Activation of Neurons in the Rat Medulla Following a Gag Reflex Stimulus

Donna Scarborough[1*] and Lori Isaacson[2]

[1]*Department of Speech Pathology and Audiology, Miami University, Oxford, OH 45056, USA*

[2]*Department of Biology, Center for Neuroscience and Behavior, Miami University, Oxford, OH 45056, USA*

Abstract: Despite the fact that a number of individuals present with a hypersensitive gag reflex, research in this area has been limited to clinical applications. An animal model that describes the neurologic underpinnings of the gag reflex has not been reported. Thus, the objective of the current study was to localize activated neurons in the rat medulla and to investigate their neurochemistry following mechanostimulation to the posterior pharyngeal wall eliciting a gag reflex response. Activated neurons, determined using c-fos immunohistochemistry, were observed in subnuclei of the nucleus tractus solitarius (NTS) and motor regions such as dorsal motor vagus, hypoglossal, and nucleus ambiguus. The lateral and dorsal paragigantocellular nuclei and the medial rostral ventrolateral nucleus involved in bitter taste processing, also showed robust activation. Tyrosine hydroxylase and/or choline acetyltransferase immunoreactivity was localized in many activated neurons. The results of the present study provide the first documentation of activated neurons and preliminary neurochemistry in brainstem nuclei that are unique to mechanostimulation to the posterior pharyngeal wall. Our results support postulated medullary structures involved with the gag reflex and propose new regions to be added to the circuitry model. Further, our results reveal an activation pattern within the NTS unique to the gag reflex.

Keywords: Brain stem, Nucleus of the solitary tract, neuroanatomy, animal model, pediatric feeding.

INTRODUCTION

The gag reflex is considered a "simple" (i.e. short latency) reflex that protects the pharynx from unwanted material [1]. A rudimentary neurological pathway of the gag circuitry has been postulated [2-4]; however, the specific brainstem nuclei as well as the neurochemicals involved are not well understood. Furthermore, the current depth of understanding of the mechanisms involved with the reflex does not explain the large variability (diminished to hypersensitive) in afferent processing within the general population [5, 6], concomitant responses such as vomiting, nausea, diaphoresis, and lacrimation [7], or reports of different degrees of efferent responses, from absent to hyperactive [5, 8-10].

A hypersensitive gag reflex in neurologically intact children and adults within general and special populations can lead to interference with dental examinations, food avoidance behaviors, and difficulty in swallowing pills [7, 11-13]. Severe food restrictions leading to gastrointestinal issues, failure-to-thrive, and malnutrition have also been observed as a direct result of a hypersensitive gag. We have proposed the existence of a hand pressure point that, when activated, alters the afferent limb of the gag reflex in hypersensitive adult individuals, and subsequently alleviates the hypergag reflex [5]. However, the underlying mechanisms for these alterations are unknown.

*Address correspondence to this author at the Department of Speech Pathology and Audiology, Miami University, Oxford, OH 45056, USA; Tel: (513) 529-2506; Fax: (513) 529-2502; E-mail: scarbod@miamioh.edu

Indeed, despite the fact that a hypersensitive gag reflex affects a number of individuals in the general and clinical populations, there is little known regarding the neurological mechanisms underlying this aberrant reflex. We recently developed a theoretical model that involves changes in the brainstem region, nucleus tractus solitarius (NTS), during development, to explain the existence of a hypersensitive gag reflex within a special population of children [14]. Yet in order to understand the underlying mechanisms in an altered gag reflex, we must first determine the basic processes involved following a pharyngeal stimulus. In the present study, we conducted experiments in adult rats to determine the location of activated neurons in the medulla following a stimulus that consistently elicits a gag reflex. We report here the existence of activated neurons in the rat medulla following mechano-stimulation of the posterior pharyngeal wall and a response that, based on motor output, resembles a gag response. Further, a preliminary report describing the neurochemistry of some of the activated neurons is presented. These findings represent the first report describing the location neurons that are involved specifically in the gag reflex resulting from mechano-stimulation of the posterior pharyngeal wall.

MATERIALS/METHODS

Procedures to Elicit the Gag Reflex

The procedures used in this study were approved by the Miami University Institutional Animal Care and Use Committee (IACUC). Male Sprague Dawley rats

E-ISSN: 2311-1917/14

(sp. Rattus rattus; n = 12), approximately 4 months of age, were sedated with acepromazine (0.05 mg/kg) administered subcutaneously. Prior to data collection and analysis, this dosage was determined through a series of pilot studies. This dosage sedated the animal just enough to tolerate the procedure while still allowing for successful responses to stimulation of the posterior pharyngeal wall. The time frames developed for the study were adapted from Rogers and colleagues [15].

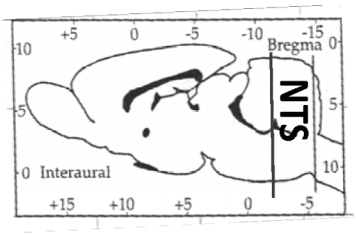
The subject was placed in a supine position in the lap of a trained handler. Immediately preceding each stimulus, the subject was propped upright and the jaw was stabilized. Stimulation to the posterior pharyngeal wall was carried out by an individual with extensive experience in eliciting the gag reflex in human subjects. Stimulation was carried out using a 20 gauge, 1.5" curved feeding needle with an attached 2.25 mm ball (Braintree Scientific, Inc., Braintree, MA, USA) that was passed laterally along the right or left side of the oral cavity. The side of activation was randomized. A positive response was recorded when simultaneous wide jaw excursion, vigorous anterior tongue protrusion, abdominal contraction and thoracic fixation were observed. The stimulus was attempted at 30 s intervals over a 30 min testing period. This timing was chosen to provide a rest period between gag-like responses (30 s), thus minimizing any potential extinction or fatigue effects. Following an additional 30 min rest period, the animals (n=7) were sacrificed by aortic perfusion for histological analysis of the brain. Sham animals (n=3) were treated as described above except that only the hard palate just posterior to the incisors was stimulated with the feeding needle. The stimulation pattern for the sham group included intraoral stimulation in order to replicate any potential cardiovascular changes that might occur following the insertion of a foreign object (a probe) intraorally. Control animals (n=2) were housed and weighed with the other animals, but received no stimulation, and otherwise were not handled.

