RESEARCH ARTICLE

c-Fos expression in rat brainstem following intake of sucrose or saccharin

Ke Chen[1], Jianqun Yan (✉)[1,2], Jinrong Li[1], Bo Lv[1], Xiaolin Zhao[1]

[1] Department of Physiology and Pathophysiology, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China; [2] Key Laboratory of Environment and Genes Related to Diseases, Ministry of Education, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China

Abstract **To examine whether the activation of brainstem neurons during intake of a sweet tastant is due to orosensory signals or post-ingestive factors, we compared the distribution of c-Fos-like immunoreactivity (c-FLI) in the nucleus of the solitary tract (NST) and parabrachial nucleus (PBN) of brainstem following ingestion of 0.25 M sucrose or 0.005 M saccharin solutions. Immunopositive neurons were localized mainly in the middle zone of the PBN and four rostral-caudal subregions of the NST. Intake of sucrose increased the number of FLI neurons in almost every subnucleus of the PBN ($F_{(2,13)} = 7.610$, $P = 0.023$), in addition to the caudal NST at the level of the area postrema ($F_{(2,13)} = 10.777$, $P = 0.003$) and the NST intermediate zone ($F_{(2,13)} = 7.193$, $P = 0.014$). No significant increase in the number of c-Fos positive neurons was detected in response to saccharin ingestion, although there was a trend towards a modest increase in a few select NST and PBN nuclei. These results suggest that the PBN and NST may be involved in sweet taste perception and modulation of sweet tastant intake, but the significantly enhanced intensity of Fos expression induced by sucrose indicates that PBN/NST neuronal activity is driven by the integrated effects of sweet taste sensation and post-ingestive signals.**

Keywords c-Fos; parabrachial; the nucleus of the solitary tract; sweet tastant; rat

Introduction

c-Fos is a ubiquitous immediately-early gene that presents specific, rapid, and transient expression in response to a myriad of extracellular stimuli. Expression of c-Fos is an anatomic landmark of activated neurons in the central nervous system [1], and countless studies have detected unique patterns of *c-Fos* expression in response to specific stimuli. Previous investigations demonstrated that gustatory stimulation induces c-Fos-like immunoreactivity (c-FLI) in different brain areas, including the nucleus of the solitary tract (NST) [2–4], parabrachial nucleus (PBN) [4–6], and amygdala [7]. In the process of tastant ingestion, both the pure taste sensory information from the oral cavity and post-ingestive factors are thought to participate in c-FLI induction [8, 9].

Sucrose, an energy-enriched carbohydrate, is hydrolyzed and absorbed as component glucose and fructose in the gastrointestinal tract. Glucose is essential for survival and development of animals and serves as the main source of immediately available energy for brain and muscle. Though binding to the same sweet receptor and inducing related sensory responses, saccharin has very weak post-ingestive effects because it contains almost no usable energy [10, 11]. Therefore, it is of interest to compare the distributions of c-FLI in the brainstem after ingestion of sucrose or saccharin as a means of differentiating the brain stem response to sweet taste sensation and post-ingestive effects of sweeteners.

Using standard immunohistochemical staining methods, we detected c-Fos expression in the rat brainstem after ingestion of sucrose and saccharin solutions to analyze the unique patterns of neural activity induced by different sweet tastants, the common neural responses to sweet taste perception, and the neural activity associated with post-ingestive effects (sucrose).

Materials and methods

Animals were treated following the *Guidelines of Animal Experiments* from the Committee of Medical Ethics, Experimental Animal Center, Medical Center, Xi'an Jiaotong

University. All efforts were made to minimize the number of animals used and to limit their suffering.

Animals

Male Sprague Dawley rats (provided by the Medical Experimental Animal Center of Shaanxi Province, China) with an initial weight of 200–220 g were caged individually and had *ad libitum* access to standard laboratory chow and tap water unless otherwise stated. Room temperature was maintained at (21 ± 2) °C under a 12:12 h light-dark cycle (lights on at 7:00 and off at 19:00). Body weight, food intake, and water intake of each animal were measured daily at 9:00 during the experiment.

Sweetener intake test

Male SD rats were fed *ad libitum* for 3 days. After that, rats were habituated to the animal facility for 7 days. Again, rats had *ad libitum* access to standard laboratory chow, but were allowed water from one bottle for two hours (9:30–11:30). On experimental days, the rats ($n = 15$) were divided into three subgroups: (1) control rats ($n = 5$) given distilled water from 9:30–11:30, (2) sucrose intake rats ($n = 5$) given 0.25 M sucrose solution, and (3) saccharin intake rats ($n = 5$) given 0.005 M saccharin solution. Sucrose and saccharin (Sigma Chemical Co., St. Louis, MO) solution were mixed with commercial sweeteners and distilled water at least 24 h before use. Liquid consumption was measured by weighing the bottles before and after each day's test.

