $p > .20$, Trial 3 $p > .20$, and test trial $p = .08$). Confident interpretation of these latter statistics as a lesion-induced deficit of COA is, however, compromised by the low levels of saccharin consumption in the LHX-saline rats (~50% lower than the corresponding group in Experiment 2A) that may be masking genuine learning in the LHX-LiCl rats. We therefore examined the CS intake of the LHX-LiCl rats on Trial 1 compared with each of the subsequent trials to determine if these rats acquired a COA. For each of these comparisons, there was a significant difference ($p < .001$). Thus, after a single odor-illness pairing, the LHX-LiCl rats displayed a significant odor aversion, reducing their consumption by 50% on Trial 2 relative to Trial 1 (see Figure 4B). On the basis of this final analysis, we are inclined to conclude that despite the severe hypodipsia, the LH does not have a major role in the acquisition of COA as determined under the present conditions.

Experiment 3: Insular Cortex

Presumably because strong CTAs can be acquired following one CS-US pairing, many neurobehavioral studies, including those involving IC lesions, have employed a design with only a single acquisition trial. However, this practice can create problems of interpretation in certain cases. For example, a lesion might diminish the magnitude of the initial neophobic reaction because the rat incorrectly perceives the novel stimulus to be familiar. In this case, one might also expect the lesion to retard CTA acquisition because aversions to familiar gustatory stimuli are less readily acquired than aversions to novel gustatory stimuli (Best, 1975; Reilly, Harley, & Revusky, 1993; Revusky & Bedarf, 1967; for a review, see Lubow, 1989). Because there is some evidence that IC lesions may disrupt neophobia (e.g., Braun, Lasiter, & Kiefer, 1982; Kiefer, Rusiniak, & Garcia, 1982), an interpretational problem may arise in experiments that use a one-trial CTA procedure. The likelihood that a lesion-induced neophobia deficit is misinterpreted as a disruption of aversion learning is inversely related to the number of CTA acquisition trials. Thus, our standard, multiple-trial design is well suited for the assessment of the acquisition of CTA in IC-lesioned (ICX) rats.

Method

Subjects

The subjects were 20 male Sprague-Dawley rats purchased from Charles River Laboratories (Wilmington, MA). They were housed and maintained under the same conditions as the rats in Experiments 1 and 2.

Surgery

Twelve rats received bilateral 0.15-M NMDA lesions of the IC (Group ICX). The surgeries were identical to those performed in Experiment 2, except for the location of the lesions. A trephine hole was drilled through the skull overlying the IC of both hemispheres, and NMDA was iontophoretically infused via a glass micropipette (tip diameter ~75 μm) at the sites in each

hemisphere. The lesion coordinates (and infusion duration) for the two lesions were AP +1.2, ML \pm 5.2, DV -5.0 (9 min), and AP +1.2, ML \pm 5.2, DV -4.3 (5 min). Eight rats were anesthetized but received no surgical treatments (Group SHAM).

Procedure

In Experiment 3A, the procedure was identical to that described in Experiment 1A, except that a third day of water access was given after the first conditioning trial because the ICX rats did not return to baseline fluid consumption during the first 2 water days. Additionally, there were three (not two) conditioning trials.

For Experiment 3B, the procedure was identical to that of Experiment 1B, except that there were two (not three) conditioning trials.

Histology

Histological processing was the same as that described in Experiment 1.

Results and Discussion

Anatomical

Figure 5 shows serial reconstructions of the smallest and largest IC lesions. The locus and extent of the lesions were identified by the loss and shriveling of neuronal cell body and the presence of gliosis. One ICX rat died following the water deprivation onset. Behavioral data of 3 rats were discarded from the statistical analysis because the NMDA lesions were either incomplete ($n = 2$) or unilateral ($n = 1$). The remaining 8 ICX rats had well-placed, symmetrical lesions of the IC. The lesions spread from the outer cortical layers to the external capsule and caused some minor damage to the claustrum.

