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Review Article

Basic brainstem taste responsivity: effects of perinatal influences

Respuestas gustativas del tallo cerebral: efectos de influencias perinatales

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Abstract

In altricial newborns the gustatory system is fundamental for survival and for establishing mother-litter bonds in the nest environment. The chemosensory experience is initiated in the uterus by the actions of chemical stimulants in the amniotic fluid. After birth, maternal care prevails, and the gustatory system is surprisingly enriched by breast milk suction. In Norway rats at 12 days of age pups make a transition from milk to solid food. At this time, when the gustatory experience is broadly developed, both the receptors and the central nervous system (CNS) sensory relay systems undergo a remarkable reorganization to permit the integration of the sensory and hedonic characteristics of the gustatory cues. The current review analyzes the morphofunctional organization of the taste buds and the afferent projections, the neuronal organization of the first CNS relay, as well as how perinatal food deprivation interferes with the plastic properties of the rostral portion of the brainstem solitary tract nucleus in the rat.

Keywords: Gustatory system, Development, Solitary tract, Rats.

Resumen

En los recién nacidos altriciales, el sistema gustativo juega un papel fundamental para la supervivencia, y establecimiento de nexos con la madre en el contexto del ambiente del nido. La experiencia quimiosensorial se inicia en el hábitat uterino por la acción de la estimulación química vía del fluido amniótico. Al nacimiento, los cuidados maternos prevalecen y el sistema gustativo es ahora fortalecido en su función por la succión de leche materna. En ratas Norway después de los 12 días de edad, las crías de entran a un periodo de transición entre la leche y la ingesta de alimento sólido. En este momento, la experiencia gustativa se desarrolla ampliamente, tanto los receptores como los relevos sensoriales del sistema nervioso central (SNC) están bajo una notoria reorganización que permite la integración de las características sensoriales y hedónicas de las señales gustativas. En la presente revisión se analizan la organización morfológica y funcional de los botones gustativos, las proyecciones aferentes, los cambios neuronales en el primer relevo dentro del SNC y cómo la restricción perinatal de alimento interfiere con las propiedades plásticas de la porción rostral del núcleo del tracto solitario de ratas Wistar en desarrollo.

Palabras clave: Sistema gustativo, Desarrollo, Tracto solitario, Ratas.

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I. Introduction

Altricial newborns must face numerous and continuous environmental demands with remarkably immature sensorial, motor, and autonomic systems in order to survive with the help of maternal care. During the neonatal period the newborn undergoes a series of complex morphofunctional brain changes in order to generate neurons, interconnect them, complete circuits, progressively increase neuronal interactions, and adapt the intracellular neuronal machinery to the diverse transitory or long-term plastic functional changes occurring throughout the life span. In mammals, brain development occurs in two different, predictable environments: the uterine habitat, in which a direct chemical link between mother and pup is established by means of the fetus-placenta circulation; after birth, pups encounter the nest environment, where maternal care prevails, and the mother constitutes the main source of food and sensory signals.

From several studies it is known that life in the uterus is constantly modified within a very narrow range of conditions and that the fetus is primarily exposed to somatosensory, vestibular, and chemosensory stimulation in preparation for a less stable nest environment. Thus, the altricial newborn must adapt to a highly variable external habitat with well-known sensorial, motor, chemosensory, and homeostatic deficiencies that are only overcome with intense maternal care.[1-4] Among the signals that the fetus receives and responds to in the uterus are the chemo-sensorial cues; the gustatory and olfactory systems in particular begin to develop early in gestation, are well advanced by the time of delivery, and undergo a neonatal period of rapid development.[5-8] The developing olfactory system in the uterus prepares the fetus for respiration and initiates the transduction of signals from maternal odor stimuli included in the amniotic fluid that reach the fetal olfactory mucous area.[9]

Initial studies of the gustatory system were made in sheep, where the deglutition rate of amniotic fluid was found to change, depending on its chemical composition. For instance, when sucrose was injected into the amniotic fluid, the rate of fetal deglutition was greater than when a neutral gustatory stimulant was injected as observed by the reduction of mouth movements and licking lips. Therefore, it was proposed that chemical stimulation of the fetus, via the gustatory cues in the amniotic fluid, may stimulate deglutition as a mechanism to provide nutrients and to promote gastro-intestinal tract development.[10]

At birth the gustatory experience is surprisingly rich; thus, with breast milk suction, the young satisfies two primary needs, nourishment and fluid balance, during the first 17 days of age. Behavioral studies have demonstrated that the newborn can distinguish at least three basic flavors, and that the gustatory response is modified until the subject acquires the adult behavioral pattern at the end of the lactating period, when the young has free access to solid food.[11] From the pioneering study of Galef and Henderson[12] it is known that at the end of the second postnatal week, the young rat already has the gustatory ability to discriminate among chemical cues, which allows it to obtain essential sensorial experience through the different components of mother's milk.

The present review focuses on studies using a variety of research tools in order to provide information about the complex integration of the gustatory system; we also include data on the development of gustatory behavior the basic elements of the gustatory system: taste buds, afferent fibers carrying the information to the first CNS relay, the solitary tract nucleus (STN) and its general anatomic characteristics at early stages of development.