Animal Sacrifice and Immunohistochemical Processing and Analysis

Animals were anesthetized with an overdose of sodium pentobarbitol (125 mg/kg, administered intraperitoneally) and then perfused through the heart with 50 ml 0.9% saline followed by 250 ml of 4% paraformaldehyde (Acros Organics, Somerville, NJ, USA) in 0.1M PO_4 buffer (PB). The brain was removed and blocked 2 mm posterior to the cerebellum (to incorporate the NTS) and stored in PB until use. In preparation for sectioning, the block was placed in 30% sucrose in PB overnight. Frozen sections (45 µm) in the coronal plane were cut using a sliding microtome and stored in PB. Specific brain levels used for immunohistochemical staining were identified in adjacent Nissl-stained sections using anatomical landmarks as described in Paxinos and Watson [16]. Sections from similar brain levels were processed together and each immunohistochemical experiment included sections from both gag-like and sham treatments.

The localization of c-fos neurons was initially carried out using diaminobenzadine (DAB) immunohistochemistry. For staining of activated neurons, floating frozen sections in individually labeled vials were treated with a solution of 0.5% Triton-X and 1% normal goat serum (NGS; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) diluted in 0.1M phosphate-buffered saline (PBS) at 4 °C for 24 h. Sections were incubated at room temperature in 3% NGS-PBS for 30 min and then placed in primary antibody (rabbit anti-c-fos; 1:750; EMD Chemicals, Gibbstown, NJ, USA) for 48 h at 4°C. Following several washes with PBS, sections were incubated in goat anti-rabbit biotinylated secondary antibody (45 min; Vector Laboratories, Burlingame, CA, USA) diluted in PBS, rinsed with PBS, and incubated with Avidin-Biotin Complex (45 min; Vector Laboratories, Burlingame, CA, USA). Following rinses in PB, the reaction product was visualized using a diaminobenzadine glucose oxidase (Polysciences, Inc., Warrington PA, USA; Sigma-Aldrich, St. Louis, MO, USA) reaction. Sections were mounted on labeled glass microscope slides and coverslipped. As a control, the primary antibody was omitted in selected sections, but otherwise the sections were treated similarly.

Immunofluorescence was carried out on additional sections to simultaneously localize activated (c-fos) neurons and neurochemical markers using confocal microscopy. Two different neurochemical markers were used in this study: 1) tyrosine hydroxylase (TH), the rate limiting enzyme for catecholamine biosynthesis; 2) choline acetyltransferase (ChAT), an enzyme that catalyzes the biosynthesis of acetylcholine. The procedure was similar to the c-fos technique described above but with some modifications. Sections were treated with a solution of 0.5% Triton-X, 1% normal donkey serum (NDS; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) and then incubated at room temperature in 3% NDS-PBS for 30 min prior to application of the primary antibody. Sections then were incubated for 48 h in a cocktail of



