Quinine and citric acid elicit distinctive Fos-like immunoreactivity in the rat nucleus of the solitary tract

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Travers, Susan P. Quinine and citric acid elicit distinctive Fos-like immunoreactivity in the rat nucleus of the solitary tract. Am J Physiol Regulatory Integrative Comp Physiol 282: R1798–R1810, 2002. First published February 21, 2002; 10.1152/ajpregu.00590.2001.—The present experiment investigated Fos-like immunoreactivity (FLI) in the nucleus of the solitary tract (NST) after intraoral infusions of 0.1 M acid, 0.3 M NaCl, and 0.3–30 mM quinine monohydrochloride (QHCl) in awake, behaving rats. Increases in QHCl concentration produced increases in the numbers of FLI-labeled neurons in the rostral part of the intermediate (i) and rostral (r) NST, but the topographic distribution of FLI was consistent across QHCl concentrations and distinctive compared with effects of citric acid. Quinine elicited FLI concentrated in the medial third of the nucleus; acid elicited more broadly distributed FLI concentrated farther laterally. Surprisingly, in contrast to QHCl and citric acid, NaCl produced FLI that was indistinguishable from that produced by water. Although the functional significance of these patterns is unknown, citric acid and QHCl are nonpreferred stimuli but produced different oromotor behaviors. QHCl (30 mM) elicited ~3.2 times as many gapes as citric acid (0.1 M), and acid elicited more ingestive responses. Parallel differences in FLI expression suggest that different NST regions may have distinctive roles in triggering oromotor behaviors.

nucleus tractus solitarius; chemotopy; sodium chloride; parabrachial nucleus; gaping

AN ORDERLY TOPOGRAPHIC REPRESENTATION of stimulus properties is a ubiquitous feature of sensory systems. The most obvious topography in the gustatory system is the systematic representation of information arising from taste buds in different parts of the mouth. This orotopy has been demonstrated throughout the neuraxis (for review see Ref. 69) but has been best documented in the first-order gustatory relay, the nucleus of the solitary tract (NST) (26, 28, 71), where it appears to be a rostral continuation of a topographic representation of the entire gastrointestinal tract (1). However, a more functionally important stimulus feature in the taste system is quality. In the olfactory system, particularly in the olfactory bulb, an orderly representation of the chemical properties of the stimulus is a salient feature of organization (for review see Ref. 74). However, in the gustatory system, a systematic topography for this parameter has been more dubious. A few neurophysiological studies have reported that responses to particular taste stimuli are more numerous or larger at certain locations (25, 43, 44, 55), but others report a lack of a chemotopy (14, 43, 54). Most often, there is simply no comment about such a level of organization, implying that it was not obvious. Using a different approach, we recently used Fos immunohistochemistry to demonstrate a differential pattern of activation in the first-order gustatory relay, the NST, for sucrose and quinine monohydrochloride (QHCl), two tastants that differ dramatically along qualitative, hedonic, and behavioral dimensions (31, 70). Both stimuli evoked Fos maximally in the rostral central subnucleus, the NST region that receives the densest primary afferent taste projections (73), but the Fos-like immunoreactivity (FLI) exhibited different topographic patterns. After sucrose stimulation, FLI neurons were distributed evenly along the mediolateral axis, but after QHCl, labeled neurons exhibited a prominent medial clustering (31, 70). However, because stimuli representing only two taste qualities were tested, it is unclear how unique these patterns are. In addition, the use of single concentrations raises the question of how Fos expression changes across the intensity domain. The intensity question is critical in the gustatory system, because single taste neurons are broadly tuned across quality and tend to become more so at higher concentrations (30). The purpose of the present study was thus twofold: 1) to determine whether other classical taste stimuli elicited distinctive patterns of Fos expression and 2) to determine whether the location of quinine-elicited Fos was stable across the intensity domain. Thus we compared FLI expression over 2 log steps of QHCl concentrations with FLI produced by NaCl and citric acid. We demonstrate that although the number of FLI neurons increases with QHCl concentration, the pattern of expression remains circumscribed and distinctive relative to the pattern produced by acid. Surprisingly, despite its potent neurophysiological effectiveness, NaCl did not elicit FLI expression in the NST.
METHODS

Animals, Surgery, and Behavioral Testing

All procedures involving animals conformed to guidelines set forth by the National Institutes of Health and were approved by The Ohio State University Animal Care and Use Committee. In addition, experiments conformed with the guiding principles published by the American Physiological Society (2). Thirty-nine male Sprague-Dawley rats, 188–456 (351 ± 8.7) g body wt, were used. Although the weight range was rather large, only one rat weighed <280 g, and the mean weights for the different groups (see below) were similar: 344 ± 17.4 g for unstimulated rats; 352 ± 29.1 g for water-stimulated rats; 351 ± 15.5 g for NaCl-stimulated rats; 351 ± 17.4 g (0.3 mM), 364 ± 36.9 g (3 mM), and 355 ± 24.8 g (30 mM) for QHCl-stimulated rats; and 340 ± 11.3 g for citric acid-stimulated rats. Rats were singly housed in plastic cages, exposed to a ~12:12-h light-dark schedule, and given free access to food and water throughout the study. All procedures were performed during the light phase.

