Chem. Senses 29: 209–215, 2004 DOI: 10.1093/chemse/bjh024

Developmental Changes in Sugar Responses of the Chorda Tympani Nerve in Preweanling Rats

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Abstract

To clarify developmental changes in the gustatory system of the rat, integrated taste responses from the chorda tympani (CT) nerve were recorded and analyzed at different postnatal ages. The response magnitude was calculated relative to the response to the standard, 0.1 M NH_4Cl. Even at 1 week of age, the CT responded well to all tested 0.1 M chloride salts (NH_4Cl, NaCl, LiCl, KCl, RbCl and CsCl). The responses to 0.1 M NaCl and LiCl increased with increasing age of the rat while response magnitudes to KCl, RbCl and CsCl did not change up to 8 weeks. At 1 week, the integrated response pattern was quite similar to that in adult rats for NaCl, HCl and quinine hydrochloride (QHCl). The concentration–response functions for NaCl, HCl, QHCl and sucrose at 2 weeks were essentially the same as those at 8 weeks. These results suggest that taste buds in the 2-week-old rat are functionally mature for the detection of the four basic taste stimuli. The relative magnitude of the responses to the various sugars was smaller at 1 week compared to the adult rat and reached a maximum at weeks 3–4, then decreased gradually with age. Among the six sugars, sucrose was the most effective followed by lactose. From weeks 1–4, the magnitude of the integrated taste response to fructose was smaller than that to lactose except at 3 weeks of age. Maltose, galactose and glucose were less potent stimuli than the other sugars tested. The response magnitude to lactose at 4 weeks had decreased compared to that for the other sugars. Taste responses to the sugars in preweanling and adult rats were not cross-adapted by the individual sugars. These results suggest that after 1 week of age during postnatal development in the rat, taste information from the CT rapidly increases in its importance for feeding behavior.

Key words: chorda tympani, development, rat, taste, weanling

Introduction

Newborn mammals must be able to distinguish taste differences between preferable and aversive sapid solutions in order to continue their development (Steiner, 1973, 1987; Ganchrow *et al.*, 1986). Such gustatory function must depend on the development of 'taste buds' both before and after birth. The appearance and maturation of taste buds are different among the subpopulations (fungiform, FF; foliate, FL; circumvallate, CV; and soft palate, SP) occurring within the oral cavity of the rat (Mistretta, 1972; Steiner, 1973, 1987; Ganchrow *et al.*, 1986; Hosley and Oakley, 1987; Harada *et al.*, 2000), hamster (Belecky and Smith, 1990) and marmoset (Yamaguchi *et al.*, 2001). The maturation of taste buds within the SP in the rat precedes that within the three types of taste-bud containing tongue papillae, suggesting the importance of the SP taste buds at birth. The FF taste buds develop during the first week after birth (Harada *et al.*, 2000). As for functional development, taste responses recorded from the chorda tympani (CT) to salts were analyzed in the rat (Hill and Almili, 1980; Hill and Bour, 1985) and mice (Ninomiya *et al.*, 1991). These studies revealed that the relative magnitude of the CT responses to NaCl and LiCl increased during development and were dependent upon an increase in the number of amiloride sensitive Na fibers. There are, however, only a few neurophysiological studies of taste responses to sugars during development in the rat (Yamada, 1980). Moreover, although 70% of FF taste buds in the rat matured at 1 week of age (Harada *et al.*, 2000), characteristics of CT responses to sugars during this time period were not investigated previously in this species. The present investigation is designed to identify the developmental changes occurring in the CT responses to different kinds of taste stimuli from early postnatal age through to the adult rat.

Methods

Animals

Pregnant female rats (Sprague–Dawley) were purchased from the Kyudo Laboratory Animal Center and were kept in separate cages. The rats were checked twice a day at 9 a.m. and 5 p.m.; the day in which a litter was found was defined as day 0. Pups were fed by the mother until 4 weeks of age. Male and female pups were placed into separate cages after weanling. Data were obtained from 36 rats of both sexes. Animals were killed at postnatal ages of days 0, 1, 2, 3 and 4 and at 8 weeks (n = 5–8 for each age). All animals were maintained on a 12:12 light:dark cycle and had chow pellets and water available *ad libitum*.

