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c-Fos Induction in the Rat Nucleus of the Solitary Tract by Intraoral Quinine Infusion Depends on Prior Contingent Pairing of Quinine and Lithium Chloride

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HOUPT, T. A., J. M. PHILOPENA, T. H. JOH AND G. P. SMITH. *c-Fos induction in the rat nucleus of the solitary tract by intraoral quinine infusion depends on prior contingent pairing of quinine and lithium chloride.* PHYSIOL BEHAV **60**(6) 1535–1541, 1996.—Intraoral infusions of sucrose or saccharin induce c-Fos-like immunoreactivity (c-FLI) in the intermediate nucleus of the solitary tract (iNTS) of rats after acquisition of a conditioned taste aversion (CTA). The induction of c-FLI in the iNTS may be a consequence of the shift in behavioral response from ingestive to aversive behaviors that characterize acquisition and expression of a CTA. To test this hypothesis, rats were intraorally infused with 0.3 mM quinine sulfate, an aversive taste, 1. prior to conditioning, 2. after 3 noncontingent (unpaired) infusions of quinine and toxic lithium chloride (LiCl) injections, 3. after conditioning with 3 contingent pairings of quinine and LiCl, and 4. after extinction with repeated unpaired infusions of quinine. Intraoral infusions of quinine induced c-FLI in the iNTS only after acquisition of a CTA against quinine; quinine failed to induce c-FLI in the iNTS of unconditioned, noncontingently treated, or extinguished rats. The pattern of c-FLI in the iNTS induced by expression of a CTA against quinine was quantitatively and anatomically similar to that elicited by sucrose in rats expressing a CTA against sucrose. We conclude that aversive responses per se are not sufficient to induce c-FLI in the iNTS. Furthermore, contingent pairing of quinine and LiCl does not cause a shift in behavioral response from palatable, ingestive behaviors to aversive behaviors as in acquisition of a CTA against sucrose. Thus, we also conclude that a shift in behavior from ingestive to aversive responses is not required for increased c-FLI expression in the iNTS during CTA expression. Therefore, the induction of c-FLI in the iNTS during expression of a CTA may be correlated with neuronal processes specific to acquisition and expression of a CTA. *Copyright © 1996 Elsevier Science Inc.*

Bitter Conditioned taste aversion Learning Memory Gene expression Immediate-early gene

IT HAS recently been demonstrated that intraoral infusions of sucrose or saccharin induce c-Fos-like immunoreactivity (c-FLI) in the intermediate nucleus of the solitary tract (iNTS) of rats after acquisition of a conditioned taste aversion (CTA) (11,20). Because sucrose infusions alone did not induce c-FLI in the iNTS prior to acquisition of the CTA (11), c-FLI induction was not a correlate of the unconditioned effect of sucrose. Furthermore, when the CTA to sucrose was extinguished by repetitive infusions of sucrose without LiCl injections, the CTA disappeared and the number of c-FLI-positive cells in the iNTS returned to preCTA levels (11). The induction of c-FLI by sucrose persists for as long as the behavioral expression of the CTA persists (at least 6 months), but the number of c-FLI-positive cells declines with forgetting of the CTA (9). Thus, these experiments demonstrated that induction of c-FLI in the iNTS was correlated with some aspects of the expression of the CTA.

The expression of a CTA includes 1. a shift in behavior response from ingestion of palatable sucrose to rejection of sucrose after CTA acquisition; 2. the aversive behaviors per se that are displayed during rejection of the sucrose solution (e.g., gaping, forelimb flailing, and chin-rubbing); 3. visceral responses (e.g., diarrhea displayed by most rats during expression of the CTA); or 4. activation of neurons participating in the associative mechanisms mediating expression of the CTA.

We have previously ruled out vagally mediated conditioned visceral responses during CTA expression, because total subdiaphragmatic vagotomy does not attenuate induction of c-FLI in the iNTS after CTA expression (5). The role of a shift in behav-

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ioral response or the expression of aversive behaviors during CTA expression remain possible correlates of c-FLI induction. Therefore, in these experiments we used intraoral infusions of unconditioned and conditioned quinine solutions to test the hypothesis that c-FLI in the iNTS induced by an intraoral infusion after CTA acquisition is correlated with the associative mechanisms mediating CTA expression, and not the shift from ingestive to aversive behaviors or the aversive behaviors per se that occur during the expression of a CTA.

If induction of c-FLI in the iNTS requires a shift in behavior from ingestive to aversive responses, then expression of a CTA against quinine should not be sufficient to induce c-FLI in the iNTS because there is no shift in behavior to quinine: the rat responds aversively to intraoral infusions of quinine before and after CTA acquisition. If the induction of c-FLI in the iNTS is exclusively correlated with the expression of aversive behaviors, however, an infusion of quinine sufficient to elicit aversive behaviors should also be sufficient to induce c-FLI in the iNTS of an unconditioned rat. Conversely, if the induction of c-FLI in the iNTS is independent of the expression of aversive behaviors, but is correlated with CTA acquisition, intraoral infusions of quinine should induce c-FLI in the iNTS only if rats have prior contingent experience of quinine and LiCl.