1. Ontogeny of facial responses to taste

An important tool to assess gustatory discrimination in the newborn rat has been the characteristics of facial responses elicited by exposure to different chemical solutions.[13, 14] Functionally, it is known that newborn rats exhibit three facial reflexes to taste. Thus, a sweet stimulus applied on the top of the tongue starts a reaction that may be delightfully, because the pup licks its lips and

moves its tongue laterally without showing an aversive facial expression. On the other hand, the application of a bitter or sour stimulant initiates a displeasure reaction with head drawback movements, torsion of the neck and tongue, and active mouth movements. A salty stimulation causes a facial response intermediate between those mentioned above.[15, 16]

Investigations made by Hall and Bryan,[13] using the facial reflex evoked by a taste stimulant applied on the back of the tongue, showed that the young clearly distinguished between water and sucrose when they were 3 days old, although the full facial response was not obtained until postpartum day 6. More recently, it was observed that the oral infusion of citric acid or quinine in 1-day-old rats elicited an aversive or reduced response, respectively; however, certain patterns of the adult rat's general stereotypical behavior (chin scratching, mouth opening, and body movements) did not appear in rats until 12 days after birth. These findings do not mean that before day 12 they cannot detect the gustatory cues of the solutions. Instead, the fact that they do not show such an efficient response may reflect the immaturity of the neuronal substrates that regulate the motor control of this aversive response.[17, 18]

Thus, newborn mammals can respond in a different way to basic flavors, and this response varies with the CNS developmental stage. For example, the response to a salt cue has been studied in rats from 3 to 18 days old by placing a catheter into the oral cavity to provoke a facial response. Rats, ranging from 6 to 18 days of age, showed a U-shaped curve of response with time, where the newborn has a high initial preference for salt that gradually declines over the following weeks, and then returns in the adulthood. According to several authors this is clear evidence that discrimination through the gustatory system already occurs with a pre-functional activity very soon after birth.[11-19] To study the sucrose-induced appetite ontogeny, rats from 3 to 15 days old were implanted with and stimulated via an oral cannula through which different concentrations of sucrose and polycose (0.03 M and 0.3 M, respectively) were applied. After assessing the general motor activity, it was concluded that the gustatory discrimination between 0.3 M sucrose and water is achieved at 6 days and between the polycose solution and water at 9 days of age.[20] Using the same experimental paradigm as for sucrose and salt, it has been shown that taste discrimination between water and quinine is already present at birth, although a consistent response is not observed until 9 days of age. Likewise, the stereotypical reaction to quinine seems to be shown at 12 days after birth, and by 15 days it can be used to discriminate taste preference or aversion in the young.[15, 18]

Thus, it is possible that the three motor mechanisms for facial expression are present at birth, although they are not yet fully developed, this is the concept of prefunctionality previously described to chemosensory systems described elsewhere.[21] These findings suggest that the gustatory system is well organized and functionally active before the structural development of the gustatory papillae is completed.

2. Taste bud development

The gustatory system is regulated by specialized receptive cells that are organized in groups of 50-100 cells, forming a spherical structure named the taste bud (Figure 1). The taste buds are located in three different types of gustatory papillae on the surface of the tongue: the circumvallate papillae located in the medial and posterior zone of the tongue and made up of hundreds of taste buds, the foliate papillae located on the lateral posterior area of the tongue where there are hundreds of buds, and the fungiform papillae distributed over the front two-thirds of the tongue's surface and which usually contain one taste bud (Figure 1). There are also taste buds on other structures of the oral cavity, such as the soft palate and the naso-incisor duct.[22, 23]

In the rat, the formation of the circumvallate and foliate papillae is initiated around gestational days 14 and 15, when the epithelium covering the tongue invaginates into the mesenchyme, and the nerves can be observed at the center of the circumvallate papillae on gestational day 16. On day 20 of

gestation the immature buds can be clearly identified morphologically.[24-27] As development proceeds, the papilla epithelium is taking shape, while the slot that shapes it becomes wider, and more taste buds appear in this area. In mammals, the morphological and functional study of receptors located on the tongue allows us to recognize the heterochronic development of receptors in the oral cavity.

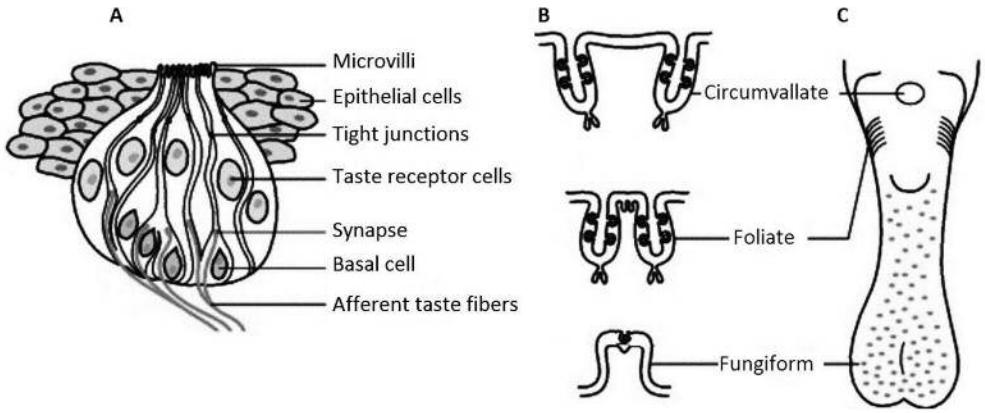

Figure 1. A). Taste buds consist of different types of small cells involved in a morphofunctional recycling process. The taste receptor cells, contains microvilli that project into the taste pore and contains the sites for sensory transduction. Basal cells derived from the surrounding epithelium that is in an initial phase of changing to taste receptor cells. The cells are continuously in a renewing process. B). Taste buds are contained within three major classes of papillae. Circunvallate papillae in the rat are unique structures placed in the posterior part of the tongue that contain receptors to sour and bitter stimuli and contain approximately 400 taste buds; foliate papillae are located at the border of the posterior tongue and are mainly responsive to sour stimuli and contain around 100 taste buds and fungiform papillae are located on the most anterior part of tongue and they are primarily sensitive to sweet and salt cues and contain only one taste bud. C) Surface to the tongue rat that show the distribution of the different papillaes (Modified of Munger, 2006).

