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REVIEW ARTICLE

ONTOGENY OF TASTE SENSE

B. S. RAO*, NILIMA SHANKAR AND K. N. SHARMA

**Department of Physiology, S.V. Dental College, Bangalore – 560 076*

and

Department of Physiology, University College of Medical Sciences, Delhi – 110 095

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Abstract : Developmental changes in the taste receptors and CNS, in physiological and behavioural patterns in fetus and postnatal animals constitute the ontogeny of taste. Tongue epithelial cells are modified into taste buds only with innervation and are seen to degenerate on denervation. On removal of a portion of the tongue, with its taste buds, the central projections of gustation also atrophy, indicating an interdependence of taste receptors and central areas for their development. Gradual transitions in electrical responses to taste stimuli are shown from fetal to adult stage. While responses to ammonium chloride are marked in early fetus, with advancements in age responses to a wide variety of chemicals are shown. Again, early in the development, fibers responsive to chloride are predominant while at a later stage fibers responsive to sodium increase in number. Behaviourally, human fetus and neonatal mammals showed increased swallowing movements on sweet taste and decreased movements on bitter taste which is somewhat at variance with electrophysiological evidence and reflects on the multimodal involvement of taste cell functional and behavioural dynamics with age.

Key words : ontogeny, developmental electrophysiology, chemosensory signals, taste

INTRODUCTION

Chemosensory signals from food provide the sensory basis of the hedonic matrix that controls food acceptance, choice and intake (1). With the recognition of the importance of taste to life and consequent unfolding of its mechanisms the question as to how it develops assumes significance. The answer to it is important according to Descartes as "the nature of things is much more easily conceived when they are only considered as produced at once in a finished and perfect state" (2). Using recent biophysical, biochemical and immunological techniques, studies on the ontogeny of taste have been conducted in several species of animals, such as amphibians, reptiles and mammals including humans. In addition, embryological and postnatal studies on the alterations in morphology of taste cells, neural responses to taste (peripheral and central) and taste behaviour from conception to the adult stage have been reported. A

*Corresponding Author and present address : 272, 4th Main, Brindavan Extn., Arakere Mico Layout, Bennerghatta Road, Bangalore - 560 076

brief account and relevance of those investigations forms the theme of this paper.

General plan of development of special senses

The mechanism that guide the ontogeny of sense organs are worked out in greater detail for the sense of vision (3). From it one can gather that the ontogeny of any sensory system includes the development of receptors, the receptive brain areas and the neural connections between those two. Initially the central and peripheral mechanisms develop independently and later their development is dependent on one another. The most amazing aspect of ontogeny of sense organs is the laying down of the connecting links between central areas and peripheral systems as the linking neurons, winding their way through millions of neuron assemblies, finally make contact with the appropriate target areas. It is thought that neural connections between receptors and the brain areas are directed by "molecular sensing". The molecules may be released from cells located along the pathway or the target area itself. However, after the axon arrives at the target area, it sprouts elaborate terminal branches which enter into the area. Several axons send their endings into target area and synapse with it, but only the synapses that are appropriate (Hebb synapses) are strenghtened while the inappropriate ones are weakened and subsequently lost. The strengthening depends on the synchronisation of electrical activity in pre- and postsynaptic cells. Absence of synchronised pre- and postsynaptic activity tends to weaken transmission and thus the synapses. It may be said, therefore, that cells which "fire" together "wire" together. The electrical activity in the receptors is spontaneous. If the activity is prevented from reaching the target brain area, the terminal axonic branches, which normally are confined to a restricted zone, are spread all over the brain area in a disorderly fashion resulting in the non-function of sense organ. Further even after the right contacts are made the synapses need to be adequately stimulated within a critical period inorder to make the contact firm. If the adequate stimulus is not given during the critical period the sense organ fails to function normally.

The developmental changes in taste sense may also be following principles similar to those that govern ontogeny of vision. The changes are continuous, sometimes changes in one area are simultaneous with alterations in another area and may even be dependent on them. Hence it is not easy to delineate the transitional stages and study the underlying mechanisms in isolation. Yet for the convenience of studying, the ontogeny of taste is arbitrarily divided into three portions: (a) the development of taste cells, (b) the development of electrophysiology of taste, and (c) the development of taste behaviour.