Immunohistochemical staining

Sixty minutes following sweetener intake, all rats were anesthetized with an overdose of 10% chloral hydrate (0.3 ml/100 g) by intraperitoneal injection, and perfused transcardially with 100 ml 0.9% saline followed by 400 ml 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.2–7.4). The brains were removed immediately and immersed in the same fresh fixative for 3–4 h before being soaked in ice-cold 30% sucrose in 0.1 M PB (pH 7.2–7.4) at 4 °C for 48 h. Subsequently, the brains were cut coronally at 40 μm on a freezing microtome (Leica, Germany) and collected in turn in three small trays containing 0.01M phosphate-buffered saline (PBS, pH 7.2–7.4).

The first set of sections was used to detect c-Fos protein immunoreactivity using the streptavidin-peroxidase conjugated method. All immunohistochemical procedures were performed at room temperature unless otherwise noted and 0.01 M PBS (pH 7.4) was used for all rinses. Sections were first incubated with PBS containing 3% H_2O_2 for 10 min to block endogenous peroxidase activity, rinsed 3 times (5 min/rinse), incubated in 0.3% Triton X-100 diluted in 0.01 M PBS (pH 7.4) for 10 min, and rinsed again 3 times. Sections were then incubated in 10% normal goat serum (A,

SP-9001, Zhongshan Bio-tech Co., Ltd., Beijing, China) for 2 h and then incubated for 72 h at 4°C in rabbit polyclonal c-Fos antiserum (Abcam, AB7963-1, Cambridge, UK) diluted 1:1000 in 0.01 M PBS (pH 7.4). After 3 rinses (10 min/rinse), the slices were incubated in biotinylated goat anti-rabbit IgG (B, SP-9001, Zhongshan Bio-tech Co., Ltd., Beijing, China) for 30 min at 37°C. Slices were rinsed and incubated in horseradish peroxidase(HRP)-avidin (egg white) (C, SP-9001, Zhongshan Bio-tech Co., Ltd., Beijing, China) according to the manufacturer's instructions. The immunoreactive products were visualized using the HRP chromogen 3,3′-diaminobenzidine tetrahydrochloride (ZLI-9032, DAB kit, Zhongshan Bio-tech Co., Ltd.). Sections were mounted onto poly-L-lysine-coated glass slides, air-dried, dehydrated, cover-slipped, and observed under a light microscope (Olympus BX-51, Japan).

Microscopic analysis of sections

The specific locations of neurons expressing FLI were determined bilaterally in subnuclei of the PBN and NST. Images were captured by a digital camera attached to the microscope. The number of c-Fos-positive neurons in different brain areas was counted at $\times$ 40 magnification on both sides of the PBN and NST using Image-Pro Plus software (Version 6.0; Media Cybernetics Inc., MD) by an observer blind to the purpose of the experiment.

The second set of sections was mounted onto gelatin-coated glass slides and processed for Nissl staining. The third set of sections was used for negative controls. In the control experiments, the primary c-Fos antibody was omitted or replaced with normal rabbit serum; no positive staining for the omitted antibodies was detected. Cytoarchitectural subdivisions of the NST and PBN were delineated according to the maps described by Yamamoto [12], Fulwiler [13], and a rat stereotaxic atlas (Paxinos and Watson, 2005 edition).

Data analysis

All values are expressed as mean $\pm$ SEM. One-way analysis of variance (one-way ANOVA) was used to evaluate differences in physiological parameters and numbers of c-Fos-positive neurons. SigmaPlot 10.0 was used to draw pictures and figures. A $P < 0.05$ was considered to be statistically significant.

Results

Physiologic assessments

Fifteen male SD rats (200–220 g) were habituated to water restriction for 7 days. In the first week, rats were given one bottle of distilled water for 2 h in the morning (9:30–11:30) and water was deprived in the rest of the time.

Weight, weight gain, food intake, and water intake decreased significantly during the first few days of water restriction, but recovered to baseline by the fifth day (Fig. 1), suggesting that the rats had adapted physiologically to the water restriction regime.

c-FLI in the NST following intake of sweet tastants

We observed the distributions of diaminobenzidine-labeled FLI neurons in the NST following sweetener ingestion. Fos-like immunoreactivity with peroxidase-DAB visualization appeared as dark-brown staining within cell nuclei. We delineated the different NST subregions according to the stereotaxic atlas of Paxinos & Watson and the results of Yammoto et al. [12], and observed and counted the number of neurons expressing c-FLI at 4 representative rostral-caudal subregions: N1, a region 1 mm caudal to the obex; N2, a region circumscribing the area postrema (AP) 0.5 mm rostral to the obex; N3, the intermediate nucleus; and N4, a region 2.0 mm rostral to the obex. The majority of neurons within

N1, N2, and N3 receive afferent fibers carrying general visceral information, while the taste-sensitive neurons are located mainly within the rostral NST (N4 region).