Rats were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip, with 2.5–5 mg supplements as needed) to achieve a surgical level of anesthesia characterized by the absence of the pedal withdrawal reflex and placed in a stereotaxic frame for implantation of intraoral cannulas for subsequent fluid delivery. Bilateral intraoral cannulas, constructed of polyethylene tubing (PE-100) coupled to small lengths of stainless steel tubing, were inserted through the buccal mucosa, just lateral to the first maxillary molars, and exited on the lateral surface of the skull (20). The intraoral cannulas were secured to the skull with dental acrylic anchored by screws. When necessary, the incision was closed with wound clips or surgical suture. The rat was injected with penicillin (24,000 U in 0.08 ml im) immediately after surgery and usually for 2 days postoperatively to prevent infection. For several days after surgery, the rat’s diet was supplemented with ground rat pellets mixed with Crisco to aid in weight gain.

On the 3rd day following surgery, the rat was placed in the testing chamber to begin acclimatization. The rat was then adapted to the stimulation procedure for 6–8 days before the test day. The animal was placed in the chamber, tubing was attached to one of the cannulas, and the rat was left undisturbed for ~1 h before fluid delivery. Distilled water (7 ml) was then delivered over a 30-min period through the cannula using a syringe pump. After fluid delivery, the rat was left in the testing chamber for ~45 min before being returned to the home cage. On the test day, the animals were assigned to one of seven groups: unstimulated (n = 5) or stimulated with water (n = 5), 0.3 M NaCl (n = 6), 0.1 M citric acid (n = 6), 0.3 mM QHCl (QHCllo, n = 5), 3 mM QHCl (QHClmed, n = 6), or 30 mM QHCl (QHClhi, n = 5). (One additional rat was also stimulated with 3 mM QHCl but was not part of the main group used for quantification, because the brain was cut in an alternate plane for illustrative purposes.) The concentrations of NaCl and citric acid were chosen to optimize the chances of observing clear Fos expression. Thus we used concentrations that appeared highly effective on the basis of behavioral (11, 48, 53, 58) and electrophysiological (3, 17, 45, 72) data. The quinine concentrations included (3 mM) and bracketed (0.3 and 30 mM) the concentration successfully used in previous reports of Fos expression (16, 31, 40, 41, 67, 70). For the animals in the stimulation groups, rats were placed in the testing chamber, tubing was attached to one of the two cannulas, and the rat was left undisturbed for 1 h before delivery of 7 ml of the appropriate fluid over a 30-min period.

After stimulation, the rat was left in the test chamber for an additional 45 min.

Tissue Processing and Immunohistochemistry

After the 45-min poststimulation period, rats were injected with a lethal dose of pentobarbital sodium (100–150 mg/kg ip) and perfused through the left ventricle or ascending aorta with phosphate-buffered saline (PBS, pH 7.4) and then with a mixture of 4% paraformaldehyde and 1.25% acrolein. The brain was removed from the skull, sometimes postfixed in 4% paraformaldehyde for a few hours, and stored in 20% sucrose-phosphate buffer (PB, pH 7.4) overnight. A sliding microtome was used to freeze brain sections in the coronal plane at 40 μm, and the brain was divided into three series: one series was used for immunohistochemistry, a second was reserved in the event that the immunohistochemistry needed to be repeated, and the third was usually stained with cresyl violet to aid in identifying the borders and cytoarchitectonics of the NST. An additional brain from one animal stimulated with 3 mM QHCl was cut in the horizontal plane for illustrative purposes. Occasionally, immunohistochemistry immediately followed sectioning, but, more commonly, sections were stored in cryoprotectant at −20°C (34).

To minimize variation in immunohistochemical processing, except in one case, tissue from animals in different experimental groups was processed simultaneously. Processing was performed on free-floating sections beginning with thorough rinsing in PBS. All subsequent steps were separated by rinses with PBS or PB. Sections were reacted with 1% sodium borohydride and sometimes quenched in 5% H2O2 before being placed in 10% normal sheep serum diluted in PBS. Sections were then incubated in the primary antibody diluted in a solution of 0.4% Triton X-100 in PB at 4°C for ~66 h. The primary antibody was a polyclonal antibody (AB-5, Oncogene Science) directed at amino acids 4–17 of the Fos protein. Two different lots of this antibody were used over the course of the experiments, and the optimal dilutions varied by a factor of 2: lot 1 (lot 60910501) was used at a dilution of 1:12,000, and lot 2 (lot DO83530) was used at 1:25,000. Tissue from slightly fewer animals (n = 17) was processed with lot 1 than with lot 2 (n = 21), and similar numbers of animals in different groups were processed with lots 1 and 2. The pattern of Fos expression was virtually identical for lots 1 and 2. After the sections were removed from the primary antibody, they were incubated in goat anti-rabbit IgG diluted 1:600 in PB with 0.4% Triton X-100 and 0.1% BSA and then in an avidin-biotin mixture (Elite Kit, Vector) diluted in PB-0.1% BSA. The final chromagen reaction began with incubation in 0.05% 3,3′-diaminobenzenidene-HCl (DAB) with 0.02% nickel ammonium sulfate followed by the final oxidation stage, achieved by addition of H2O2 to a final concentration of 0.003%. Reacted sections were mounted on chrome-alum subbed slides, dehydrated through ascending alcohols, cleared with Hemo-De (Fisher Scientific), and coverslipped.

