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Increased c-*fos* expression in nucleus of the solitary tract correlated with conditioned taste aversion to sucrose in rats

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Abstract

The pattern of neuronal activation in the rat nucleus of the solitary tract (NTS) in response to a standard gustatory stimulus was examined using c-Fos-like immunoreactivity (c-FLI) before and after conditioned taste aversion (CTA) formation. While unconditioned oral infusions of sucrose solution did not induce c-FLI in the NTS, after three pairings of sucrose with lithium chloride injections, sucrose induced c-FLI in the medial intermediate NTS 1 h after oral infusion. Extinction of the CTA by repeated infusions of sucrose alone reversed the induction of c-FLI.

Key words: Gustatory nerve; Immediate-early gene; Gene expression; Immunoreactivity; Learning; Memory

Conditioned taste aversion (CTA) is a form of associative learning and classical conditioning in which an animal avoids and reacts aversively to the taste of a food that has previously been paired with illness. Conditioned taste aversion has been described in many species, from invertebrates to humans, and has important clinical implications in drug and radiation therapies [3]. In this experiment, the pattern of neuronal activation in the rat nucleus of the solitary tract (NTS) in response to a standard gustatory stimulus was examined using c-Fos-like immunohistochemistry before and after CTA formation.

c-Fos expression has been observed following many interventions, including sensory stimulation such as light exposure [1,14,19] and food ingestion [11,18], as well as systemic administration of lithium chloride (LiCl) [9,18,20]. We examined the responsiveness of the NTS to a standard gustatory stimulus, 6 ml of 5% sucrose delivered by intraoral catheter, before and after pairing with systemic LiCl injections.

Adult male rats ($n = 8$) were implanted with anterior sublingual intraoral catheters [10]. Cannulas were led subcutaneously from the submental region and externalized through the skin on the dorsal surface of the neck. Rats were allowed 5–7 days recovery with one deionized water infusion during that time to confirm cannula patency. Following overnight food deprivation, rats were infused intraorally with 6 ml of 5% sucrose solution over 6 min. Thirty minutes after the sucrose infusions, 12 ml/kg of 0.15 M LiCl was injected intraperitoneally. The conditioning procedure was performed a total of three times on alternate days. Two days after the last pairing, the rats were overnight food deprived before a final 6 ml of 5% sucrose infusion without LiCl injections. One hour after the sucrose infusions, they were sacrificed and the brains processed for c-Fos-like immunoreactivity (c-FLI). Rats were overdosed with pentobarbital, and then perfused intracardially with 100 ml of 0.15 M saline, 0.5% $NaNO_2$ containing 1000 U heparin, followed by 400 ml of 4% formalin in 0.1M phosphate buffer. After dissecting and blocking, the brains were postfixed for 1–2 h and cryoprotected overnight in 30% sucrose. Coronal whole brain sections were cut at 40 μm thickness on a sliding microtome through the medulla, pons, and forebrain at the level of the optic chiasm. Membranes of free-floating sections were solubilized in 0.02% Triton

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X-100 for 30 min, and incubated overnight at room temperature with sheep c-Fos peptide antiserum (Cambridge Biochemicals). After 1 h incubation with biotinylated anti-sheep secondary antibody, immunoreactivity was visualized with Vector Vectrastain ABC Elite kit and 5 min reaction with 0.05% diaminobenzidine. The expression of c-FLI was quantified by counting positive-staining cells within the NTS.

Three control groups of 6–7 rats each were also examined. One group received a single, 6 ml oral infusion of 5% sucrose one hour before sacrifice. The second control group received only the unconditioned stimulus of a single LiCl injection (12 ml/kg of 0.15 M LiCl) one hour before sacrifice. The third control group received 3 LiCl injections and 3 intraoral infusions of sucrose, but injections and infusions were administered on alternate days in a non-contingent manner so that no CTA was formed in order to control for the effects of repeated exposure to sucrose and LiCl. This non-contingent group received an oral infusion of 6 ml of 5% sucrose one hour before sacrifice.