Surgical procedures

The surgical procedure to dissect the CT was similar to that described previously (Harada and Smith, 1992; Harada *et al.*, 1997). The trachea was cannulated with polyethylene tubing. Three different sizes of non-traumatic head holders made of Plexiglas were prepared for animals at different ages. For each experiment, the head of a rat was fixed within the appropriate head holder. An incision was made ventrally along the angle of the right mandible and the ventral wall of the right tympanic bulla was removed. The CT was cut at the entrance of the wall of the tympanic bulla and was detached carefully from the malleus.

Electrophysiological recording

The exposed CT was placed on a 100 μm Ag–AgCl hook electrode and an indifferent electrode was placed on the inner wall of the bulla. These electrodes were soaked in petroleum jelly that was mixed with an equal amount of liquid paraffin. The animal was grounded by an alligator clip attached to the surgical margin. Neural activity from the whole nerve was led to a high impedance probe (JB-101J; Nihon Kohden) and an A/C amplifier (ABV-11; Nihon Kohden), monitored on an oscilloscope and an audio monitor and recorded on a PCM data recorder (RD-111T; TEAC) for later analysis. Responses of the whole nerve were integrated (RC = 0.3 s; EI-600G; Nihon Kohden) and displayed on a thermal array recorder (RTA-1100M; Nihon Kohden) at a speed of 1 mm/s.

Taste stimulation

An outlet of polyethylene tubing (2.5 mm i.d.) was placed adjacent to the tongue for application of taste stimuli; rinsing distilled water (DW) flowed constantly over the tongue at 2 ml/s. For stimulation, a three-way electromagnetic valve controlled by a microcomputer (PC9801RX; NEC) switched the flow from DW to a taste stimulus for 10 s. Stimulus solutions were made with reagent grade chemicals (Nacalai Tesque Inc.) in DW. The stimuli were 0.1 M salts (NaCl, LiCl, NH_4Cl, KCl, RbCl and CsCl), four basic taste stimuli [10^{-5}–10^{0} M NaCl, 10^{-6}–10^{-2} M HCl, 10^{-6}–10^{-2} M quinine hydrochloride (QHCl), 10^{-3}–100 M sucrose (Suc)] and 0.5 M sugars [Suc, lactose (Lac), maltose (Mal), D-fructose (Fru), D-glucose (Glu) and D-galactose (Gal)]. Sugar solutions were prepared weekly and stored at 5°C. All stimuli and rinsing water were presented to the tongue at room temperature. In the cross-adaptation experiment, rinsing DW was switched to the adapting solution (AS) which was applied for 10 s prior to the application of the stimulus solution (SS) for 10 s.

Data analysis

The magnitude of the peak response during the first five s after the stimulation onset was measured because: (i) rats can discriminate taste quality within 250–600 ms (Halpern and Tapper, 1971); (ii) the time course of the phasic portion of the taste response depends on the taste quality (Harada *et al.*, 1983); and (iii) the taste stimulation system employed in this experiment produced no appreciable mechanical response (Figure 1). The 0.1 M NH_4Cl stimulus was employed as the standard because the responsiveness of single peripheral taste neurons in the rat to this standard was similar during development (Hill *et al.*, 1982). The standard solution was applied between every three or four test stimuli. Differences in the effects of age on the taste responses to the different stimuli were analyzed by ANOVA with stimuli as the variables; a multiple comparison post test (Bonferroni–Dunn) tested for statistical significance between each possible pair of mean response magnitudes. To test the rank order of the response magnitudes to the 0.1 M chloride salts and the six 0.5 M sugars, Kendall's concordance coefficient (Siegel, 1956) was calculated and the statistical significance was tested.

