Korean J Physiol Pharmacol
Vol 4: 169-175, June, 2000

Developmental Changes of Gustatory Neurons in Nucleus of Solitary Tract in Rats

Miwon Kim[1], Wonjae Kim[2], and Charlotte Mistretta[3]

[1]Department of Nursing and Research Institute of Nursing Science and [2]Department of Oral Physiology, Chonnam National University, Kwangju 501-746, Korea; [3]Department of Materials Science, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, U.S.A.

To learn the developmental changes in intrinsic electrophysiological properties of the second order taste neurons, whole cell recordings from the developing nucleus of the solitary tract neurons were done in brainstem slices of postnatal rats. Rats aged from postnatal 0 to 21 days (P0-P21) were used, being divided into 3 age groups: postnatal first week (P0-P7 days), second week (P8-P14 days), and third week (P15-P21 days). Slices containing gustatory NTS were cut horizontally in the thickness of 300 μm. Whole cell recordings were obtained from neurons in response to a series of hyperpolarizing and depolarizing current pulses. The intrinsic electrophysiological properties of the rostral NTS (rNTS) neurons were compared among the age groups. Depolarizing current pulses evoked a train of action potentials in all neurons of all age groups. The resting membrane potential and input resistance of the neurons did not show any significant differences during the postnatal 3 weeks. The time constant, however, decreased during the development. Duration of action potential measured at half maximum amplitude was longer in younger age groups. Both the maximum rate of rise and the maximum rate of fall in the action potential increased during the first 3 weeks postnatal. Electrophysiologically more than half neurons were type III. In summary, it is suggested that developmental changes in electrophysiological properties in rNTS occur during the first three weeks in rats.

Key Words: Gustatory neuron, Development, Brain slice, Taste, Whole cell recording

INTRODUCTION

Input signals from taste nerve branches of the seventh, ninth and tenth cranial nerves are transmitted to the gustatory portion of the nucleus tractus solitarius (NTS) (Whitehead, 1988; Bradley, 1994). Central projections of these afferent fibers project from the geniculate, petrosal, and nodose ganglia into the medulla to form the solitary tract (Mistretta, 1991). Fibers within the tract have terminal fields within NTS where they synapse with second-order taste neurons. Although there is an overlap in the projection of fibers within the tract, taste fibers enter the solitary tract in a topography that reflects innervation of the tongue and oral cavity: seventh-nerve fibers that innervate taste buds on the anterior tongue and soft palate comprise what is termed the nervus intermedius centrally and enter the most rostral portions of the gustatory solitary tract; ninth-nerve fibers that innervate taste buds on the posterior gongue enter the solitary tract caudal to seventh fibers; and tenth-nerve fibers that innervate taste buds on the epiglottis enter still more caudally (Mistretta & Hill, 1990).

Recently the whole cell recording technique has made it possible to study intrinsic properties of the central taste system (Blanton et al, 1989; Bradley & Sweazey, 1990). Whole-cell recordings have demonstrated that neurons in the adult rostral (r)NTS can be classified electrophysiologically into 4 groups on the basis of their repetitive discharge patterns (Bradley & Sweazey, 1992). In Group I neurons, the repetitive

Corresponding to: Miwon Kim, Department of Nursing and Research Institute of Nursing Science, Chonnam National University, 5 Hak-dong, Kwangju 501-746, Korea. (Tel) 82-62-220-4346, (Fax) 82-62-227-4009

discharge pattern initiated by membrane depolarization is changed to an irregular spike train by prior hyperpolarization. In Group II neurons, hyperpolarization either delays the occurrence of the first action potential or increases the length of the first interspike interval in the action potential train produced by membrane depolarization. In Group III neurons, hyperpolarization has little effect on the discharge pattern. In Group IV neurons, the discharge pattern consists of a short burst of action potentials that is often shortened by prior hyperpolarization.

Bao et al (1995) have shown that the developmental changes in resting membrane potential, action potential, and descharge properties of the gustatory NTS neurons between postnatal 5 and 15 days in rats. However, few studies have been done on the maturation of the basic intrinsic neural properties of NTS cells. The present study was carried out to learn developmental changes in intrinsic electrophysiological properties of the second order taste neurons in NTS. Whole cell recordings were made in brainstem slices of postnatal rats.