As a general rule, a taste bud reaches full maturation when a gustatory pore appears in its apical part. This pore is the link between the chemical substances contained in foods and the internal medium. At birth, the taste buds of the soft palate (SP) and of the fungiform papillae (FP) are partially mature; by contrast, in buds of the front part of the tongue, some gustatory pores are not observed until the second postnatal week.[28] At birth the rat has approximately 127 taste buds in the SP, but only 53% of them have a gustatory pore. In the case of the FP 110 buds were observed, but only 14% of them had a gustatory pore. At the end of the first postnatal week the number of buds in the FP increases rapidly, and 90% of them have pores, while 80% of the buds in the FP have pores at this time. In the foliate (FoP) and circumvallate (CP) papillae, some taste buds with pores appear during the second postnatal week when 52% of the 132 taste buds have a pore (Figure 2). In the rat at early postnatal stages, the fastest addition of taste buds clearly occurs during the early postnatal stages.[29, 30, 22]

The taste buds are in a continuous cycle of regeneration due to a local phenomenon of death and differentiation of the taste receptors throughout the animal's entire life.[31] The role of this cyclic death/regeneration of taste buds and their reinnervation under normal and pathological conditions is, at present, poorly understood. In addition, it is still not known how gustatory information is modulated or how it influences brain development.

Nevertheless, the plastic gustatory properties of the early brain may bypass the taste bud-recycling process in order to maintain homeostatic fluids and food-intake balance.

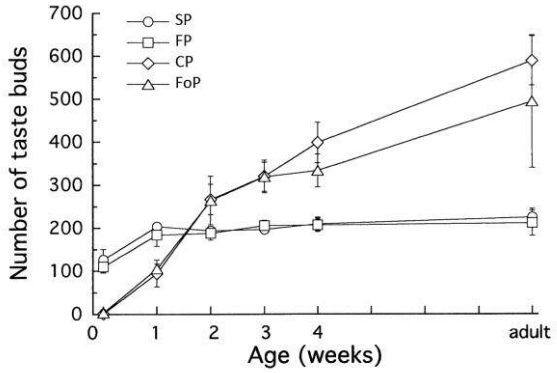

Figure 2. Number of taste buds at different ages of rat's development. SP, soft palate; FP, fungiform papillae; CP, circunvallate papillae, and FoP, folliated papillae. (Modified of Harada et al., 2000).

From other studies it is known that the maintenance and differentiation of the taste buds in the adult rat depend on afferent innervations and that the interaction among specific nerves and areas of the tongue epithelium regenerates the buds.[32-35] The gustatory nerve endings release trophic factors, activating a program that promotes basal cell differentiation in the epithelium of the gustatory papilla.[23]

3. Development of CNS afferents and early properties of the gustatory system

Several studies indicate that all morphogenetic events that characterize the appearance and maturation of the buds and their neuronal afferent elements influence gustatory function. Thus, at the peripheral level, changes can be expected in the expression and regulation of transduction mechanisms and in the development of afferent responses.[36]

Previous electrophysiological studies have analyzed the ontogeny of the electrical gustatory responses in the afferent fibers that convey the information from the taste buds to the brainstem STNr. Thus, when the electrical activity elicited by a sweet oral cue is recorded from the tympanic chord (TC) and compared to the magnitude of the reference response provoked by a 10 M NH_4Cl solution that does not change with the age, the responses for glucose and fructose do increase significantly with age. In 14- to 20-day-old hamsters, the responses to glucose and fructose are significantly smaller than those in adults, and in addition, the magnitude of the response measured at 25 to 35 days old is intermediate between those of newborn and adult subjects. When compared, the response of the neuronal system to monosaccharide and polysaccharide showed that the sensitivity to monosaccharide gradually increases during postnatal development, whereas the response to disaccharide rises more sharply at the end of this period.[37] In general, these results show that the response properties of the TC mature in a different way, with the response to monosaccharide appearing earlier than the response to more complex sugar compounds.[37] On the other hand, the responsiveness of the TC could be related to the postnatal changes in the intracellular membrane components involved in the transduction of the gustatory stimulus. The age-related changes in the TC response to NaCl and LiCl are largely attributed to changes in the sensitivity of individual fibers to salts. Approximately 90% of the TC fibers in 14- to 20-day-old rats respond to 0.10 and 0.5 M NaCl and LiCl; in addition, the average frequency of response to NaCl or LiCl increases about two-fold more than that to NH_4Cl. However, when the TC fibers were classified according to the salt to which they best respond, the number of fibers that are sensitive to NaCl and LiCl rises sharply between the neonatal and adult period. During that same period, there is a reduction in the number of fibers with a preferential response to NH_4Cl. Therefore, the increase in TC sensitivity to NaCl and LiCl during development may be due to an increase in the ratio of fibers that respond more strongly to NaCl and LiCl, as well as to the increased sensitivity of individual fibers to these stimulants.[38]

Specifically, the TC electrical responses to NaCl and LiCl in 13- and 23-day-old rats is not significantly affected by lingual pre-treatment

with 100 μM amyloride (a substance that promotes membrane input resistance). By contrast, in rats that are 29-31 or 90-100 days old, amyloride suppresses responses to NaCl and LiCl. From this study Hill and Bour[39] concluded that the increased sensitivity to Na+ and Li+ and to amyloride, are due to the gradual increase in the functional expression of amyloride-sensitive Na+ channels in the apical membranes of the gustatory cells.

The changes associated with the role of Na+ during the development of taste have also been documented in mice, rat, hamster, and sheep. Bradley and Mistretta[25] showed in pregnant ewes that the TC responds to a variety of gustatory stimuli. Later, it was also shown that TC sensitivity to LiCl and NaCl in sheep increases progressively during pre- and postnatal development.[40] The increased gustatory sensitivity to Na+ and Li+ was attributed by these authors to changes that depend on the age of the gustatory cells with apical membranes that contain functional transduction systems, which were recognized later as amyloride-sensitive Na+ channels.[41] However, it is still unclear what modulates the gustatory response to these salts. The work of Hill and Bour[39] showed that the increased sensitivity to NaCl and LiCl occurs in parallel with an increased sensitivity to the inhibitory effects of the amyloride.