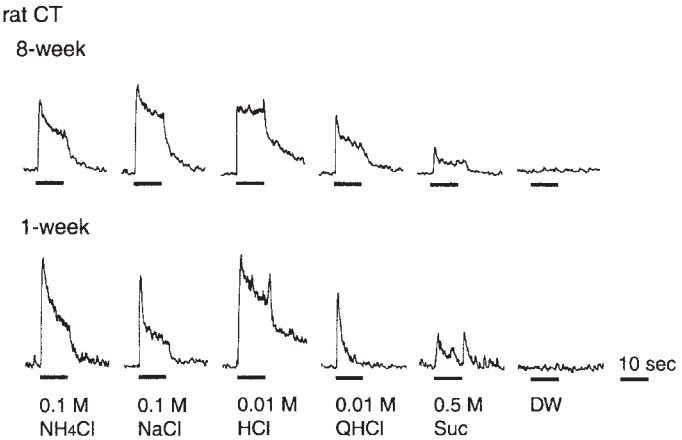

Figure 1 Integrated taste responses to 0.1 M NH_4Cl, 0.1 M NaCl, 0.01 M HCl, 0.01 M QHCl and 0.5 M sucrose (Suc) at 1 and 8 weeks of age. A horizontal bar indicates the stimulus duration of 10 s.

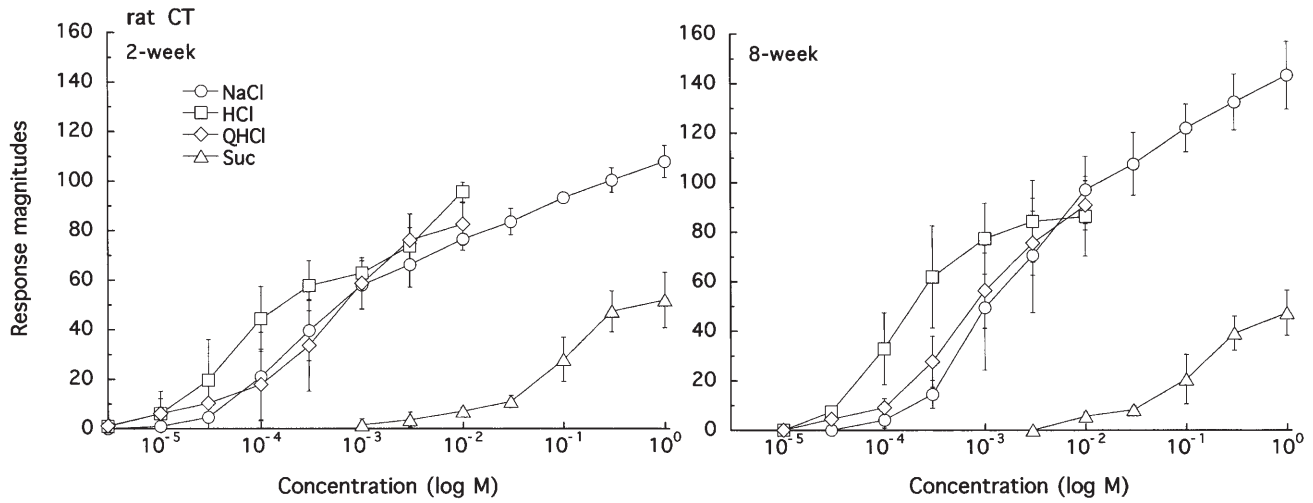

Figure 2 Relation between concentration and response magnitudes for NaCl, HCl, QHCl and sucrose relative to the response to 0.1 M NH_4Cl. The data were obtained from eight 2-week-old, and seven 8-week-old rats. Error bar shows SD.

Results

Responses to the four basic taste stimuli

At 1 week of age, the rat CT showed robust phasic and tonic responses to 0.1 M NH_4Cl, NaCl and 0.01 M HCl. The response to QHCl was also phasic, but it was followed by a smaller tonic portion of the response. A small 'off' response occurred to 0.01 M HCl and 1 M sucrose. In the adult rat, all four stimuli resulted in robust phasic and tonic responses (Figure 1).