METHODS

Animals

Sprague-Dawley rats (n=25) aged from postnatal 0 to 21 days (designated as P0 to P21) were used. They were divided into 3 age groups: postnatal 0~7 days (n=10), 8~14 days (n=10), and 15~21 days (n=5). The animals were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). The brainstem was rapidly removed and cooled for 2 min in an oxygenated artificial cerebrospinal fluid at 4°C. They were fixed on the vibratome stage with cyanoacrylate glue. Slices containing gustatory NTS were cut horizontally at the thickness of 300 μm. The slices were kept in oxygenated physiological saline for at least one hour at room temperature. A slice was then transferred to a recording chamber with a wide-mouthed pipette and covered with a piece of mesh. The slice was maintained at room temperature and superfused with oxygenated artificial cerebrospinal fluid at a rate of 1.5~2.0 ml/min. The composition of the artificial cerebrospinal fluid used was (in mM): NaCl 124, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.3, $NaHCO_3$ 26, KH_2PO_4 1.25, and glucose 2.5. The solution was gassed with 95% O_2, 5% CO_2 to give a pH of 7.4.

Neurophysiology

Whole cell recordings were made using the technique described by previous investigators (Blanton et al, 1989). A petri dish containing the neural tissue was placed on the stage of an upright microscope and the tissue was superfused with oxygenated artificial cerebrospinal fluid at room temperature. Positive pressure was applied to the back of a recording pipette using a 10 ml syringe connected by polyethylene tubing to the electrode holder. While positive pressure was maintained, the electrode pipette tip was passed into the tissue and could be driven to any desired depth. Small current steps (−0.1 nA) were applied and a small decrease in the voltage deflection signaled that the electrode tip was approaching a cell. A slight negative pressure was then applied with the syringe, resulting in the formation of stable gigaohm seals. In this configuration, it was possible to record voltage from spontaneous action potentials. The membrane patch was ruptured to obtain whole-cell recordings by applying additional negative pressure.

Patch electrodes were pulled in two stages on an electrode puller (Narishige PP83) using borosilicate filament glass of outside diameter measuring 1.5 mm. These electrodes had a tip resistance of 5~8 megaohm (bubble number of 5.8~6.2). The electrode were filled with a solution containing (in mM) K-gluconate 130, HEPES 10, EGTA 10, $CaCl_2$ 1, APT 2, adjusted to a pH of 7.2 with KOH. They were positioned over the rNTS through the dissecting microscope while transluminated from below. The indifferent electrode was connected to the extracellular solution.

Data analysis

Once a gigaohm seal had been obtained and the patch ruptured, current was injected into the neurons using the bridge circuit of an Axoclamp 1A in current clamp mode. Biophysical properties of neurons responding to a series of hyperpolarizing and depolarizing current pulses were recorded using the Clampfit program. The current pulses were injected to the neurons from 100 pA to 125 pA with an interval of 25 pA. The neuronal input resistance was monitored by injecting negative constant current pulses (100 ms, −0.1 nA) into the neurons at 0.15 Hz, being continuously recorded on a pen recorder at a slow speed. This pulse length was sufficient to charge the mem-

brane to a steady-state level.

Successful recordings were made from 25 neurons: 10 neurons at P0-7, 10 at P8-14, and 5 at P15-21. Resting membrane potential, input resistance, amplitude and duration of action potential, maximum slope of the rising and falling phases of the action potential, and time constant were measured and compared among the age groups, using analysis of variance. Duncan tests were used to make comparisons between the groups.

RESULTS

The sites where the whole cell recordings were made in rNTS are shown in Fig. 1. Stable recordings were obtained in response to a series of hyperpolarizing and depolarizing current pulses from 100 to +125 pA. The recordings from the neurons of which resting membrane potential was more negative than 40 mV were used for data analysis. Some developmental differences were noted in resting membrane and action potential properties among the age groups (Fig. 2).

Resting membrane potentials of rNTS cells ranged from 51 to 67 mV. They did not significantly differ among the three age groups (F=0.2, p=0.8185). Input resistance at the older age group was smaller than that at the younger ones, but did not show significant differences among the three age groups. The range of input resistance was from 300 to 1100 MΩ (F=1.94, p=0.1623). The membrane time constant, however, decreased during the development from 73 msec at the first week to 39 msec at the third week postnatal (F=6.58, p=0.0044; Fig. 3).

The amplitudes of action potentials were not significantly different among the three age groups, ranged from 58 to 90 mV (F=3.25, p=0.0534). Duration of the action potential was longer in younger age groups.

