Centrifugal Inputs Modulate Taste Aversion Learning Associated Parabrachial Neuronal Activities

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Centrifugal inputs modulate taste aversion learning associated parabrachial neuronal activities. J Neurophysiol 92: 265–279, 2004. First published February 18, 2004; 10.1152/jn.01090.2003. Our previous studies have demonstrated that gustatory neurons in the parabrachial nucleus (PBN) show altered responses after the acquisition of conditioned taste aversion (CTA) to NaCl. The present study was conducted 1) to examine centrifugal influences on the altered gustatory activity of CTA-trained rats, and 2) to evaluate the role of amiloride-sensitive (ASN) and -insensitive NaCl (AIN) best units in coding the taste of NaCl. Animals were separated into 2 groups: a CTA group that had acquired taste aversion to 0.1 M NaCl and a control group that underwent pseudoconditioning before the recording experiment. Single-neuron activity, in 2 separate series of experiments, was extracellularly recorded in anesthetized rats. In the stimulation studies, the effects of electrical stimulation of the gustatory cortex (GC) or the central nucleus of amygdala (CeA) were examined on firing of PBN taste units. CeA stimulation produced excitatory effect in significantly more neurons in the CTA group (n = 8) than in the control group (n = 1). Furthermore, ASN-best units in the CTA group showed larger responses to NaCl than similar units in the control group. In the decerebration experiment, there was no statistical difference among the taste responses between the 2 groups in any best-stimulus category. These results suggest that CTA conditioning uses an effective central amygdaloid input to modulate activity of gustatory neurons in the PBN. Data also substantiate that amiloride-sensitive components of NaCl-best neurons play a critical role in the recognition of distinctive taste of NaCl.

Introduction

Conditioned taste aversion (CTA) occurs when animals experience a novel taste [conditioned stimulus (CS)] with a gastrointestinal illness-inducing toxin [unconditioned stimulus (US)], and then they show a robust rejection to that taste. CTA helps animals to survive by preventing the repeated ingestion of food or fluid that contains toxic substances (Bures et al. 1998).

It has been well established that the pontine parabrachial nucleus (PBN), the second central relay for taste in rodents, is critically involved in the formation of CTA. Lesion-behavior studies have repeatedly shown that bilateral electrolytic (Di Lorenzo 1988a; Reilly et al. 1993; Sakai and Yamamoto 1998; Spector et al. 1992) or ibotenic acid (Grigson et al. 1998; Scalera et al. 1995; Yamamoto et al. 1995) lesions of the PBN severely disturb the acquisition of CTA. Because bilateral lesions of the nucleus of the solitary tract (NTS), the first central gustatory relay, fail to disturb the acquisition of CTA (Grigson et al. 1997; Shimura et al. 1997a), the critical region for CTA appears to be located rostral to the NTS.

Several factors including learning have been reported to alter the responsiveness of gustatory neurons in the PBN (Baird et al. 2001; Hajnal et al. 1999; Shimura et al. 1997b,c, 2002). In the case of CTA, aversive conditioning resulted in an increase in the magnitude of response to the conditioned taste when NaCl served as the CS (Shimura et al. 1997c), suggesting that neural coding of the CS taste changes by learning. However, it is not clear whether the alteration of taste responses in the PBN occurs at the brain stem level, or reflects the altered responsiveness of the forebrain areas that send descending fibers to the PBN.

Anatomical studies show that the cells in the PBN project to the gustatory cortex (GC) by the ventroposteromedial nucleus of the thalamus (Norgren and Leonard 1971, 1973), and to the limbic structures including the central nucleus of the amygdala (CeA), the bed nucleus of the stria terminalis, the substantia innominata, and the lateral hypothalamic area (LHA) (Fulwiler and Saper 1984; Norgren 1974 1976; Saper and Loewy 1980). The PBN, in turn, receives descending fibers from several higher gustatory relays such as the GC, CeA, and LHA (Halsell 1992; Krettek and Price 1978; Lasiter et al. 1982; Moga et al. 1990; Saper 1982; van der Kooy et al. 1984; Veening et al. 1984; Whitehead et al. 2000; Zahm et al. 1999).

Recent electrophysiological studies revealed that some of these forebrain areas are functionally as well as anatomically connected with the PBN. Electrical stimulation or reversible chemical lesions of the GC (Di Lorenzo 1990; Di Lorenzo and Monroe 1992) and electrical stimulation of the CeA (Lundy and Norgren 2001) altered gustatory activities of parabrachial neurons. Apart from the PBN, the NTS also receives descending inputs from the GC, CeA, and LHA (Cho et al. 2002 2003; Di Lorenzo and Monroe 1995; Li et al. 2002; Matsuo and Kusano 1984a; Matsuo et al. 1984b; Smith and Li 2000). These studies suggest that in these 2 brain stem taste relays gustatory information is processed by close interactions with various forebrain structures.

Among the forebrain gustatory structures mentioned above, both the GC and CeA are known to be involved in CTA (Berman et al. 1998; Lamprecht and Dudai 1996; Navarro et al. 1998).
animals were cared for in accordance with institutional, national, and international regulations.