Thresholds at 2 weeks estimated from the mean integrated taste responses to NaCl, HCl, QHCl were 3×10^{-6} M–10^{-5} M and to Suc was 10^{-3} M. Thresholds at 8 weeks to these chemicals were 0.5–1.0 log units higher in concentration than at 2 weeks (Figure 2). There were, however, no fundamental differences between the concentration–response functions for each stimulus for rats of 2 and 8 weeks of age except for the larger magnitude of response to high concentrations of NaCl (Figure 2).

Documented changes in the relative magnitude of the taste responses to the basic taste stimuli occurring during development were (i) the magnitude of the response to 0.1 M NaCl increased from 80% of the standard at 1 week to 120% at 8 weeks (Figure 3); (ii) the responses to 0.01 M HCl and QHCl increased during 1–2 weeks of age, then decreased until week 5 (Figure 3); (iii) the responses to 0.5 M Suc increased during weeks 1–3, reached a maximum, then decreased until 8 weeks of age (Figure 3).

Responses to mono-chloride salts

The relative magnitudes of the responses to the six chloride salts, including NH_4Cl as a control, tested at 0.1 M were approximately the same at 1 week of age (Figure 4). Although the response magnitudes to KCl, RbCl and CsCl were stable from 1 to 8 weeks of age, those to 0.1 M NaCl

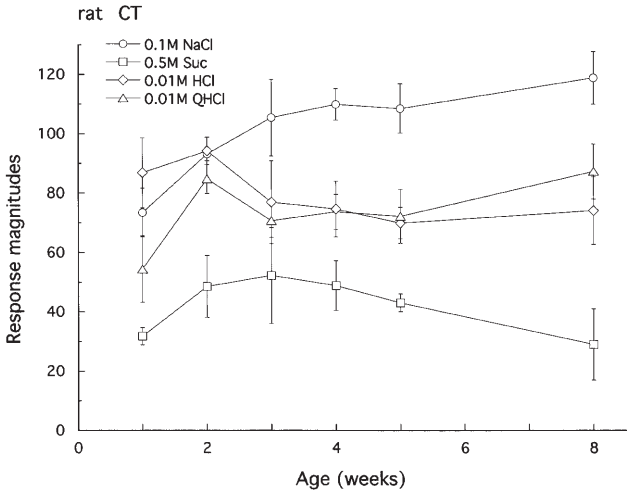

Figure 3 Developmental changes in the integrated response magnitudes for NaCl, HCl, QHCl and sucrose from 1 to 8 weeks of age. Response magnitudes are relative to the response to 0.1 M NH_4Cl. Error bar shows SD.

and LiCl increased with increasing age (Figure 4). At 2 weeks of age, the relative taste response to 0.1 M NaCl was significantly larger than that to KCl, RbCl and CsCl (ANOVA, $P < 0.0001$, $F = 37.54$, df = 5; Bonferroni, KCl, RbCl and CsCl, $P < 0.0001$). Further, the taste responses to LiCl were significantly smaller than that to NH_4Cl, but larger than those to KCl, RbCl and CsCl (Bonferroni, NH_4Cl, $P < 0.0008$; KCl, RbCl and CsCl, $P < 0.0001$). At 8 weeks of age, the taste response to NaCl was significantly larger than that to either KCl, RbCl, or CsCl (ANOVA, $P < 0.0001$, $F = 23.56$, df = 5, Bonferroni, $P < 0.0001$)

Of the six 0.1 M chloride salts tested, all rank orders of relative effectiveness were significant ($P < 0.01$). At 1 week, there was no significant difference in the relative magnitudes

of the responses to the six 0.1 M salts. From weeks 3 to 8, the relative taste responses to 0.1 M NaCl, which was previously less effective at 1 and 2 weeks than NH_4Cl, became most stimulatory (Table 1). Further, during this time, the stimulatory effectiveness of LiCl increased from being third most potent to second below that to NaCl (Table 1). In addition, through these 8 weeks, taste responses to NaCl, LiCl and NH_4Cl remained significantly larger than those to KCl, CsCl and RbCl (Table 1).