Immunohistochemical techniques were used to demonstrate that amyloride-sensitive Na+ channels are present in 2-day-old rats and that they are located in the apical membrane of the gustatory cells; the next question was: "Are they functional?" Mc Pheeters et al.,[42] reported that Na+ currents sensitive to amyloride are present in approximately 40% of gustatory cells isolated from the FP of 2-day-old rats, and that, following a dose of 30 μM amyloride, the input resistance of the membrane significantly increases. However, apical sensitivity to amyloride is not apparent until later in development, and amyloride-sensitive Na+ channels may be present in the basolateral membrane of neonatal gustatory cells. This distribution is consistent with the immunoreactivity for Na+ channels in the basolateral membrane that is observed in the FP gustatory cell membranes.[43]

The temporary discrepancy between the appearance and function of the buds and the expression of their sensitivity to amyloride suggests that, after birth, endocrine and exocrine events may activate the development of previously quiescent Na+ transport or transduction of Na+ signals in the rat's gustatory system.[43] Therefore, the morphology of taste buds constitutes the basis for understanding the changes in the response properties of the gustatory peripheral system. Recent studies attempt to identify the mechanisms that regulate these changes, and they focus on specific, G-protein-coupled membrane receptors related to sweet and bitter solutions that transduce the gustatory stimulation into mechanisms or signals that regulate the development of the peripheral gustatory system. Likewise, these studies also aim to identify specific membrane receptors coupled to G-proteins that transduce signals to the second neuronal relays. The resulting electrophysiological changes may reflect alterations of the affinity or density of the neuronal gustatory system receptors or changes of the second messenger.[44]

Information regarding electrophysiological development of glossopharyngeal and vagus nerves is still scarce, because the stimulation that generates the response of these nerves is more complex. Another reason is the technical difficulty of performing the same timeframe study that has been made on the TC. However, authors such as Hill[37] mention that each cranial nerve may have a fundamental influence that can modulate the gustatory function.

4. The first relay of the gustatory system in the CNS

The STN is the first relay of the gustatory system and it conveys information from afferent axons that innervate the taste buds in the tongue. The STN is a very complex nucleus because at this level information is combined related to basic respiratory, gastro intestinal and gustatory systems necessary for newborn

survival in the nest. This nucleus is generated during gestation, and at birth it exhibits rapid neuronal growth, making it a good model for studying the development of the plastic properties and their age-related changes.

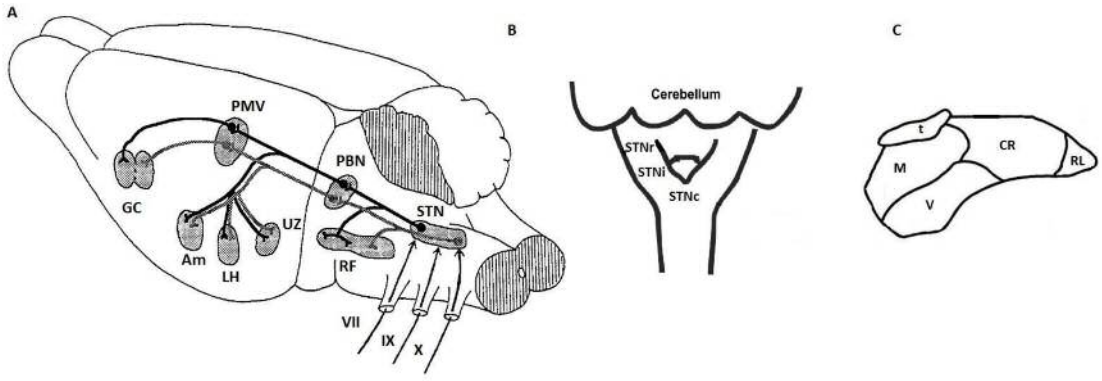

Figure 3. A). Schematic drawing of the central taste pathways in the rat. VII, facial nerve, IX, glossopharyngeal nerve, X, vagus nerve; STN, solitary tract nucleus; RF, reticular formation; PBN, parabrachial nucleus; UZ, uncertain zone. LH, lateral hypothalamus; Am, amygdala; PMV posteromedial ventral thalamic nucleus, GC, gustatory insular cortex (Modified of Yamamoto et al. 1998). B) Schematic representation of the dorsal surface of the medulla indicating the different areas of the nucleus of solitary tract. STNr, i and c; solitary tract nucleus rostral, intermediate and caudal. C). Horizontal subdivision of the STNr showing the subnuclear organization, t, solitary tract; M, medial subnuclei; CR, rostral central subnuclei, RL, rostral lateral subnuclei and V, ventral subnuclei.

4.1 Anatomical characteristics of the STN

The STN is located in the bulbar area of the brainstem (Figure 3). Taking the vertex of the head as reference it lies in the rostral portion and at coordinates –10.52 to 13.24.[45] The somatic sensitive column of trigeminal and glossopharyngeal nerves is adjacent to the STN; the vestibular lateral nerve as well as the vestibular medial nerve are dorsal, and the reticular parvocellular nucleus is ventral to the STN.

In the adult rat, the STN is considered an integrative station of very complex information, and for research purposes it has been divided into three main areas: the most rostral part, denominated the STNr, receives special visceral afferent information from the gustatory receptors in the tongue and epiglottis and conveys it to the facial, glossopharyngeal, and vagus nerves. The intermediate (STNi) and caudal (STNc) areas receive information from the cranial glossopharyngeal (IX), vagus (X), and trigeminal (V) nerves, which are responsible for the general visceral afferents, including information from chemoreceptors (IX), baroreceptors (X), pulmonary distension receptors (X), intestinal receptors (X), and mechanoreceptors (V).[46-50] The gustatory portion of STNr can be defined electrophysiologically by the location of neurons that respond electrically to taste stimulation or anatomically by distributing neuronal axonal branches that convey gustatory information. The gustative area is expanded from the rostral end of the STNr to the medial edge of the nucleus as far as the fourth ventricle (lateral 2.72 mm).[45]

Recent studies have shown that the STNr is made up of 4 sub-nuclei that are named with reference to the solitary tract (ST), which crosses exactly over the center of the nucleus from the caudal to the rostral portions. The sub-nuclei that constitute this structure are: the central rostral (CR), the lateral rostral (LR), the ventral (V), and the medial (M) (Figure 3).[50]