(a) Taste cell development

The development of gustatory sense begins with the appearance of taste buds. In amphibians the taste organ is shown to originate from the epithelium of oropharyngeal cavity as indicated by dye injection studies on early salamander gastrules (4). Similarly in tadpoles of *Rana*

temporaria, the taste organs appear at the apices of oral premetamorphic papillae (5).

In the 15 day rat fetus, the taste bud development is indicated by slight elevation of a group of lingual epithelial cells and presence of gustatory nerve fibers in the vicinity (5). The epithelial cells then produce small dense membrane-bound granules followed by growth of nerves towards granular cells. Interestingly the denervated adult taste cells before regeneration also follow similar stages of granule formation and innervation (6). The intracellular vesicular substance probably helps in the formation of close relation between nerve and cell and lead to differentiation of epithelial cell into the gustatory cell. Following innvervation each taste bud develops several vertically elongated gustatory cells and supporting cells. Each gustatory cell has bidirectional synapses and synaptic vesicles (5). Significantly the denervation at this stage of initial innervation of epithelial cells irreversibly prevents the formation of taste buds locally in contrast to reemergence of taste buds after reinnervation of denervated adult gustatory epithelium. It suggests that initial innervation provides protective integumental covering that replaces any superficial secondary sensory cells rendered useless by denervation (7). In contrast, the 14 day fetus tongue fragments organ culture showed that morphogenesis of fungiform papillae is independent of innervation, though maintenance of papillae requires trophic neuronal influence (8). In hamsters also, the fungiform eminences begin to form in the absence of innervation, but the subsequent differentiation of papillae depends on innervation as in the rat. The hamster is precocious as compared to rat in terms of lingual nerve development and structural maturity of anterior tongue at birth (9).

In general the mammalian taste bud differentiation depends on innervation contrasting with amphibian taste bud morphogensis which is independent of innervation (10) or can be induced by non-gustatory fibers (11). The mammalian taste bud stimulated to undergo changes by innervation, exerts in its turn a trophic effect on central and peripheral nerve fibers. This was revealed by investigations on 14 day rat fetus. Epithelial cells of the taste bud influence growth of the chrodatympani and the lingual branch of trigeminal, though the responses of the nerves are varied (8). It is also known that destruction of the anterior tongue of 0-10 postnatal day (PND) rats (but not of later day rats tongue) results in defective axonic and dendritic growth in medullary taste neurons located in the nucleus tractus solitarius (NTS) which is similar to arrest of taste bud growth following its denervation (12). The influence of taste cells on growing neurons in central receptive areas may be mediated by granules present in the taste cells (13). Incidentally the PND's 0-10 appear to be critical period for the rat's taste cell neuronal interaction. Denervation during this period permanently restricts the gustatory competence of vallate epithelium (14) and recovery of reinnervation is about 50% only (15,16). After this initial interaction, the complexity of neural connections appears to increase as shown by increasing convergence of the afferent taste fibers on to multipolar second-order neurons in NTS during fetal and postnatal

development in sheep (17). It reinforced the earlier report on the rabbit type III cells (18), showing the converging and diverging synaptic input/output from type III cells the intragemmal nerve fibers with varying sizes of active synaptic zones.

In humans, presumptive taste buds are reported to the present in 7-9 week old fetus and adult buds in 13-15 week fetus (19). Some reports indicated that adult taste buds appear as late as in 4-7 month fetus (6).

Ultra-structure studies of the human fetus showed that the tongue is completely formed during 8-9 months of prenatal development (20). Anyway, all the studies indicated that the human child is born with functional taste buds. This seems to be the rule in all animals with long gestation period (21). Contrastingly in the animals with short gestation period the taste cells mature postpartum though presumptive buds are formed during intrauterine life (6). A notable exception is the chicken with a short gestation period and yet born with mature gustatory apparatus (22). In rabbit pups, the taste bud precursors are reported to be present in the second half of the short intrauterine life (5) and completely get differentiated in 7 days post-partum. A similar pattern of taste bud development in rat is reported (23). However, some reports on the time of appearance of taste buds in the intrauterine life of animals are contradictory, which may be due to uncertainty regarding the stage of development at which the collection of cells can be called taste buds. This contradiction may be reduced to some extent by using antibodies against gustatory receptor molecules in intragemmal cells. Such antibodies against cytokeratins 7, 8 or 19 are already developed (24).