Data Analysis and Photomicroscopy

Analyzed sections were chosen from standard levels of the rostral and intermediate NST (rNST and iNST). Figure 1A depicts a schematic diagram in the horizontal plane that illustrates these anatomic divisions of the NST and the position of the analyzed levels. The rNST is anterior to where the nucleus abuts the IVth ventricle, and the iNST extends from the caudal limit of the rNST to the obex (the most caudal section with the area postrema) (33). The rNST sections are referred to as r1–r4; r1 is the caudal limit of rNST,
and r2–r4 are at successively more rostral locations separated by equal intervals. The major afferent projections to the rNST are from the mouth. The predominant afferent terminations in r4 are from gustatory fibers in the VIIth nerve, supplying taste buds on the anterior tongue and hard and soft palates. In contrast, r1 receives taste information mainly from the IXth nerve, which innervates taste buds in the circumvallate and foliate papillae. The two sections from the midregion (r2 and r3) receive overlapping VIIth and IXth nerve projections. Sections r1–r3 also receive terminations from somatosensory afferents in the IXth nerve and oral branches of the Vth nerve. Somatosensory input tends to be lateral to gustatory input (71). Two sections for analysis were taken from the iNST. One was caudal to the rNST by a distance equal to the separation between sections in the rNST or ~40% of the distance from the caudal limit of the rNST to the rostral pole of the area postrema. This level, referred to as i NST, receives afferents from the IXth and Xth nerves (28). Finally, we analyzed a section from a midpostremal level of the iNST (i NST) where visceral afferents from the Xth nerve primarily terminate (28).

Tissue sections were examined under a light microscope, at ×20–600; most plotting was done at ×100–200. The NST outlines were identified, and labeled neurons were plotted relative to anatomic structures using a videocamera and software (Neurolucida, Microbrightfield). This system allowed sections to be viewed and traced on an image of a computer screen superimposed over the microscopic field. After the appropriate levels were identified, NST outlines were drawn using background staining from immunohistochemistry, dark-field optics, and adjacent sections from cresyl-stained material if necessary. The NST was split into "subfields," as originally described by King et al. (41); a line was drawn parallel to the mediolateral axis of the NST, the nucleus was split into three equal sections, and then each section was split into dorsal and ventral halves. The subfields were originally numbered I–6, but we have chosen to use anatomically descriptive terms. Thus the lateral-dorsal, lateral-ventral, middle-dorsal, medial-dorsal, and medial-ventral subfields correspond to subfields 1, 3, 2, 4, 5, and 6, respectively (40, 41). The positions of the subfields can be seen in Fig. 1B. Dividing the nucleus in this manner made it possible to identify differential distributions of FLI neurons according to stimulus. Similar patterns are also clear if the nucleus is divided on a cytoarchitectonic basis (31, 70), but the subfield method is more efficient. The location of nuclei with FLI was plotted by an investigator blind to the experimental condition, usually on the side of the brain ipsilateral to stimulation.

Data were analyzed using separate ANOVAs for the orosensory NST (i NST and rNST) and visceral NST (i rNST), with stimulus as a between-groups factor. These were followed by ANOVAs for each of the six subfields and for each of the five levels of i NST/rNST. If significant ANOVAs were obtained, significant differences between individual stimuli were determined using Fisher’s least significant difference test; only the significant differences between water and the other stimuli are noted. The level for statistical significance was set at P < 0.05; differences approaching this level (P < 0.1) are sometimes also mentioned. The similarities in the mean topographic patterns of FLI across subfields that were associated with the different stimuli were summarized using correlational statistics (Pearson’s r) followed by multidimensional scaling in two dimensions (Systat).

Photomicrographs were taken with a digital camera (Nikon DMX1200, resolution = 3,840 × 3,072). Files were imported into Canvas (Deneba Systems), brightness and contrast were adjusted, sharpening filters were applied, and labels were added.