The CR sub-nucleus contains neurons that receive information from taste buds whose

peripheral afferent fibers convey information via the glossopharyngeal, facial, and major superficial petrosal nerves. Studies using neuronal stains have shown that neurons of this area relay gustatory information, since they send axonal ascending fibers and information to the next neuronal relay, the parabrachial nucleus (PBN). The LR subnucleus is the main site of tactile input, which is carried by the trigeminal nerve that innervates the taste buds of the tongue, bringing somatosensorial information to this portion of the nucleus. Sub-nucleus V is the main origin for STNr projections to motor centers in the brainstem such as the facial motor nucleus, the glossopharyngeal, and the vagus dorsal motor nucleus. The M sub-nucleus plays an important role in intra-nuclear communication between the caudal and gustatory areas, suggesting that the information coming from the external medium into the gustatory area interacts with that generated in the internal medium (Figure 3).[50]

4.2 STN afferents and projections

Gustatory receptor activity is transmitted into the brainstem along three cranial nerves. One is the tympanic chord, which is an anastomotic union between cranial nerves VII and VIII that gather the information from the front two-thirds of the tongue, and specifically, from the fungiform gustatory papillae, from a population of small buds in the buccal wall of the sublingual organ, and from some of the foliated papillae. The second is the glossopharyngeal nerve, which transmits the information of the back third of the tongue, coming from the circumvallate gustatory papillae and the rest of the foliated papillae. The third is the vagus nerve, which gathers the information from the epiglottis, part of the palate, and the upper portion of the esophagus.[51, 52]

The first order neurons gathering the different gustatory modalities originate in the papillae and have their cell bodies in the peripheral ganglia: the geniculated, petrosal, and nodose ganglia located at the cranial cavity entrance. The central branches of these ganglionic neurons penetrate the brainstem at the bulb level where they make the first synaptic contact with the STNr neurons.[53]

The STNr efferent neurons in rodents project ipsilaterally to the PBN dorsal middle area at the pontine level, where a topographic layout in this nucleus has also been described[53] The PBN efferents project ventrolaterally to the uncertain area over the internal capsule to connect with the ventral portion of the forebrain, to the central nucleus of the amygdala, to the red nucleus, and to the terminal groove. Other neurons project ipsilaterally to the thalamus as far as the ventral posteromedial nucleus, and the thalamic neurons project to the agranulocytic part of the insular cortex near the zone of the tongue (Figure 3).

Some of the STNr neuronal axons cross to the opposite side near the thalamus and the pontine area, giving a counterlateral character to the gustatory tract. Like other nuclei involved in the gustatory tract, ascendant neuronal relays also show a "taste-topic" distribution of the gustatory information.[54]

4.3 Types of neurons in the STNr

The STN is a reticular-shaped structure formed by different types of neurons. Knowing the cell types of any structure helps to understand the relationship between the morphology and the function of the cells of neuronal circuits. Diverse methodological strategies have been used to classify the STNr cells. However, the staining strategies that have been used (Nissl, biotin, Golgi-Cox, and rapid-Golgi procedures) to visualize the shape, size, orientation, dendritic distribution, projection site, etc, are still controversial. These stains can determine the morphology of cells in the STN but cannot show whether these cells respond to gustatory stimulation or how differences between cells may affect the functional properties of the gustatory response. In order to solve this problem, electrophysiological studies were made to identify which neurons respond to chemical stimulants placed on the back of the tongue; responsive neurons can also be marked by

using neurobiotin, which allows visual recognition of the cell morphology.[55]

The cell types which have been described by these techniques are: multipolar neurons, which are triangular or polygonal in shape with 3 to 5 primary dendrites; fusiform neurons, characterized by an elongated soma and two main primary dendrites arising at opposite poles; and small ovoid neurons having 2-4 thin primary dendrites (Figure 4). Using the Nissl or Golgi techniques, multipolar neuronal subgroups can be identified, and these are subdivided into large, small, and ovoid shapes which have a similar subdivision.[54, 56-61]

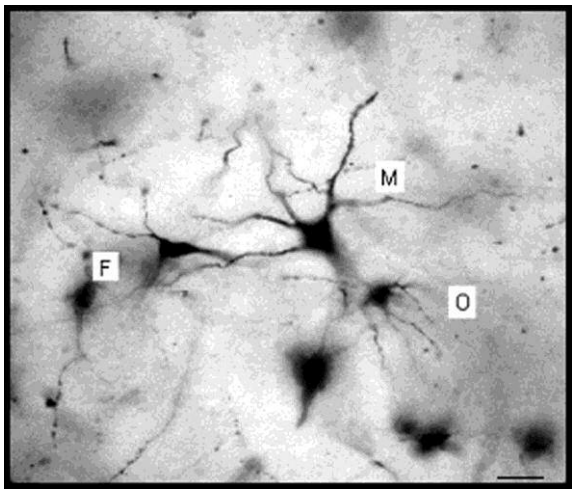

Figure 4. Photomicrography of multipolar (M); ovoid (O) and, fusiform (F) neurons of the STNr, stained with the Golgi-Cox technique. Calibration, 50μm.

The most common neurons in the STNr are ovoid (63%) and fusiform (19%), and the remaining 18% are multipolar.[58] Based on techniques with neuronal tracers, multipolar and fusiform neurons have been observed projecting to the PBN.[54, 57] Likewise, there are multipolar neurons in the ventral part of the STN that send information to the reticular formation and to the motor nuclei of the cranial nerves (V, VII, IX, X, and XII).[62-64] It has been suggested that the neurons projecting rostrally are involved in the processing and relaying of gustatory information. Meanwhile, the caudal projections are thought to be involved in the reflex control of saliva secretion and food ingestion.[64] Ovoid neurons are believed to be local, interconnecting interneurons that modulate the nucleus output.[57]

4.4 Neurogenesis and neurochemical development of the STN

The STN layout in adult mammals has been widely studied,[65-69] but to our knowledge there is little information about the development of the morphofunctional properties of this structure. Studies of STN development are important, because the neuronal information comes from critical areas that are physiologically necessary for newborn survival, such as the respiratory, cardiovascular, and digestive systems.[70]