The necessary changes in the presumptive taste cells and their afferent fibers which transform them into functional units are worked out thoroughly in the case of salt (Na-and Licl) responsive units, using amiloride which selectively blocks cellular response to Na^+ and Li^+ taste. However, amiloride does not completely block the neural responses to NaCl taste in adult. Further the NaCl response in PND 12-13 rats is unaffected by amiloride (25). Hence residual response to NaCl in adults and all the response to NaCl in young rats (PND 12-13) ought to be due to amiloride resistant Cl^- ion channels (25–26). It appears that the fibers in young rats responsive to Cl^- ions continue into adult life. Meanwhile the adult develops amiloride sensitive Na channels. Similar changes in cells and nerve fibers were shown in sheep (27). In sheep and rat, the peripheral nerve responses to NaCl are of low magnitude during early development. Progressively, the taste system acquires an increasing proportion of fibers that respond maximally to NaCl. The sodium responsiveness emerges in the context of shifting peripheral innervation patterns and the apparent addition of functional receptor membrane channels sensitive to the sodium transport blocker, amiloride. It appears that these developmental processes may be altered by early manipulation of sodium in the diet (28). The number of fibers responsive to salts of Na and Li increase gradually with concomitant decrease in the receptive field of single chorda tympani fibers while the fibers responsive to NH_4Cl remain unaltered (29). Additionally, there may be an increase

in NaCl sensitive "patch" and other patches responsive to sweet (30), bitter and sour tastes on the individual cell membrane. This increases in taste responsive patches could be the effect of innervation or independent of it. Specific taste sensitive membrane and the invading afferent fiber develop independently and "match" during innervation process (28). Contrastingly in the hamster adapted to desert conditions, the number of Na sensitive fibers show decrease with age (28) probably because they do not need to identify NaCl for their survival but rather have the problem of getting rid of NaCl ingested along with its food. Morphogenesis of taste bud appears to be accompanied by formation of carrier protein for transportation of taste molecules to receptors. This is suggested by detection of high concentration of Van Ebner's gland proteins in the clefts of circumvallate and foliate papillae along with development of taste buds. It is thought that Van Ebner's gland proteins may control the access of lipophylic sapid molecules (e.g. bitter) to the gustatory receptors (31).

Maturity of the taste bud is indicated by appearance of the taste pore, although taste cells may continue to undergo changes even after the appearance of the pore. In the rat vallate taste bud, for instance, the pore appears at 0-10 PND and cells continue to increase in length and width and number for 2 months PND (15). The taste pore facilitates interaction of chemical stimulants with taste cells. Probably the testants reach the taste bud cells even before the appearance of the pore as there is no permeability barrier in the epithelium overlaying taste buds (32). Once formed the taste cells in papillae undergo maturation process by interacting with substances present in mother's milk (33), or in the amniotic fluid (34) or in the food that adults eat to which pups are constantly exposed. Exception to this appears to be newly hatched snake's species-specific preferences for certain prey extracts without any previous exposure to it (35).

(b) Developmental electrophysiology

Most of the electrophysiological data related to ontogeny of taste sense are obtained from the sheep as the sheep fetus can be exteriorised without damaging its neural and vascular connections to the mother, and its gestational period is sufficiently long (150 days) to facilitate the developmental studies. Interestingly, the sheep taste bud development and maturation are similar to the development and maturation of taste buds in man (19). Scarcity of electrophysiological studies on the fetus of other laboratory animals (e.g. rat and rabbit) are probably due to their short gestational period, appearance of taste buds late in their fetal life (21 days) and their maturation postnatally (21 days after birth).