We used a concentration of quinine sulfate (0.3 mM) that elicits an aversive response without prior conditioning in rats when it is infused intraorally (2). Thus, the gross behavioral response of rats is not changed by pairing intraoral infusions of 0.3 mM quinine with LiCl during acquisition of a CTA against quinine. We exploited intraoral catheters to present an aversive quinine solution directly into the oral cavity of nonwater-deprived rats. [Others have demonstrated taste aversions to quinine and unpalatable tastants, but only by using water-deprived rats to ensure consumption during conditioning and 1-bottle intake tests (12–14,19).]

We report here that infusions of quinine did not induce c-FLI in the iNTS of unconditioned rats, confirming the earlier report by Swank et al. (21), or in rats with noncontingent experience of quinine and LiCl, but infusions of quinine into conditioned rats did. Furthermore, induction of c-FLI in the iNTS of conditioned rats can be extinguished by repeated unpaired infusions of quinine, supporting an associative mechanism underlying acquisition of the c-FLI response. Because intraoral infusions of quinine were sufficient to elicit aversive behavioral displays in both conditioned and unconditioned rats, we conclude that neither the shift in behavioral responses from ingestive to aversive, nor the aversive behaviors per se seen during expression of a CTA, are sufficient for induction of c-FLI in the iNTS. Rather, induction of c-FLI in the iNTS may be correlated with the neural mechanisms of association mediating CTA expression. A preliminary report has appeared (10).

MATERIALS AND METHODS

Experiment I: Conditioned and Unconditioned Quinine

Animals and surgery. Adult male Sprague–Dawley rats (Taconic, 300 g) were individually housed under a 12-h light, 12-h dark cycle at 25°C. Food (Purina® rodent chow) and water were provided ad lib, except as noted below.

Anterior sublingual intraoral catheters were implanted under metofane anesthesia by a modified version of the technique described previously (22). Intraoral catheters were prepared from 10 cm of PE-50 polyethylene tubing; one end of the catheter was heat-flared to form a 2 mm diameter annular end. A small incision was made on the ventral midline between the mandibles, and a bent 23-gauge syringe needle pushed between the mandibles until the needle projected into the mouth midway between the root of the lower incisors and the base of the tongue. The unflared end of the catheter was affixed to the end of the syringe needle; the needle was retracted to pull the tubing along the needle tract and out the incision on the ventral submental surface until the flared end of the catheter rested on the floor of the mouth beneath the tongue.

An incision was then made from the caudal extent of the skull to midway between the scapulas on the dorsal surface of the rat's neck. A blunt wire probe was threaded between the skin and the musculature from the dorsal incision to the ventral submental incision. Then, the end of the intraoral catheter was attached to the wire probe, which was pulled back with the intraoral catheter under the skin and externalized through the dorsal incision. The intraoral catheter was held in place by threading it through an outer sleeve of 0.040 silastic tubing attached to a 15 mm diameter Marlex® mesh disk (Bard-Parker, Billerica, MA) sutured to the dorsal neck musculature. A 5-cm length of sleeve and catheter projected from the dorsal surface of the rat for attachment to an infusion catheter. The neck incision was closed with wound clips on either side of the catheter sleeve and the submental incision was sutured closed. Rats were allowed 4 days recovery prior to testing.

Conditioning. Six rats were implanted with intraoral catheters as described above and conditioned by pairing intraoral infusions of 0.3 mM quinine sulfate (quinine) with LiCl. Rats were deprived of food, but not water, for 17 h prior to intraoral infusions. Individual rats were weighed and placed in 26-cm wide by 17-cm deep by 30-cm tall test chambers (formed by subdividing a 40-gallon glass aquarium with Plexiglas walls). Syringe pumps (Harvard Apparatus) infused quinine from 20-ml syringes at a rate of 1.1 ml/min for 6 min (setting 10) through 0.0040-gauge Silastic® catheters attached to the externalized end of the implanted intraoral catheters. Rats and any feces produced in the test chamber during the infusion were weighed immediately after the 6-min infusion; the weight gained during the infusion procedure was recorded as a measure of consumption of quinine. The rats were returned to their home cages and injected with LiCl (0.15 M, 12 ml/kg, IP) 30 min after the start of the intraoral infusion. Intraoral quinine infusions were paired 3 times with LiCl injections at 48-h intervals. All tests were conducted 2–6 h after lights on.

Seventy-two hours after the third pairing of quinine and LiCl, the rats were 17-h food-deprived and received a final intraoral infusion of 0.3 mM quinine (6.6 ml over 6 min). No LiCl injection was administered. The rats were weighed immediately before and after the test infusion, and returned to their home cages at the end of the infusion. One hour after the start of the quinine infusion, the rats were sacrificed and processed for c-FLI as described below.

A control group of 5 rats was also processed after an unconditioned intraoral quinine infusion. The control rats had intraoral catheters implanted as above. The rats were 17-h food-deprived and then received a single intraoral infusion of 0.3 mM quinine (6.6 ml over 6 min). They were weighed immediately before and after the infusion, and returned to their home cages. One hour after the start of the infusion, the rats were sacrificed and processed for c-FLI.