During the conditioning period, the rats' behavior after intraoral sucrose changed from ingestive responses in which the sucrose was readily consumed to aversive behaviors such as head-shaking, chin-rubbing, and mouth-gaping [4,8]. When these aversive behaviors appeared, the sucrose solution was no longer ingested – rats let the infused sucrose drain out of the mouth. The change from ingestive to aversive responses was already evident after the first pairing of sucrose and LiCl. LiCl injections induced a transient, toxic reaction, characterized by lying prone, piloerection, and diarrhea lasting 1–2 h. By the final sucrose infusion, sucrose alone elicited similar symptoms, including diarrhea, in the conditioned rats. In contrast, the rats that received the sucrose infusion once and the rats that received sucrose infusions after prior non-contingent sucrose infusions and LiCl injections expressed only ingestive responses to the intraoral sucrose.

The two control groups receiving sucrose showed little or no c-FLI anywhere in the dorsal vagal complex of the medulla. Sucrose alone did not induce c-FLI anywhere in the NTS, area postrema (AP), or dorsal motor nucleus of the vagus, although c-FLI was induced in the dorsal raphe, locus coerulus, paraventricular nucleus of the hypothalamus, and piriform cortex. In contrast, LiCl induced strong c-FLI in the caudal (subpostremal and behind) and intermediate (where the NTS abuts the IVth ventricle) portions of the NTS, both medially and laterally, as well as in scattered cells of the AP. Dorsal raphe neurons, hypothalamic paraventricular and supraoptic nuclei, and piriform cortex also expressed high levels of c-FLI following LiCl.

Oral infusion of sucrose following CTA formation induced c-FLI in some of the parts of the NTS where LiCl did (Figs. 1,2). Although c-FLI-positive cells were almost completely absent from the rostral NTS and the AP, robust c-FLI was seen in the medial part of the intermediate NTS and less c-FLI was seen in the caudal NTS. This c-FLI, however, was less intense than that following LiCl administration. It is not clear if the pattern of c-FLI shows a similar increase in other brain regions. Experiments are in progress to analyze this point further. In the control group which received sucrose and LiCl in a non-contingent manner, the pattern of c-FLI was identical to that following unconditioned sucrose infusions alone.

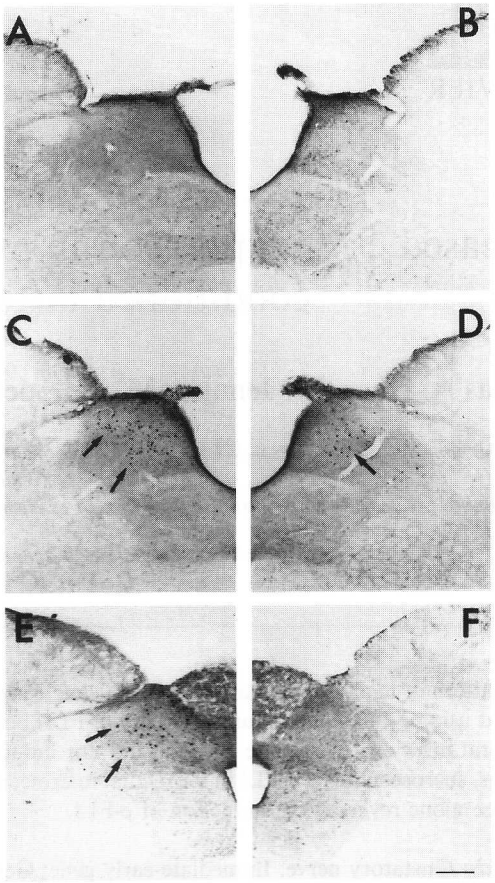

Fig. 1. c-Fos-like immunoreactivity in the NTS 1 h after oral infusion of 6 ml of 5% sucrose in unconditioned (A), non-contingent treated (B) or conditioned rats (D,F) and 1 h after i.p. administration of 12 ml/kg 0.15 M LiCl (C,E). Sucrose infusions in unconditioned or non-contigent treated rats induced very few or no c-FLI-positive cells in either the intermediate (A,B) or caudal (not shown) NTS. In contrast, sucrose infusion did induce robust c-FLI in the medial intermediate NTS of conditioned rats (D), but little or none in the caudal NTS (F). LiCl injections induced c-FLI in large numbers of cells throughout the intermediate (C) and caudal NTS, along the NTS-AP border, and within the AP (E). Arrows indicate punctate c-FLI limited to cell nuclei.