Fig. 2 shows the mean number of FLI neurons within N1, N2, and N3 of the NST for each treatment group. The distribution of immunoreactive neurons was distinct for the two sweeteners. There was a tendency for the control group (14.50 ± 9.31) and saccharin-intake group (10.40 ± 7.72) to have fewer c-FLI neurons in the N2 region of the NST than the sucrose-intake group (97.67 ± 27.82). In the N3 region, the control group (5.50 ± 3.07) and saccharin-intake group (19.60 ± 13.42) had much fewer FLI neurons than the sucrose-intake group (144.00 ± 38.59). Additionally, c-Fos was strongly expressed in the caudal NST (cNST) in area N2 ($F_{(2,13)} = 10.777$, $P = 0.003$) and the intermediate part of the N3 region ($F_{(2,13)} = 7.193$, $P = 0.014$) in response to ingestion of 0.25 M sucrose solution. The elevated FLI expression extended forward to the rostral part of the NST (Fig. 2). In contrast, very few neurons in N4 showed FLI in response to sucrose or saccharin solution (the numbers would not be
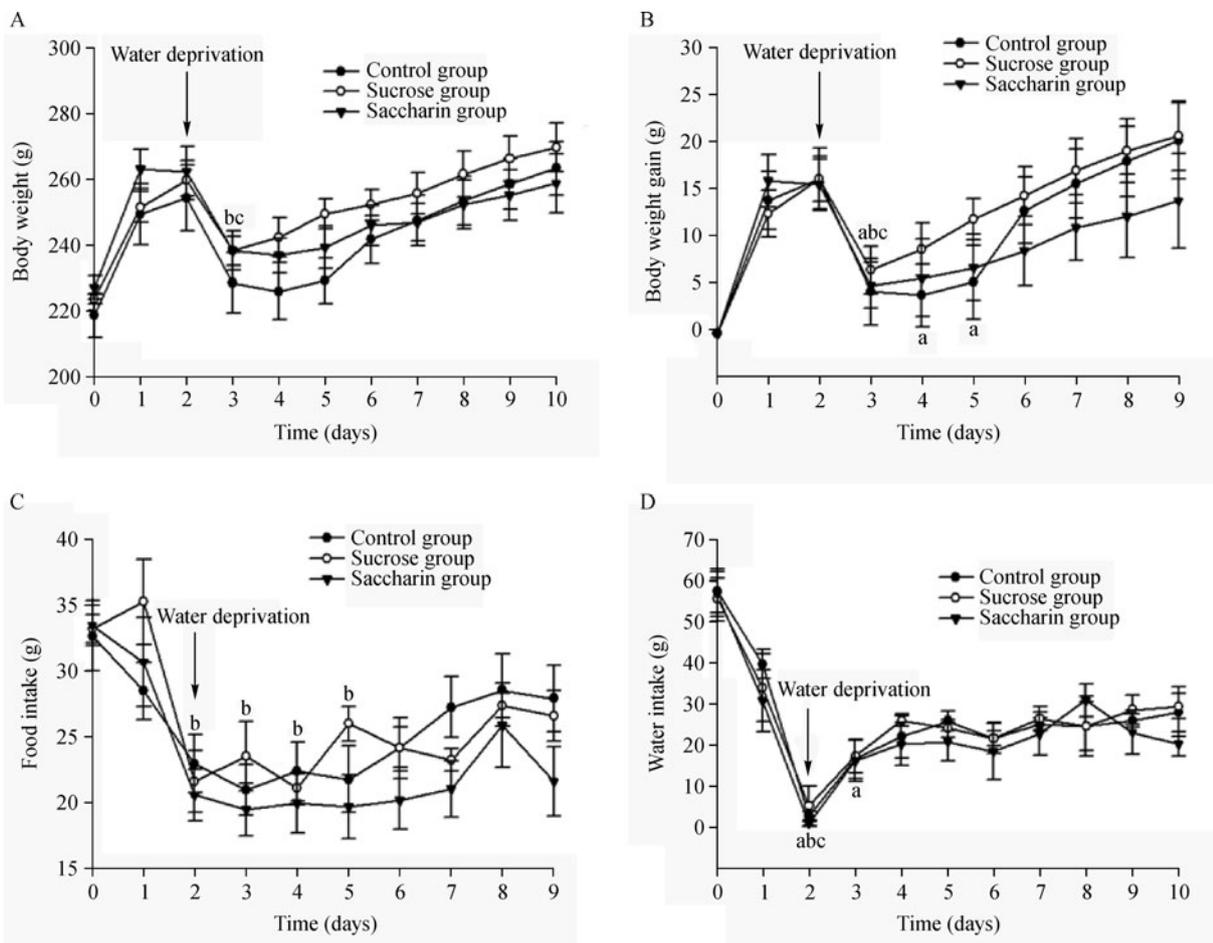