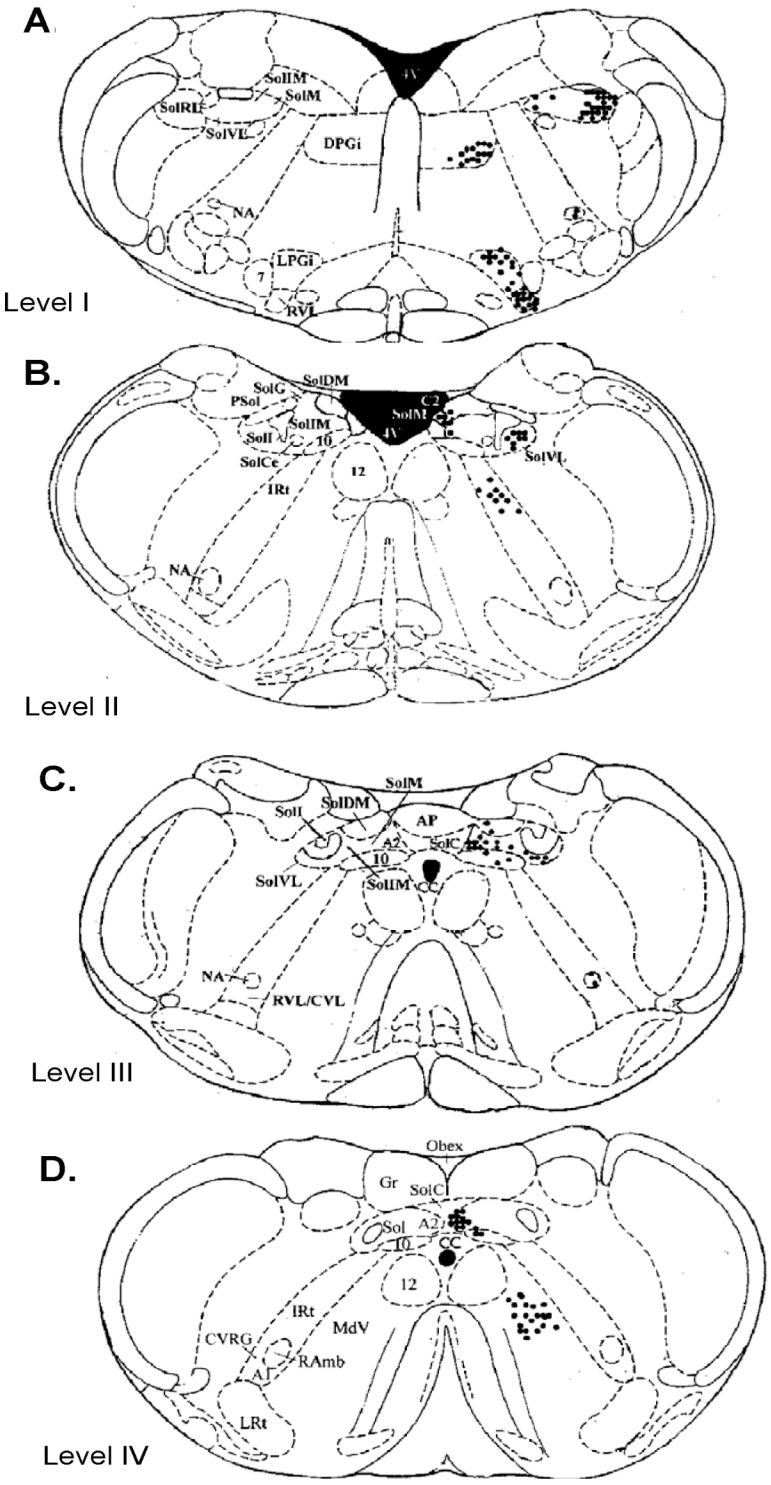


Figure 1: Line drawings depicting the location of activated neurons at four rostral-caudal levels of the medulla oblongata. As shown in the inset, these four Bregma levels define the rostral-caudal limits of NTS [16]. Note drawings only depict activation on one side although activation was observed bilaterally.

A. The most rostral level (Level I) analyzed was Bregma -11.80 to -11.30 mm. Activated neurons were observed in subnuclei of NTS, and DPGI, as well as, ventrolateral regions such as LPGi and RVL.

B. Level II, from Bregma -13.24 to -13.30 mm, showed activated neurons in the hypoglossal nucleus (12), dorsal vagal complex (10), as well as in subnuclei of NTS, IRt, and NA.

C. At Level III, Bregma -13.68 to -14.08 mm, activation was scattered in 10, as well as in subnuclei of NTS. Activated neurons also were observed in NA and RVL.

D. The caudal level was taken from Bregma -14.30 to -14.60 mm. Activation was robust in Sol, nuclei of 10, 12, as well as in MdV and LRt. See Table **1** and text for details and explanation of abbreviations. Based on the atlas of Paxinos and Watson [16].

primary antibodies (goat anti-ChAT, Chemicon AB144P, Billerica, MA, USA; 1:500; rabbit anti-c-fos, Calbiochem, San Diego, CA, USA; 1:750, mouse anti-TH, BD Transductions Laboratories, San Diego, CA, USA; 1:1000). Following washes, sections were incubated for 2 h in a cocktail of appropriate secondary antibodies (Alexa 488 donkey anti-goat; Alexa 555 donkey anti-mouse; Alexa 694 donkey anti-rabbit; all at 1:200 dilution; Invitrogen Corporation, Carlsbad, CA, USA) were diluted in PBS. Sections were rinsed with PBS, mounted on labeled glass microscope slides and coverslipped with Gel/Mount medium (Biomeda Corp, Foster City, CA, USA) and viewed using confocal microscopy.

Analysis of Activated Neurons

Medullary sections taken through the rostral caudal extent of the nucleus tractus solitarius (NTS; Bregma levels -11.00 mm to -14.60 mm; Figure **1** [13] were processed for c-fos immunohistochemistry to localize activated neurons in the gag, sham, or control animals. Positive activation was restricted to neurons showing nuclear immunostaining. Documentation of activated neurons was carried out at four rostral-caudal levels of the medulla oblongata as these levels provided the most accurate and thorough representation of the NTS subnuclei: **Level I**: rostral level (Bregma -11.80 to -11.30 mm; Figure **1A**), **Level II**, mid-medullary level (Bregma -13.30 to -13.24 mm; Figure **1B**), **Level III**, level of the area postrema (Bregma -14.08 mm to -13.68 mm; Figure **1C**), and **Level IV**, caudal level (Bregma -14.30 mm to -14.60 mm; Figure **1D**). The accuracy of each level was verified by examining the cytoarchitecture of the adjacent Nissl stained sections.

Activated neurons were documented using bright field (for DAB sections) and confocal (for immunofluorescence) microscopy. Similar activation patterns were observed using both the DAB and immunofluorescence procedures. Maps of c-fos immunolabeled neurons were generated based on activation patterns that were found in the experimental animals above our sham and control groups using templates based on the atlas of Paxinos and Watson [16], from which Figure **1** was generated. Significance testing was not considered due to the type of data we reported.

RESULTS

Stimulation of Gag Animals

Stimulation of the posterior pharyngeal wall elicited a gag reflex motor response that was characterized by simultaneous wide jaw excursion, vigorous anterior tongue protrusion, abdominal contraction and thoracic fixation. The number of gag responses per animal ranged from 16 to 24 (average= 19.6, SD 2.7) over a 30 min period following pharyngeal stimulation at 30 s intervals. Data were not collected from one of the 7 gag animals as it became progressively too sedated to respond to the pharyngeal stimulation. Sham animals showed no such response to stimulation of the hard palate just posterior to the incisors.