Behavioral Analysis

Because 0.1 M citric acid and 30 mM QHCl (QHClhi) resulted in similar numbers of FLI neurons in the NST but distinct topographic distributions (see Results), we analyzed the oromotor behaviors elicited by these stimuli in six additional animals that were videotaped for this purpose. Rats were implanted with intraoral cannulas and adapted to the testing procedure as described above. Testing proceeded in the same manner, except all rats were tested with each of the two stimuli on separate days; half of the rats were tested with citric acid first and half with QHCl. An additional session with water stimulation separated the gustatory tests. The number of gapes in the first 2 min of stimulation was counted and expressed as gapes per second, with adjustment for the amount of time the rat’s mouth could be viewed clearly in the videotape. In addition to this quantitative analysis, a qualitative analysis of the entire session noted the occurrence of gapes and other aversive behaviors (chin rubs and passive rejection), ingestive behaviors (mouth movements, tongue protrusions, and lateral tongue protrusions) (20), and the condition of the test cage floor (i.e., wet or dry)
for each 5-min block. Differences in behaviors evoked by the
two stimuli were compared with paired t-tests (Excel). Error
terms in all analyses are presented as standard errors of the
mean.

RESULTS

Figure 2 shows a series of photomicrographs that
summarize the principal findings of the present inves-
tigation. Stimulation with 0.3–30 mM QHCl (Fig. 2,
A–C) produced increasing numbers of FLI neurons,
distributed in a circumscribed topographic pattern
that was characterized by a pronounced medial clus-
tering. This medial clustering is also strikingly appar-
ent in the horizontal photomicrograph in Fig. 3, which
depicts an animal stimulated with QHCl_med (3 mM).
The topography of the QHCl pattern was distinctive
compared with the more broad distribution of FLI
observed after 0.1 M citric acid (Fig. 2F). Surpris-
ingly, stimulation with 0.3 M NaCl did not produce
FLI in the NST that could be distinguished from that

Fig. 2. Photomicrographs of the NST at a level between r1 and r2 showing the distribution of Fos-like immuno-
reactivity (FLI) for each of the 6 stimulation groups. The borders of the NST are depicted with a solid line. Division
of the NST into subfields is shown with dotted lines. Scale bar, 200 μm. QHCl, quinine monohydrochloride.
elicited by water stimulation on the basis of the number or pattern of distribution of labeled neurons (Fig. 2, D and E).

Stimulus-Evoked FLI Expression in Orosensory vs. Visceral NST

Figure 4A shows the mean number of FLI neurons summed across the four levels of the rNST and iNST for unstimulated and water-stimulated animals and each of the taste-stimulated groups. The numbers of FLI neurons in the iNST and rNST were markedly different between groups. An ANOVA supported a significant effect of stimulus \[ F = 29.2, \text{df} = 6,31, P < 0.0001 \]. Compared with the water group, the unstimulated group had only about one-third as many FLI neurons in the iNST and rNST, but the numbers of FLI neurons in the water group were quite variable, and this difference just approached statistical significance \( P < 0.1 \). Nevertheless, as observed in several other studies, this suggests that the somatosensory aspects of fluid stimulation are effective in eliciting Fos expression in iNST and rNST, making water-stimulated animals the appropriate control group for assessing the efficacy of gustatory stimuli (31, 41, 70).

Some, but not all, of the taste stimuli in the present study were effective in evoking Fos in the iNST and rNST. Citric acid was a potent stimulus, eliciting FLI in >3.6 times as many neurons as did water \( P < 0.0001 \). QHCl \( \text{hi} \) (30 mM) was similarly effective \( P < 0.0001 \). The QHCl \( \text{med} \) (3 mM) also was associated with a significant increase in FLI \( P < 0.05 \), but the log-step decrease in concentration reduced the efficacy of this stimulus by about one-half. With an additional log-step decrement in concentration, QHCl \( 0.3 \text{ mM}, \text{QHCl}_{\text{lo}} \) was no longer an effective stimulus. NaCl was also without detectable effect.

Figure 4B shows similar results for the visceral NST for a single midpostremal section (iNST level). Effects of intraoral infusion of taste stimuli also were apparent for this region (ANOVA, \( F_{\text{stimulus}} = 4.2, \text{df} = 6,31, P < 0.003 \)) but were more restricted. Although the mean number of FLI neurons in the water group was nominally greater than in the unstimulated group, this difference did not even approach significance. In addition, only citric acid \( P < 0.02 \) and QHCl \( \text{hi} \) \( P < 0.01 \) produced significant increases in Fos expression relative to water; QHCl \( \text{med} \), QHCl \( \text{lo} \), and NaCl were ineffective, and the relative increases evoked by the effective tastants were smaller than in the iNST and rNST. Stimulation with citric acid and QHCl \( \text{hi} \) produced 2.8- and 2.6-fold increases in the midpostremal NST compared with the 3.6-fold increases observed rostrally.