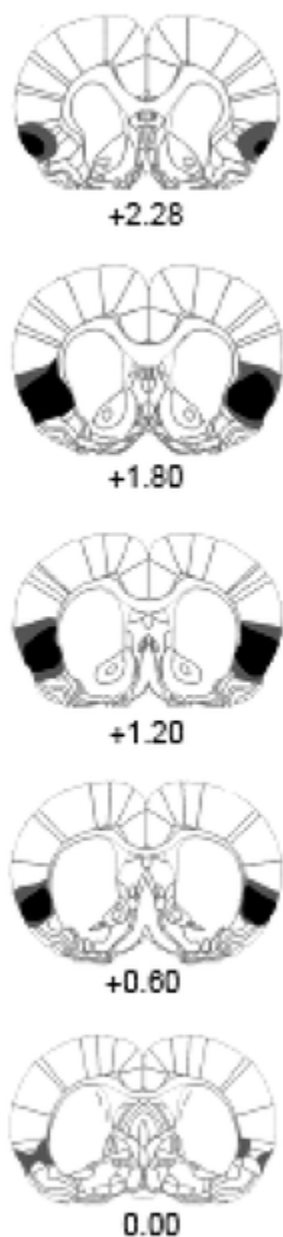


Figure 5. Serial reconstructions of the largest (gray) and smallest (black) *N*-methyl-*D*-aspartate lesions of the insular cortex in rats that provided behavioral data for the statistical analyses in Experiment 3. The areas of cell loss are shown at five levels (+2.28, +1.80, +1.20, +0.60, 0.00 mm relative to bregma) on diagrams from *The Rat Brain in Stereotaxic Coordinates* (5th ed., Figures 14, 18, 23, 28, & 33, respectively), by G. Paxinos and C. Watson, 2005, San Diego, CA: Academic Press. Copyright 2005 by Academic Press. Adapted with permission from Elsevier

Behavioral

In Experiment 3A, as is evident from inspection of Figure 6A, IC lesions had a substantial influence on saccharin intake during the experiment. On the first conditioning trial, the ICX rats consumed twice as much saccharin as the SHAM subjects, an intergroup difference that persisted until the test trial, when all rats drank minimal amounts of the CS. Unsurprisingly, then, an

ANOVA found a significant main effect of lesion, $F(1, 14) = 74.35, p < .001$; a significant main effect of trials, $F(3, 42) = 198.56, p < .001$; and a significant Lesion \times Trials interaction, $F(3, 42) = 32.11, p < .001$. Further analysis with Fisher's LSD test revealed that the ICX rats drank more saccharin than the SHAM subjects on Trial 1, Trial 2, and Trial 3 ($ps < .05$). There was, however, no significant group difference on the test trial ($p > .50$). Because the ICX rats consumed significantly more fluid on Trial 1 (i.e., before the US was administered) than did the SHAM subjects, the deficit must be related to some feature of the taste cue rather than of learning per se. That is, the attenuation of CTA likely is a secondary consequence of overconsumption of the taste cue on the first conditioning trial. Because IC lesions appear to have no major influence on taste perception (e.g., Braun et al., 1982), we are currently investigating the possibility that the Trial 1 deficit in the present experiment reflects a lesion-induced disruption of gustatory neophobia.