The first ontogenetic studies were made by Altman and Bayer[71] using the ^{3}H-thymidine-radiographic technique. They reported that neurons reaching the STN are generated between gestational day 11 (E11) and E14, with a peak of neurogenesis on E12. From recent studies it is known that at birth, the primary afferents to the STNr are organized in a viscerotopic pattern equivalent to the adult stage. The afferents of the facial and vagus nerves reach the STNr by embryonic day 17 (E17), and on E19 they show a mature, organized pattern.[70] In the case of the glossopharyngeal nerve there is a controversy, since Lasiter[72] mentioned that in the rat the glossopharyngeal does not reach the STNr until 9 or 10 days after birth. Recently, the Zhang's group was unable to mark the glossopharyngeal nerve during the embryonic period because of its proximity to the vagus nerve; they suggest that it may follow a developmental pattern similar to that of the facial and vagus nerves, but this has not yet been demonstrated. These differences may be due to the anatomical techniques used by the two groups. The afferents of most of the information sources to the STN are well represented before the last differentiation period (E17 to E19), when the chemical properties are established.[70] It is possible that the input from these afferents may be responsible for triggering the rapid STN differentiation.

Immunohistochemistry and histochemical studies of neurochemical development have demonstrated the presence of acetylcholinesterase in the STN between E15 and E17. Immunoreactivity for calbindin and calretinin appears in later stages of gestation with a peak at postnatal day P10. Neuron immunoreactivity for tyrosine hydroxylase was recognized on E15, showed rapid differentiation on E17, and reached the adult pattern on day E19. Immunostaining for substance P showed an adult distribution pattern on E19.[70]

These results indicate that the patterns of immunohistochemical development differentiate rapidly between E15 and E17, remaining more stable by E19. This suggests that the morphologic and chemical features of the STN are present even before birth; thus, the nucleus is prepared to be involved in its vital functions at the time of birth.[5, 6, 70, 73-75]

The peaks that are observed for each marker may represent an accelerated development of the connections and essential circuits in the STN associated with the primary necessities for postnatal survival (for instance, respiratory, cardiovascular, and digestive tract functions). Furthermore, the postnatal peaks may suggest additional elements that are required to establish paths with essential connections, or formation of routes related to non-essential behaviors during postnatal life, for example in the gustatory system, the plastic changes underlying the switching of pups from liquid to solid food intake.

4.5 Synaptogenesis in the STNr

Although some synaptic buttons have been observed on E17, these structures in the afferents to the STN develop mainly on E19, followed by the beginning of chemical differentiation as described by Zhang and Ashwell.[69] This developmental period corresponds to the initial architectonic differentiation of the STN. It is possible that the maturation of the synaptic terminals may be related to the neurophyllum organization.

The gustatory glomeruli are highly preserved units constituted by the afferents that convey information into the STN neurons from different brain sources, including the pulmonary, laryngeal, and taste afferent fibers.[48, 70, 76, 77] Zhang and Ashwell[69] did not observe any taste glomeruli during the embryonic period or the first weeks of life in the rat. Therefore, they speculate that at birth, most STN primary functions, including cardiovascular control, may be modulated by simple circuits that have not matured to form synaptic glomeruli. The postnatal development of the synaptic glomeruli may lead to numerous changes in the layout of STN connections, which may be modified in accordance with postnatal needs and the plasticity of the organism.[69] The glomeruli units, whose formation and function begin prenatally and whose maturation is complete after the first postnatal weeks, are a feature of the adult stage and are used for chemosensory functions. In this regard, the pre- and neonatal STN functions may be regulated by means of axodendritic afferent signals from the gustatory receptors to the STN neurons.

4.6 Neurotransmitters involved in STN function

Neurotransmitters and their precursors in the STN have been identified by immunostaining techniques, revealing that both neurons of the peripheral ganglia where the cellular bodies are located and the neurons carrying information into the STNr contain substance P, tyrosine hydroxylase, vasoactive intestinal polypeptide, calcitonin gene-related peptide, galanin, glutamate, and aspartate.[78-82] It is not surprising that glutamate and GABA are found as neurotransmitters and neuromodulators in the STNr.[83] Glutamate is released from gustatory afferents,[84] and it is also contained in STNr neuronal bodies and in some of the neuronal projections to the PBN.[85] Immunostaining for GABA can also be detected in the STNr, mainly in the small ovoid neurons, which are thought to be inhibitory interneurons.[56, 86, 87]

Through retrograde labeling techniques, it has been shown that dextran injection into the central nucleus of the amygdala (CeA) labeled

fibers ending at different locations in the STFNr,[88] in the medial, central, and ventral part of this structure.[89] It also was found that after the injection of cholera B toxin into the STN, many cells in the central amygdala are labeled. Generally, the influences of the descending fibers to the fore brain are excitatory. However, it has been shown that these amygdala fibers have an inhibitory effect in the rat.[90-92] In the rat there is evidence that this projection is GABAergic, suggesting that in some way it may be modulating primarily local connections, with less effect in the tract that carries the gustatory information to upper neuronal relays.[93]

The gustatory process may be modulated by descending information from different nuclei of the fore brain as a result of sensorial experience. Opioid receptors, which receive information from central amygdala neurons, are also expressed in the STNr, suggesting another possible modulation of the gustatory information in the STN. Also found in the STN are receptors for oxytocin and the catecholamines that come from the hypothalamic structures and possibly modulate the hedonic aspects elicited by taste cues.[92, 94-96]

4.7 Gustotopic organization in the STN

In mammals, most parts of the CNS are constituted by maps that represent the receptor layout. These maps in the cortex, as in other parts of the brain, arise during ontogeny as a result of interactions between numerous factors. Several behavioral investigations indicate that altricial mammals have a functional gustatory system at the time of birth, before the neuronal substrates attain full anatomical maturation. The gustatory system develops during gestation and acquires a well-advanced organization in the days just prior to birth, then passes through a neonatal period of rapid development. The most evident feature of this system is the receptor layout in different parts of the tongue.