Conditioning procedure

Before the conditioning procedure, the animals were acclimated to an 18-h water deprivation schedule, and trained to lick distilled water (DW) from a spout for 20 min in the morning (1000 h) and 60 min in the afternoon (1500 h). The 20-min morning period served as the training and testing sessions during which all behavioral manipulations were performed. The afternoon session was always a DW presentation to provide adequate hydration of the animals. They were allowed free access to normal rat chow (dry pellets, MF, Oriental Yeast, Osaka, Japan) except from 1000 h (morning session starts) to 1600 h (afternoon session ends). Once baseline water intake was established in the morning session, CTA animals were presented with 0.1 M NaCl, which served as a CS. Immediately after the drinking period animals were injected intraperitoneally (ip) with 0.15 M LiCl (2% of body weight) as a US. This procedure was repeated 3 times on consecutive days. The control group was composed of the 2 subgroups; the first control group in which animals were similarly treated as CTA animals except that they were given physiological saline instead of LiCl after the CS, and the second control group in which animals were injected with LiCl 3 h before the CS presentation. In the control groups, as in case of the conditioned animals, the CS-US pairing was also repeated 3 times on consecutive days. Because the preliminary analyses revealed that there was no fundamental difference in behavioral and neural data (i.e., the mean intake of the CS), proportions of each best-stimulus unit, spontaneous firing rates, magnitude of responsiveness, and breadth of tuning were similar between the first and second control groups, the data were pooled together in the subsequent analyses. Overnight food and water deprivation was used after the final afternoon session to ensure the stable induction of anesthesia.

Surgery

At ≥24 h after the last injection of LiCl or saline, each rat was anesthetized with urethane (1.3 g/kg body weight, ip). Supplemental anesthetic (0.3 g/kg) was administered as necessary throughout the experiment to maintain the animals under deep anesthesia. Bilateral hypoglossal nerves were cut to prevent undesirable movement of the tongue during the recording session. After tracheotomy was performed to permit respiration, each rat was fixed in a stereotaxic instrument equipped with nontraumatic ear bars. The scalp was opened with a midline incision, and the skull was leveled between bregma and lambda by adjusting the bite bar. The body temperature was monitored and maintained at 36.8°C using an infrared lamp. A hole (3.0–4.0 mm in diameter, centered about 12.0 mm posterior to the bregma), serving access to the PBN, was unilaterally drilled through the skull.

In the stimulation experiments, bipolar stimulating stainless steel electrodes (80 μm in diameter, insulated except at the edge of the tip) were placed in the ipsilateral (to recording) GC (antero-posterior = +1.5 mm, mediolateral = 5.4 mm, dorsoventral = −6.3 mm from bregma) and CeA (antero-posterior = −2.5 mm, mediolateral = 4.4 mm, dorsoventral = −7.8 mm from bregma). Coordinates for both structures were chosen according to the brain atlas of the rat (Paxinos and Watson 1998). Each electrode was fixed firmly to the skull with dental acrylic.

In the decerebration experiment, a 1.0-mm-thick groove was made across the skull. After removing the dura, decerebration at the suprasylvian segment was performed using an L-shaped spatula. The skull and brain surface were kept moist throughout the experiment with physiological saline.

METHODS

Subjects

Fifty-five male Wistar rats (Nihon Dobutsu, Osaka, Japan) for the stimulation experiment and 34 for the decerebration experiment were used. They weighed between 240 and 340 g at the start of the experiments. The animals were individually caged with 12 h light/12 h dark cycle (lights on at 0700 h, off at 1900 h) and constant temperature (23 ± 2°C) and humidity (60%). They were arbitrarily divided into control (Control; n = 27 for the stimulation; n = 18 for the decerebration experiment) and conditioned (CTA; n = 28 for the stimulation; n = 16 for the decerebration experiment) groups. All

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**Test solutions**

In both experiments, the taste stimuli used were 0.1 M NaCl, 0.5 M sucrose, 0.01 M HCl, 0.02 M quinine-HCl, 0.1 M KCl, and 0.1 M NaCl mixed with 10^-4 M amiloride. All stimuli were prepared from reagent-grade chemicals and dissolved in DW. Taste solutions were maintained at room temperature during testing.

**Electrophysiological recording**

Extracellular single-neuron recordings from gustatory neurons in the PBN were made using dura-piercing, glass-insulated tungsten microelectrodes (Z = 1.5–3.0 MΩ at 1 kHz). With the skull flat, the PBN was ventral to the transverse sinuses and therefore the electrode was inserted in the parasagittal plane in a rostrocaudal direction at an angle of 20° off vertical (tip pointing rostrally). The electrode was advanced through the cerebellum into the PBN with a micromanipulator (MO-81, Narishige, Japan). Neuronal activity was amplified with a conventional method, monitored with a computer-aided data-acquisition and analysis system (CED 1401, Spike2; Cambridge Electronic Design, Cambridge, UK), and stored on a DAT recorder for off-line analysis.

A mixture of the 4 basic tastants (0.5 M sucrose, 0.3 M NaCl, 0.02 M HCl, and 0.02 M quinine-HCl) was used as a search stimulus. After isolating a single unit in the PBN, taste stimuli were presented at room temperature (23–24°C). To ensure stimulating the entire oral cavity, we used a method similar to that described by Chang and Scott (1984). Briefly, fluid stimuli were delivered through a length of intraorally inserted slender tubing, closed at the end and extensively perforated along its final 2 cm. The perforated end was slipped into the mouth, held just slightly agape, so that tastants, delivered under mild pressure from a 5-ml syringe, could irrigate the entire oral cavity. Each stimulus trial consisted of a 10-s rinse of DW, 10-s stimulus, and 10-s rinse of DW. The flow rate was 0.5 ml/s for all stimuli, including rinse. When taste responses persisted after the 10-s poststimulus rinse of DW, we continued the water rinse until the neural activity returned to the prestimulus level. The gustatory stimuli and the DW were cleared from the tubing by air pressure. At least 90 s were allowed to elapse between stimuli to avoid the effects of adaptation.