Location of Activated Neurons

Consistent background activation was observed in sham animals in the rostral and caudal ventrolateral medulla and the dorsal hypoglossal nucleus in more caudal levels (Level II, III, IV), DM nucleus of 10, parasolitary nucleus and DM NTS in Level II, and retroambiguus in Level IV. Comparable areas were also activated in the animals showing a gag response. Thus this pattern of activation is likely a result of the general movements and cardiorespiratory changes that took place during the experimental procedures. No activation was observed in the medulla from control, untreated animals. In addition, no activated cells were found in any sections in which the primary antibody was omitted from the staining procedure. Reported below are the specific areas of activated neurons that were observed only from animals that received mechanostimulation to the posterior pharyngeal wall, but not observed in the other conditions.

In rats in which a gag motor response was elicited, activated neurons were localized in specific medullary subnuclei of the nucleus tractus solitarius (NTS) as well as in select motor, reticular, and respiratory nuclei as described below. The maps shown in Figure **1** reveal the typical pattern of activation within these nuclei at four select rostral caudal levels through the medulla. A listing of the nuclei in which activated neurons were observed is provided in Table **1**.

In the NTS, activated neurons following the gag stimulus were located in several subnuclei (Table **1**; Figure **1**). Within more rostral medullary levels, distinct clusters of small activated neurons were observed in the ventral lateral subnucleus (SolVL; Levels I, II, III). Clusters of activation also were noted in the adrenaline (C2; Level II) and noradrenaline (A2; Level III) cells of the medial solitary subnucleus (Sol M). Activated neurons also were scattered throughout the intermediate (SolIM; Levels I, II, and III) and the dorsomedial (Sol DM; Level III) subnuclei. At more

Table 1: Location of Activated Neurons in NTS Subnuclei, Motor Nuclei, and Reticular, Respiratory, and other Nuclei Following Pharyngeal Stimulation. These Nuclei Showed Activation in Addition to Baseline Areas which were Seen in both Sham and Experimental Animals

NTS subnuclei:			
Level I (-11.80 to -11.30)	**Level II (-13.24 to -13.30)**	**Level III (-13.68 to -14.08)**	**Level IV (-14.30 to 14.60)**
Sol VL	Sol VL	Sol VL	A2 of Sol
Sol IM	Sol IM	Sol IM	
Sol M	C2 of Sol M	A2 of Sol M	
		Sol DM	
Motor nuclei:			
Level I (-11.80 to -11.30)	**Level II (-13.24 to -13.30)**	**Level III (-13.68 to -14.08)**	**Level IV (-14.30 to 14.60)**
NA		NA	
		DM 10	DM 10
Reticular, respiratory, and other nuclei:			
Level I (-11.80 to -11.30)	**Level II (-13.24 to -13.30)**	**Level III (-13.68 to -14.08)**	**Level IV (-14.30 to 14.60)**
Bo and C1 of RVL	IRt		MdV
LPGi			
DPGi			
MRVL			

Abbreviations: A2=noradrenaline cells; C1=adrenaline cells; C2=adrenaline cells; Bo=Botzinger complex; Sol= nucleus of the solitary tract; DM 10=dorsal motor nucleus of the vagal complex; NA=nucleus ambiguus RVL=rostrolateral reticular nucleus; IRt=intermediate reticular nucleus; MdV=ventral medullary reticular nucleus; LPGi=lateral paragigantocellular nucleus; DPGi=dorsal paragigantocellular nucleus; MRVL=medial rostral ventrolateral nucleus.

caudal levels a distinct cluster of activated neurons was observed in the nucleus of the solitary tract (Sol; Level IV), in the medial region known to contain noradrenaline cells (A2).

Neuronal activation in motor nuclei was observed throughout the medulla (Figure **1**; Table **1**). The nucleus ambiguus (NA) contained activated neurons at Levels I and III. Activation also was observed in the dorsal motor nucleus of the vagal complex (DM10) at more caudal levels (III, IV). At Level III clusters of activated neurons were observed in the ventral aspects of the hypoglossal nucleus (XII) with scattered activation throughout the other areas of the nucleus.

Several of the reticular nuclei showed activation as a result of the gag response. In the rostral medulla clusters of activated neurons were observed in the Botzinger complex and the adrenaline region C1 of the rostroventrolateral reticular nucleus (RVL; Level I). At this level, other clusters of activated neurons were observed in the lateral paragigantocellular nucleus (LPGi), the dorsal paragigantocellular nucleus (DPGi), and medial rostroventrolateral nucleus (MRVL). In addition, the intermediate reticular nucleus (IRt; Level II) and the ventral medullary reticular nucleus (MdV; Level IV) showed a cluster of activated neurons.

Co-Localization Studies

Co-localization studies of c-fos and ChAT (Figure **2**) revealed that a majority of the activated motor neurons in NA, DM 10, and XII also showed ChAT immunoreactivity. ChAT-immunoreactive (-ir) activated neurons also were observed in DPGi (Level I) and MdV (Level I). Co-localization of c-fos with TH (Figure **3**) revealed that all of the activated neurons neurons in Sol VL, Sol DM, and Sol IM (Level III) were TH-ir. A small proportion of the activated neurons observed in A2 of Sol M (Level IV) and in DPGi (Level I) also were TH-ir. Triple labeling studies for c-fos, ChAT and TH revealed that frequent activated neurons in the C2 region of Sol M were both ChAT and TH immunoreactive. In addition, activated neurons were present that did not co-localize ChAT or TH.