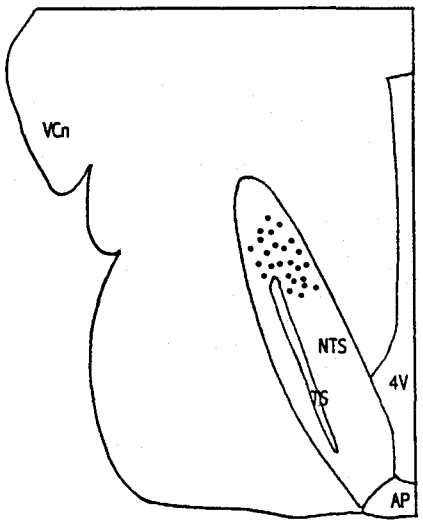

Fig. 1. The sites of the whole cell recordings in rNTS in rats. AP, area postrema; NTS, nucleus tractus solitarii; TS, tractus solitarius; VCn, ventral cochlear nucleus; 4V, 4th ventricle.

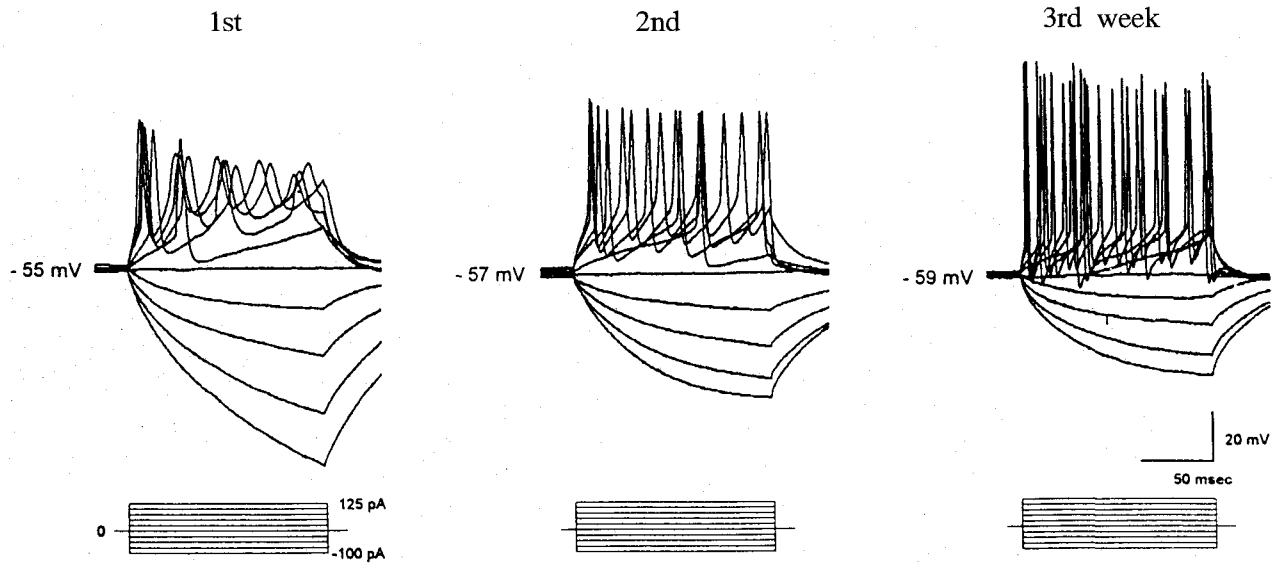

Fig. 2. Responses of rNTS in the three different age groups to a series of hyperpolarizing and depolarizing current pulses from −100 pA to 125 pA.

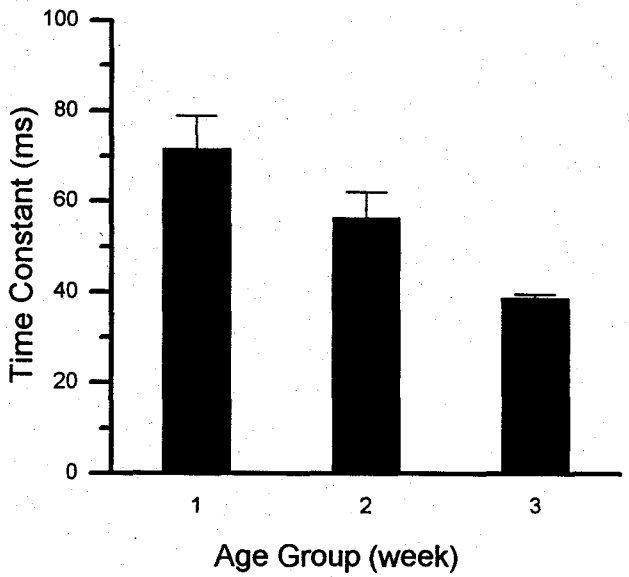

Fig. 3. Membrane time constant of rNTS cells in the three age groups (F=6.58, p=0.0044).