Topography of Stimulus-Evoked FLI Expression in Orosensory NST

Our previous studies suggested that different gustatory stimuli, namely, 1.0 M sucrose and 3 mM QHCl, evoked differential patterns of Fos expression across the coronal plane in the rNST (31, 70). We therefore examined the distribution of FLI in the six subfields collapsed across the iNST and rNST to determine whether citric acid also evoked a pattern different from QHCl. In addition, it was of interest to more closely scrutinize NaCl and QHCl \( \text{lo} \) stimuli that appeared ineffective.
when analyzed across the entire i,rNST. Figure 5 shows the mean number of FLI neurons for the unstimulated and water-stimulated, as well as the gustatory, groups for the six subfields. The ANOVAs performed for each subfield with stimulus as a factor were highly significant ($P < 0.000001$ for all). However, significant differences between experimental groups varied according to stimulus and subfield. Compared with unstimulated animals, water was associated with significantly more FLI neurons only in the lateral-dorsal ($P < 0.02$) and middle-dorsal ($P < 0.03$) subfields, but the mean number of FLI neurons was nominally greater in all subfields. Thus water served as the control condition for each subfield. For expression in the coronal plane was strikingly heterogeneous according to stimulus. Relative to water, citric acid significantly increased FLI in each subfield ($P < 0.0001$ for all). The subfield with the greatest numbers of FLI neurons, the middle-ventral subfield, had 2.5 times as many FLI neurons as that with the fewest, the lateral-dorsal. Thus, although there was preferential expression of FLI across subfields, the distribution was quite broad. This broad topography contrasted dramatically with the pattern of FLI expression for QHCl. Although QHCl$_{hi}$ produced very similar numbers of FLI neurons across the entire i,r NST, significant increases were restricted to only three subfields. The medial two subfields, the medial-dorsal and medial-ventral, exhibited the largest increases in FLI neurons relative to water ($P < 0.0001$ for both), and there was a smaller increase in the middle-ventral subfield ($P < 0.0001$). There also were tendencies for FLI to be greater in the lateral-ventral ($P < 0.06$) and actually less in the lateral-dorsal subfield ($P < 0.09$). Thus, in contrast to the 2.5:1 ratio of FLI expression for the most heavily to the least labeled subfield for citric acid, this ratio was $\sim 17:1$ for QHCl$_{hi}$. In other words, the pattern was much more spatially specific. The pattern of FLI expression for QHCl$_{med}$ was similar to that for QHCl$_{hi}$, but significant increases only occurred in the medial two subfields: medial-dorsal ($P < 0.0004$) and medial-ventral ($P < 0.001$). In addition, although the increases did not even approach significance, the topographic pattern of FLI expression for QHCl$_{lo}$ resembled that for both of the higher concentrations of QHCl. The only two subfields in which QHCl$_{lo}$ produced nominally greater numbers of FLI neurons than elicited by water were the medial-dorsal and medial-ventral subfields. In contrast, no subfields in the i,rNST of the NaCl group exhibited significant or even nominal increases in FLI compared with the water group.

The difference between the distribution of FLI in the coronal plane was further quantified by calculating correlation coefficients (Table 1) between the average patterns evoked across subfields depicted in Fig. 5 and summarizing these relationships in two dimensions using multidimensional scaling (Fig. 6). All three QHCl concentrations evoked highly similar mean patterns of FLI across subfields. Figure 6 shows that these stimuli are tightly clustered in the multidimensional scaling space, reflective of their high correlations ($r > 0.93$ for all; Table 1). Furthermore, the pattern produced by QHCl was very distinct from that associated with citric acid. The correlations between QHCl$_{hi}$ and QHCl$_{med}$ vs. citric acid were nearly zero ($r = 0.02$ and 0.03, respectively). Compared with QHCl$_{lo}$ the pattern for citric acid was still distinct, albeit less so ($r = 0.29$). All three QHCl concentrations produced patterns markedly different from those observed in the unstimulated group or with the marginally effective stimuli (water and NaCl). QHCl$_{med}$ and QHCl$_{hi}$ correlated...
negatively with the patterns for the unstimulated and water- and NaCl-stimulated rats. The QHCl_lo pattern correlated at slightly higher levels, but still very weakly ($r_{\text{QHCl}_{\text{lo}}} = 0.17$ for all) with patterns from these groups. Although citric acid produced a large increase in the number of NST FLI neurons, that increase was not associated with a topography that was very distinct compared with the pattern associated with low levels of FLI in the unstimulated and water- and NaCl-stimulated groups. All correlations between citric acid and these other groups were $r_{\text{citric acid}} = 0.59$. Although the patterns of FLI expression for citric acid and QHCl were distinctive in the orosensory NST, these stimuli produced similar distributions in the visceral NST. At the ip level, the average patterns for citric acid and QHCl across subfields were strongly correlated ($r = 0.88$).