Experiment II: Contingent vs. NonContingent Experience

Experiment I compared the induction of c-FLI in the NTS by an intraoral infusion of quinine in conditioned rats after 3 pairings of quinine and LiCl to c-FLI induction in unconditioned rats receiving their first intraoral infusion of quinine. To control for the

effects of multiple intraoral infusions of quinine and multiple injections of LiCl, we examined the induction of c-FLI in a noncontingent control group of rats. The noncontingent rats were unconditioned rats that received the same number of intraoral infusions of quinine and the same number of LiCl injections as conditioned rats. The noncontingent group received intraoral infusions of quinine and LiCl noncontingently on alternate days, however, so that no conditioned association would be formed.

Ten rats were implanted with intraoral catheters as described above. Five rats were conditioned against intraoral quinine by contingent pairing of intraorally infused 0.3 mM quinine (6.6 ml over 6 min) and, 30 min after the start of the infusion, 12 ml/kg of 0.15 M LiCl IP (contingent experience of quinine and LiCl). The other 5 rats received noncontingent experience of quinine and LiCl: they were administered a LiCl injection (0.15 M, 12 ml/kg IP) 24 h prior to an intraoral infusion of 0.3 mM quinine (6.6 ml over 6 min). All rats were 17-h food-deprived prior to each intraoral infusion, and received a total of 3 infusions and 3 injections at 48-h intervals.

Seventy-two hours after the third intraoral infusion of quinine, both contingent and noncontingent groups of rats were 17-h food-deprived, and received a final intraoral infusion of 0.3 mM quinine (6.6 ml over 6 min). No LiCl injection was administered. The rats were weighed immediately before and after the test infusion, and returned to their home cages at the end of the infusion. One hour after the start of the quinine infusion, the rats were sacrificed and processed for c-FLI as described below.

Experiment III: Extinction

A critical feature of CTA expression is that conditioned responses acquired by contingent pairing of taste and toxin can be extinguished by repeated noncontingent exposure to the taste. We have previously shown, in rats conditioned by 3 sucrose-LiCl pairings, that both the behavioral expression of a CTA and induction of c-FLI in the iNTS elicited by an intraoral infusion of sucrose can be extinguished by 28 unpaired sucrose infusions (11). Experiments I and II demonstrated that the induction of c-FLI by an intraoral infusion of quinine depends on prior contingent experience of quinine and LiCl. To establish that the altered response of the iNTS to quinine in quinine-conditioned rats paralleled the associative properties seen in sucrose-conditioned rats, we attempted to extinguish the conditioned response to quinine with 28 unpaired infusions of quinine.

Sixteen rats were implanted with intraoral catheters as described above. All 16 rats were conditioned against intraoral quinine by pairing intraorally infused 0.3 mM quinine (6.6 ml over 6 min) and, 30 min after the start of the infusion, 12 ml/kg of 0.15 M LiCl IP. Rats were 17-h food-deprived prior to each pairing, and received a total of 3 pairings at 48-h intervals. After 3 pairings of quinine and LiCl, the rats were divided into 2 groups of 8 rats each: an extinction group and a sham-extinction group.

Seventy-two hours after the third pairing of intraoral quinine and LiCl, the extinction group received 4 intraoral infusions of 0.3 mM quinine (6.6 ml over 6 min). The 4 infusions were separated by 1 h, and the rats were weighed before and after each infusion. The 4 infusions of quinine were repeated daily for 7 days, for a total of 28 intraoral quinine infusions. We have previously shown that 28 intraoral infusions of sucrose is sufficient to extinguish a CTA acquired by 3 pairings of intraoral sucrose and LiCl (11). The rats were not food-deprived during this week. After the last (28th) unpaired intraoral quinine infusion, the rats were 17-h food-deprived, and then received a final intraoral infusion of 0.3 mM quinine (6.6 ml over 6 min). One hour after the start of the intraoral infusion, the rats were sacrificed and processed for c-FLI.

As the extinction group, the sham-extinction group received 4 daily intraoral infusions for 7 days beginning 72 h after the third pairing of intraoral quinine and LiCl. The sham-extinction group, however, was infused with deionized water (6.6 ml over 6 min) a total of 28 times. After the last water infusion, the rats were 17-h food-deprived, and then received a final intraoral infusion of 0.3 mM quinine (6.6 ml over 6 min). One hour after the start of the intraoral infusion, the rats were sacrificed and processed for c-FLI.

Tissue Collection and Immunohistochemistry

Rats were overdosed with sodium pentobarbital and, when completely unresponsive, transcardially perfused, first with 100 ml heparinized isotonic saline containing 0.5% $NaNO_2$, then with 400 ml 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB). The brains were dissected, blocked, postfixed for 2 h, and transferred into 30% sucrose for cryoprotection. Forty micron coronal sections were cut on a freezing, sliding microtome through the rostral-caudal extent of the NTS. Thirty-six sections were cut from the caudal subpostremal NTS (bregma, 14 mm) to the caudal extent of the rostral NTS (bregma, 12.8 mm). All coordinates were based on Paxinos and Watson (15). Every other section collected from the NTS was processed for c-FLI.