In the stimulation experiment, recording sessions composed of taste only, taste + GC stimulation, and taste + CeA stimulation trials. After taste stimulation trials using the 6 taste stimuli were over, electrical stimulations of the GC or CeA (200-μs duration, rectangular pulses, 10 Hz, 0.3 mA, generated from an electronic stimulator, SEN-3201, Nihon Kohden, Tokyo, Japan) were applied during the period of 10-s taste stimulation and spontaneous activity. Effects of electrical stimulation of either GC or CeA on taste responses were examined using 4 basic taste stimuli (0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.02 M quinine-HCl). The parameters of effective electrical stimulation were determined in a preliminary experiment. The intensity of electrical stimulation of the present experiment is comparable with that (0.4 mA) of Lundy and Norgren (2001).

**Data analysis**

Neuronal activity was counted using a computer-aided data-acquisition and analysis system (CED 1401, Spike2; Cambridge Electronic Design). All data analyses were based on neural activity in 10-s samples. Spontaneous activity and responses to prestimulus water were calculated from multiple samples. The spontaneous rate was determined during the periods just before the prestimulus water rinse. Water and taste responses were calculated during the first 10-s period after the onset of stimulation with prestimulus water or a taste solution. An adjusted score (a net response rate), obtained by subtracting the immediately preceding raw water responses from the raw taste responses, was used for data analyses. A response to taste stimulus was considered to be significant if the neuronal activity increased or decreased ≥2 SD from the mean of the spontaneous activity of the neuron.

With the use of adjusted response data, a breadth-of-response measure was derived for each neuron from the formula for entropy (Smith and Travers 1979)

\[ H = - K \sum_{i=1}^{4} p_{i} \log p_{i} \]

where \( p_{i} \) represents the response to each of the 4 basic taste stimuli. The constant \( K = 1.661 \) for 4 stimuli. Values of entropy (\( H \)) close to zero indicate sensitivity to a single stimulus (narrow tuning); values close to 1.0 indicate sensitivity to all 4 stimuli (broad tuning).

The similarity among taste stimuli, based on responses of each neuron to the 6 stimuli, was further analyzed by the use of hierarchical cluster analysis. The primary cluster analysis used the Pearson product–moment correlation coefficients and the Ward method (Statistica version 5.5, StatSoft, Tulsa, OK). Units were classified as excited or inhibited if their neuronal activities (taste responses to the 4 basic stimuli and spontaneous activity) in electrical stimulation trials were different by ≥25% from those in taste-stimulation trials.

**Histology**

At the end of the last experimental session, a small electrolytic lesion (20 μA for 20 s, electrode positive) was made at the final recording site in the PBN. Marking lesions in the GC and CeA were also made at the tip of one electrode by passing anodal current (20 μA for 20 s). Then, rats were given a lethal dose of urethan and perfused transcardially with phosphate-buffered saline and 10% formalin. The brains were removed and placed in 10% formalin and then transferred for 20 s). Then, rats were given a lethal dose of urethan and perfused transcardially with phosphate-buffered saline and 10% formalin. The brains were removed and placed in 10% formalin and then transferred to a 30% buffered sucrose solution and stored at 4°C for at least 1 wk. Coronal sections of 60 μm thickness were serially cut using a freezing microtome, then stained with cresyl violet. The location of each recording and stimulation site was histologically verified and reconstructed. In the present study, electrophysiological data of the stimulation experiment were exclusively processed from those animals in which the tips of the stimulating electrodes were confirmed to be located in the targeted stimulating sites (GC or CeA).

**RESULTS**

**Stimulation experiment**

**BEHAVIORAL RESULTS.** Figure 1 shows mean fluid intake of animals used in the stimulation experiment during the last water training and subsequent 3 conditioning sessions. The mean

**FIG. 1.** Intake of distilled water (DW) on the last day of water training before conditioning and 0.1 M NaCl across 3 acquisition (Acqu) trials in conditioned taste aversion (CTA) (closed bars) and control (open bars) groups. Animals in the CTA group acquired a robust aversion to 0.1 M NaCl. *P < 0.01 compared with Acqu 1 of the CTA group.
intake of the CS (0.1 M NaCl) in the CTA group progressively decreased by repeated pairings of the CS and US. A 2-way (Group × Session) ANOVA with repeated measures revealed a significant main effect of group [F(1,53) = 87.161, P < 0.01] and session [F(3,159) = 89.613, P < 0.01], and a significant Group × Session interaction [F(3,159) = 127.532, P < 0.01]. Post hoc analyses using Tukey’s test showed that there were significant differences between intake of the CTA and control groups on the 2nd and 3rd acquisition day (P < 0.01).

HISTOLOGY. Locations of stimulating and recording electrodes in animals of both groups (n = 55) are reconstructed in Fig. 2. Filled and open symbols represent stimulating and recording sites for the CTA and control groups, respectively. Distribution of stimulating sites and effects of electrical stimulation of the GC were similar in both groups (A). On the other hand, CeA stimulation produced excitatory responses in only one neuron in the control group, whereas it produced excitatory responses in 5 neurons in the CTA group (B). In the present study, a given