In contrast to the topography in the coronal plane, there was little evidence for a similar phenomenon across anteroposterior levels in the ir/rNST. ANOVAs for each level supported the hypothesis that stimulus condition affected FLI across the entire rostral-caudal extent of the orosensory NST ($P < 0.005$ for all). However, the number of FLI neurons varied across this axis (Fig. 7). In general, for QHCl and citric acid, FLI neurons were most numerous at the two most caudal levels and declined rostrally. This trend was most pronounced for citric acid and QHCl_hi. However, except for QHCl_hi at the most rostral level, both of these stimuli elicited significant increases in FLI relative to water at each anteroposterior level ($P < 0.02$ for all). The medium QHCl concentration did not produce significant increases in FLI at any single level, but the increases at r1–r3 approached significance ($P < 0.07$ for all). The lowest QHCl concentration was associated with a nominal increase relative to water only at r1. Stimulation with NaCl never elicited even a nominal increase relative to water at any level of the ir or rNST.

Because FLI expression was more robust caudally, the topographic pattern of FLI expression across subfields was more pronounced in the rostral-caudal extent of the orosensory NST. The medium QHCl concentration did not produce significant increases in FLI at any single level, but the increases at r1–r3 approached significance ($P < 0.07$ for all). The lowest QHCl concentration was associated with a nominal increase relative to water only at r1. Stimulation with NaCl never elicited even a nominal increase relative to water at any level of the ir or rNST.

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Table 1. Pearson’s $r$ values between mean patterns of FLI across subfields ($r_{iNST}$)

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Values in italic type indicate correlations between quinine monohydrochloride (QHCl) and the other stimuli; values in bold type indicate correlations between various concentrations of QHCl, FLI, c-Fos-like immuoreactivity; NST, nucleus of solitary tract; rNST, rostral NST; iNST, rostral level of intermediate NST; U, unstimulated; W, water; N, 0.3 M NaCl; C, 0.1 M citric acid; QHCl_hi, 30 mM QHCl; QHCl_med, 3 mM QHCl; QHCl_lo, 0.3 M QHCl.
fields was examined separately for each level of orosensory NST for citric acid and QHCl by inspecting correlations between the average pattern of FLI expression across subfields for each level. The correlations between the patterns for each QHCl concentration exceeded those between each of these stimuli and citric acid only at i NST, r1, and r2. Thus the distinctive topography in the coronal plane was most obvious in the caudal portion of orosensory NST, where FLI neurons were most numerous.

Behavioral Results

The oromotor behaviors elicited by citric acid and QHCl were markedly different. During the first 2 min of stimulation, 0.1 M citric acid produced significantly fewer gapes (0.36 ± 0.11 gapes/s) than QHClhi (1.14 ± 0.16 gapes/s, t = 4.6, df = 5, P < 0.01). Although the occurrence of gapes or chin rubs was noted in response to both stimuli during each of the six 5-min blocks of the stimulation period, other aversive and ingestive measures differentiated oromotor responses to QHClhi and citric acid across the entire session. Passive rejection was most likely underestimated because of the difficulty of viewing the mouth through the wet floor but was observed during more 5-min periods during QHClhi (3.2 ± 0.79) than acid (0.5 ± 0.34) stimulation (t = 3.7, df = 5, P < 0.02). In addition, QHClhi stimulation always resulted in notable fluid rejection by the end of the first 5-min block. Specifically, by this time (or sooner), the floor of the testing chamber was very wet. In contrast, stimulation with 0.1 M citric acid never produced notable fluid rejection by the end of the first 5-min period. In fact, in three of six rats, the floor was still dry (except for urine) at the end of the entire 30-min session. In the other three cases, significant fluid rejection occurred by the end of 10, 20, and 25 min of acid stimulation. Thus, even though both stimuli produced some aversive behaviors, QHClhi was much more potent. In contrast, citric acid produced more ingestive behaviors. Most strikingly, on average, tongue protrusions (lateral or midline) accompanied by low-amplitude mouth movements occurred in 5.2 ± 0.31 of six 5-min blocks during acid stimulation, but only in 0.5 ± 0.22 during QHClhi stimulation (t = 14.0, df = 5, P < 0.0005).

DISCUSSION

The present study clearly demonstrates that citric acid and QHCl elicit topographically distinct spatial patterns of Fos expression within the orosensory NST. The distinctive medial clustering of FLI in the NST produced by QHCl appeared virtually identical to that in several previous reports (16, 31, 40, 41, 67, 70). In addition to consistency across experiments, the present results demonstrate that the pattern for QHCl is stable and distinctive relative to citric acid across 2 log steps of QHCl concentration. These results extend our earlier observations, which demonstrated a differential distribution of FLI in NST evoked by QHCl (3 mM) compared with a stimulus representative of a different classical taste quality, sucrose (1.0 M) (31, 70).

Concentration Effects

In the i NST, the numbers of FLI neurons increased dramatically with QHCl concentration, but the pattern of expression retained its characteristic spatial distribution. Similarly, in the olfactory bulb, stable topographic patterns of activation have been observed across concentration for several chemicals using a variety of imaging techniques (10, 24, 39). However, a recent study revealed that certain olfactory stimulants did produce changes in pattern with concentration shifts (39). Thus the impact of stimulus intensity on FLI needs to be examined for other tastants to determine whether fixed topographic patterns are a general rule.