Free-floating tissue sections were washed twice for 15 min in 0.1 M PBS, then permeabilized in 0.2% Triton, 1% bovine serum albumin (BSA) in PBS for 30 min. After washing twice in PBS-BSA, sections were incubated overnight with a sheep anti-c-Fos peptide antibody (Cambridge Research Biochemicals) at a dilution of 1:3000. Sections were washed in PBS-BSA twice and incubated for 1 h with a biotinylated antisheep rabbit antibody (Vector Laboratories); bound secondary antibody was then amplified with the Vector Elite ABC kit. Antibody complexes were visualized by a 5-min 0.5% diaminobenzadine reaction.

Cells expressing positive, nuclear c-FLI were quantified in the NTS. Only dark, punctate nuclear staining was counted; diffusely stained cell bodies were not counted. The NTS was divided into 3 subregions: caudal [ventral and caudal to the area postrema (AP)], intermediate (abutting the IVth ventricle), and rostral (where the NTS separates from the IVth ventricle). Each of these 3 subregions was represented by approximately 6 sections of the 18 NTS sections collected from each rat. Cell counts for all sections within each subregion of each rat were averaged, and the individual mean counts for each region averaged across rats by subregion within experimental groups. All data were analyzed by analysis of variance, and subsequent post hoc analyses were performed using Fisher's post hoc test.

RESULTS

Experiment I

During intraoral infusions of 0.3 mM quinine, both conditioned and unconditioned rats expressed typical aversive orofacial movements and behavioral responses (e.g. gaping, chin-rubbing, forearm-flailing, and head-shakes). There was no apparent qualitative change in behavior of the rats after pairing intraoral quinine infusions with LiCl. No rats gained weight during the intraoral quinine infusions, indicating that they rejected all quinine infused. LiCl produced diarrhea and piloerection within 10–30 min after injection. Diarrhea after intraoral infusions of quinine was observed in some rats after 2–3 pairings of LiCl and intraoral quinine infusions.

After 3 pairings with LiCl, intraoral infusion of quinine induced significantly more c-FLI-positive cells in the intermediate