FIG. 2. Distribution of stimulating and recording sites. A and B: location of stimulating electrodes in animals of both groups plotted in the standard atlas sections of the gustatory cortex (GC) (A) and central nucleus of the amygdala (CeA) (B) of rat (Paxinos and Watson 1998). Symbols indicate the effects produced by electrical stimulation on the parabrachial nucleus (PBN) gustatory neurons. Triangles indicate sites that evoked excitatory responses, inverted triangles indicate sites that produced inhibitory responses, and squares indicate sites that produced no effect. Filled symbols, the CTA group; open symbols, the control group. Values under each section show distances from the bregma. C: anatomical reconstruction of recording sites in the PBN. Sections a-d are arranged rostral to caudal throughout the extent of the taste-responsive region of the parabrachial pons separated by about 200 μm. Filled symbols, CTA group; open symbols, control group; circles, S-best units; triangles, amiloride-sensitive N-best units; inverted triangles, amiloride-insensitive N-best units; squares, H-best units. BC, brachium conjunctivum; MesV, mesencephalic trigeminal nucleus. Scale bars = 0.5 mm.
site in the GC or CeA was classified as effective (satisfying the +25% criterion described in METHODS) if stimulating that site facilitated or suppressed ≥1 neuron in the PBN. Therefore stimulating a site could be effective for a neuron but not for another. However, there was no site that produced opposite effects on different neurons (i.e., the effects of electrical stimulation of a given site were uniform across neurons). In the PBN (C), recording sites of 5 neurons (3 from CTA and 2 from control group) were not reconstructed because they could not be histologically identified. However, the data of these neurons were included in the subsequent analyses. A χ² test revealed that there was no difference in the proportion of recording sites anterior (a + b) and posterior (c + d) level between the groups (P > 0.1). There was also no difference in the dorsoventral and mediolateral distribution of recording sites between the groups. There was also no difference in the dorsoventral and mediolateral distribution of recording sites between the CTA and control groups (P > 0.4 and P > 0.9, respectively). However, significantly more HCl-best neurons in both groups were located in the dorsal region (n = 11) than in the ventral region (n = 2) of the brachium conjunctivum (P < 0.05).

**BASIC CHARACTERISTICS.** A total of 84 taste-responsive neurons were recorded from the PBN: 41 from the CTA and 43 from the control group. All the neurons showed excitatory responses to at least one of the 4 basic taste stimuli (sucrose, NaCl, HCl, or QHCl). The mean spontaneous firing rates (spikes/s) were 2.75 ± 0.44 for the CTA group and 1.96 ± 0.38 for the control group, respectively. There was no significant difference between the spontaneous firing rates of the 2 groups (t-test, P > 0.1).

**RESPONSE PROFILES AND CLASSIFICATION.** Based on their largest responses to the 4 standard taste stimuli, we classified PBN neurons as follows: 7 S-best, 24 N-best, and 10 H-best in the CTA group; 8 S-best, 29 N-best, and 6 H-best in the control group. In both groups, no Q-best unit was recorded in the stimulation experiment. N-best units were further classified by their sensitivity to amiloride. Figure 3A indicates examples of taste responses of ASN- and AIN-best units to NaCl with and without amiloride. Amiloride substantially suppressed the NaCl-elicited taste responses of the ASN-best unit, whereas the responses of the AIN-best unit remained unaffected. Figure 3B shows distribution of all ASN- and AIN-best units from both groups in terms of their amiloride-induced response magnitude inhibition ratio. We classified a neuron as amiloride-sensitive if its responses to NaCl were inhibited by amiloride by more than 40% compared with the response magnitude without amiloride. Forty units (18 from the CTA and 22 from the control group) were classified as ASN-best and 13 (6 from the CTA and 7 from the control group) as AIN-best. The bimodal distribution of salt-sensitive neurons without any intermediate type is apparent. The total gustatory response profiles of PBN neurons of both groups are shown in Fig. 4. Taste neurons are ordered by best-stimulus category (S, sucrose; N, salt; H, acid; Q, quinine) and, within categories, by response magnitude. S-best neurons are on the left, followed by N-best units, then H-best units. In the N-best category, ASN- and AIN-best units are separately shown. Fisher’s exact probability test revealed that the proportion of units responding to any of the standard taste stimuli was not significantly different between the CTA and control groups (P > 0.6).

**FIG. 3.** Effects of amiloride on taste responses to NaCl of N-best neurons in the PBN. A: examples of responses of ASN- (amiloride-sensitive) and AIN- (amiloride-insensitive) best units to NaCl with and without amiloride. A marked inhibition of taste responses to NaCl was observed in the ASN- but not in the AIN-best unit. Arrows indicate stimulus onset. B: numbers of ASN- and AIN-best units of both groups. ASN-best units consist of 38 (17 from CTA and 21 from control group) and AIN-best units, 8 units (4 from each group). Bimodal distribution of ASN- and AIN-best neurons without any intermediate type is apparent.

**BREADTH OF RESPONSIVENESS.** Because there were some inhibitory responses in our data, the entropy measure was calculated from the absolute value of the responses to the 4 standard taste stimuli. There was no significant difference between the mean entropy of the 2 groups: 0.68 ± 0.03 for the CTA, 0.70 ± 0.03 for the control groups (t-test, P > 0.5).

**COMPARISON OF TASTE RESPONSES.** Figure 5 presents a comparison of mean response profiles of best-stimulus categories of units in both groups. Taste responses to NaCl were significantly higher in the CTA group than in the control group in the ASN-best category, whereas taste responses in the AIN- and other best-stimulus categories were almost identical between the groups. A 2-way ANOVA with repeated measures (Group × Stimulus) indicated a significant main effect of stimulus in the ASN-best category [F(6, 228) = 60.325, P < 0.01]. A Group × Stimulus interaction was also significant [F(6, 228) = 4.578, P < 0.01]. Post hoc analyses using Tukey’s test showed that
the NaCl-elicited response to NaCl of ASN-best units was significantly higher in the CTA group than in the control group ($P < 0.01$).

HIERARCHICAL CLUSTER ANALYSIS. Figure 6 shows the results of a hierarchical cluster analysis derived from Pearson product-moment correlations. A cluster distance of 0 on the
abscissa indicates that stimuli or groups of stimuli joined at this level had identical response profiles. Larger values of distance represent more dissimilar relationships. Labels indicate the types of the neuron. Note that ASN-best units in the CTA group form a distinct cluster apart from the cluster of AIN-best units. S, S-best; ASN, ASN-best; AIN, AIN-best; H, H-best.