The concentration data in the present study provide a novel opportunity to assess the sensitivity of Fos immunohistochemistry in NST. Behavioral studies suggest an absolute detection threshold for QHCl at ∼0.01–0.02 mM (48, 59), and just slightly higher concentrations are clearly sensed as aversive on the basis of their gustatory properties (12, 57). These same con-
centrations are close to the threshold for producing oromotor rejection (gaping) (20, 52). The lowest QHCl concentration we tested, 0.3 mM, elicited recognizable Fos expression in the NST. However, the effects were weak, suggesting that lower concentrations would have been ineffective. Thus, at the present level of sensitivity, the concentration of QHCl necessary to produce FLI in the NST appears to be \(~1\log\) step greater than that which elicits aversive behavioral effects.

**Differential Efficacy of Taste Stimuli in Eliciting FLI in the NST**

NaCl did not elicit FLI expression in the NST. This was surprising, since 0.3 M NaCl is a potent stimulus neurophysiologically (17, 27, 72) and behaviorally (48, 53, 58). The lack of NaCl-induced Fos expression does not appear to extend to other levels of the gustatory system. In contrast to the NST, NaCl has been reported to evoke FLI in the parabrachial nucleus (PBN) (78), even though these same investigators apparently did not observe NaCl-evoked FLI in the NST (77). In fact, although the data were not quantified, we also observed a distinct cluster of FLI neurons in the PBN of five of six NaCl-stimulated rats, despite the lack of such expression in the NST of the same animals (Fig. 8). This cluster was located in the caudal third of the PBN in the central medial subnucleus, similar to the location reported by Yamamoto et al. (78). Thus an overall weakness for NaCl in our experiments cannot explain its ineffectiveness in the NST. Instead, it would appear that the capacity for Fos expression is preferential in particular subpopulations of NST taste cells. A similar phenomenon has been well documented in the somatosensory system. Nociceptive signals conveyed by small-diameter afferent fibers are much more effective in evoking FLI than innocuous signals conveyed over large-fiber systems (6, 38). The lack of NaCl-induced FLI in the NST highlights the limited nature of using FLI for mapping central patterns of neural activation. Although three classical gustatory qualities (31, 70), umami tastants (56), and chorda tympani electrical stimulation (32) evoke Fos expression in the NST, these immunohistochemical maps may not completely capture the neural activation pattern for any stimulus. Nevertheless, within these constraints, Fos immunohistochemistry has revealed a property of NST organization that has been elusive using electrophysiological techniques.

**Functional Significance of FLI Gustatory Topography**

The functional significance of the chemotopy that we observed in the NST is not certain. In the PBN, Yamamoto and colleagues (78) also reported a differential distribution of FLI after different gustatory stimuli. The PBN topography partially reflects stimulus hedonics. For example, the external PBN subnuclei express FLI after aversive but not appetitive stimuli. Furthermore, FLI is expressed in the external region after multiple aversive tastants, both bitter QHCl and sour HCl. In contrast, the differential topography in the NST does not break down along simple hedonic lines. The pattern evoked by QHCl is different from that evoked by sucrose (31, 70), and the QHCl pattern...
is also different from that produced by an aversive (11, 21) sour stimulus, citric acid. Citric acid and sucrose elicit FLI that is more evenly dispersed across the mediolateral axis than the medial clustering elicited by QHCl (31, 70). In other words, chemotopy is more striking for QHCl than for sucrose and citric acid, stimuli that produce somewhat similar patterns but represent a hedonically positive/aversive pair. The lack of a clear distinction between Fos patterns for sucrose and citric acid also makes it unlikely that chemotopy is a ubiquitous principle of anatomic organization in the NST, let alone that it serves as a general coding mechanism.

On the other hand, the chemotopy observed strongly suggests some type of functional segregation. As proposed by King and colleagues (40, 41), the parallel disruption of QHCl-elicited oromotor rejection (22, 65) and Fos expression (40) by IXth nerve lesions suggests that those NST cells that express Fos after quinine are important for the gape response. The present study supports this hypothesis and further suggests the critical substrate. Previous studies show that each QHCl concentration used in the present study elicits gapes but that the number is a positive function of concentration (65, 66). This parallels the consistent topographic distribution of FLI neurons maintained in the face of their increasing numbers. It was particularly interesting that, with our stimulation paradigm, QHCl elicited about three times as many gapes as did citric acid but a similar total amount of FLI in the nNST. Significantly, QHCl elicited more FLI in a circumscribed NST location, the medial subfields, and, furthermore, the FLI patterns for citric acid and QHCl were most distinct caudally where the IXth nerve input dominates. Thus we propose that the medial third of the caudal orosensory NST is a critical afferent link for gaping. Furthermore, it is interesting that 0.1 M citric acid also produced less passive rejection and many more ingestive responses. This suggests that the medial orosensory NST may likewise be involved in passive rejection, perhaps by inhibiting circuits giving rise to ingestive responses. Similarly, the NST regions that express markedly more FLI after citric acid, the lateral third and middorsal regions, may be preferentially involved in ingestive responses. These hypotheses are admittedly tentative. Further support will require a more rigorous correlation between behavior and FLI and direct assessment of the behavioral effects of perturbing specific NST regions.