The taste sense is reflected in the electrical responses of gustatory nerves to chemical stimuli applied over taste buds. The taste responses were obtained from single fibers as well as group of a few fibbers from the chorda tympani of sheep fetus of 109-137 days of gestation (27). Responses to salt taste (NH_4Cl,NaCl,KCl), sweet (sugars, glycerol and saccharin), bitter (quinine and urea) and sour (acetic acid) and the taste of amniotic fluid were obtained. It was found that electrical

responses of single chorda tympani fibers of fetus in the last third of gestation (> 100 day) to NH_4Cl and glycerol were greatly increased, to sour taste of acetic acid and sweet taste of saccharin the response was mild; and to NaCl, glutamate and amniotic fluid it was poor. Surprisingly, the responses to taste of the calorie-rich sugars as well as to life-saving bitter taste were not even poor but depressed. The integrated response recorded from a small bundle of nerve fibers indicated an almost similar response pattern. Significantly, the single fiber responses of 128 day fetus, 12 day lamb and adult sheep to monochloride salts (NH_4Cl, NaCl, KCl and HCl) were identical. Additionally the multifiber responses to increasing concentration of NH_4Cl of 137 day fetus, 12 day lamb and adult were also similar. Hence it appears that neuroanatomical substrate related to taste sense in sheep are laid down and ready to function nearly 50 days before the birth. The stimulus for organisation of gustation before birth could only come from frequent alterations in the taste of amniotic fluid due to the addition of fetal urine to it in the final 1/3 of gestational period (34).

The absence of electrical responses in chorda tympani of fetus to sugar taste and bitter taste of quinine, contrasting with its presence in the lamb and adult is difficult to explain. However, absence of sugar taste response is explained as due to difficulty of recording from small diameter "sweet" fibers which in fetus might have been damaged easily (34). The absence of neural responses to bitter taste is not explained as yet.

An investigation of sheep medulla for taste responses (36) showed that the tractus solitarius units responded to a broader range of stimuli with increasing age of fetus and lamb. In the fetal sheep at 84 days gestation the tractus solitarious units respond to NH_4Cl, KCl and citric acid only. At the final third term of gestation (122-126 days) the tractus solitarius units responded to NaCl, LiCl and HCl also, in addition to NH_4Cl, KCl and citric acid. Tractus solitarius responses in the final term foetus are similar to the tractus solitarius responses of lamb and adult sheep The results suggested the increasing convergence of taste afferents on to medullary receptors (17).

The rat fetus chorda tympani responses to taste stimuli are not known. However, responses were recorded from PND2 to 110 day old rats (37). Though 2 day old rats had 1-7% mature fungiform papillae they responded differently to qualitatively different chemical stimuli (NH_4Cl, LiCl, NaCl, citric acid, and sucrose) thus indicating the capability of neonatal rat to distinguish different taste qualities. Like the sheep fetus the neonatal rat also showed least responses to sucrose. Another interesting finding is that the responsiveness to taste stimuli varies throughout postnatal development and it is more dramatic for citric acid. Recording of integrated responses from the chorda tympani nerve revealed that taste buds with a pore at 1 week of age responded to NaCl, HCl and quinine-HCl as well as in adult rats. However, the response magnitudes for various sugars at 1 week of age were smaller compared to those in the adult rat. These results suggest that functional changes occur in the gustatory processing of sugars during postnatal development in the rat

chorda tympani nerve (38). In addition, the neural responses were approximately proportional to concentration of taste stimuli (0.1, 0.25m, 0.5, and 1.0m, NH_4Cl) throughout developmental stages starting with 6-7 day old rats. Finally the proportion of units in neonatal rat responding to NaCl was less as compared to units responsive to NH_4Cl initially but increased gradually with the age (39). It has also been shown that tractus solitarius activity both in rat and in sheep (36) is similar to the activity seen in axons innervating the taste cell indicating that tractus solitarius is not intimately involved in analysis of the taste information further than the analysis achieved by taste cells.

Developmental changes in cortical analysis of taste sense if any are unknown though significant changes in taste cortical areas are demonstrated (40).