Fig. 1 The changes in weight, weight gain, food intake, and water intake after water restriction (on day 2). (A) Body weight; (B) Body weight gain; (C) Food intake; (D) Water intake. Values are presented as means ± SEM. a, b and c indicate significant difference from baseline after water restriction in the control group (a), sucrose group (b), and saccharin group (c) (n = 5 per treatment group).
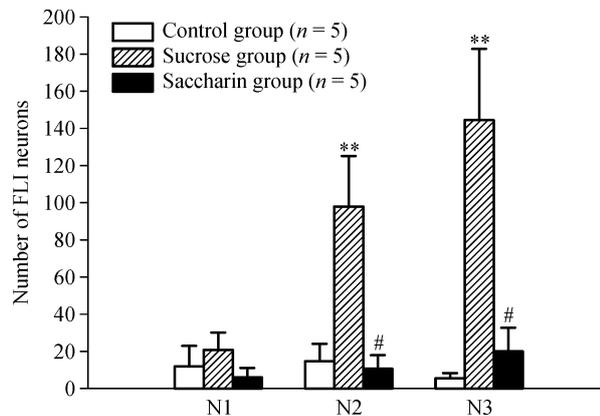
Fig. 2 The number of FLI-positive neurons in the NST induced by sweetener intake. After sucrose solution intake, the number of FLI-positive neurons in the N2 and N3 regions increased significantly. Values are presented as means ± SEM. **$P < 0.01$, the difference between the sucrose group and control group; #$P < 0.05$, the difference between the saccharin group and the sucrose group. There were 5 rats in each treatment group.

visible on the histogram of Fig. 2).

Fig. 3 presents photomicrographs of sections through the NST-N3. There were very few intensely stained nuclei in the NST of control rats (water-intake group) or the saccharin-intake group, whereas every rat in the sucrose-intake group had sizable numbers of intensely stained cell nuclei.

FLI in the PBN following intake of sweet tastants

We also assessed the distribution of diaminobenzidine-labeled FLI neurons in the PBN and counted the number of neurons expressing c-Fos within subregions of the PBN according to the stereotaxic atlas of Paxinos & Watson: vl, ventral intermediate nucleus; dl, dorsal lateral nucleus; cl, lateral central nucleus; m, medial nucleus; exm, external medial nucleus.

Like the NST, the distribution of immunoreactive neurons in the PBN was distinct for each treatment group (Fig. 4). In the PBN of the pontine area, FLI-positive neurons were localized mainly in the middle zone. There was a tendency for the control group and saccharin-intake group to have fewer FLI neurons in the PBN than the sucrose-intake group. There was a significant increase in FLI labeling in almost every subnucleus of the PBN in rats that ingested 0.25 M sucrose ($F_{(2, 13)} = 7.610$, $P = 0.023$). c-Fos was strongly expressed in the vl ($F_{(2,13)} = 5.866$, $P = 0.032$), the dl ($F_{(2,13)} = 17.459$, $P = 0.002$), and the medial nucleus ($F_{(2,13)} = 6.760$, $P = 0.023$), while there was only a slight increase in the external medial nucleus. There was also a slight elevation in the number of c-FLI neurons in rats fed saccharin, but the increase did not reach statistical significance compared to the control group.

Fig. 5 presents photomicrographs of sections through the

PBN with the subnuclei demarcated (lower right). There were very few intensely stained cell nuclei in the PBN of control rats or rats in the saccharin-intake group, whereas every rat in the sucrose-intake group had sizable numbers of intensely stained cell nuclei.

Discussion

The present study compared c-Fos expression profiles in gustatory brainstem nuclei following ingestion of saccharin or sucrose to examine neural activation patterns in response to a simple sweet tastant (saccharin) and a sweet tastant with post-ingestive effects (sucrose) on brain physiology.

We used two sweet tastants, sucrose and saccharin. The sweet taste perception, hedonic value, and behavioral representation induced by a real high-energy disaccharide and an artificial sweetener with little energy are different markedly [2, 14]. The concentrations of sucrose and saccharin solutions used in our study come from the most preferred concentration determined by our previous behavioral tests [15].

Saccharin was used as a simple sweet taste stimulus in our study. It is known that T1R2/T1R3 sweet taste receptors are expressed in the taste buds, gut and pancreas [16, 17], so in addition to acting as a sweet taste stimulus, saccharin also binds to its receptors in the gut and pancreas, and triggers some related physiologic responses. As a non-caloric sweetener, however, saccharin passes directly through the digestive system unabsorbed. It has less post-ingestive effects compared to caloric sweeteners, and would thus act as a simple gustatory stimulus in our experiment [10, 11].

Energy-rich sucrose not only evokes a sweet taste sensation in the oral cavity, but also produces post-ingestive effects. Previous studies reported that compared with water, 0.6 M sucrose elicited more Fos protein production in each of the central gustatory nucleus [18]. Additionally, the results of Yamamoto and his colleagues showed that ingestion of sucrose induced strong c-FLI in both the NTS and PBN, and that this strong expression was mainly due to post-ingestive signals rather than gustatory (sensory) signals [12]. In our study, rats had *ad libitum* access to sucrose solution. Therefore, we concluded that *fos* gene expression induced by sucrose results from the integrated effect of the sweet taste sensation and post-ingestive signals, rather than a simple sweet taste stimulation.