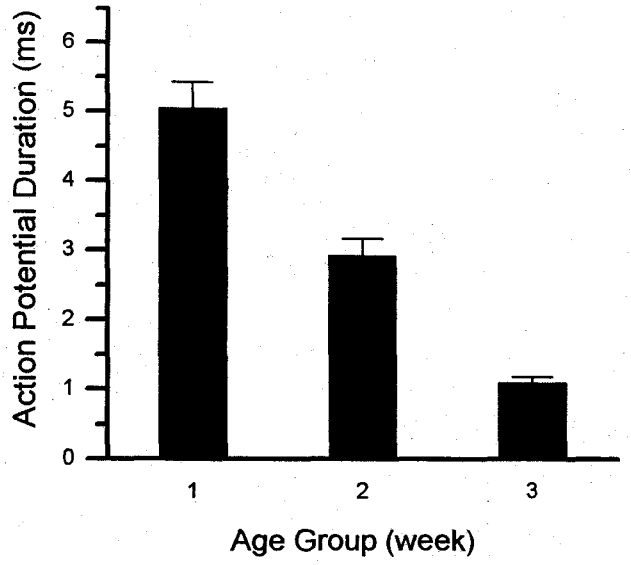

Fig. 4. Duration of the action potential of rNTS cells measured at half maximum amplitude in the three age groups (F=42.69, p=0.0001).

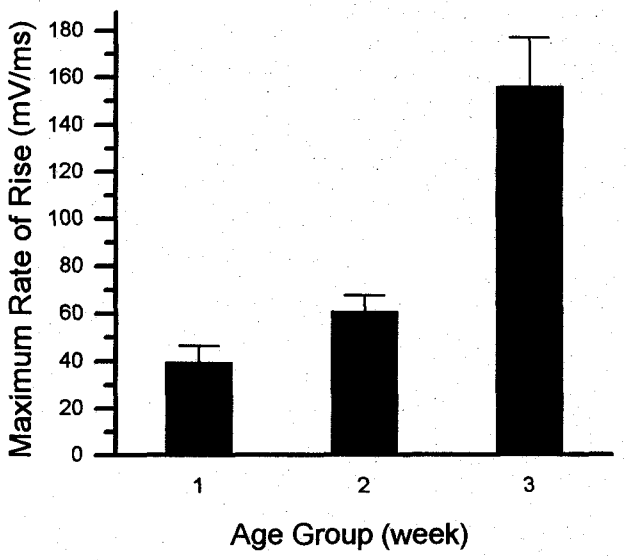

Fig. 5. Slopes for the maximum rate of rise of the action potential of rNTS cells in the three age groups (F=26.36, p=0.0001).

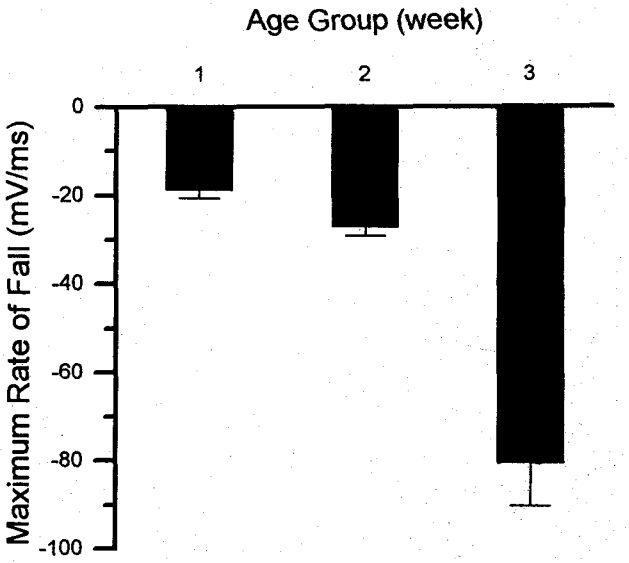

Fig. 6. Slopes for the maximum rate of fall of the action potential of rNTS cells in the three age groups (F=46.58, p=0.0001).