EFFECTS OF ELECTRICAL STIMULATION. An example of taste responses to the 4 basic stimuli and spontaneous activity of a
neuron with and without CeA stimulation are shown in Fig. 7. The responses to all of the taste stimuli and the spontaneous activity itself were similarly inhibited in this unit. In general, the effects of electrical stimulations in the present study were nonspecific; that is, electrical stimulation produced similar effects on taste responses to different stimuli and on spontaneous firing rates.

Antidromic activation was observed in 2 neurons in the CTA group and one neuron in the control group by GC stimulation, and 2 neurons in the CTA group and 3 neurons in the control group by CeA stimulation. Responses induced by antidromic activation were subtracted from the 10-s responses and none of these 8 neurons met the criterion to be classified as excited or inhibited.

Figure 8 summarizes the effect of electrical stimulation on the taste responses to the basic 4 tastes and spontaneous activity.

Nine units (2 S-best, 5 ASN-best, and 2 H-best) were excited and 4 units (3 ASN-best and 1 H-best) were inhibited by GC stimulation in the CTA group. In the control group, GC stimulation resulted in excitation of 10 units (2 S-best, 6 ASN-best, and 2 H-best) and inhibition of 6 units (3 ASN-best, 1 AIN-best, and 2 H-best). There was no significant difference in the number of neurons affected by GC stimulation between the groups (Fisher’s exact probability test, \( P > 0.5 \)) (Table 1).

In case of CeA stimulations, 8 neurons (6 ASN-best, 2 H-best) were excited and 13 neurons (3 S-best, 5 ASN-best, 2 AIN-best, 3 H-best) were inhibited in the CTA group. On the other hand, 1 ASN-best unit was excited and 15 units (2 S-best, 5 ASN-best, 5 AIN-best, 3 H-best) were inhibited by CeA stimulation in the control group. Fisher’s exact probability test revealed that the number of neurons affected by CeA stimulation was significantly different between the 2 groups (\( P < 0.05 \)) (Table 2).

Figure 9 shows mean response profiles of each neuron type that were affected by GC or CeA stimulation. We adopted an analysis that classifies all units into different neuron types because the effects of CTA training on the PBN neuronal activity were specific for ASN-best units. Only the best-stimulus categories whose number of samples was 5 or more are shown. A 2-way ANOVA with repeated measures (Taste × Electrical stimulation) revealed that there was a significant main effect of taste in all of the neuron types that were affected by GC or CeA stimulation (\( P < 0.05 \) for \( E, P < 0.01 \) for \( A, B, C, D, \) and \( F \)). However, the main effect of electrical stimulation was significant only in ASN-best units that were excited by CeA stimulation (Fig. 9B, \( F(1,10) = 5.100, P < 0.05 \)) and in ASN-best units inhibited by CeA stimulation (Fig. 9C, \( F(1,8) = 8.035, P < 0.05 \)) in the CTA group. There was no significant interaction between taste and electrical stimulation in all of the neuron types, which means that the effects of electrical stimulation were not stimulus selective.

**Decerebration experiment**

**BEHAVIORAL RESULTS.** Behavioral results of the decerebration experiment proved to be very similar to those observed in the stimulation experiment. The mean intake of the CS (0.1 M NaCl) in the CTA group severely decreased by repeated pairings of the CS and US (from 13.0 ± 0.98 to 4.3 ± 0.59 to 0.6 ± 0.31 g in the CTA group; from 12.3 ± 1.56 to 12.8 ± 0.88 to 13.1 ± 0.64 g in the control group). A 2-way (Group × Session) ANOVA with repeated measures revealed a significant main effect of group (\( F(1,132) = 52.349, P < 0.01 \)) and session (\( F(3,96) = 39.021, P < 0.01 \)), and a significant Group × Session interaction (\( F(3,96) = 51.357, P < 0.01 \)). Post hoc analyses using Tukey’s test showed that the intake of the CS significantly decreased across trials in the CTA group.

**FIG. 7.** Taste responses to the 4 basic tastes and spontaneous activity of a PBN neuron with and without concurrent electrical stimulation in the CeA. Note that CeA stimulation similarly inhibits the responses to different taste stimuli and spontaneous activity. Thin bar corresponds to 10-s taste stimulation and the thick bar corresponds to concurrent electrical stimulation. QHCl, quinine hydrochloride.
(P < 0.01). These results clearly show that the CTA animals have acquired a strong aversion to NaCl.

HISTOLOGY. A photomicrograph of a representative sagittal section of a decerebrated rat’s brain is shown in Fig. 10. A complete transection at the supracollicular level can be seen. Reconstructed recording sites in the PBN were similarly located to those in the stimulation experiment (data not shown). There was no fundamental difference in the distribution of recording sites between the CTA and control groups.

BASIC CHARACTERISTICS. A total of 84 taste-responsive neurons were recorded from the PBN: 42 from each group. All the neurons showed excitatory responses to at least one of the 4 basic taste stimuli (sucrose, NaCl, HCl, or QHCl). The mean spontaneous firing rates (spikes/s) were 1.79 ± 0.36 for the CTA group and 2.19 ± 0.30 for the control group, respectively. There was no significant difference between the spontaneous firing rates of the 2 groups (t-test, P > 0.4).