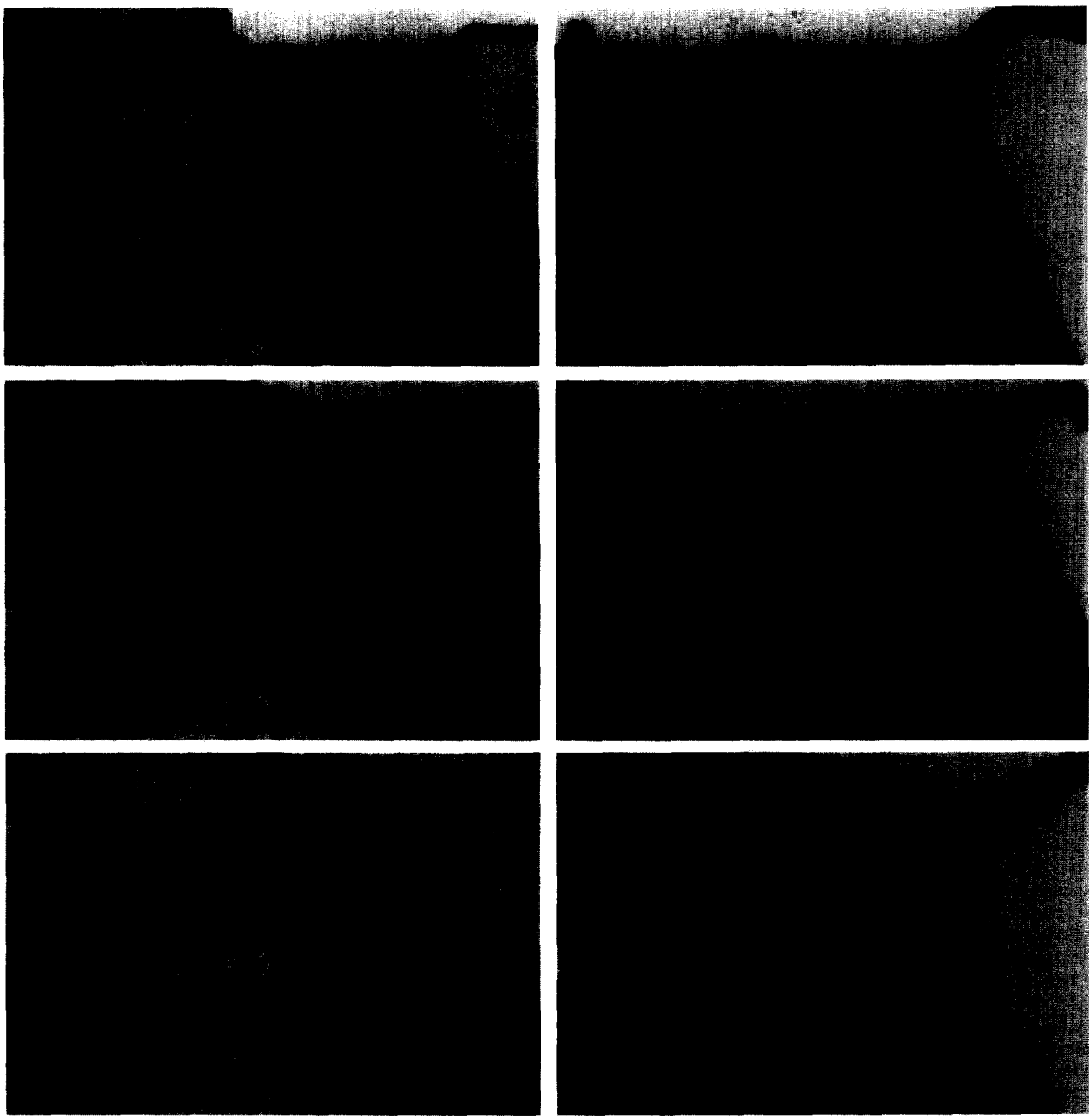

FIG. 1. Examples of c-FLI induced 1 h after intraoral infusions of 0.3 mM quinine (6.6 ml over 6 min) in the intermediate NTS of an unconditioned rat (A, B); a rat conditioned by 3 prior pairings of intraoral quinine infusions with LiCl (C, D); and a rat conditioned against quinine and then extinguished with 28 unpaired intraoral infusions of quinine (E, F). Panels on the right are closeups of the medial intermediate NTS. Only the rat conditioned against quinine expresses large numbers of c-FLI-positive cells after an intraoral infusion of quinine. st, solitary tract; mNTS, medial NTS; IV, fourth ventricle. Scale bar = 0.1 mm.

($p < 0.005$) and caudal ($p < 0.05$) subregions of the NTS than did an unconditioned intraoral infusion of quinine (Figs. 1A,B,C,D, 2). The largest number of cells was observed in the medial part of the iNTS; fewer c-FLI-positive cells were seen in the caudal NTS along the ventral edge of the medial NTS immediately dorsal to the dorsal motor nucleus of the vagus. No c-FLI-positive cells were observed in the area postrema. In both the intermediate and caudal NTS, occasional cells were seen medially and laterally adjacent to the solitary tract and in the lateral part of the NTS. Diffusely scattered c-FLI-positive cells were seen in the rostral NTS ventral to the solitary tract.

Experiment II

As in Experiment I, rats gained no weight during intraoral infusions of quinine in either the contingent or noncontingent

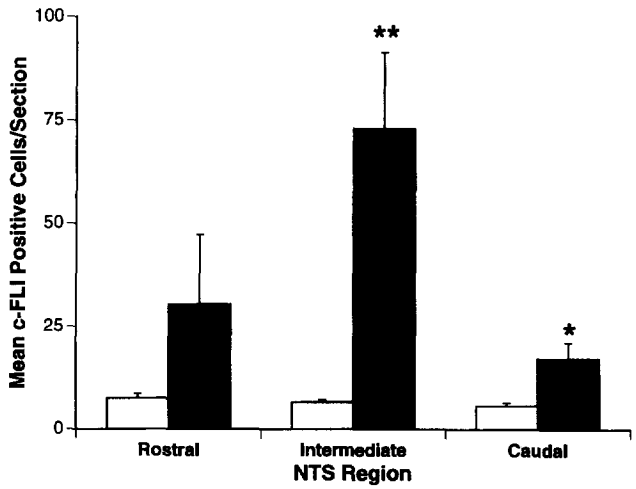

FIG. 2. Mean (± SEM) number of c-FLI-positive cells in the NTS 1 h after intraoral 0.3 mM quinine infusion (6.6. ml over 6 min) in unconditioned rats (n = 5; white bars) and 3 days after the third pairing of intraoral quinine infusion with LiCl (n = 6; black bars). * $p < 0.05$; ** $p < 0.005$ significantly different from unconditioned group.

groups. Neither group gained weight (i.e., consumed any quinine) during the final test infusion. Intraoral infusion of quinine induced significantly more c-FLI-positive cells in the medial NTS of rats that had received 3 contingent intraoral infusions of quinine and LiCl injection (quinine infusions preceding LiCl injections by 30 min) than in rats that had received the same number of quinine infusions and LiCl injections noncontingently (LiCl injections preceding quinine infusions by 24 h). The number of c-FLI-positive cells was significantly greater in all 3 subregions of the medial NTS in the contingent group, and was maximal in the intermediate medial NTS (see Fig. 3).

Experiment III

As in Experiments I and II, rats gained no weight during intraoral infusions of 0.3 mM quinine during pairing with LiCl. None of the rats in the extinction group consumed any quinine during any of the 28 unpaired quinine infusions. The rats in the sham-extinction group consumed little or no deionized water during the 28 unpaired water infusions they received; they appeared to allow most of the water to passively drip from their mouths during the infusions without displaying active aversive reactions. (Note that during the week of water infusions, rats were neither food- nor water-deprived and, thus, might not be expected to ingest water during the water infusions.) Neither the extinction group nor the sham-extinction group gained weight (i.e., consumed any quinine) during the final test infusion.