Responses to sugars

Similar to the response to 0.5 M Suc, the five other sugars resulted in larger integrated taste responses at 3 weeks of age (Figure 5). At 1 week of age, the relative mean magnitude of the response to Suc was significantly larger than that to either Fru, Mal, Glu, or Gal (ANOVA, $P < 0.0001$, $F = 66.09$, df = 5; Bonferroni, $P < 0.0001$). Further, the response to Fru was significantly larger than those to Mal, Glu and Gal (Bonferroni, $P < 0.0001$). The relative mean magnitudes of the responses to all six sugars increased during weeks 1–3. The response magnitudes to four sugars (Suc, Lac, Fru and Mal) reached maximum at 3 weeks and responses to Gal and Glu maximized at 4 weeks (Figure 6). At 3 weeks of age, the relative mean response magnitude to Suc was significantly larger than that to the other five sugars (ANOVA, $P < 0.0001$, $F = 33.66$, df = 5; Bonferroni, $P < 0.0001$). Also, the response to Fru was significantly larger than that to either Mal, Glu, or Gal (Bonferroni, Mal $P = 0.03$, Glu, and Gal, $P < 0.0001$). After reaching maximum, the magnitude of the taste responses to all the sugars tested decreased until week 8 (Figure 6). At 8 weeks of age, the relative mean magnitude of the response to Suc was significantly larger than that to the four sugars except for Fru (ANOVA, $P < 0.0001$, $F = 37.06$, df = 5; Bonferroni, $P < 0.0001$); however, the response to Fru was significantly larger than that to Mal, Glu and Gal (Bonferroni, $P < 0.0001$). With the exception of week 3, the rank order of the response magnitudes from weeks 1 to 4 was approximately the same (Table 2); however, during weeks 4–8, the responsiveness to Lac decreased (Figure 6 and Table 2).

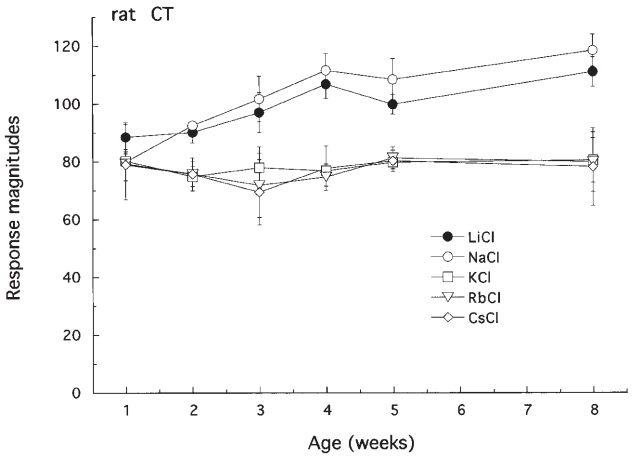

Figure 4 Developmental changes in the integrated response magnitudes for 0.1 M NaCl, LiCl, KCl, RbCl and CsCl from 1 to 8 weeks of age. Response magnitudes are relative to the response to 0.1 M NH_4Cl. Error bar shows SD.

Cross-adaptation between Suc or Lac and other sugars

Both at 2 and 8 weeks of age, adaptation to 0.5 M Suc did not depress the response to 0.5 M Lac and 0.5 M Lac adaptation did not depress the response to 0.5 M Suc (Figure 7). Also, adaptation to 0.5 M Suc or Lac did not depress taste responses to the other four 0.5 M sugars (Figure 8). Following sucrose adaptation, however, responses to the other sugars were significantly ($P < 0.0001$) larger than to Suc (2 weeks ANOVA, $P < 0.0001$, $F = 47.45$, df = 5; 8 weeks ANOVA, $P < 0.0001$, $F = 34.48$, df = 5). A similar effect occurred following adaptation to Lac (2 weeks ANOVA, $P < 0.0001$, $F = 18.0$, df = 5; 8 weeks ANOVA, $P < 0.0001$, $F = 25.01$, df = 5).