Fig. 4 illustrates the duration of action potential measured at half maximum amplitude, being decreased from about 4.9 msec at the 1st week to 1.0 msec at the 3rd week postnatal (F=42.69, p=0.0001). Slopes for the maximum rate of rise of the action potential increased from an average of 35 mV/ms at the 1st week to 136 mV/ms at the 3rd week postnatal (F=26.36, p=0.0001; Fig. 5). The slopes for the maximum rate of fall increased from an average of 17 mV/ms at the 1st week to 70 mV/ms 3rd week postnatal (F=46.58, p=0.0001; Fig. 6).

Depolarizing current pulse evoked a train of action potentials in all neurons of all age groups. Electrophysiologically more than half neurons at the 1st week were type IV cells, at the 2nd week, type I cells, and

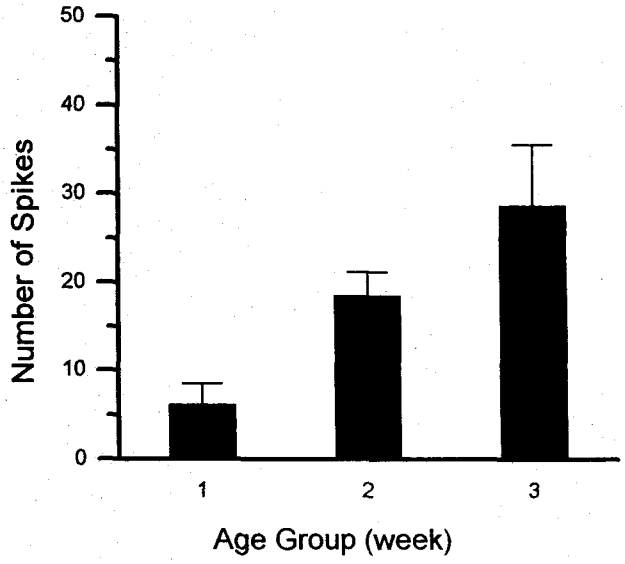

Fig. 7. Discharge frequencies in response to 100 pA depolarizing current for one second in the age grous (F= 9.57, p=0.0006).

at the 3rd week, type III cells. Discharge frequencies in response to 100 pA depolarizing current for one sec were significantly different among the age groups (F= 9.57, p=0.0006; Fig. 7).

DISCUSSION

The rNTS is the first central relay in the gustatory pathway (Bradley, 1995). In the late 1950s, multi- and single-unit recordings in the medulla were made while stimulating the tongue with taste stimuli (Pfaffmann et al, 1961). In these early studies, similarities between recordings made from second-order neurons and the chorda tympani nerve innervating the anterior tongue were noted. It was believed that there was little processing of responses to chemical stimuli past the first synapses in the taste pathway and the gustatory NTS was a relatively simple relay nucleus (Doetsch & Erickson, 1970; Travers et al, 1987). However, there is a growing body of evidence based on anatomical, electrophysiological, and biophysical experiments suggesting that the rNTS is capable of considerable synaptic processing and morphologically and functionally complex (Bradley, 1994).

Whole cell recordings from neurons of the gustatory NTS in rats demonstrated that there were four different classes on the basis of responses to a current injection consisted of membrane hyperpolarization immediately followed by a depolarizing pulse (Bradley & Sweazey, 1992). In the present study the four groups of neurons were found. Hyperpolarization of Group I neurons altered the repetitive discharge pattern initiated by membrane depolarization to an irregular pattern. Hyperpolarization of Group II neurons either delayed the occurrence of the first action potential, or increased the length of the first interspike interval in the action potential train produced by membrane depolarization. Hyperpolarization least affected the discharge pattern of Group III neurons. Hyperpolarization of Group IV neurons shortened the length of the action potential burst produced by membrane depolarization. Among the three age groups, Group IV neurons were most frequently observed in the postnatal first week, Group I neurons in the second week, and Group III neurons in the third week. These findings may reflect the neuronal position in rNTS or Group IV neurons which have one action potential are shown in earlier developmental stage.