Extinction of conditioning against quinine did not appear to alter the behavioral response of rats to intraoral infusion of quinine as measured by intake, but it did abolish the induction of c-FLI in the NTS. Following 28 intraoral infusions of quinine, a final intraoral quinine infusion administered to extinguished rats elicited aversive behaviors and was not ingested, but induced significantly fewer c-FLI positive cells in the intermediate ($p < 0.0005$) and caudal subregions of the NTS ($p < 0.05$; Fig. 3) than in sham-extinguished rats or in conditioned rats (Fig. 1E and F; compare Fig. 3 to Fig. 2).

Sham-extinction of conditioning against quinine did not abolish expression of c-FLI in the iNTS in response to intraoral infusion of quinine. Following 28 intraoral water infusions, intraoral infusion of 0.3 mM quinine elicited aversive behaviors, was not ingested, and produced a pattern of c-FLI in the NTS of sham-extinguished rats very similar to that seen in quinine-conditioned rats described above. Although significantly fewer c-FLI-positive cells were seen in the iNTS of sham-extinguished rats compared to the conditioned quinine rats of Experiment I ($p < 0.005$; compare Figs. 2 and 3), sham-extinguished rats expressed significantly more c-FLI-positive cells in the iNTS than did extinguished rats.

DISCUSSION

Our major result is that intraoral infusions of 0.3 mM quinine induce large numbers of c-FLI-positive cells in the iNTS of rats only after quinine infusions have been previously paired with LiCl injections. Intraoral infusions of quinine do not induce c-FLI in the iNTS prior to the pairing of quinine and LiCl. This confirms the report of Swank et al. that an unpaired intraoral infusion of quinine does not induce c-FLI in the NTS (21). Intraoral infusions of quinine do not induce c-FLI in the iNTS after extinction of quinine conditioning by repeated infusions of quinine without LiCl administration. Furthermore, intraoral infusions of quinine are not ingested, and elicit aversive behaviors before conditioning, after conditioning, and after extinction. Therefore, we conclude that an aversive behavioral response elicited by quinine, the prototypical bitter and aversive taste quality, is not sufficient to induce c-FLI in the iNTS. Rather, the induction of c-FLI in the iNTS by quinine depends upon prior contingent pairing of quinine and LiCl, so as to condition the rats against quinine infusions.

These results parallel our initial observation that intraoral sucrose infusions induced c-FLI in the iNTS only after acquisition of a CTA against sucrose (11). Acquisition of a CTA against

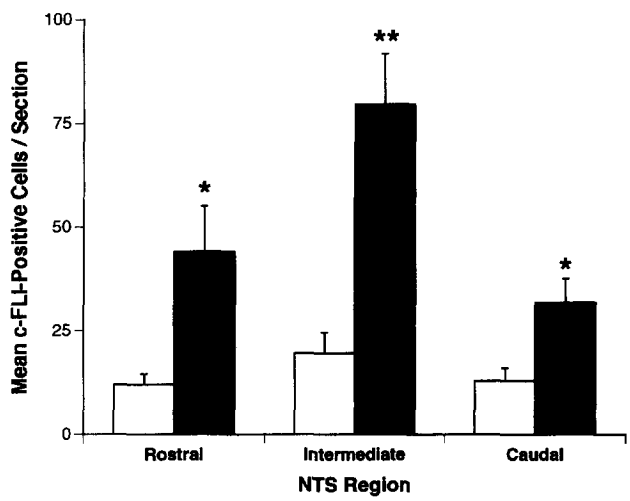

FIG. 3. Mean (± SEM) number of c-FLI-positive cells in the NTS 1 h after an intraoral 0.3 mM quinine infusion (6.6. ml over 6 min) administered 3 days after the last intraoral infusion of quinine in a noncontingent group (n = 5; white bars) or contingent group (n = 5; black bars). Both groups received a total of 3 intraoral infusions of quinine and 3 injections of LiCl prior to the final test infusion of quinine; rats in the noncontingent group received unpaired intraoral infusions of quinine and LiCl injections (24-h apart). Rats in the contingent group received paired intraoral infusions of quinine and LiCl injections (30 min apart). * $p < 0.05$l; ** $p < 0.005$ significantly different from noncontingent group.

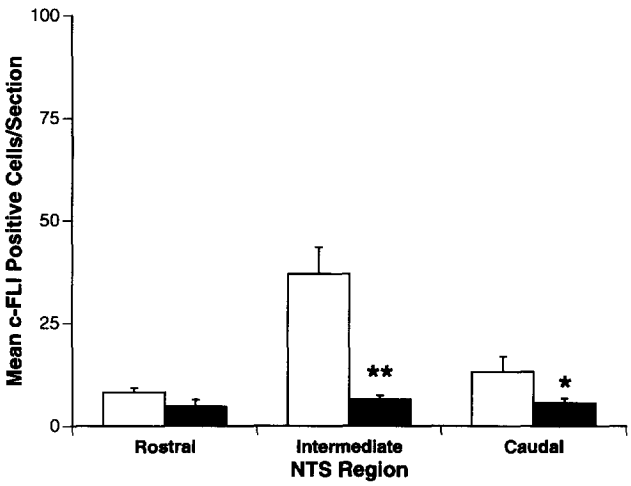

FIG. 4. Mean (± SEM) number of c-FLI-positive cells in the NTS 1 h after intraoral 0.3 mM quinine infusion (6.6. ml over 6 min) in rats (n = 8) conditioned against quinine and then sham-extinguished with 28 intraoral water infusions (white bars) or in rats (n = 8) conditioned against quinine and real-extinguished with 28 intraoral quinine infusions (black bars). * $p < 0.05$; ** $p < 0.005$ significantly different from sham-extinguished group.

sucrose is accompanied by a change in behavioral response from acceptance to rejection. Quinine, however, is actively rejected by the rat before and after pairing with LiCl; thus, a change in behavioral response from acceptance to rejection is not required for the change in activation of the iNTS.