Discussion

Histological experiments in newborn rats revealed that <<14% of FF taste buds, but >>53% of SP taste buds possessed taste pores (Mistretta, 1972; Harada *et al.*, 2000). The maturation of taste buds within the FF suggests that CT responses in rats before 1 week of age may not be sufficiently stable to mediate taste information. The only report concerning CT taste responses in 2–3-day-old rats showed responses of only a few units which prevented the recording

Table 1 Rank order of response magnitudes for six 0.1 M chloride salts at different ages

Age (weeks)	Order						*W*	*n*
2	NH_4Cl	NaCl	LiCl >	CsCl	RbCl	KCl	0.808	5
3	NaCl	NH_4Cl	LiCl >	KCl	RbCl	CsCl	0.883	4
4	NaCl >	LiCl	NH_4Cl >	CsCl	KCl	RbCl	0.856	4
5	NaCl	NH_4Cl	LiCl >	RbCl	CsCl	KCl	0.897	4
8	NaCl	LiCl	NH_4Cl >	KCl	CsCl	RbCl	0.893	5

The rank order was obtained in each animal, then Kendall's concordance coefficient *W* (Siegel, 1956) was calculated. A multiple comparison post test (Bonferroni–Dunn) was made for the mean response magnitudes; significant difference between neighbouring pairs is shown by '**>**' ($P < 0.05$).

of reproducibly stable integrated responses (Hill and Almili, 1980).

During the first week after birth, the number of mature taste buds increased rapidly, with 80% of the FF taste buds containing taste pores (Harada *et al.*, 2000). Taste responses to 0.5 M NH_4Cl and 0.1 M citric acid were recorded from the rat CT at 1 week of age (Hill and Almili, 1980). Similarly, in the present report, the rat CT at 1 week of age produced stable integrated responses to 0.1 M salts and 0.01 M HCl (see Figures 1 and 5). These findings confirm that the rat CT develops during the first week after birth and becomes sufficiently mature to mediate taste information to the central nervous system.

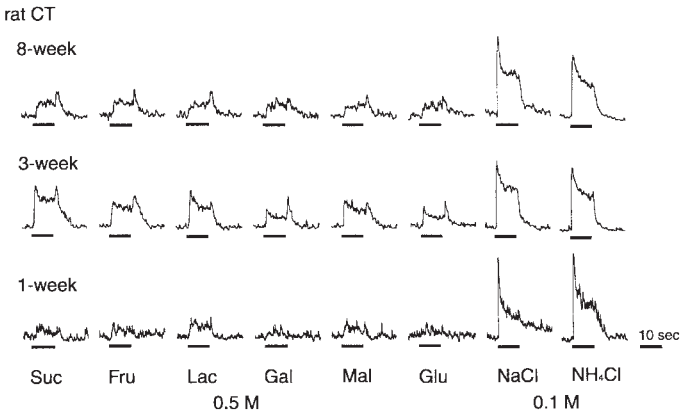

Figure 5 Integrated taste responses to six 0.5 M sugars (sucrose, Suc; fructose, Fru; lactose, Lac; maltose, Mal; glucose, Glu; galactose, Gal), 0.1 M NaCl and 0.1 M NH_4Cl at 1, 3 and 8 weeks of age. A horizontal bar indicates the stimulus duration of 10 s.

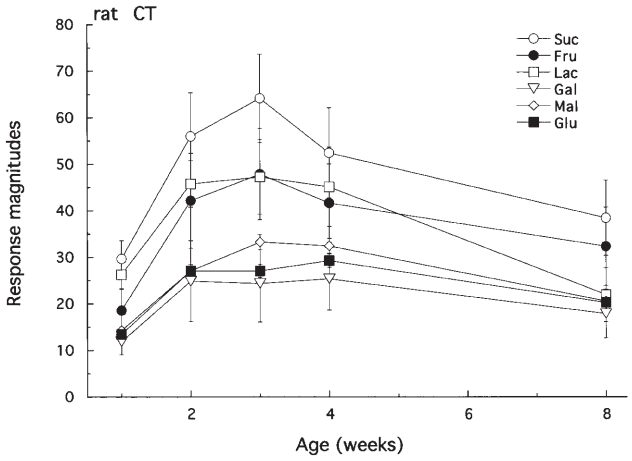

Figure 6 Developmental changes in the integrated response magnitudes for six 0.5 M sugars (sucrose, Suc; fructose, Fru; lactose, Lac; maltose, Mal; glucose, Glu; galactose, Gal) from 1 to 8 weeks of age. Response magnitudes are relative to the response to 0.1 M NH_4Cl. Error bar shows SD.