RESPONSE PROFILES AND CLASSIFICATION. Based on their largest response to the 4 standard taste stimuli, we classified PBN neurons as follows: 11 S-best, 17 ASN-best, 4 AIN-best, 8 H-best, and 2 Q-best in the CTA group; 8 S-best, 21 ASN-best, 4 AIN-best, 7 H-best, and 2 Q-best in the control group. The criteria to classify N-best units into ASN- and AIN-best were

**FIG. 8.** Effects of electrical stimulation of the GC and CeA on the PBN gustatory neurons. Neurons whose activity was affected by electrical stimulation are shown. Gray and black areas indicate taste responses and spontaneous discharge that were excited or inhibited by ≥25% by electrical stimulation compared with those without electrical stimulation. White areas indicate taste responses and spontaneous discharge unaffected by electrical stimulation. S, sucrose; N, NaCl; H, HCl; Q, quinine hydrochloride.

### TABLE 1. Effects of GC stimulation on PBN neurons

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>CTA</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Excitation</td>
<td>Inhibition</td>
</tr>
<tr>
<td>S-best</td>
<td>2 (29)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ASN-best</td>
<td>5 (28)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>AIN-best</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>H-best</td>
<td>2 (20)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (22)</td>
<td>4 (10)</td>
</tr>
</tbody>
</table>

Percentage values are in parentheses. AIN-best, amiloride-insensitive NaCl-best; ASN-best, amiloride-sensitive NaCl-best; CTA, conditioned taste aversion; GC, gustatory cortex; H-best, HCl-best; PBN, parabrachial nucleus; S-best, sucrose-best.
as described in the stimulation experiment. As in the stimulation experiment, ASN- and AIN-best units distributed bimodally. The response profiles of PBN neurons of both groups are shown in Fig. 11. Taste neurons are ordered by best-stimulus category and, within each category, by response magnitude. S-best neurons are on the left, followed by N-best units, H-best units, then Q-best unit. In the N-best category, ASN- and AIN-best units are separately shown. Fisher’s exact probability test revealed that the proportion of units responding to each standard taste stimulus was not significantly different between the 2 groups (P > 0.8).

**BREADTH OF RESPONSIVENESS.** Because there were some inhibitory responses in our data set (as seen in Fig. 11), the entropy measurements were calculated from the absolute value of the responses to the 4 standard taste stimuli. There was no significant difference between the mean entropies: \(0.69 \pm 0.03\) for the CTA, \(0.77 \pm 0.04\) for the control groups (t-test, \(P > 0.1\)).

**COMPARISON OF TASTE RESPONSES.** Figure 12 presents a comparison of mean response profiles of best-stimulus categories of units in both groups. The ASN-best panel includes data from the intact animals of the stimulation experiment because this is the group of neurons for which evidence for changes in neuronal activity after CTA training was found. A 2-way ANOVA with repeated measures (Group × Stimulus) indicated no significant main effect of group and Group × Stimulus interaction in S-, AIN-, and H-best-stimulus categories. In the ASN-best category, ANOVA indicated a significant main effect of stimulus \([F(6,444) = 103.603, P < 0.01]\) but not of group \([F(3,74) = 1.152, P > 0.3]\). A Group × Stimulus interaction was also significant \([F(18,444) = 2.7896, P < 0.01]\). Post hoc Tukey’s test

### Table 2. Effects of CeA stimulation on PBN neurons

<table>
<thead>
<tr>
<th>Neuron Type</th>
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<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Excitation</td>
<td>Inhibition</td>
</tr>
<tr>
<td>S-best</td>
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<td>3 (43)</td>
</tr>
<tr>
<td>ASN-best</td>
<td>6 (33)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>AIN-best</td>
<td>0 (0)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>H-best</td>
<td>2 (20)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (20)</td>
<td>13 (32)</td>
</tr>
</tbody>
</table>

Percentage values are in parentheses. AIN-best, amiloride-insensitive NaCl-best; ASN-best, amiloride-sensitive NaCl-best; CeA, central nucleus of the amygdala; CTA, conditioned taste aversion; H-best, HCl-best; PBN, parabrachial nucleus; S-best, sucrose-best.

**FIG. 9.** Mean response profiles of each neuron type that were affected by GC or CeA stimulation. Both GC and CeA stimulation produced similar effects on taste responses to different tastes and spontaneous activity.

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revealed that the NaCl elicited taste responses of the ASN-best neurons in the stimulation experiment were significantly higher in the CTA group than in the other 3 groups ($P < 0.01$).

The ANOVA revealed that spontaneous firing rates of the 4 groups in the decerebration and stimulation experiments (1.79 ± 0.36 for the CTA group and 2.19 ± 0.30 for the control group in the decerebration experiment; 2.75 ± 0.44 for the CTA group and 1.96 ± 0.38 for the control group in the stimulation experiment) were not statistically different [$F(3,164) = 1.235, P > 0.2$].

There was also no significant difference in the values of entropy among the 4 groups [0.69 ± 0.03 for the CTA group and 0.77 ± 0.04 for the control group in the decerebration experiment; 0.68 ± 0.03 for the CTA group and 0.70 ± 0.03 for the control group in the stimulation experiment, $F(3,164) = 1.584, P > 0.19$].

**DISCUSSION**

In the present study, for the first time, we have demonstrated that forebrain gustatory areas contribute to the CTA induced altered taste responses in the PBN. Furthermore, increased taste responses were observed only in ASN-best units after the acquisition of taste aversion to NaCl. These results suggest that interactions between the PBN and higher taste relays play an important role in processing information of a taste conditioned to be aversive, and that ASN-best units in the PBN are critically involved in the distinctive taste of NaCl.