(c) Behaviour

Morphological and electrophysiological changes during development form the basis for behaviour in the growing animal or man. Intake behaviour involves swallowing for which several muscles need to work in a sequential order. In the fetus of several warm blooded animals including the human fetus deglutition occurs as random brief episodes that could be part of spontaneous movements (41). The fetus swallows amniotic fluid which stimulates the tongue receptors on its way to alimentary canal. Hence, to identify the gustatory status of fetus, the test substance is added to amniotic fluid and swallowing movements observed. Such procedures have shown that the swallowing movements in human fetus are increased on sweet saccharin and decreased on bitter taste (21). Surprisingly the sheep fetus does not decrease spontaneous swallowing movements on bitter taste, though the naive neonatal lamb (2-7 days old) is known to reject bitter tasting milk (21). The lack of conscious control over random swallowing movements or immaturity of bitter taste cell at that stage of fetal development or both may be the causative factors for absence of responses to bitter taste in sheep fetus (21).

Compared to studies on fetal responses, investigations on intake behaviour of post natal animals, specially the rat, are extensive. In a recent study (42), the PND 1 rat pups are shown to discriminate between sweet and bitter taste though taste cells development (5) and electrophysiological evidences (37) indicate the contrary. Further as they advance in age the rat pups (PND 3-6) in a two-bottle study showed increased intake on sucrose over water intake and still later (PND 15) the increase on sucrose was shown to be porportional to its concentration. Recent studies indicate that neonatal and adult rats are attracted to the taste of sugar as well as to starch - derived polysacharides. The preference for sweet taste, relative to that for starchy taste, increases with age and the sweet taste preference is somewhat stronger in the male rats than in the female rats (43). In contrast the intake on the taste of mineral oil and corn oil emulsions showed a decreases as compared to water intake in PND 3-6 rats, though as late as PND 21 the intake on oils was increased. At PND 21 (i.e. at the time of weaning) the rats are known to exhibit adult salt appetite (44). Unlike rats of the Sprague-Dawley or Wistar

strains, Fischer 344 (F344) rats and Syrian hamsters exhibit an aversion to dilute NaCl solutions as adults while showing preference for the same at both 6 and 18 days of age (45). Another interesting study is on the taste behaviour of adult rats which were taste deprived (fed intragastrically) or given asymetrical input to their taste system during their early postnatal days (46). Rat pups aged 4-18 days were used for the above study as they show rapid changes in peripheral taste cells. The study showed that neither the taste deprivation nor asymetrical input to taste system of PND 4-18 rats could influence their taste responses after they grew into adults. Hence it was concluded that certain events occuring before the period PND 4-18 firmly established the direction of the taste development in rats. Such a critical period during which irrevocable changes might have occured for salt taste is speculated to be the period between conception and embryonic day 8 or even earlier (47). NaCl deprivation initiated on or before embryonic day 8 but not later (embryonic day 10) resulted in decreased chorda tympani responses to salt taste in the adult rat. This is explained as due to the permeability of placenta to some maternally produced agents (sex hormones, growth factors or their metabolic substrates) at fetal age of 8-10 days and not later. These maternally produced agents may be necessary to initiate a cascade of events that finally result at a later stage in the appearance of NaCl sensitive areas on taste cells. However, neither the agents nor the permeability of placents to these agents are identified. Moreover, the rat embryos deprived of NaCl from day 3, when allowed to drink isotonic saline on PND 40 and returned to low NaCl diet for additional 30 days showed "recovery" of chorda tympani fiber responses to NaCl to control levels (47). Hence, it appears that effects of early deprivation of NaCl are completely reversable (28). In view of this the idea of well defined "sensitive period" for development of salt taste sense is in doubt. Probably the NaCl channels may remain in immature state in the NaCl deprived rats and mature into adult forms only after the rat ingests NaCl. It is possible that such interactions also play an important role in the maturation of sweet, sour, bitter and umami taste as well.