Behavioral responses to taste stimuli in newborn rat pups during the first 4 postnatal days showed that the gustatory system becomes functionally mature during the first postnatal days (Ganchrow *et al.*, 1986). Further, rat pups at 6–18 days of age showed similar preference–aversion curves for NaCl (Moe, 1986). Neurophysiological experiments revealed that the response to NH_4Cl in the rat CT was robust and stable during early postnatal age (Yamada, 1980; Hill and Bour, 1985; Hendricks *et al.*, 2000). However, the taste response magnitudes to NaCl and LiCl increased with increasing age from 1 to 5 weeks after birth, which was dependent upon the association with amiloride sensitive fibers during development (Hill and Almili, 1980; Ninomiya *et al.*, 1991; Hendricks *et al.*, 2000). During 1–8 weeks, 0.1 M KCl, RbCl and CsCl resulted in approximately the same response magnitudes relative to 0.1 M NH_4Cl as indicated in previous reports (Hill and Almili, 1980; Hendricks *et al.*, 2000), while responses to 0.1 M NaCl and LiCl increased with increasing age (Figure 4). Also, single fiber analysis of the rat CT revealed that the response frequency to NH_4Cl remained the same during development while that to NaCl and LiCl increased (Hill *et al.*, 1982).

The present experiments revealed that various sugars resulted in discernible integrated taste responses in the rat, even at 1 week of age (Figures 1 and 5). Interestingly, the magnitude of taste responses to the tested sugars increased until 3–4 weeks and reached maximum, then decreased until 8 weeks of age. This latter decline in the responses to the sugars cannot be explained by an increase in the taste response to 0.1 M NH_4Cl since the response magnitude to 0.01 M HCl and QHCl did not decrease during the same time period. It is plausible that the decrease of the sugar response was caused by a decrease in the number of sugar

Table 2 Rank order of response magnitudes for six 0.5 M sugars at different ages

Age (weeks)	Order						*W*	*n*
1	Suc	**Lac >**	Fru	Mal	Glu	Gal	0.885	5
2	Suc **>**	**Lac**	Fru **>**	Glu	Mal	Gal	0.855	9
3	Suc **>**	Fru	**Lac >**	Mal	Glu	Gal	0.894	5
4	Suc	**Lac**	Fru	Mal	Glu	Gal	0.855	9
8	Suc	Fru	**Lac >**	Mal	Glu	Gal	0.923	8

The rank order was obtained in each animal, then Kendall's concordance coefficient *W* (Siegel, 1956) was calculated. All rank orders were significant ($P < 0.01$). A multiple comparison post test (Bonferroni–Dunn) was made for the mean response magnitudes; significant difference between neighbouring pairs is shown by '>' ($P < 0.05$).

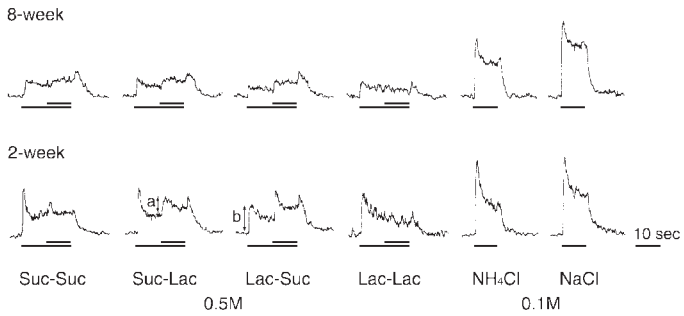

Figure 7 Integrated taste responses of cross-adaptation experiment in the rat at 1 and 8 weeks of age. Adapting solution was applied for 10 s, then test solution dissolved in adapting solution was applied for 10 s. Adapting solution was 0.5 M Suc or 0.5 M Lac and test solution was 0.5 M Suc or 0.5 M Lac dissolved in 0.5 M Suc or 0.5 M Lac. **(a)** Lac response with Suc adaptation. **(b)** Lac response.