**Acquisition of conditioned taste aversion**

Animals in the CTA group in the both experiments showed significantly reduced intake of NaCl solution across trials. On the day of the last CS–US paring, they almost completely avoided ingesting the CS. Increased orofacial aversive taste reactivity (Grill and Norgren 1978) to the CS was also observed in the CTA group, although it was not quantified. These results clearly show that rats in the CTA group had acquired a strong CTA to NaCl by the day of single-neuron recording experiments. Therefore it is reasonable to suppose that some plastic changes took place in neuronal activity reflecting taste aversion learning in CTA animals.
Spontaneous firing rate

Mean spontaneous firing rates of the parabrachial gustatory neurons (2.75 spikes/s for the CTA and 1.96 spikes/s for the control groups in the stimulation experiment; 1.79 spikes/s for the CTA and 2.19 spikes/s for the control groups in the decerebration experiment) were relatively low compared with those reported in previous studies using rats under urethan anesthesia (e.g., over 5 spikes/s; Di Lorenzo 1990; Di Lorenzo and Monroe 1992). However, there were also studies reporting low spontaneous firing rates (Ogawa et al. 1987; Perrotto and Scott 1976; Scott and Perrotto 1980) compatible to those in the present study. Anesthesia might have suppressed the spontaneous firing rates by blocking the descending excitatory inputs to the PBN because spontaneous firing rates of the PBN neurons in conscious rats were found to be much higher (ranging from 6.1 to 11.2 spikes/s; Hajnal et al. 1999; Nishijo and Norgren 1997).

Taste response profiles

The proportion of S-best units in the present study is somewhat larger, resulting in the smaller proportion of N-best units, than in our previous studies in which N-best units dominate the sample (Shimura et al. 1997c, 2002). This difference may reflect the fact that the entire oral cavity was sufficiently stimulated and/or that a search stimulus consisted of a mixture of the 4 basic chemicals more successfully detected taste responses in the PBN (0.3 M NaCl was used as a search stimulus in our previous studies).

The proportion of each best-stimulus unit of rats used in the stimulation and decerebration experiments was not fundamentally different. Although no Q-best unit was recorded in the stimulation experiment, this result should not be overemphasized because the sample sizes were relatively small in both experiments.

In the present study, ASN-best units constituted about 75–85% of the N-best units in the 4 groups in the both experiments. These results are comparable with those of a recent electrophysiological study that also classified N-best units into ASN- and AIN-best by their sensitivity to amiloride in the PBN (Lundy and Norgren 2001).

The value of entropy of neurons in the PBN is not statistically different among the 4 groups used in the decerebration and stimulation experiments, which indicates that the breadth of tuning is unchanged by CTA or decerebration. These results are also in line with previous reports (Di Lorenzo 1988b; Shimura et al. 1997c).

Recording site

The location of recording sites was almost identical to that shown by previous electrophysiological experiments (Norgren and Pfaffmann 1975; Scott and Perrotto 1980; Shimura et al. 1997c, 2002). Although there was no fundamental difference in the distribution of ASN- and AIN-best neurons in the PBN, H-best units were preferentially located in the dorsal region of the brachium conjunctivum, as previously reported by Ogawa et al. (1984, 1987).

Taste responses after conditioned taste aversion

In the decerebration experiment, there was no significant difference in the magnitude of taste responses between the 2 groups in all best-stimulus categories. Because the experimental procedure was practically the same as that of our previous experiments, which show that gustatory activities in the PBN are altered after the acquisition of CTA (Shimura et al. 1997c, 2002), it is reasonable to suppose that the decerebration prevented the development of altered gustatory activities induced by CTA. This, furthermore, means that interconnections with forebrain structures are an indispensable requirement of adaptive taste information-processing mechanisms.

The same recording condition for the CTA and control groups ensured that the different response characteristics between the 2 groups were primarily reflecting some plastic changes that developed as a result of taste aversion learning.
the stimulation experiment, responses to NaCl significantly increased only in the ASN-best units in the CTA group. Furthermore, responses to other taste qualities did not differ between the groups. Therefore aversive conditioning appears to increase the responsiveness only to behaviorally relevant taste stimuli in the PBN, which may help the animals to avoid the ingestion of aversive, potentially harmful fluids. Similar results have been reported by a previous study in which CTA to sodium saccharin resulted in an increased responsiveness to the CS only among the sweet-sensitive subset of gustatory neurons in the NTS (Chang and Scott 1984).

Cluster analyses also detected altered responsiveness in the CTA group in the stimulation experiment. ASN-best units in the CTA group formed a distinct cluster apart from the cluster of AIN-best units, whereas in the control group ASN- and AIN-best units were nearly clustered. The shift of the cluster is thought to reflect the fact that in the CTA animals NaCl becomes a more salient stimulus for the ASN-best units. The above data suggest that specific activity changes of these ASN-best neurons of the PBN may represent the underlying neural substrate of plastic changes resulting in the taste of NaCl CS becoming more distinct and sharply discriminated from the other taste qualities.

It should be noted that taste quality of the CS may influence the effects of CTA training on gustatory responses in the brain stem. When sweet saccharin was used as the CS, the changes in taste activity in the NTS were relatively modest (Chang and Scott 1984) compared with those of our earlier (Shimura et al. 1997c) and the present studies in which the salty NaCl CS was used. Moreover, when nonpreferred sour HCl was used as the CS, PBN neuronal responses to the CS decreased, although the same conditioning procedures were used as in the present study (Shimura et al. 2002). These results suggest that neural mechanisms underlying the CTA are different for various tastes serving as the CS.