Surprisingly though NaCl is essential for life the development of taste for salt appears late in fetal or neonatal life of mammals. Most of the mammals including man are born without the functionally active NaCl taste sense as is evidenced by low sensitivity to NaCl taste, absence of amiloride-sensitive Na channels and very few number of chorda tympani fibers responsive to NaCl in the fetus and the new born mammals. As the animals advance in age, the taste system becomes more sensitive to NaCl, amiloride sensitive Na channels are added to taste cells and the chorda tympani fibers responding to NaCl increase in number (28, 48). Correlated change in salt taste behaviour is also reported. For instance PND5 rat does not show preference for NaCl over water whereas at PND10 preference for NaCl is exhibited and at PND25 they show three fold increase in NaCl intake as compared to intake of adult rats. However, these post-weaning rats (PND25) are unable to discriminate between the taste of NaCl, NH_4Cl and KCl though their response to sucrose and citric acid were similar to adult rats, indicating quantitative and qualitative

alterations in salt taste perception, with age (49). Magnitude of aversion to concentrated NaCl solution exhibited by adults is not shown till the rats attain the age of 48 days. All these studies show that the taste of NaCl to young rats is not the same as it is for adult rats. It is possible that alterations in salt taste appreciation may have to wait for certain physiocochemical changes in CNS as evidenced by precocious and specific sodium appetite developement in 3 day old rat pup, on activation of its brain angiotensin (50). Such transitions in NaCl taste in human infants are not known. However, the human infant's response to NaCl taste appears as late as the age of 4 years though the effective responses to sweet, sour and bitter tastes are known (28). Even the response to bitter taste appears a little later after birth. While the newborns do not reject bitter taste (urea) a few days old infants exhibit aversion to it (51).

It is not clear as to the role of experience with gustatory substances in the development of taste sense. Some studies have indicated that diet selection pattern of adult rats are atleast partially established as a result of early experiences with food related stimuli present in mother's milk. An interesting example is the evidence of preweanling rat's enhanced ethnol intake on earlier pairing of ethnol with milk during suckling (52). Even the probable basis for learned preference for ethnol is indicated as unique neurochemical changes during post-natal development (53). In contrast to it the neonate rats tested from PND 1-21 showed increased intake on saccharin with a sharp rise at PND 7-9 days (54). This increased intake was also shown by the 18 day preweanlings without previous experience of saccharin taste. A similar increased aversion for bitter taste of quinine when tested for the first time on PND 14 was also shown. The above evidence indicate that taste development is independent of previous exposure to taste stimuli (54). Evidences for and against learned preferences/aversions support the idea that the gustatory competence develops with the age and is innate. But for establishing the preferences/aversions and for regulating intake based on taste, the innate taste sense needs to be reinforced as partially happens with the intake of mother's milk or random sampling of adult food that is usually available in the vicinity.

CONCLUSIONS

In conclusion it may be said that the studies on taste ontogeny have contributed substantially to our knowledge of taste sense. The central and peripheral mechanisms are known to develop independently and "seek" one another. After the connection is established, further development of the central and peripheral mechanisms becomes interdependent. The development of taste sense is under multiple controls which come into existence at appropriate time and space. Further, the sensing of different chemcials appear in an orderly manner and may change with age. However, as at present, the ontogeny of multimodal responses of taste cells, and mechanisms of transduction of taste stimulus are not clear. There is a need for further research on those unsolved problems.