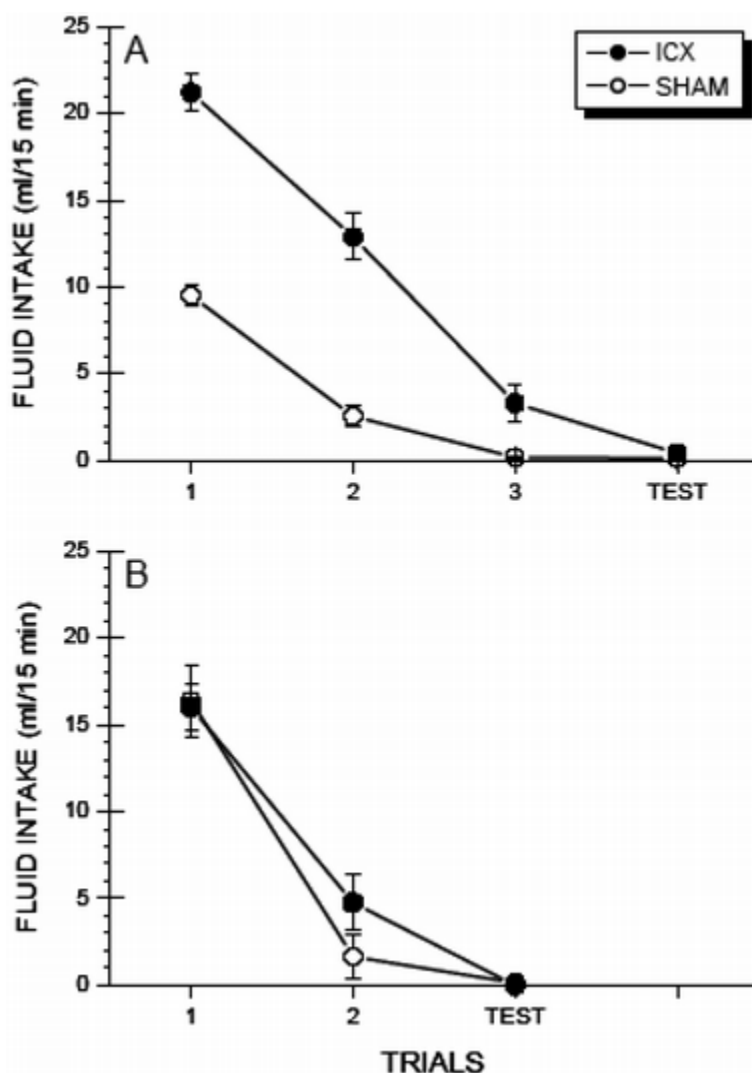


Figure 6. Mean (\pm SE) conditioned stimulus consumption for nonlesioned control (SHAM) and insular cortex-lesioned (ICX) subjects during the 15-min conditioning and test trials in the conditioned taste aversion (Panel A) and conditioned odor aversion (Panel B) procedures of Experiment 3

In Experiment 3B, as shown in Figure 6B, the ICX rats acquired a COA as rapidly as the SHAM subjects. This impression of the data was confirmed with an ANOVA that found a significant main effect of trials $F(3, 39) = 109.35, p < .001$, but no main effect of lesion ($F < 1$) or Lesion \times Trials interaction ($p > .25$).

General Discussion

The findings that decerebrate rats (Grill & Norgren, 1978) and rats with lesions of the PBN (e.g., Reilly, Grigson, & Norgren, 1993) are each incapable of acquiring CTAs support the view that the PBN must interact with at least one forebrain structure for successful taste aversion learning to occur. The present experiments are part of an attempt to identify forebrain nuclei that are interacting with the PBN during CTA acquisition. Using a standardized behavioral procedure, the results show that BNST lesions have no effect on CTA or COA acquisition and that LH lesions, despite inducing severe hypodipsia, do not prevent the across-session LiCl-induced reduction of CS intake that is the hallmark of CTA and COA. Finally, lesions of the IC resulted in a significant overconsumption of the taste cue on the first conditioning trial (~100% more than the SHAM subjects). However, this deficit did not prevent ICX rats from acquiring CTA as assessed by virtually complete suppression of CS intake on the test trial. Furthermore, this deficit, whatever its nature, was selective to CTA, with there being no influence of the lesion on COA acquisition.

To aid interpretation of CTA data, we characterize the process as involving five broadly defined stages (e.g., Reilly & Bornovalova, 2005). First, the brain must detect and process the taste stimulus that will (following pairing with the US) become the CS. Second, the information that constitutes the illness US must be detected and processed. Third, the neural representations of taste and illness must be integrated to form the learned association. Fourth, the knowledge embodied in this association must be retrieved when the CS is encountered again. Finally, that knowledge must be expressed in performance. As explained in detail elsewhere (e.g., Reilly, 1999), we believe that lesions of the medial, gustatory region of the PBN disrupt the associative mechanism that links the taste CS with the illness US (i.e., Stage 3 in the model outlined above). As inferred from the decerebrate rat data, at least one forebrain nucleus is also a component of the associative mechanism that underlies taste aversion learning.

The major forebrain recipients of ascending gustatory information from the medial PBN are the GT, BNST, amygdala, LH, and IC. The GT does not appear to be involved in first-order CTA learning (Flynn, Grill, Schulkin, & Norgren, 1991; Scalera, Grigson, & Norgren, 1997), although there is some suggestion that it may have a role in the acquisition of CTAs when multiple-taste CSs are employed (Reilly, Bornovalova, Dengler, & Trifunovic, 2003). Work recently completed in our lab (St. Andre & Reilly, 2006) found no influence of CNA lesions on CTA, and the deficit obtained in rats with lesions of the BLA seems best characterized as a dysfunction of taste-CS processing. Finally, the results of the present study indicate that lesions of the BNST, LH, and IC do not render rats incapable of acquiring CTAs.