DISCUSSION

The results of the present study show for the first time that specific neurons in the rat brainstem are activated following mechanical stimulation of the

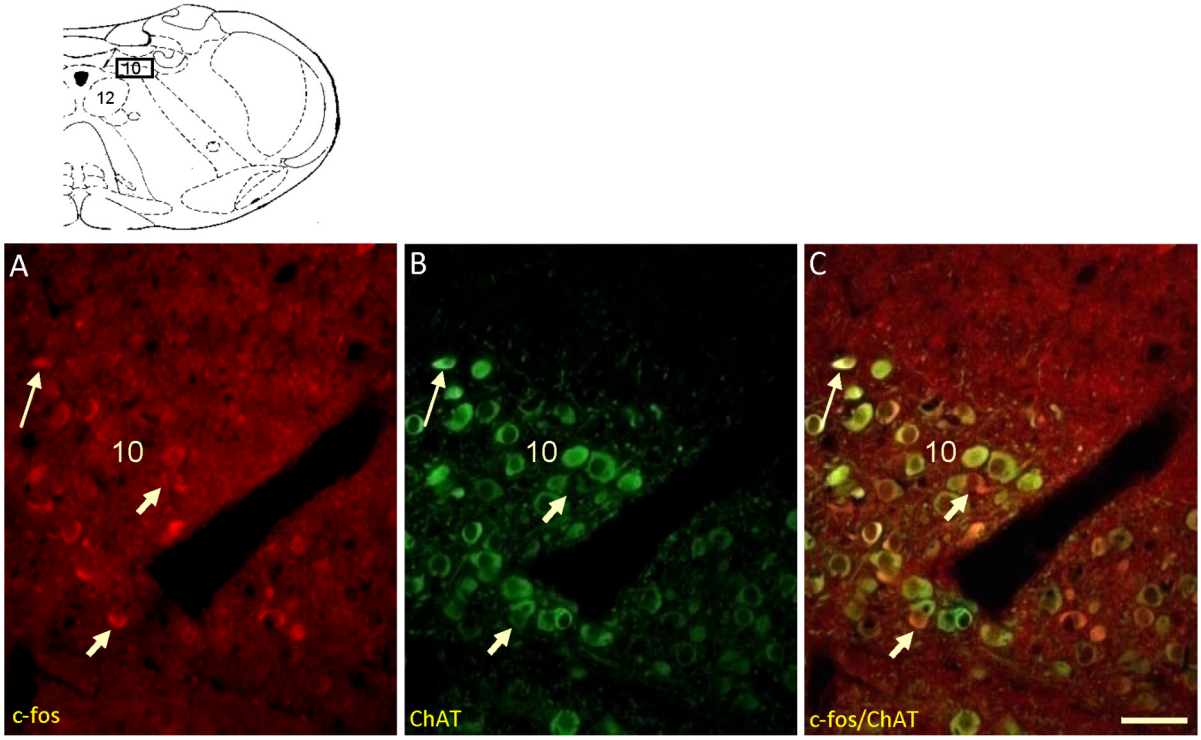


Figure 2: Activated neurons (arrows; c-fos, **A**) in 10 that also were ChAT-ir (**B**). Merged image in **C**. provides details regarding the neurochemistry of the activated neurons. Occasional activated neurons that were not ChAT-ir (arrowheads) also were observed. However a number of c-fos-ir neurons were only lightly ChAT-ir. **A-C**. Scale = 10 μm. Taken from Level III, Bregma -13.68 mm.

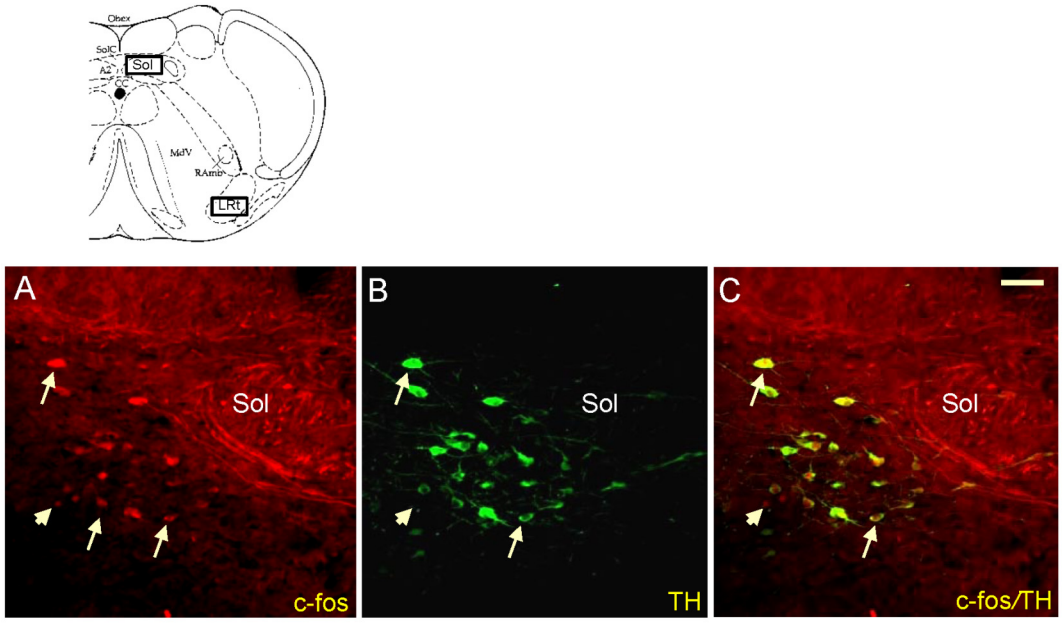


Figure 3: Activated neurons in NTS (Inset, upper box; **A-C**) were TH-ir. **A.-C.** Arrows show activated neurons (c-fos, **A**) in the Sol subnucleus of NTS that also were TH immunoreactive (**B**). Merged image in **C**. provides details regarding the neurochemistry of the activated neurons. Occasional activated neurons that were not TH-ir (arrowheads) also were observed. **A-C**, Scale = 20 μm. Taken from A2 region of Sol, Level IV, Bregma -14.30 mm.