REFERENCES

1. Sharma KN, Jacobs HL, Gopal V, Dua-Sharma S. Nutritional state/taste interaction in food intake: behvioural and physiological evidence fo gastric taste-modulation. In: Kare MR, Maller O, eds. Chemical senses and nutrition. New York: Academic Press 1977; 167-188.
2. Adolph EF. Origin of Physiology regulations. Academic Press, New York, 1968.
3. Shatz CJ. The developing brain. *Scientific American*, 1992; 267:60–67.
4. Barlow LA, Northcutt RG. Embryonic origin of amphibian taste buds. *Dev Biol* 1995; 169(1):273–285.
5. Zuwa AK, Jakubowski M. Development of taste organs in Rana temporaria. *Transmission and scanning* electron microscopic study. *Anat Embryol (Berl)*. 1991; 184(4):363–369.
6. Ferbman AI. Development of taste bud. In:Beidler LM, ed. Chemical senses 2, Taste. New York: Springer Verlag; 1971.
7. Oakley B. Neuronal - epithelial interactions in mammalian gustatory epithelium. *Ciba Found Symp* 1991; 160:277–287: duscussion 287–293.
8. Farbman AI, Mbiene JP. Early development and innervation of taste bud bearing papillae on the rat tongue. *J Comp Neurol* 1991; 304(2): 172–186.
9. Whitehead MC, Kachele DL. Development of fungiform papillae, tastebuds, and their innervation in the hamster. *J Comp Neurol* 1994; 340(4): 515–530.
10. Stone LS. Independence of taste organs with respect to their nerve fibers demonstrated in living salamanders. *Proc Soc Exptl Biol (N.Y.)* 1933; 30: 1256–1257.
11. Robbins N. The role of nerve in maintenance of frog taste buds. *Exp Neurol* 1967; 17: 364–380.
12. Lasiter PS, Kaachela DL. Effects of early postnatal receptor damage on deveopment of gustatory recipient zones within nucleus of solitary tract. *Devt Brain Res* 1992; 55: 57–71.
13. Hamberger V, Yip JW. Reduction of experimentally induced neuronal death in spinal gangilia of the chick embryo by nerve growth factors. *J Neurosciences* 1984; 4(3): 767–774.
14. Oakley B. The gustatory competence of the lingual epithelium requires neonatal innervation. *Brain Res Dev Brain Res* 1993; 72(2): 259–264.
15. Oakley B, Labelle DE, Riley RA, Wilson D, Lab Hsain WK. The rate and locus of development of rat vallate taste buds. *Dev Brain Res* 1991; 58: 215–221.
16. Hosley MA, Hughes SE, Morton LL, Oakley B. A sensitive period for the neural induction of taste buds. *J Neurosci* 1987; 7: 2075–2080.
17. Mistretta CM, Labyak SE. Maturation of neuron types in nucleus of solitary tract associated with functional convergence during development of taste circuits. *J Comp Neurol* 1994; 345(3): 259–376.
18. Royer SM, Kinnamon JC. HVEM serial-section analysis of rabbit foliate taste buds:I. Type III cells and their synapses. *J Comp Neurol* 1991; 306(1): 49–72.
19. Bradley RM, Stern IB. The development of human taste bud during fetal period. *J Anat* 1967; 101: 743–752.
20. Alekseev ON. Embryogenesis of the human tongue. *Arkh, Anat Gistor Embriol* 1991; 100(6): 87–90.
21. Bradley RM, Mistretta CM. The developing sense of taste. In: Denton, D.A., ed Olfaction and taste V. New York; J.P. Academic Press Inc; 1975.
22. Ganchrow D, Ganchrow JR, Goldstein RS. Ultrastructure of palatal taste buds in the perihatching chick. *Am J Anat* 1991; 192(1): 69–78.
23. Farbman AI. Electron microscope study of the developing taste bud in rat fungiform papilla. *Devel Biol* 1965; 11: 110–135.
24. Knapp L, Lawton A, Oakley B, Wong L, Zhang C. Keratins as markers of differentiated taste cells of the rat. *Differentiation* 1995; 58(5): 341–349.
25. Garty H, Benos DJ. Characteristics and regulatory mechanisms of the amiloride-blockable Na^+ channels. *Physiol Rev* 1988; 68: 309–373.
26. Heck GL, Mierson S, De simone JA. Salt taste transdution occurs through an amiloride-sensitive sodium transport pathway. *Science* 1984; 223: 403–405.
27. Bradley RM, Mistretta CM. The gustatory sense in fetal sheep during the last third of gustation. *J Physiol (Lond)* 1973; 231: 271–282.
28. Hill DL, Mistretta CM. Developmental neurobiology of salt taste sensation. *Trends Neurosci* 1990; 13(5): 188–195.

29. Nagai T, Mistretta CM, Bradly RM. Developmental decrease in size of peripheral receptive fields of single chords typani nerve fibers and relation to increasing taste sensitivity 1988; 8(1): 64–72.

30. Waldrop MM. Bitter sweet findings. *Sciences* 1990; 248: 817.

31. Kock K, Blaker M, Schmale H. Postnatal development of von ebner's glands: accumulation of a protein of the lipocalin super-family in taste papillae of rat tongue. *Cell Tissue Res* 1992; 267(2): 313–320.

32. Mbiene JP, Farbman AI. Evidence for stimulus access to taste cells and nerves during development: an electron microscopic study. *Microsci Res Tech* 1993; 26(2): 94–105.