Given that we have examined the most likely forebrain candidate structures that might interact with the PBN during CTA acquisition, how, then, are the CTA results from decerebrate and PBNX rats to be reconciled? We see three potential interpretations.

First, perhaps there is an as yet unidentified forebrain structure that, when lesioned, will, like the loss of medial PBN neurons and decerebration, prevent CTA acquisition. This remains a possibility and cannot be ruled out until an exhaustive examination of all forebrain structures that are connected to the medial PBN have been properly investigated for their role in CTA. At the present time, we are not convinced that a search for this missing link will yield positive results. A variant of this first interpretation suggests that two or more forebrain structures act in concert with the PBN during CTA acquisition. Indeed, suggestions of this type are evident in the literature. For example, Yamamoto (1993) proposed that the amygdala and IC functionally interact during CTA acquisition. An immediate difficulty for this particular combination of forebrain structures is that the behavioral design involved only a single conditioning trial. As explained in detail elsewhere (Reilly & Bornovalova, 2005), lesion-induced deficits observed after a single conditioning trial are difficult to interpret with confidence because it is possible to confuse elimination with the attenuation of CTA. This problem of the correct identification of the CTA deficit is directly related to the number of conditioning trials. As shown by St. Andre and Reilly (2006), using a design that involved multiple conditioning trials, CNA lesions have no influence on CTA acquisition, whereas BLA lesions attenuate acquisition to a novel, but not a familiar, taste CS (also see Morris, Frey, Kasambira, & Petrides, 1999). Also, as noted above, IC lesions do not prevent CTA acquisition. Rather, they slow the speed of acquisition because of a deficit in taste or CS processing. Thus, we are unconvinced that the amygdala and IC are the critical forebrain structures involved in CTA acquisition. Nonetheless, this version, like the single-structure version, cannot be dismissed until an exhaustive investigation of all possible combinations of forebrain nuclei has been completed.

A second interpretation focuses on differences in the procedures employed to induce CTAs. As used in all our research, the standard CTA procedure involves voluntary consumption of a fluid that the rat can avoid simply by moving away from and not drinking the CS solution. Because decerebration renders the rat adipsic, aphagic, and incapable of any spontaneous behavior (e.g., Grill & Kaplan, 1990), the voluntary intake method cannot be used with the decerebrate rat. For these rats, the CS solution is infused directly into the oral cavity via a chronically implanted cannula (Grill & Berridge, 1985; Grill & Norgren, 1978). The dependent measures used with this CS delivery procedure involve stereotypical orofacial responses that are categorized as acceptance responses if the fluid is swallowed or rejection responses if the fluid is allowed to passively drip from the mouth. In other words, the voluntary intake method is an active avoidance procedure, whereas the involuntary, infusion method is a passive avoidance procedure. Bernstein and colleagues (e.g., Schafe, Thiele, & Bernstein, 1998; Spray, Halsell, & Bernstein, 2000) have provided evidence that suggests that qualitatively different neural substrates are involved with voluntary and involuntary intake procedures. If this analysis is correct, then the CTA data from decerebrate rats and PBNX rats may simply be explained in terms of the different methods used to present the CS solutions.

The third and admittedly most speculative way to reconcile the CTA results from decerebrate and PBNX rats appeals to unidentified neural damage induced by decerebration. In this context, it might be noted that Lasiter and Glanzman (1985) found that electrolytic lesions of the CNA attenuated CTA acquisition, a deficit that does not occur following excitotoxic lesions (St. Andre & Reilly, 2006). Of particular relevance to present purposes, Lasiter and Glanzman also reported

an impressive histological analysis of remote neural degeneration consequent to CNA lesions. This analysis revealed degeneration of neurons in the PBN. That is, electrolytic lesions of a single forebrain recipient of ascending gustatory information from the PBN caused significant degeneration of neurons in the PBN. We speculate that transection of the neuraxis, which disconnects all ascending and descending gustatory pathways between the PBN and forebrain, occurs in decerebrate rats, resulting in a greater degree of PBN degeneration than that observed following electrolytic lesions of the CNA. That is, we are suggesting that the absence of CTA acquisition in decerebrate rats is a consequence of retrograde degeneration of the PBN. As noted by Reilly, Grigson, and Norgren (1993), this analysis remains viable until it is established that decerebration does not cause retrograde degeneration of neurons in the PBN that are involved in CTA acquisition.

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