posterior pharyngeal wall and the subsequent gag response. Using c-fos immunohistochemistry, activated neurons that participate in the gag reflex were localized in identified subnuclei of the NTS as well as in motor areas and nuclei of the ventrolateral regions of the medulla. The location of many of the activated neurons

correlated relatively well with our proposed model of gag reflex connectivity, including those in the NTS, dorsal motor 10, hypoglossal nucleus, and NA. The majority of vagal and glossopharyngeal afferent information from the posterior pharyngeal wall is carried to the NTS [17-21]. The NTS, in turn, excites neurons in the nucleus ambiguus (NA) which then activates the pharyngeal and velar muscles (CN X) and the muscles of the tongue (*via* CN XII) [14].

The presence of activated neurons in the ventral medullary reticular nucleus (MdV) of Level IV, the intermediate reticular nucleus (IRt) of Level II, and activated neurons in the LPGi, DPGi, and MRVL in the most rostral portion of the medulla oblongata (Level I) was unexpected. These regions have been implicated in cardiovascular function, analgesia, memory, and conditioned fear response. Connections to such systems may explain concomitant responses such as nausea and diaphoresis [7] and the clinical phenomenon of food refusal after only one or two episodes of gagging or gagging to visual stimuli [11, 22, 23]. These unexpected regions of activation are also similar to those reported by DiNardo and Travers [24], who studied c-fos immunoreactivity in rats following quinine stimulation. Quinine is known as a bitter taste quality that has also produced oral projections behaviors (such as wide jaw excursion, termed gapes, and simultaneous rhythmic protrusions of the tongue) [25, 26]. Thus, it would appear that both mechanostimulation of the posterior pharyngeal wall and bitter receptors are important for airway and/or ingestion protection, regardless if from a bitter taste stimulus or a pharyngeal stimulation unrelated to feeding. Further bitter processing and mechanostimulation share afferent processing, thus overlap is to be expected. However, because no reports of a gag reflex have been made following quinine stimulation, it appears that pharyngeal stimulation elicits a somewhat different response. Further research is needed to explore the detailed relationship between the two different stimuli and their subsequent motor responses.

The results of the present study reveal that, although regions of the NTS were activated at all medullary levels examined, not all of the NTS subnuclei showed activation. However, the pattern of activation that was observed in the NTS subnuclei seems logical based on the functional differences reported for each area. For example, SolVL has been linked to cessation of breathing [27], Sol M to baroreceptor reflexes with a decrease in parasympathetic innervations of the heart [28], and SolIM to laryngeal and pharyngeal sensation [21, 29]. These are activities that take place during the gag motor response [1, 7-9, 14].

Many of the neurons in the NTS, particularly in SolDM and Sol subnuclei were also TH immunoreactive. Most of these activated neurons comprise the A2/C2 catecholamine groups previously described by others [30, 31]. These regions have been linked to homeostatic regulation of food intake [32]. In addition, portions of the rostral NTS reportedly have been linked to the melanocortin system that is involved with energy homeostasis and have specific connections to the arcuate nucleus of the hypothalamus [33, 34]. The activation in this area may be related to appetite suppression that some individuals may experience with a heightened gag response (author, personal communication). The sparcity of activated neurons in regions such as SolG, SolCe, and SolC was unexpected. These medullary areas have been implicated in gastric tone, the esophagus, and the esophageal phase of swallowing and vomiting [15, 35, 36], and were considered likely candidate regions for activation during a gag response.

Some of the activated neurons showed ChAT immunoreactivity. The ChAT-ir neurons in the dorsal motor 10 may have been activated due to cardiorespiratory changes that result from the gag reflex (author, personal comm.). Similarly, activated neurons in the hypoglossal nucleus were likely the result of the strong patterned tongue responses observed with the gag reflex response.

It appears that the observed activation exceeds that expected in a simple gag reflex [2-4]. Other reflexes may also be elicited, such as the laryngeal adductor reflex, accounting for some of the other regions that were activated in the present study. In addition, responses related to the emetic reflex may also be activated. Our model is simplified by the fact that rats do not vomit [37], allowing us to focus more specifically on the identification of gag activated neurons. However, other autonomic aspects of the emetic response may be activated, including the gastric motor response, increased salivary secretion, taste aversions, and elevated plasma vasopressin levels [38]. The purpose of this study was not to separate each potential physiologic response, but instead to report activated neurons following mechanostimulation of the pharyngeal wall which produced a gag motor response. Further the methodological approach that we used in this preliminary experiment only allowed us to report

medullary areas that were consistently found to be activated in the animals that had stimulation to the posterior pharyngeal wall (above the control and sham), future work will explore the "degree" of activation observed within each of these levels. As we continue to explore the gag reflex, future studies will also be necessary to clearly define all of the physiologic responses that may concomitantly be involved. However, establishment of the complex physiology that may be related to the gag reflex is outside of the scope of this paper and series of experiments.