In mammals the somatosensory cortex is organized according to the location of the receptors over the body surface; this representation seems to arise during development as a result of experience or local factors that participate to different extents. It is also known that the cortical representation is retained in other subcortical relays. This conclusion comes mainly from studies of electrical stimulation of the somatosensory and motor cortex and from cortical lesions.[97, 98] More recently, such investigations have been extended to the auditory and visual pathways, and the results are very similar to those in the somatosensory and motor cortical representations.[99-100] Currently, the gustatory pathway is considered an important model to study the anatomical and functional organization of the chemosensory systems. Electrophysiological studies show that the nuclei involved along the gustatory tract maintain an organization in response to taste stimulation.[50]

Immunohistochemistry techniques that detect expression of early genes such as c-Fos in response to specific stimuli have been used to show the topographic organization in the olfactory, somatosensory, and visual system areas. The first studies using these techniques in the gustatory system showed that sucrose and quinine induced c-Fos expression in the STNr, with expression greater in the medial part of the nucleus in response to quinine, and greater in the lateral part in response to sucrose.[101-103] Recently, a group of investigators sought to identify the specific area that induces c-Fos in response to quinine and to determine the extent to which its expression is modified by the intensity of the gustatory cue. After application of quinine at three concentrations, immunostain was again observed in the STNr medial area, and it was similar at all three concentrations.[104]

In similar experiments, it was found that the STNr lateral area responds to 0.1 M citric acid, and by means of a correlation analysis, it was determined that c-Fos is expressed in completely different areas for quinine and citric acid. The data showed that the correlation of c-Fos expression with location at the different quinine concentrations is very high (between 0.95 and 0.99), whereas between quinine and citric acid it is much lower (0.29), suggesting

that the expression areas for these stimulants are different. On the other hand, when applying 0.3 M NaCl and counting the distribution of c-Fos immunoreactive neurons in the STNr, the number of stained neurons is quite similar to or lower than number labeled when water was applied. Areas labeled in response to NaCl and citric acid showed a higher correlation (0.84) (Figure 5).[104]

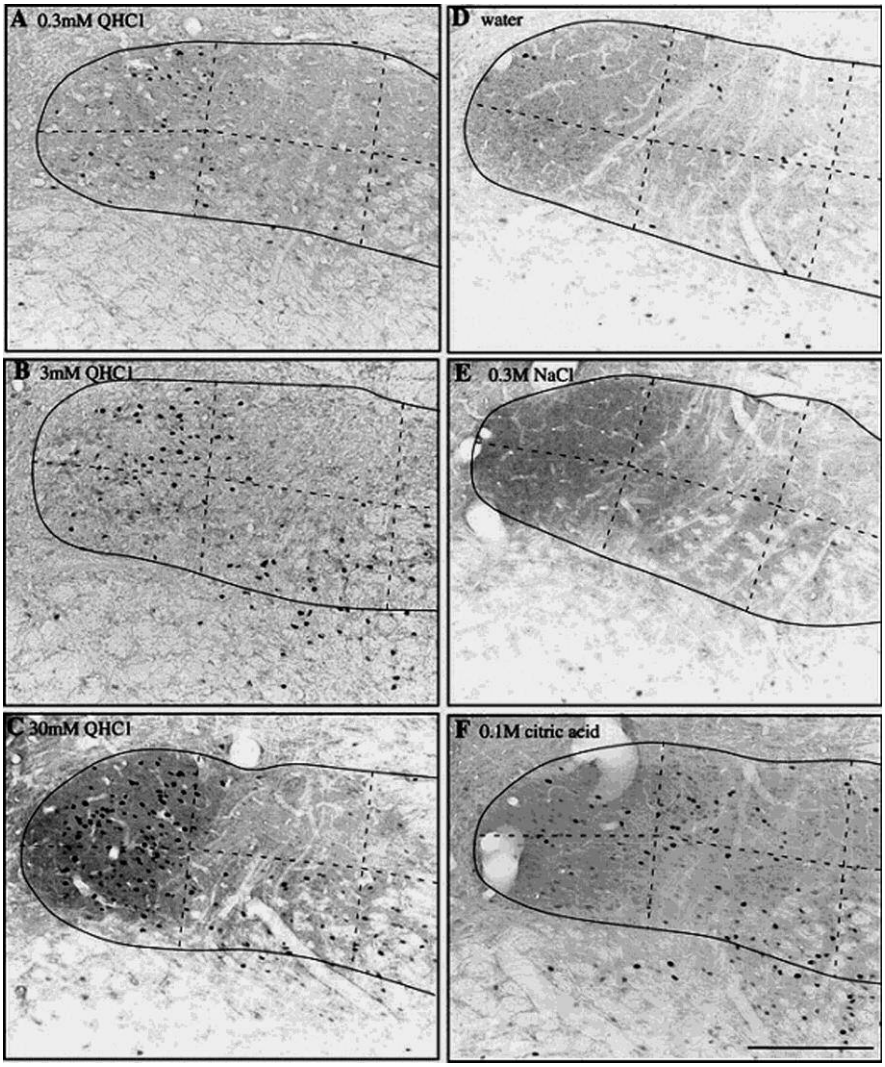

Figure 5. Distribution of immunoreactivity for c-Fos in the STNr after a period of 45 min. of different gustatory exposures. Calibration, 200 μm. Extracted from (Travers et al., 2002).

These findings indicate that water may serve as a control stimulant, since it is an insipid stimulation that maintains the fluidity characteristics of other cues. They also show that cells marked in the STNr after stimulation with water are cells that respond to mechanical stimulation applied to the back of the tongue. It is noteworthy that NaCl does produce lower c-Fos expression, which suggests that not all cells of the STNr respond to a specific stimulation; this has also been shown in the somatosensorial system, since it has been observed that signals that travel along small-diameter adherent fibers induce more Fos

expression than signals following a more complex axonal system. This suggests that Fos expression varies with the complexity of the neuronal connections.[105]

5 Early environmental influences on the gustatory system

In many sensorial systems, the normal function and morphologic maturation along the ascending neuronal relays depend on the appropriate type of stimulation and the selective experience obtained during well-defined developmental periods.[30, 106] It is also important to highlight the information obtained about the underlying processes that are necessary for normal development.