These complex firing patterns are mediated by different ionic conductance. Voltage-clamp recordings of Group II neurons revealed that the firing delay is due to a transient outward K^+ current partially inactivated around the resting membrane potential. Blocking Ca^{++} currents with Co^{++} depressed the excitability of rNTS neurons. After a Ca^{++} channel blockade, the discharge pattern of Group III neurons resembles that of Group IV neurons, suggesting that some Group IV neurons may be a subset of Group III (Bradley, 1994).

It has been suggested that some of the electrophysiological properties from cells in the younger animals may differ as a consequence of the viability of the slice preparation or neurons. However, the immature neurons in the present study were apparently healthy based on measures of membrane properties and ability to fire a discharge in response to the injected current. Intracellular or whole cell recordings from slice preparations of the postnatal rat neocortical or hippocampal cells also showed similar developmental trends (McCormick & Prince, 1987; Zhang et al, 1991). Immature rat central neurons have a higher input resistance and longer membrane time constant, a slower rate of rise and fall, and longer duration in the action potential.

There are some developmental trends in membrane and action potential properties (Bao et al, 1994). In the present study, the resting membrane potential and the amplitude of action potential were not signifi-

cantly different among the three age groups. However, the time constant in the third week was significantly shorter than that in the younger groups, whereas the duration of action potentials was longer. The maximum rates of rise and fall of the action potentials in third week were significantly faster than those in younger groups. The amplitude, duration, and maximum rates of rise and fall of the action potentials in the third week were similar to those observed in adults by other investigators (Bao et al, 1994).

The mechanisms underlying the altered membrane and action potentials and discharge properties have not been established. However, based on data from other systems in the brain, developmental differences in ion channel density or in the dominant ion that acts as a carrier for depolarization may underlie the neurophysiological maturation in NTS cells (Kriegstein et al, 1987; McCormick & Prince, 1987). The increasing maximum rate of rise in the action potential may relate to an increase in density of voltage-dependent sodium channels in the membrane (McCormick & Prince, 1987). A similar increase in the rate of rise in action potential has been observed in cultured spinal cord neurons, in parallel with an increase in estimated sodium channel density (MacDermott & Westbrook, 1986). Data from non-mammalian embryonic neurons indicate that generation of early action potentials is primarily calcium-dependent, although sodium currents are also present at the onset of electrical excitability (Fitzgerald & Fulton, 1992). Data on ionic currents in neurons from the adult rat gustatory NTS indicate that specific combinations of potassium and calcium conductances mediate discharge patterns in these cells (Tell & Bradley, 1994). Taken together, these finding suggest that there are some developmental changes in sodium, potassium and calcium currents in NTS.

Golgi-stained neurons in the postnatal rat NTS exhibit developmental changes in morphology (Lasiter et al, 1989). There is a rapid period of growth for the first and second order dendrites of two NTS cell types, elongate and ovoid types, from P6 to P20. The first order dendrites of multipolar cells also grow rapidly during this period, and the second order dendrites for these types of neurons continue to increase in length to at least 70 days postnatal. Therefore, a period of major growth in dendritic extent of NTS cells (P6 to P20) apparently parallels the period of maturation of electrical properties of these neurons, whereas some dendrites continue to grow well past the time of electrophysiological maturation and presumably are involved in the gustatory circuit formation.

In summary, it is suggested that developmental changes in electrophysiological properties in rNTS occur mainly in first three weeks in rats.

ACKNOWLEDGEMENTS

This work was supported by a research grant from Chonnam National University (1996).

REFERENCES

Bao H, Bradley RM, Mistretta CM. Development of intrinsic electrophysiological properties in neurons from the gustatory region of rat nucleus of solitary tract. *Brain Res Dev* 86: 143−154, 1995

Barraco R, El-Ridi M, Ergene E, Parizon M, Bradley D. An atlas of the rat subpostremal nucleus tractus solitarius. *Brain Res Bul* 29: 703−765, 1992

Blanton M, Turco J, Kriegstein A. Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J Neurosci Methods* 30: 203−210, 1989

Bradley RM, Sweazey RD. In vitro intracellular recordings from gustatory neurons in the rat solitary nucleus. *Brain Res* 508: 168−171, 1990

Bradley RM, Sweazey RD. Separation of neuron types in the gustatory zone of the nucleus tractus solitarii on the basis of intrinsic firing properties. *J Neurophysiol* 67: 1659−1668, 1992