One challenge to undertaking this type of research involves the complexity of differentiating boundaries of the medullary nuclei. For example, regarding the NTS, we have noted frequent nomenclature related to rostral to caudal organization [28, 29], while others differentiate the NTS *via* subnuclei [20, 39]. Because the mapping in this study required multiple levels and detailed boundary differentiation both within and outside of the NTS, we selected a single rat atlas source to as our reference guide [16]. In addition, the neurochemistry associated with certain brain regions was helpful in determining the boundaries of specific medullary nuclei. In addition, immunofluroscence techniques were very beneficial in allowing more specific delineation of nuclei at each of the levels. Organization of brainstem regions using cholinergic and adrenergic neurons to assist with mapping have been successfully reported by others [40]. Further, our findings were based on function rather than anatomic criteria and circuitry; thus some of our activation patterns (i.e. IRt, MdV) were isolated to only one or two levels rather than the entire rostral to caudal extent of a nucleus.

The results described in this report are the first to yield information regarding the location of activated neurons in the rat medulla following a gag stimulus. Although the physiological mechanisms are unknown at this time, it is clear that specific neurons in the rat brainstem are activated following mechanostimulation to the posterior pharyngeal wall. Activation within many regions (i.e. NTS, dorsal motor 10, NA) was consistent with our proposed theoretical model. However, other regions of activation such as the LPGi and MRVL were not expected and have resulted in an expansion of the detailed reflex circuitry involving the gag reflex. As we continue to explore detailed brain regions dedicated to eliciting the various actions and behaviors involved with the gag reflex, we will begin to answer the complex clinical questions regarding the underlying mechanisms involved in the hypersensitivity to the gag reflex and offer insight into the differential diagnosis of the gag reflex with appropriate treatment options.

REFERENCES

[1] Miller AJ. Oral and pharyngeal reflexes in the mammalian nervous system: Their diverse range in complexity and the pivotal role of the tongue. Crit Rev Oral Biol Med 2002; 13: 409-25. http://dx.doi.org/10.1177/154411130201300505

[2] Martin JH. Neuroanatomy text and atlas. 2nd ed. Stamford (Conn): Appleton & Lange 1996; pp. 392-393.

[3] Nolte J. The human brain, 3rd ed. St. Louis: Mosby-Year Book 1993; pp. 198-199.

[4] Schulze-Delrieu K, Miller RM. Clinical Assessment of Dysphagia. In: Perlman AL, Schulze-Delrieu K, Eds. Deglutition and its disorders: anatomy, physiology, clinical diagnoses, and management. San Diego: Singular Publishing Group 1983; 144-5.

[5] Scarborough DR, Van Kuren M, Hughes M. Altering the gag reflex via a hand pressure point. J Am Dent Assoc 2008; 139: 1365-72. http://dx.doi.org/10.14219/jada.archive.2008.0048

[6] Akarslan ZZ, Bicer AZY. Utility of the gagging problem assessment questionnaire in assessing patient sensitivity to dental treatments. J Oral Rehabil 2012; 39: 948-55. http://dx.doi.org/10.1111/j.1365-2842.2012.02321.x

[7] Bassi GS, Humphris GM, Longman LP. The etiology and management of gagging: a review of the literature. J Prosthet Dent 2004; 91: 459-67. http://dx.doi.org/10.1016/j.prosdent.2004.02.018

[8] Leder SB. Gag reflex and dysphagia. Head Neck 1996; 18: 138-41. http://dx.doi.org/10.1002/(SICI)1097-0347(199603/04)18:2<138::AID-HED5>3.0.CO;2-2

[9] Perlman AL, Luschei ES, Du Mond CE. Electrical activity from the superior pharyngeal constrictor during reflexive and non-reflexive tasks. J Speech Lang Hear Res 1989; 32: 749-52.

[10] Saita N, Fukuda K, Koukita Y, Ichinohe T, Yamashita S. Relationship between gagging severity and its management in dentistry. J Oral Rehabil 2013; 40: 106-11. http://dx.doi.org/10.1111/joor.12014

[11] Byars KC, Burklow KA, Ferguson K, O'Flaherty T, Santoro K, Kaul A. A multi-component behavioral program for oral aversion in children dependent upon gastrostomy feedings. J Pediatr Gastroenterol Nutr 2003; 37: 473-80. http://dx.doi.org/10.1097/00005176-200310000-00014

[12] Scarborough DR, Boyce S, McCain G, Oppenheimer S, August A, Neils-Strinjas J. Abnormal physiological responses among children with persistent feeding difficulties. Dev Med Child Neurol 2006; 48: 460-64. http://dx.doi.org/10.1017/S0012162206000995

[13] Armfield JM. Towards a better understanding of dental axiety and fear: cognitions *vs*. experiences. Eur J Oral Sci 2010; 118: 259-64. http://dx.doi.org/10.1111/j.1600-0722.2010.00740.x

[14] Scarborough DR, Isaacson LG. Hypothetical anatomical model to describe the aberrant gag reflex observed in a clinical population of orally deprived children. Clin Anat 2006; 19: 640-44. http://dx.doi.org/10.1002/ca.20301

[15] Rogers RC, Travagli RA, Hermann GE. Noradrenergic neurons in the rat solitary nucleus participate in the esophageal-gastric relaxation reflex. AJ Physiol Regul Integr Comp Physiol 2003; 285: R479-89.

[16] Paxinos G, Watson C. The rat brain: In stereotaxic coordinates. 4th ed. San Diego: Academic Press 1998.