To determine if c-FLI in the NTS paralleled the change in behavior during extinction of a CTA, two other groups ($n = 7$) were conditioned with 3 pairings of sucrose and LiCl as previously. One group was then extinguished by administering 4 oral infusions of 5% sucrose (6 ml) daily (separated by 1–4 h) for 7 consecu-

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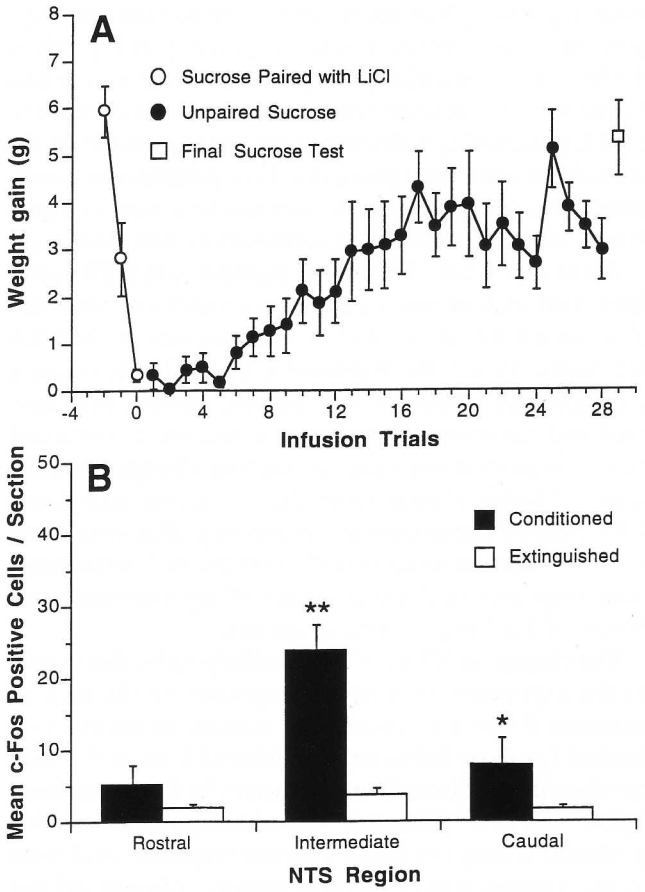

Fig. 3. A: extinction of CTA by repeated conditioned stimulus exposure. Closed circles show CTA formation by three pairings of i.p. injections of LiCl (12 ml/kg, 0.15 M) 30 min after 5% sucrose infusion. Open circles show the rapid decline in mean intake ($n = 7$) during conditioning trials. The CTA was extinguished with 4 daily oral infusions of 6 ml of 5% sucrose for 7 consecutive days. Filled circles show the gradual increase in intake during the extinction period. By the final, pre-sacrifice infusion of sucrose, intakes were close to preconditioning baseline (open square). A conditioned control group ($n = 7$) were sham-extinguished by 4 daily oral infusions of deionized water for 7 consecutive days, and rejected sucrose on the final test day (data not shown). B: c-FLI induction in the NTS by CS in conditioned and extinguished rats from A, quantified by counting c-FLI positive cells in three regions of the NTS. Conditioned rats showed significantly more c-FLI in the NTS in the intermediate and caudal regions of the NTS than extinguished rats: $*P < 0.05$, $**P < 0.005$, Mann-Whitney U-test.

identifying cellular and molecular mechanisms of this type of conditioning. c-Fos transcription is regulated by cAMP-dependent second messengers, in particular protein kinase A (PKA) and cAMP response element binding protein (CREB) [17]. Thus conditioning of a gustatory stimulus may sensitize and upregulate cAMP pathways in NTS neurons. This suggests parallels with the mechanisms of conditioning in some invertebrate species. For example, Kandel and his colleagues have demonstrated that long-term sensitization of the *Aplysia* gill withdrawal reflex is mediated by presynaptic cAMP-dependent gene transcription, resulting in increased PKA catalytic subunit activation [2,13]. Several *Drosophila* mutations with associative learning deficits also point to a role for cAMP, such as *rutabaga* which has limited ability to produce cAMP due to a lack of functional adenylyl cyclase and has impaired associative learning and short-term memory [15]. Since c-*fos* induction is a marker of neuronal cAMP pathway activation, the increase in c-FLI observed after CTA suggests a similar role in mammalian CTA learning.

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