The brainstem is an important relay in the taste information transmission pathway. The sweet taste information produced by the binding of sweeteners to heterodimeric T1R2/T1R3 receptors is transmitted to the central nervous system via the facial nerve, glossopharyngeal nerve, and vagus nerve. In the rat, the first relay is the rostral division of the nucleus of the solitary tract (rNST) [19], which was divided into several subregions based on cytoarchitectonics and functional differences [20]. Taste information from the NST transmits
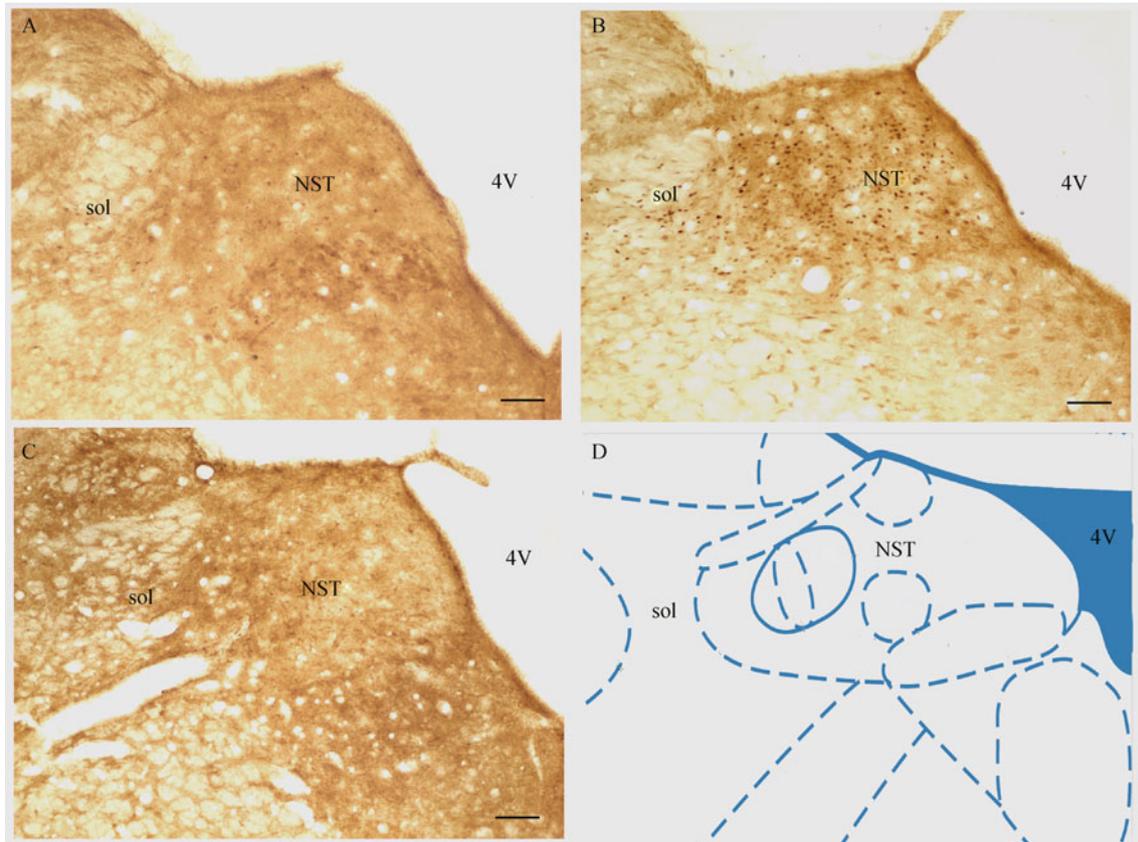
Fig. 3 The distribution of FLI-positive neurons in the NST-N3 region of rats induced by intake of sweet taste solutions. Sucrose solution elicited robust FLI in this area. A, B, and C indicate control group, sucrose group, and saccharin group, respectively. D is the schematic neuroanatomic diagram of the N3 region. NST, the nucleus of the solitary tract; sol, solitary tract; 4V, fourth ventricle ($n = 5$). Scale bar = 100 μm.

to specific subregions in the PBN [21]. The typical "taste area" in the PBN is the waist area (w), comprising the medial subnucleus (m), ventral lateral subnucleus (vl), and external medial subnucleus (exm) [22]. Additionally, the lateral subnucleus, including the superior lateral subnucleus (sl), external lateral subnucleus (el), and dorsal lateral subnucleus (dl), also receive afferent taste information [23].

Previous studies found that sweet taste stimulation evoked hedonic behavioral responses in rats, and induced the activities of taste neurons in the central nervous system [24]. The taste-specific c-Fos immunoreactive neurons induced by sucrose solution were mainly located in the medial part of the NST, and the lateral and medial part of the PBN, which were known to participate in the transmission of sweet taste information [25]. Our study found that the expression pattern and proportion of FLI-neurons was similar in the subnuclei of the NST and PBN. The number of FLI-positive neurons increased markedly in the sucrose intake group relative to the control group, while there were no significant changes in the number of FLI-positive neurons in the saccharin group (rather there was a slight increase in some subnuclei that did not reach statistical significance).