33. Galeff Jr. BG, Sherry DF. Mother's milk: A medium for transmission of cues reflecting flavour of mother's diet. *J Comp Physiol* 1973; 83: 374–378.

34. Mellor DJ, Slater JS. Daily changes in foetal urine and relationships with amniotic and allantoic fluid and maternal plasma during last two months of pregnancy in conscious unstressed ewes with chrnonically implanted cacheters. *J Physiol* 1972; 227: 503–525.

35. Burghardt GM. The ontogeny evolution and stimulus control of feeding in human and reptiles. In: Kare MR Maller O, eds. The chemical senses and nutrition. New York: Academic Press; 1977.

36. Mistretta CM, Bradley RM. Taste responses in sheep medulla: changes during development. *Science* 1978; 202:535–537.

37. Hill DL, Almli RC. Ontogeny of chorda tympani nerve responses to gustatory stimuli in the rat. *Brain Res* 1980; 197: 27–38.

38. Maeda S. Developmental changing of rat taste responses form chorda tympani nerve. *Nippon Seirigaku Zasshi* 1990; 52(3): 67–83.

39. Hill DL, Mistretta CM, Bradley RM. Developmental changes in taste response characteristics of rat single chorda tympani fibres. *J Neuroscience* 1982; 2: 782–790.

40. Ogawa H, Ohgushi M, Hasegawa K, Murayama N. Differetial development of cortical taste areas in granular and dysgranular insular cortices in rats. *Brain Res Dev* 1991; 60: 271–274.

41. Hamberger V. The journey of neuroembryologist. *Ann Rev Neurosci* 1989; 12: 1–12.

42. Ackerman SH, Albert M, Shindledcker RD, Gayle C, Smith GD. Intake of different concentrations of sucrose and corn oil in pre wearing rats. *Am J Physiol* 1992; 262: R 624–627.

43. Perez C, Sciafani A. Developmental changes in sugar and starch taste preferences in young rats. *Physiol Behav* 1990; 48(1): 7–12.

44. Leshem ML, Yetuda I. The ontogeny of salt appetite aroused by depletion in the rat. *Physiol Behav* 1991; 49: 161–163.

45. Rowland NE. Ontogeny of preference and aversion to salt in Fischer 344 rats and Syrian hamsters. *Dev Psychobiol* 1991; 24(3): 211–218.

46. Bernstein IL, Fenner DP. Diaz J. Influence of taste stimulation during suckling period on adult taste preference in rats. *Physiol Behav* 1986; 36: 913–919.

47. Hill DL. Prezekop PR Jr. Influence of dietary sodium on functional taste receptor development:A sensitive period. *Science* 1988; 241: 1826–1828.

48. Settles AM, Mierson S. Ion transport in rat tongue epithelium *in vitro*: a developmental study. *Pharmacol Biochem Behav* 1993; 46(1): 83–88.

49. Formaker BK, Hill DK. Alterations of salt taste perception in the developing rat. *Behav Neurosci* 1990; 104(2): 356–364.

50. Leshem M, Del Canno S, Epstain AN. Intercerebroventricular injection of renin in the neonatal rat reveals a precocious sodium appetite that is dissociated from renin– aroused thirst. *Dev Psychobiol* 1994; 27(3): 185–193.

51. Kajiura H, Cowart BJ, Beauchamp GK. Early development change in bitter taste responses in human infants. *Dev Psychobiol* 1992; 25(5): 375–386.

52. Hunt PS, Kraebel KS, Rabine H, Spear LP, Spear NE. Enhanced ethanol intake in preweanling rats follwoing exposure to ethanol in a nursing context. *Dev Psychobiol* 1993; 26(3): 133–153.

53. Witt ED. Mechanism of alcohol abuse and alcoholism in adolescents: a case for developing animal models. *Behav Neural Biol* 1994; 62(3): 168–177.

54. Jacobs HL, Smutz ER, DuBose CN. Comaprative observations on the ontogeny of taste preference. In: Taste and development: the genesis of sweet preference. Bethesda, Maryland: NIH-DHEW publications 1977; 99–107.