Bradley RM. Neurobiology of the gustatory zone of nucleus tractus solitarius. In: Barracto R, Boca Raton ed, *Nucleus of the Solitary Tract.* CRC, p 51−62, 1994

Bradley RM. *Essentials of Oral Physiology.* St. Louis: Mosby, p 86−123, 1995

Del Rio J, Soriano E, Ferrer I. Development of GABA-Immunoreactivity in the neocortex of the mouse. *J Comp Neurol* 326: 501−526, 1992

Doetsch GS, Ganchrow JJ, Nelson LM, Erickson RP. Information processing in the taste system of the rat. In: *Olfaction and Taste III.* Pfaffmann C, ed, Rockefeller University Press, New York, p492, 1969

Doetsch GS, Erickson RP. Synaptic processing of taste-quality information in the nucleus tractus solitarius of the rat. *J Neurophysiol* 33: 490−507, 1970

Du J, Bradley RM. Electrophysiological and morphological properties of neurons acutely isolated from the rostral gustatory zone of the rat nucleus of the solitary tract. *Chem Senses* 21: 729−37, 1996

Fitzgerald M, Fulton BP. The physiological properties of developing sensory neurons. In: Scott SA, ed, *Sensory Neurons, Diversity, Development and Plasticity*. New York Oxford, p 287−306, 1992

Hamilton RB, Norgren R. Central projections of gustatory nerves in the rat. *J Comp Neurol* 222: 560−577, 1984

Hill DL, Mistretta CM. Developmental neurobiology of salt taste sensation. *Trends Neurosci* 13: 188−195, 1990

King M, Wang L, Bradley R. Substance P excites neurones in the gustatory zone of the rat nucleus tractus solitarius. *Brain Research* 619: 120−130, 1993

King M, Bradley R. Relationship between structure and function of neurons in the rat rostral nucleus tractus solitarii. *J Comp Neurol* 344: 50−64, 1994

Kriegstein AR, Suppes T, Prince DA. Cellular and synaptic physiology and epileptogenesis of developing rat neocortical neurons in vitro. *Dev Brain Res* 34: 161−171, 1987

Lasiter PS, Kachele DL. Organization of GABA and GABA-transaminase containing neurons in the gustatory zone of the nucleus of the solitary tract. *Brain Res Bull* 21: 313−321, 1988

Lasiter PS, Wong DM, Kachele DL. Postnatal development of the rostral solitary nucleus in rat: dendritic morphology and mitochondrial enzyme activity. *Brain Res Bull* 21: 313−321, 1989

MacDermott AB, Westbrook GL. Early development of voltage-dependent sodium currents incultured mouse spinal cord neurons. *Dev Biol* 113: 317−326, 1986

McCormick DA, Prince DA. Postnatal development of electrophysiological properties of rat cerebral cortical pyramidal neurons. *J Physiol* 393: 743−762, 1987

Mistretta CM. Developmental neurobiology of the taste system. In: Getchell TV, Doty RL, Bartoshuk LM, Snow JB Jr, ed, *Smell and Taste in Health and Disease*. New York, Raven Press, p 35−64, 1991

Mistretta CM, Hill D. Developmental neurobiology of salt taste sensation. *Trends Neurosci* 13: 188−195, 1990

Pfaffmann C, Erickson RP, Frommer GP, Halpern BP. Gustatory discharges in the rat medulla and thalamus. In: Rosenblith WA, ed. *Sensory Communication*. MIT Press, Cambridge, p 455, 1961

Tell F, Bradley RM. Whole-cell analysis of ionic currents underlying the firing pattern of neurons in the gustatory zone of the nucleus tractus solitarii. *J Neurophysiol* 71: 479−492, 1994

Travers JB, Travers SP, Norgren R. Gustatory neural processing in the hindbrain. *Annu Rev Neurosci* 10: 595−632, 1987

Vogt M, Mistretta CM. Convergence in mammalian nucleus of solitary tract during development and functional differentiation of salt taste circuits. *J Neurosci* 10: 3148−3157, 1990

Whitehead MC. Neuronal architecture of the nucleus of the solitary tract in the hamster. *J Comp Neurol* 276: 547−572, 1988

Zhang L. Development of GABA-mediated, chloride-dependent inhibition in CA1 pyramidal neurones of immature rat hippocampal slices. *J Physiol* 444: 25−49, 1991