The pattern and distribution of c-FLI in the iNTS of quinine-conditioned rats after intraoral quinine infusion is very similar to that observed in rats receiving intraoral sucrose infusions after acquisition of a CTA against sucrose. Although c-FLI-positive cells observed following CTA expression are localized to the medial intermediate and caudal NTS in both quinine- and sucrose-conditioned rats, it remains to be determined if the neuronal populations activated by the two tastants are identical in phenotype or connectivity.

There is electrophysiological evidence that the response profile of sucrose-best neurons in the gustatory NTS comes to resemble the response-profile of quinine-best neurons after CTA acquisition (1). We and others (21) have observed very few c-FLI-positive cells in the rostral, gustatory NTS after brief sucrose or quinine infusions; the first-order neurons of the gustatory NTS may not express c-Fos in response to short intraoral infusions, either before or after CTA acquisition. In Experiment II, a significant increase in c-FLI was observed in the rostral NTS of quinine-conditioned rats (Fig. 3), but the highest density of c-FLI was consistently found in the medial iNTS. The gustatory response of the medial iNTS, where c-FLI was observed after CTA acquisition, has not been characterized electrophysiologically.

These results provide additional evidence that c-FLI induction in the iNTS by an intraoral infusion is correlated with CTA acquisition and extinction. Indeed, c-FLI expression in the NTS serves as a marker of previously contingent pairing of a taste and a toxin even when the behavioral expression of a CTA is masked by an innate, unconditioned aversive response. An unconditioned rat and a rat conditioned against quinine are apparently indistinguishable in their qualitative behavioral responses to the taste of quinine; at the neuronal level, however, only the iNTS of the conditioned rat consistently expresses a high density of c-FLI-positive cells. Thus c-FLI in the iNTS is a consistent neuronal correlate of the expression of a previously acquired CTA against either sucrose or quinine.

That c-FLI expression in the iNTS is not a correlate of the aversive behaviors displayed during CTA expression is further support for our hypothesis. That is, that c-FLI in the iNTS induced during CTA expression is correlated solely with altered neuronal activation by the taste stimulus brought about by associative processes during CTA acquisition, and not by nonassociative aspects of acquisition or expression. The other evidence for the correlation between expression of a CTA and expression of c-FLI in the iNTS is:

1. c-FLI in the iNTS is not an artifact of repeated LiCl injections, because intraoral infusions of water elicited c-FLI after pairing with LiCl, but mock-infusions previously paired with LiCl failed to elicit c-FLI in the iNTS (7).
2. The induction of c-FLI in the iNTS is not a generalized response to the intraoral infusion procedure, because it was specifically induced by the tastant previously paired with LiCl and not by intraoral infusion of other, unpaired tastants (8).
3. The altered responsiveness of the iNTS to a conditioned taste is not a transient phenomenon, because it correlated persistently and quantitatively with behavioral expression of a CTA up to 6 months after acquisition (9).
4. The induction of c-FLI is probably not correlated with gastrointestinal visceral events during expression of the CTA, because total subdiaphragmatic vagotomy did not block c-FLI induction in the iNTS by conditioned intraoral sucrose infusions (5).

In these experiments, we have attempted to eliminate nonassociative factors that may be responsible for induction of c-FLI in the iNTS during CTA expression. To date, only the prior contingent experience by rats of a tastant and LiCl injection is sufficient for c-FLI expression in the iNTS when the tastant is intraorally infused. These results strongly support our working hypothesis that c-FLI in the iNTS is correlated with the neuronal processes underlying expression of a CTA.

The induction of c-FLI in the iNTS during expression of a CTA is currently the best characterized neuronal correlate of CTA expression. Although the caudal hindbrain may be necessary for acquisition and expression of a CTA, it is not sufficient to mediate CTA expression without intact connections with brain regions rostral to the midbrain. This is demonstrated by the inability of the chronic decerebrate rat to acquire or express a CTA (3). It has recently been reported that unilateral disruption of hindbrain-forebrain connections by hemidecerebation blocks acquisition of an altered c-Fos response during CTA expression in the iNTS ipsilateral to the hemidecerebration (18).

Elucidation of the neural substrate of CTA expression will also require analysis of forebrain regions. An analysis of c-FLI in the forebrain induced by expression of a CTA is currently in progress (6). Preliminary results indicate that the central nucleus of the amygdala expresses c-FLI in response to intraoral infusion of sucrose only after acquisition of a CTA against sucrose (4). Parallel activation of the central nucleus of the amygdala and the iNTS during expression of a CTA may represent functional interactions because the NTS and the central nucleus have dense reciprocal connections (16,17). The causal interactions between the iNTS and the amygdala and other forebrain regions involved in expression of a CTA remain to be established.