The NST is not merely a relay transmitting taste information, but a complex functional area. The taste neurons in rNST integrate taste and visceral sensory information from afferent nerve fibers transmitting information about various chemical stimuli in the mouth [26]. An early study found that sucrose induced c-Fos expression in the rNST, especially in the intermedial NST [2], the subregion that receives most of the primary afferent projections transmitting taste sensation [27]. In our study, Fos expression induced by saccharin and sucrose solutions was also localized largely in the intermediate nucleus of NST-N3 area. In a recent study, however, Schwarz and his colleagues found that most c-Fos immunoreactive neurons induced by ingestion of sucrose solution were located at the level of the AP in mice [28]. This discrepancy may be partially attributed to species differences, tastant concentration, or the method of administration. In Schwarz's study, the animals were gavaged with sucrose diluted in water using a feeding needle, while our experimental rats had *ad libitum* access to sucrose solution for 2 h. Second, the concentrations used in these two studies were different; they used 0.4 g/ml sucrose diluted in water, approximately equivalent to 1.17 M, as compared with the
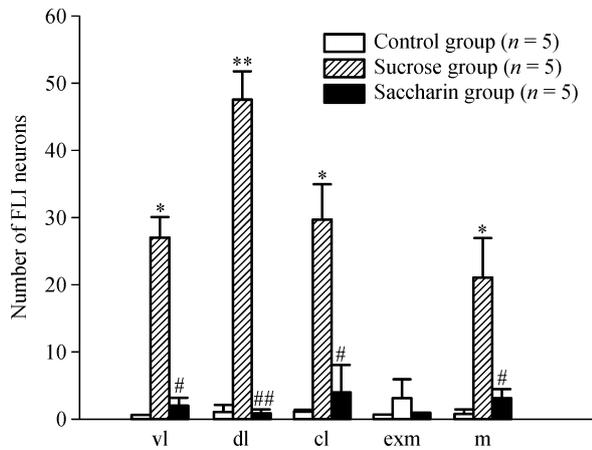
Fig. 4 The number of FLI-positive neurons in subnuclei of the PBN induced by sweet tastant intake. Sucrose elicited marked c-FLI in the vl, dl, cl, and medial nucleus of the PBN. Values are presented as means ± SEM of five rats per treatment group. *$P < 0.05$, **$P < 0.01$, compared with control rats; #$P < 0.05$, ##$P < 0.01$, compared with sucrose group.

0.25 M sucrose solution used in our experiments. Third, male C57BL/6J mice and male SD rats may have species-specific differences in taste perception, sensory processing, or physiological response to sucrose.

The sensory information projects from the NST to several specific functional subregions in the PBN [21]. Neuroanatomical studies found that the PBN waist area is the major taste transmission area. Neurons located in the medial subnucleus and ventral lateral subnucleus of PBN are the targets of many ascending taste fibers [22, 29]. Additionally, the lateral PBN also receives taste information [22, 23]. Previous studies showed that sweet taste stimuli in the oral cavity evoked c-Fos expression in the PBN [12, 30]. Electrophysiological studies also found that most of the "sucrose-best" neurons were located in the waist area of the PBN [31]. In our study, we also found a significant enhancement in c-Fos expression in this PBN subnuclei induced by sucrose intake.

Both the dl and el subregions of PBN participate in visceral and taste sensation [22, 23]. Some studies found that after deprivation of water, FLI expression in the dl subregion was
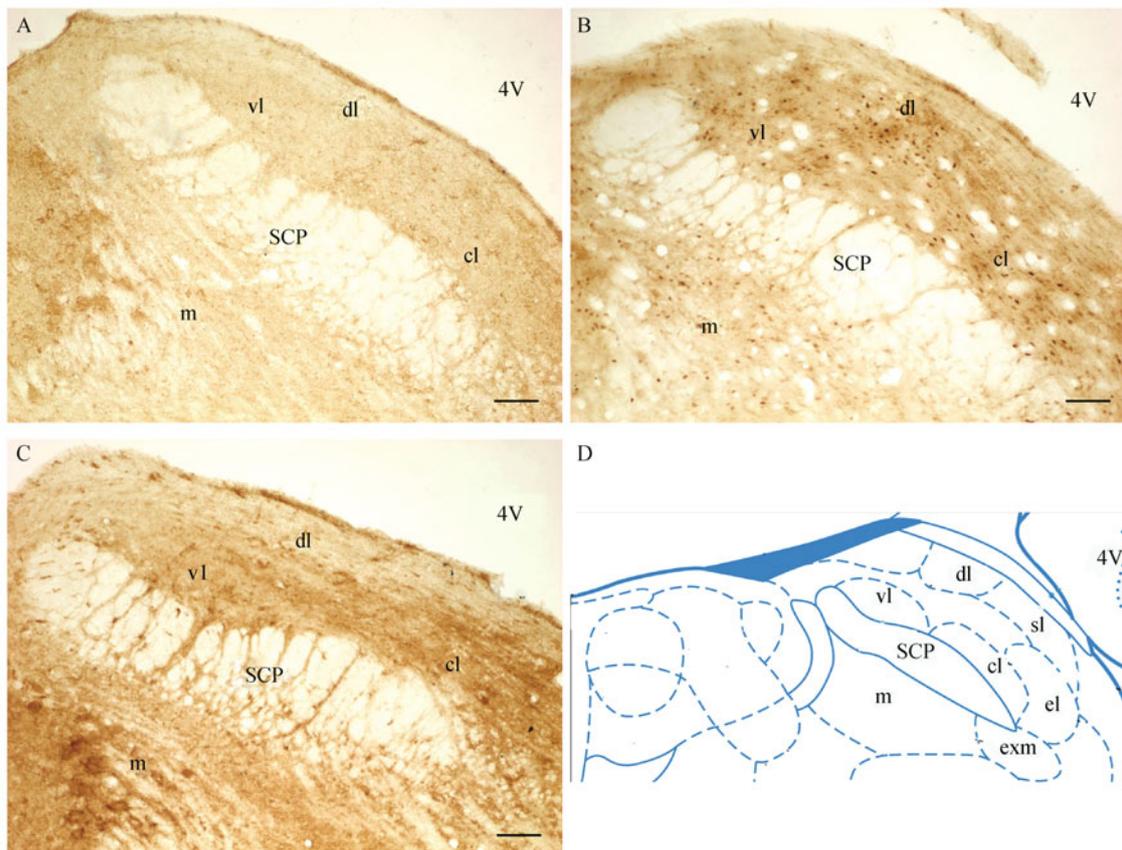