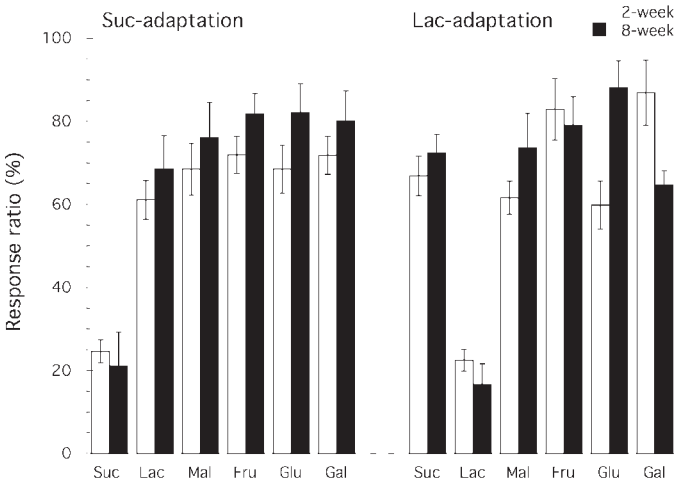

Figure 8 Adapting effects with 0.5 M Suc or 0.5 M Lac adaptation on six 0.5 M sugars at 2 and 8 weeks rats, calculated by 100 × a/b (%) as shown in Figure 7; $n = 5$. Error bar shows SD.

sensitive fibers or number of receptor sites on the respective taste cells within the taste buds. On the other hand, the concentration–response relationships for Suc and Mal did not differ in the 14–35-day-old hamsters, while these increased in 55–73-day-old adult hamsters (Hill, 1988). High responsiveness to the sugars in the rat GSP (Hill, 1988; Harada and Smith, 1992; Harada *et al.*, 1997) could compensate for the decrease in responsiveness in the CT. Also, the different responses to the sugars in the CT in the rat and hamster may produce the different developmental changes of sugar responsiveness between the two species.

Previous studies using different experimental techniques suggested the existence of different receptors for sugars and saccharin: (i) experiments utilizing taste inhibitors (Iwasaki and Sato, 1986; Vlahopoulos and Jakinovich, 1986); (ii) behavioral investigations (Faurion *et al.*, 1980); and (iii) experiments with assays for IP_3 and with Ca^{2+} imaging (Bernhardt *et al.*, 1996). Similarly, electrophysiological cross-adaptation experiments in the present study suggested that there might be individual sugar receptors on the taste buds innervated by the CT of the adult rat even at the early postnatal age of 2 weeks. This latter result indicated that different sugar receptors arise at an early stage in development and facilitate the ability of the rat pup to distinguish the taste of sugars during suckling behavior.

During the preweanling period, the magnitude of the taste response to Lac in the rat was nearly the same magnitude as that to Suc. The taste response to Lac was larger significantly than responses to the other sugars, but then declined subsequent to weanling. This change in taste response magnitude to Lac suggests that the specificity or the number of receptor sites for Lac decreased during the weanling period. This previous change along with the concomitant decrease of synthesis and activity of intestinal lactase (Tsuboi *et al.*, 1981; Johanson and Shapiro, 1986) may be related to the decline of those proteins required in taste transduction to Lac in the rat. On the other hand, no clear developmental change was observed to sugars or amino acids in the CT responses of mice, although response to Gln in 8–16 weeks was significantly smaller than that in 7–10 days (Ninomiya *et al.*, 1993). These results suggest that developmental changes of taste responses to the same taste qualities differes in the diverse species.

Acknowledgement

We thank Dr John Caprio for valuable comments and correcting the manuscript.

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Accepted January 17, 2004