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REFERENCES

1. Chang, F-C. T.; Scott, T. R. Conditioned taste aversions modify neural responses in the rat nucleus tractus solitarius. J. Neurosci. 4:1850–1862; 1984.
2. Grill, H. J.; Berridge, K. C. Taste reactivity as a measure of the neural control of palatability. In: Progress in psychobiology and physiological psychology. New York: Academic Press; 1985:1–61.
3. Grill, H. J.; Norgren, R. Chronically decerebrate rats demonstrate satiation but not bait shyness. Science 201:267–269; 1978.
4. Houpt, T. A.; Berlin, R. A.; Smith, G. P. Altered induction of c-Fos in the central nucleus of the amygdala (CeN) correlated with conditioned taste aversion expression. Soc. Neurosci. Abs. 21:1682; 1995.
5. Houpt, T. A.; Berlin, R. A.; Smith, G. P. c-Fos induction in the nucleus of the solitary tract by sucrose after conditioned taste aversion acquisition is not attenuated by subdiaphragmatic vagotomy. Chem. Senses 20:710; 1995.
6. Houpt, T. A.; Philopena, J. M.; Jahng, J. W.; Joh, T. H.; Smith, G. P. c-Fos induction in the pons and forebrain by lithium chloride and sucrose after conditioned taste aversion formation. Soc. Neurosci. Abstr. 20:1223; 1994.
7. Houpt, T. A.; Philopena, J. M.; Jahng, J. W.; Joh, T. H.; Smith, G. P. Intraoral infusions of water induce c-Fos immunoreactivity in the rat nucleus of the solitary tract after conditioned taste aversion formation. Appetite 23:308–309; 1994.
8. Houpt, T. A.; Philopena, J. M.; Jahng, J. W.; Joh, T. H.; Smith, G. P. Taste specificity of c-Fos induction in the rat nucleus of the solitary tract following conditioned taste aversion formation. Chem. Senses 19:488; 1994.
9. Houpt, T. A.; Philopena, J. M.; Joh, T. H.; Smith, G. P. c-Fos induction in the rat nucleus of the solitary tract correlates with the retention and forgetting of a conditioned taste aversion. Learning Memory 3:25–30; 1996.
10. Houpt, T. A.; Philopena, J. M.; Wessel, T. C.; Joh, T. H.; Smith, G. P. Quinine induces c-Fos immunoreactivity in the rat nucleus of the solitary tract after conditioned taste aversion formation. Soc. Neurosci. Abstr. 19:583; 1993.
11. Houpt, T. A.; Philopena, J. M.; Wessel, T. C.; Joh, T. H.; Smith, G. P. Increased c-Fos expression in the rat nucleus of the solitary tract after conditioned taste aversion formation. Neurosci. Lett. 172:1–5; 1994.
12. Kutscher, C. L.; Wright, W. A. Unconditioned taste aversion to quinine induced by injections of NaCl and LiCl: dissociation of aversion from cellular dehydration. Physiol. Behav. 18:87–94; 1977.
13. Kutscher, C. L.; Wright, W. L.; Lisch, M. NaCl and LiCl efficacy in the induction of aversion for quinine and saccharin solutions immediately following injection. Pharmacol. Biochem. Behav. 6:567–569; 1977.
14. Nowlis, G. H.; Frank, M. E.; Pfaffmann, C. Specificity of acquired aversions to taste qualities in hamsters and rats. J. Comp. Physiol. Psychol. 94:932–942; 1980.
15. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1986.
16. Ricardo, J. A.; Koh, E. T. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. Brain Res. 153:1–26; 1978.
17. Rogers, R. C.; Fryman, D. L. Direct connections between the central nucleus of the amygdala and the nucleus of the solitary tract: an electrophysiological study in the rat. J. Auton. Nerv. Syst. 22:83–87; 1988.
18. Schafe, G. E.; Seeley, R. J.; Bernstein, I. L. Forebrain contributions to the induction of a cellular correlate of conditioned taste aversion in the nucleus of the solitary tract. J. Neurosci. 15:6789–6796; 1995.
19. Scott, T. R.; Giza, B. K. A measure of taste intensity discrimination in the rat through conditioned taste aversions. Physiol. Behav. 41:315–320; 1987.
20. Swank, M. W.; Bernstein, I. L. c-Fos induction in response to a conditioned stimulus after single trial taste aversion learning. Brain Res. 636:202–208; 1994.
21. Swank, M. W.; Schafe, G. E.; Bernstein, I. L. c-Fos induction in response to taste stimuli previously paired with amphetamine or LiCl during taste aversion learning. Brain Res. 673:251–261; 1995.
22. Tyrka, A.; Smith, G. P. SCH23390, but not raclopride, decreases intake of intraorally infused 10% sucrose in adult rats. Pharmacol. Biochem. Behav. 45:243–246; 1993.