Fig. 5 The distribution of FLI-positive neurons in the parabrachial nucleus induced by sucrose or saccharin intake. Sucrose solution induced vigorous c-FLI in this area, while intake of saccharin solution elicited negligible expression compared to the control group. (A–C) Micrographs are stained slices from a control rat (A), sucrose-fed rat (B), and saccharin-fed rat (C). SCP, superior cerebellar peduncle (brachium conjunctivum). Scale bar = 100 μm.

induced by ingestion of water, saccharin, or NaCl solution, suggesting that dl activity was related to a positive hedonic response and feeding behavior [5, 6, 32]. In contrast, el activity was associated with a negative hedonic state related to gustation and ingestion [32]. In the present study, we also found that FLI expression in the dl was markedly induced by sucrose, while neurons in the el remained unreactive, supporting these previous results.

Ingestion of sucrose and saccharin solution evoked distinct patterns of c-Fos expression in the brain stem. The greater intensity of c-Fos expression induced by sucrose reflected both the sweet taste sensation transmitted from the oral cavity and tongue and neural activity related to post-ingestive effects. This conclusion is based on several additional observations: (1) Saccharin, a sweet tastant that passes through the digestive system unabsorbed evoked minimal c-Fos expression; (2) The areas where c-FLI expression was most intense (the N2 and N3 subregions of the NST and the lateral PBN) are well known targets of afferent fibers conveying general visceral information [33, 34]; (3) There are many kinds of receptors in the gastrointestinal tract, portal vein, and liver, including osmotic pressure receptors, glucose receptors, amino acid receptors, and stretch receptor that can respond to physiological changes associated with high-energy sucrose ingestion. Thus, the marked enhancement of c-FLI expression was most likely due to convergent inputs activated by post-ingestive responses rather than sweet taste sensation alone.

Acknowledgements

References

1. Sagar SM, Sharp FR, Curran T. Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 1988; 240(4857): 1328–1331

2. Harrer MI, Travers SP. Topographic organization of Fos-like immunoreactivity in the rostral nucleus of the solitary tract evoked by gustatory stimulation with sucrose and quinine. Brain Res 1996; 711(1–2): 125–137

3. King CT, Travers SP, Rowland NE, Garcea M, Spector AC. Glossopharyngeal nerve transection eliminates quinine-stimulated fos-like immunoreactivity in the nucleus of the solitary tract: implications for a functional topography of gustatory nerve input in rats. J Neurosci 1999; 19(8): 3107–3121

4. Travers JB, Urbanek K, Grill HJ. Fos-like immunoreactivity in the brain stem following oral quinine stimulation in decerebrate rats. Am J Physiol 1999; 277(2 Pt 2): R384–R394

5. Yamamoto T, Shimura T, Sako N, Sakai N, Tanimizu T, Wakisaka S. c-Fos expression in the parabrachial nucleus after ingestion of sodium chloride in the rat. Neuroreport 1993; 4(11): 1223–1226

6. Yamamoto T, Shimura T, Sakai N, Ozaki N. Representation of hedonics and quality of taste stimuli in the parabrachial nucleus of the rat. Physiol Behav 1994; 56(6): 1197–1202

7. Yamamoto T, Sako N, Sakai N, Iwafune A. Gustatory and visceral inputs to the amygdala of the rat: conditioned taste aversion and induction of c-fos-like immunoreactivity. Neurosci Lett 1997; 226 (2): 127–130