In the literature there is abundant information on the somatosensory, auditory, and visual somatotopic cortical organization obtained mainly from experiments of local electrical stimulation of peripheral receptors.[107, 108] Unfortunately, little is known concerning the effects of perinatal sensory stimulation and specifically in the gustatory system. The normal ascending patterns of sensory information are crucial for the establishment and maintenance of adequate connectivity patterns and for the integrative processes taking place at the neuronal levels.[108-109]

5.1 Alterations in the STNr by sodium restriction during gestation in the rat

In relation to the effect of sensorial stimulation on the gustatory pathway, it is known that Na+ restriction (0.03% NaCl in the diet), starting on day 8 after conception and continuing throughout development, noticiably reduces the neurophysiological response to NaCl in the tympanic cord. This response is reduced in more than 60% in the restricted animals compared to controls whose diet was normal (approximately 1.0% NaCl). For comparison, the TC responses to NH_4Cl and other stimulants were not affected by Na+ restriction in the maternal diet.[110] The same authors reported in 1991 that NaCl deprivation influences TC terminal fields and the STNr. Thus, the groups that were under sodium restriction showed irregular and larger shapes in TC terminal fields than the controls, and even after restrictions longer than 60 days, restoring NaCl to the diet can reverse the damage at the TC level. However, other studies indicate that the functional nerve recovery is not sufficient to promote anatomical restoration.[111, 112]

The lack of neuronal information reaching the TC during development may contribute to the neurophysiological changes observed in this structure, since the activity produced by sodium administration is essential to form an adequate terminal field.[59, 112]

5.2 Alterations produced in the STNr by perinatal undernutrition

Regarding the effects of sensory and food intake restriction on the gustatory sensorial channel development, the available information is scarce. In particular, the question, to what extent the effects of malnutrition may influence the anatomic and functional organization of the STN, has been ignored, in spite of the fact that it is one of the most significant relay areas of the brainstem on the route of neural impulses to the cerebral cortex.[57, 110]

When neonatal food is restricted during periods of rapid brain development, the presence of taste substances in the mouth is significantly reduced, causing (not only the lack of food, but also) a decrease of gustatory stimulation. Similar conditions of reduced content will prevail in the rest of the digestive tract, with possible consequences of reduced afferent information reaching the caudal and intermediate STN regions.

Using the model of perinatal food restriction in rats at different gestational ages by reducing the food intake of pregnant females, and neonatally by placing pups for 12 h of each day with a nipple-ligated mother and 12 h with a normally lactating mother, we found that in the malnourished group, the STNr neurons become hypotrophic compared to the controls. Furthermore, interneurons showed fewer and shorter dendritic prolongations. In a rehabilitated group with restricted food before birth but normal food intake during the

lactating period, the neuronal morphology was similar to that of controls.[113]

Prenatally malnourished subjects, fed and cared for by a pair of normal "wet nurse" mothers (rehabilitation), revealed interesting aspects of STNr neuronal plasticity. Thus, the finding of a larger number of branches in the distal parts of the dendritic trees of STNr neurons contrasts with the result obtained in malnourished animals with no postnatal rehabilitation. On the other hand, in prenatally malnourished groups either with or without rehabilitation, the dendritic extensions are larger in the distal portion of the dendritic tree than in controls, suggesting a possible compensatory mechanism of a plastic nature. This interpretation is supported by the "covering" and "tiling" phenomena by which a neuron's dendrites of the same functional group extend to cover nearby zones where neuronal death or damage occurred in an adjacent dendritic tree.[114]

Taken together, this information shows that gustatory stimulation in early stages of life is necessary to induce normal neuronal development of the STNr.[71-113] Later, during the lactating period it may accelerate taste bud development and promote neuronal maturation.[115]

II. Conclusions

The experimental findings included in this review allow us to appreciate the vast scope of the field of gustatory physiology; technological progress has generated original and novel information that has been used to identify new basic neuronal mechanisms of chemoreception. For instance, now it is undeniable that the uterine environment is an important source of sensorial experience for the fetus and that the gustatory and olfactory signals from the amniotic fluid contribute to prenatal brain development. It is also evident that in altricial species, neuronal substrates are already precociously developed at birth in order to satisfy the basic needs and survival of the newborn. The time of birth is the critical stage for obtaining early experience and plastic capabilities of brain tissue to be used later in life.

Another important contribution to the knowledge in this field is the developmental characterization of gustatory afferents, since this allows appropriate timeframes to be selected for a specific study of the structures involved in gustatory signal transduction, such as ion channels and receptors. This characterization also determines the correlation between the afferent connectivity and the specific neuronal activation by different chemical compounds at critical ontogenetic stages of the gustatory pathway.

The gustotopic organization is another important line of research that has been studied recently in order to determine whether the neuronal relays are anatomically and/or functionally organized for the different basic flavors (sweet, salty, sour, and bitter). As a result of these investigations, it has been suggested that in the STN, the cell layout that responds to basic flavor is segregated, and it may be related to the hedonic and behavioral characteristics resulting from stimulation by each flavor. On the other hand, it has been shown that not only the information from the stimulation of the oral cavity receptors by chemical stimulants, but also information coming from other sources (somatosensorial and visceral) has a complex influence upon the STNr neuronal substrate. These findings indicate that the basic mechanisms underlying the taste sensitivity for food intake are also operating during harmful or aversive food rejection as a part of the early gustatory experience.

Current studies seek to define the early stages when the STN gustatory layout is established and to determine if they can be altered by exposure to different epigenetic factors. It will also be important to discover how the dietary change from breast milk to solid food causes anatomical and functional changes of the plastic brainstem taste organization. This will help to establish the activation time of STNr neuronal sensitivity to basic flavors and critical ages for neuronal and anatomical organization, and to study the plastic neuronal properties associated with chemoreception in both normal and altered perinatal conditions.

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