**Effect of decerebration on the parabrachial neuronal activities**

In the present study, decerebration did not have a clear effect on taste responses and spontaneous activities in the PBN. A similar study by Di Lorenzo (1988b) reported that decerebration, while leaving spontaneous firing rates in the PBN unchanged, produced smaller responses to NaCl and HCl and larger responses to saccharin sodium. This discrepancy may be explained by the different experimental procedures used: that is, animals were anesthetized with urethan in the present study, whereas animals were paralyzed with Flaxedil during recording in Di Lorenzo’s study. Deep anesthesia induced by urethan in the present experiments might have reduced centrifugal influences and this could mask the effect of decerebration on neuronal activities. Nevertheless, the finding that taste responses to NaCl increase after CTA in intact but not in decerebrate animals suggests that the influence of forebrain structures is not completely suppressed even despite the deep anesthesia. This notion is supported by the recent finding showing that gustatory activities in the PBN of rats under urethan anesthesia were different before and after electrolytic lesions in the CeA (Huang et al. 2003).

**Effects of electrical stimulation**

In the present study, CeA stimulation produced mainly inhibitory effects on the PBN neurons. These results are in line with those of the previous reports that examined descending influences from the CeA to the gustatory neurons in the PBN (Huang et al. 2003; Lundy and Norgren 2001). However, the CeA stimulation did not produce a taste-quality–specific effect in the present study: in our case the effect was similar for the spontaneous activities and for the responses to various taste stimuli. This nonspecific effect may partly be attributable to the relatively strong intensity of electrical pulses (0.3 mA) used in our stimulation experiments. It is thus possible that a centrifugal influence plays a more complex role in conscious rats than in anesthetized ones.

The GC has been known to be implicated in several phases of CTA memory (Berman et al. 1998; Ramirez-Lugo et al. 2003; Yasoshima and Yamamoto 1998). In the present study, however, there was no significant effect produced by GC stimulation. Thus it is suggested that the GC is not the principal source of excitatory inputs causing increased responses to the CS in the PBN after acquisition of a CTA. These results of course do not deny the functional involvement of the GC in CTA.

Although the effect of electrical stimulation was, in general, nonspecific, we found that CeA stimulation excited 8 neurons in the CTA group, whereas only one neuron was excited by CeA stimulation in the control group. This increased excitatory effect may partly reflect the plastic changes that had occurred in the PBN during the acquisition phase of CTA.

**Possible mechanisms**

Deep anesthesia has already been suggested to block descending influences from the forebrain (Shimura et al. 1997c, 2002); thus we previously concluded that alteration of taste responses induced by CTA could occur within the PBN and/or at lower gustatory representation levels below the PBN. In the present study, however, it has been demonstrated that the decerebration effectively prevents such alteration of taste responses after CTA acquisition. This obviously means that descending inputs from the forebrain, but not ascendingly wired information, induce increased taste responses to the CS, at least in the retrieval phase of CTA. Nevertheless, it should be noted that this finding does not necessarily preclude the possibility that plastic changes occur within the PBN. In addition to forebrain areas, such plastic changes may also develop in the synapses formed by the PBN neurons and terminal buttons of neurons in the forebrain that convey descending information. Although the present study cannot conclusively rule this out, we found no evidence that the brain stem was sufficient to maintain changes in the taste responses properties of the neurons normally after CTA acquisition in intact rats. This notion is consistent with the behavioral finding that decerebrate rats can neither acquire nor retain CTA (Grill and Norgren 1978).

Which site causes enhanced responses to NaCl CS in the PBN? As already mentioned, the effect of GC stimulation on neuronal activity in the PBN was not statistically significant. By contrast, CeA stimulation produced more excitatory effects in the CTA group than in the control group, although such
effects were not exclusively confined to the ASN-best units. Thus these results suggest that at least the CeA may contribute to the altered responsiveness of PBN neurons in the CTA-trained rats. Plastic changes that occur between the CeA and the PBN might underlie increased responses to the CS. For example, direct measurement of long-term potentiation between the CeA–PBN projection is required to confirm this hypothesis. Descending inputs from the CeA to the PBN might also function as feedback systems that facilitate discrimination of the taste of the CS.

There is some evidence in the literature that centrifugal influences from the GC and CeA play a role in CTA. Schaef and Bernstein (1996, 1998) demonstrated that electrolytic lesions of both GC and CeA reduce c-Fos–like immunoreactivity in the intermediate division of the NTS, which is already well known to be correlated with the behavioral expression of CTA. Results of the present study along with these findings demonstrate that the gustatory relays in the brain stem and forebrain form a highly organized neural network that—to contribute to adaptive behavioral outputs—almost simultaneously operates with gustatory information; perceptual, motivational, and learning determinants; as well as memory constituents of these processes. Recent views in the gustatory neural coding also emphasize interactions of neurons in spatially separate brain regions (Katz et al. 2002).

It should be noted that effect of electrical stimulation in the present study can also be attributed to activation of intrinsic neurons and fibers of passage as well as that of forebrain neurons connected with the PBN and/or lower gustatory levels below the PBN. Because many changes in gustatory neurons in the NTS have been shown by stimulation of GC (Di Lorenzo and Monre 1995; Smith and Li 2000) and CeA (Cho et al. 2003; Li et al. 2002), it is possible that the present results of electrical stimulation are simply reflections of changes in the NTS that are then transferred to the PBN. Moreover, forebrain areas that send fibers to the PBN are also connected with each other (Halsell 1992; van der Kooy et al. 1984; Yamamoto et al. 1984). For example, gustatory neurons in the GC respond to CeA stimulation and, in turn, gustatory neurons in the CeA also respond to GC stimulation (Yamamoto et al. 1984). Interpreta
tion of stimulation data must therefore not be confined to the effects that were produced by monosynaptic connections.

In summary, CTA to NaCl modified ASN-best units in the PBN so that sodium taste became more salient than other tastes in these rats. The present data substantiate that this neuronal modification requires active inputs from the forebrain to facilitate a highly sensitive, adaptive gustatory discrimination of conditioned animals.

GRANTS

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