Nongustatory Contributions to QHCl- and Citric Acid-Elicited FLI

Somatosensory. Although citric acid and QHCl elicited different patterns of FLI expression in orosensory NST, the possibility that some of the difference arises from nongustatory effects needs to be entertained. The NST receives ample afferent inputs from oral somatosensory fibers (28), and neurophysiological data demonstrate a sizable population of gustatory and nongustatory rNST neurons responsive to innocuous oral mechanical stimulation (47, 71). The tendency for water to increase FLI in the NST further suggests that intraoral somatosensory effects are demonstrable with Fos immunohistochemistry (31, 41, 70). Thus it is possible that the larger number of ingestive responses elicited by acid in turn produces a higher level of oral mechanical stimulation responsible for the more widespread distribution of acid-elicited FLI. On the other hand, it is difficult to explain why QHCl produces a greater absolute number of FLI neurons in the medial subfields if differential gustatory factors are not involved.

It should also be noted that, at high concentrations, acids can stimulate somatosensory pain fibers. Citric acid, at concentrations similar that used in this study, evokes gustatory and irritant sensations in humans (13, 19). Indeed, even low concentrations of this chemical (0.3 M) weakly stimulate such afferents (49). Thus neurophysiological data suggest that citric acid can activate nongustatory receptors, but the effectiveness of the concentration used in this study (0.1 M) is unknown. Furthermore, it is unclear whether somatosensory-responsive NST neurons respond to nociceptive chemical stimuli. The available data cast doubt on this proposition. Previous Fos studies demonstrate that intraoral capsaicin (7, 15), piperine, histamine, acetylcholine, and nicotine (8) elicit FLI in the trigeminal brain stem nuclei, but not in orosensory NST. Finally, recent results in our laboratory (68) show that a concentration of citric acid (0.03 M) lower than that used in the present study elicits a very similar pattern of FLI in the NST. Thus, overall, it seems unlikely that the irritant properties of citric acid contribute greatly to the differential topography and, instead, that the gustatory properties of citric acid and QHCl make a significant contribution.

Visceral. Citric acid and QHCl elicited FLI not only in NST regions that receive input from orosensory afferents, but also in a location that mainly receives primary afferent input from the Xth nerve (28). Specifically, both stimuli induced FLI at a midpostremal level of the NST. Although the amount of FLI evoked by QHCl and citric acid at the midpostremal level was half or less than that elicited at peak individual levels of the orosensory NST, these results suggest that strong gustatory stimuli can influence visceral afferent systems. Indeed, Yamamoto and Sawa (76) recently demonstrated that intragastric infusions of 1 mM QHCl induced FLI in the visceral NST at a site where intraoral infusions did not. It is possible that our rats swallowed enough 30 mM QHCl to give rise to a postingestive stimulus capable of directly activating gastrointestinal afferents. However, the behavioral data demonstrated very few ingestive responses to QHCl and there was a large amount of fluid, presumably the rejected stimulus, on the testing chamber floor after stimulation. Thus there may be other explanations. Gustatory stimuli, including bitter- and sour-tasting chemicals, trigger cardiovascular and digestive
reflexes (29, 35, 46, 51, 79), and there are pathways from the rostral to the caudal NST that could mediate these actions (4, 62). The caudal FLI could therefore represent gustatory activation of theafferent limb of visceral reflex circuits or reafference from visceral signals activated by reflexes. Because rats appeared to ingest a significant amount of citric acid, direct postigestive effects also are conceivable. However, intragastric infusion of a comparable amount of 30 mM HCl did not evoke much FLI in the NST (75), making indirect effects more likely.

Finally, it should be recognized that FLI expression at the most caudal level included in our analysis of orosensory NST (i level) may represent combined visceral and gustatory effects, since this region receives significant input from the IXth and Xth nerves (28). With intraoral QHCl stimulation, Fos expression at a similar NST level is dependent on IXth nerve integrity (40), but the NST location with maximal Fos expression after intragastric QHCl is nearby (76). In fact, the iNST anterior to the area postrema is a transition zone between IXth and Xth nerve input and expresses FLI after a wide variety of manipulations. Effective conditions include and are not limited to ingestion of sucrose or a palatable meal (18, 31, 61), exposure to the conditioned stimulus after conditioned taste aversion (37, 63, 64), gastrointestinal malaise (23, 75), blood pressure changes (8, 9, 36, 42, 50), and morphine withdrawal (60). The heterogeneity of these effective manipulations could suggest an integrative function, a final common mechanism, or subtle topographic differences in this region.

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TOPOGRAPHY OF QHC\textsubscript{L} AND CITRIC ACID FLI IN NST