[17] Takemura M, Wakisaka S, Iwase K, *et al.* NADPH-diaphorase in the developing rat: lower brainstem and cervical spinal cord, with special reference to the trigemino-solitary complex. J Comp Neurol 1996; 365: 511-25. http://dx.doi.org/10.1002/(SICI)1096-9861(19960219)365:4<511::AID-CNE1>3.0.CO;2-7

[18] Roozendaal B, Williams CL, McGaugh JL. Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. Eur J Neurosci 1999; 11: 1317-23. http://dx.doi.org/10.1046/j.1460-9568.1999.00537.x

[19] Brining SK, Smith DV. Distribution and synaptology of glossopharyngeal afferent nerve terminals in the nucleus of the solitary tract of the hamster. J Comp Neurol 1996; 365: 556-74. http://dx.doi.org/10.1002/(SICI)1096-9861(19960219)365:4<556::AID-CNE4>3.0.CO;2-3

[20] Kalia M, Sullivan JM. Brainstem projections of sensory and motor components of the vagus nerve in the rat. J Comp Neurol 1982; 211: 248-64. http://dx.doi.org/10.1002/cne.902110304

[21] Hayakawa T, Maeda S, Tanaka K, Seki M. Fine structural survey of the intermediate subnuclues of the nucleus tractus solitarii and its glossopharyngeal afferent terminals. Anat Histol Embryol 2005; 210: 235-44. http://dx.doi.org/10.1007/s00429-005-0021-9

[22] Kramer RB, Braham RL. The management of the chronic or hysterical gagger. ASDC 1977; 44: 111-6.

[23] Murphy WM: A clinical survey of gagging patients. J Prostet Dent 1979; 42: 145-8. http://dx.doi.org/10.1016/0022-3913(79)90163-X

[24] Denardo LA, Travers JB. Distribution of fos-like immunoreactivity in the medullary reticular formation of the rat after gustatory elicited ingestion and rejection behaviors. J Neurosci 1997; 17: 3826-39.

[25] Grill HJ, Norgren R. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 1978; 143: 263-79. http://dx.doi.org/10.1016/0006-8993(78)90568-1

[26] Kinzeler NR, Travers S. Licking and gaping elicited by microstimulation of the nucleus of the solitary tract. Am J Physiol Regul Integr Comp Physiol 2008; 295: R436-48. http://dx.doi.org/10.1152/ajpregu.00189.2008

[27] Yokota S, Tsumori T, Oka T, Nakamura S, Yasui Y. GABAergic neurons in the ventrolateral subnucleus of the nucleus tractus solitarius are in contact with Kolliker-Fuse nucleus projecting to the rostral ventral respiratory group and phrenic nucleus in the rat. Brain Res 2008; 1228: 113-26. http://dx.doi.org/10.1016/j.brainres.2008.06.089

[28] Zhang X, Fogel R, Renehan WE. Relationship between the morphology and function of gastric and intestine-sensitive neurons in the nucleus of the solitary tract. J Comp Neurol 1995; 363: 37-52. http://dx.doi.org/10.1002/cne.903630105

[29] Travers SP, Norgren K. Organization of orosensory responses in the nucleus of the solitary tract of rat. J Neurophysiol 1995; 73: 2144-62.

[30] Lee A, Wissekerke AE, Rosin DL, Lynch KR. Location of α_{2c}-adrenergic receptor immunoreactivity in catecholaminergic neurons in the rat central nervous system. Neuroscience 1998; 84: 1085-96. http://dx.doi.org/10.1016/S0306-4522(97)00578-2

[31] Zhul L, Onaka T. Involvement of medullary A2 noradrenergic neurons in the activation of oxytocin neurons after conditioned fear stimuli. Eur J Neuroscience 2002; 16: 2186-98. http://dx.doi.org/10.1046/j.1460-9568.2002.02285.x

[32] Appleyard SM, Marks D, Kobayashi K, Okano H, Low MJ, Andresen MC. Visceral afferents directly activate catecholamine neurons in the solitary tract nucleus. J Neurosci 2007; 27: 13292-302. http://dx.doi.org/10.1523/JNEUROSCI.3502-07.2007

[33] Cone RD. Anatomy and regulation of the central melanocortin system. Nat Neurosci 2005; 8: 571-8. http://dx.doi.org/10.1038/nn1455

[34] Cone RD. Studies on the physiological functions of the melanocortin system. Endocr Rev 2006; 27: 736-49. http://dx.doi.org/10.1210/er.2006-0034

[35] Hornby PJ. Central neurocircuitry associated with emesis. Am J Med 2001; 111: 106S-12.

[36] Jean A. Brainstem organization of the swallowing network. Brain Behav Evol 1984; 25: 109-16. http://dx.doi.org/10.1159/000118856

[37] Borinson H, Borinson R, McCarthy LE. Phylogenic and neurological aspects of the vomiting process. J Clin Pharmacol 1981; 21: 23S-9.

[38] Uchino M, Kuwahara M, Ebukuro S, Tsubone H. Modulation of emetic response by carotid baro- and chemoreceptor activations. Auton Neurosci 2006; 128: 25-36. http://dx.doi.org/10.1016/j.autneu.2005.12.006

[39] Zhang L-L, Ashwell KWS. Development of the cyto- and chemoarchitecutral organization of the rat nucleus of the solitary tract. Anat Histol Embryol 2001; 203: 265-82. http://dx.doi.org/10.1007/s004290000151

[40] VanderHorst VGJM, Ulfhake B. The organization of the brainstem and spinal cord of the mouse: Relationships between monoaminergic, cholinergic, and spinal projection systems. J Chem Neuroanat 2006; 31: 2-36. http://dx.doi.org/10.1016/j.jchemneu.2005.08.003

Received on 20-12-2013 Accepted on 24-02-2014 Published on 27-03-2014

DOI: http://dx.doi.org/10.12970/2311-1917.2014.02.01.2