8. Yamamoto T, Sawa K. c-Fos-like immunoreactivity in the brainstem following gastric loads of various chemical solutions in rats. Brain Res 2000; 866(1–2): 135–143

9. Kobashi M, Ichikawa H, Sugimoto T, Adachi A. Response of neurons in the solitary tract nucleus, area postrema and lateral parabrachial nucleus to gastric load of hypertonic saline. Neurosci Lett 1993; 158(1): 47–50

10. Ågmo A, Marroquin E. Role of gustatory and postingestive actions of sweeteners in the generation of positive affect as evaluated by place preference conditioning. Appetite 1997; 29(3): 269–289

11. Kushner LR, Mook DG. Behavioral correlates of oral and postingestive satiety in the rat. Physiol Behav 1984; 33(5): 713–718

12. Yamamoto T, Sawa K. Comparison of c-fos-like immunoreactivity in the brainstem following intraoral and intragastric infusions of chemical solutions in rats. Brain Res 2000; 866(1–2): 144–151

13. Fulwiler CE, Saper CB. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. Brain Res 1984;7 (3):229–259

14. Travers SP, Hu H. Extranuclear projections of rNST neurons expressing gustatory-elicited Fos. J Comp Neurol 2000; 427(1): 124–138

15. Chen K, Yan J, Suo Y, Li J, Wang Q, Lv B. Nutritional status alters saccharin intake and sweet receptor mRNA expression in rat taste buds. Brain Res 2010; 1325:53–62

16. Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Ilegems E, Daly K, Maillet EL, Ninomiya Y, Mosinger B, Shirazi-Beechey SP. T1R3 and gustducin in gut sense sugars to regulate expression of Na$^+$-glucose cotransporter 1. Proc Natl Acad Sci USA 2007; 104 (38): 15075–15080

17. Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami H, Nikolaev VO, Lohse MJ, Shigemura N, Ninomiya Y, Kojima I. Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. PLoS ONE 2009; 4(4): e5106

18. Mungarndee SS, Lundy RF Jr, Norgren R. Expression of Fos during sham sucrose intake in rats with central gustatory lesions. Am J Physiol Regul Integr Comp Physiol 2008; 295(3): R751–R763

19. Hamilton RB, Norgren R. Central projections of gustatory nerves in the rat. J Comp Neurol 1984; 222(4): 560–577

20. Halsell CB, Travers SP, Travers JB. Ascending and descending projections from the rostral nucleus of the solitary tract originate from separate neuronal populations. Neuroscience 1996; 72(1): 185–197

21. Travers JB. Efferent projections from the anterior nucleus of the solitary tract of the hamster. Brain Res 1988; 457(1): 1–11

22. Halsell CB, Travers SP. Anterior and posterior oral cavity responsive neurons are differentially distributed among parabrachial subnuclei in rat. J Neurophysiol 1997; 78(2): 920–938

23. Karimnamazi H, Travers SP, Travers JB. Oral and gastric input to

the parabrachial nucleus of the rat. Brain Res 2002; 957(2): 193–206

24. Yamamoto T. Brain mechanisms of sweetness and palatability of sugars. Nutr Rev 2003; 61(Supplement s5): S5–S9

25. Streefland C, Farkas E, Maes FW, Bohus B. c-fos expression in the brainstem after voluntary ingestion of sucrose in the rat. Neurobiology (Bp) 1996; 4(1–2): 85–102

26. Travers SP, Norgren R. Organization of orosensory responses in the nucleus of the solitary tract of rat. J Neurophysiol 1995; 73(6): 2144–2162

27. Whitehead MC, Frank ME. Anatomy of the gustatory system in the hamster: central projections of the chorda tympani and the lingual nerve. J Comp Neurol 1983; 220(4): 378–395

28. Schwarz J, Burguet J, Rampin O, Fromentin G, Andrey P, Tomé D, Maurin Y, Darcel N. Three-dimensional macronutrient-associated Fos expression patterns in the mouse brainstem. PLoS ONE 2010; 5 (2): e8974

29. Norgren R, Pfaffmann C. The pontine taste area in the rat. Brain Res 1975; 91(1): 99–117

30. Tokita K, Shimura T, Nakamura S, Inoue T, Yamamoto T. Involvement of forebrain in parabrachial neuronal activation induced by aversively conditioned taste stimuli in the rat. Brain Res 2007; 1141:188–196

31. Geran LC, Travers SP. Bitter-responsive gustatory neurons in the rat parabrachial nucleus. J Neurophysiol 2009; 101(3): 1598–1612

32. Yamamoto T. Neural substrates for the processing of cognitive and affective aspects of taste in the brain. Arch Histol Cytol 2006; 69(4): 243–255

33. Norgren R, Smith GP. Central distribution of subdiaphragmatic vagal branches in the rat. J Comp Neurol 1988; 273(2): 207–223

34. Sakai N, Yamamoto T. Conditioned taste aversion and c-fos expression in the rat brainstem after administration of various USs. Neuroreport 1997; 8(9–10): 2215–2220