Physiol. Res. 53: 703-717, 2004

Brainstem Areas Involved in the Aspiration Reflex: c-Fos Study in Anesthetized Cats

J. JAKUŠ, E. HALAŠOVÁ[1], I. POLIAČEK, Z. TOMORI[2], A. STRÁNSKY

Department of Biophysics, [1]Department of Biology, Comenius University, Jessenius Faculty of Medicine, Martin and [2]Department of Pathophysiology, Faculty of Medicine, Šafárik University, Košice, Slovak Republic

Received November 24, 2003
Accepted March 23, 2004

Summary

Expression of the immediate-early gene *c-fos*, a marker of neuronal activation was employed in adult anesthetized non-decerebrate cats, in order to localize the brainstem neuronal populations functionally related to sniff-like (gasp-like) aspiration reflex (AR). Tissues were immunoprocessed using an antibody raised against amino acids of Fos and the avidin-biotin peroxidase complex method. The level of Fos-like immunoreactivity (FLI) was identified and counted in particular brainstem sections under light microscopy using PC software evaluations in control, unstimulated cats and in cats where the AR was elicited by repeated mechanical stimulation of the nasopharyngeal region. Fourteen brainstem regions with FLI labeling, including thirty-seven nuclei were compared for the number of labeled cells. Compared to the control, a significantly enhanced FLI was determined bilaterally in animals with the AR, at various medullary levels. The areas included the nuclei of the solitary tract (especially the dorsal, interstitial and ventrolateral subnuclei), the ventromedial part of the parvocellular tegmental field (FTL - lateral nuclei of reticular formation), the lateral reticular nucleus, the ambigual and para-ambigual regions, and the retrofacial nucleus. FLI was also observed in the gigantocellular tegmental field (FTG - medial nuclei of reticular formation), the spinal trigeminal nucleus, in the medullar raphe nuclei (ncl. raphealis magnus and parvus), and in the medial and lateral vestibular nuclei. Within the pons, a significant FLI was observed bilaterally in the parabrachial nucleus (especially in its lateral subnucleus), the Kölliker-Fuse nucleus, the nucleus coeruleus, within the medial region of brachium conjunctivum, in the ventrolateral part of the pontine FTG and the FTL. Within the mesencephalon a significantly enhanced FLI was found at the central tegmental field (area ventralis tegmenti Tsai), bilaterally. Positive FLI found in columns extending from the caudal medulla oblongata, through the pons up to the mid-mesencephalon suggests that the aspiration reflex is thus co-ordinated by a long loop of medullary-pontine-mesencephalic control circuit rather than by a unique "center".

Key words

Sniff-like aspiration reflex • Fos-like immunoreactivity • Brainstem mapping • Cat

Introduction

The aspiration reflex (AR), a powerful defensive reflex of the airways, is evoked by a variety of mechanical and electrical stimuli to sensory receptors of the naso- and oropharyngeal mucosa. Originally, the reflex was termed as "aspiration" for its functional role in the removal of mucus and irritants from the upper

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

airways to the hypopharynx by aspiration (Tomori 1965, 1979, Tomori and Widdicombe 1969). However, in the literature the terms as sniff-like AR (Batsel and Lines 1973) or gasp-like AR (Fung *et al.* 1994) are also used for this reflex. The reflex is characterized by solitary or several repeated brief but very strong inspiratory efforts followed by passive expiration, and it includes transient but widespread effects.

The AR consists of an inspiratory phase characterized by short but vigorous contractions of the diaphragm, external intercostal and abductor muscles of the upper airway, resulting in a sudden and strong decrease of intrapleural pressure and a rapid inspiratory airflow through the airways including the glottis (Tomori 1970, Poliaček 2000, Poliaček *et al.* 2003). The expiratory phase results from a relaxation of all inspiratory muscles, and a slight glottal narrowing which reflects a prolonged burst of electrical activity in the laryngeal adductor (Poliaček 2000), without a concomitant increase in lumbar expiratory motoneuron activity (Stránsky *et al.* 1976, Tomori 1979). AR persists under deep anesthesia, hyper- or hypothermia, during hypercapnia or in severe hypoxic coma and can even interrupt any ongoing respiratory activities such as apnoe, episodes of apneusis, hicouping, attacks of laryngo- or bronchospasms, and coughing or sneezing (Tomori *et al.* 1998, 2000). Thus the functional significance and possible implications of this powerful reflex, e.g. for cardio-pulmonary-cerebral resuscitation, far exceed its supposed mechanical action.

However, the central pathways and control mechanisms of the reflex are almost unknown. The primary site of intramedullary integration of the reflex was proposed within inspiratory neurons of the solitary tract nucleus (NTS) (Batsel and Lines 1973), where the afferent fibers of the glossopharyngeal nerve terminate. From this area, the neural activity may spread to another parts of the brainstem. Former experiments with recording of single neuronal activity in the rostral part of the ventral respiratory group (VRG) of the medulla in cats (Jakuš *et al.* 1985), and the present study indicate that inspiratory neurons generating quiet breathing also participate in the AR. During the reflex, the activity of inspiratory neurons also increased due to a regular recruitment of further (originally silent) inspiratory units. Similar behavior in activity of single phrenic motoneurons was also documented (Nail *et al.* 1979, Jodkowski *et al.* 1989, Beňačka and Tomori 1995). Fung *et al.* (1994) reported that injections of kainic acid into the lateral tegmental field of medulla completely abolished the signs of both fictive gasping and fictive „gasp-like aspiration reflex“ in midcollicularly decerebrate, vagotomized, paralyzed and artificially ventilated cats. In contrast, similar kainic acid lesions within the same medullary area did not abolish the AR in cats without decerebration. Only an additional midbrain transection at the level between the superior and inferior colliculi finally removed the signs of AR (Jakuš *et al.* 2000). This finding suggested the hypothesis that in addition to basic neurons within the medullary lateral tegmental field also another medullary and supramedullary neurons might be involved in the generation of the AR in cats. To test this hypothesis we used the method of c-Fos-like immunohistochemistry.

Expression of the nucleoprotein Fos, a product of *c-fos* immediate-early gene, was considered as a high resolution marker of neuronal activity (Dragunow and Faull 1989), providing evidence for a sensory pathway connection. The immunocytochemical detection of transcripts of the *c-fos* genes (Fos antigens) was used later to map functional neural pathways in a variety of motor and vegetative reflexes, e.g. in vomiting (Miller and Ruggiero 1994), sneezing (Wallois *et al.* 1995), coughing (Gestreau *et al.* 1997), swallowing (Amirali *et al.* 2001), as well as in the carotid baroreflex (Dampney *et al.* 1995), respiratory chemoreflexes (Berquin *et al.* 2000), cardiac sympathoexcitatory reflexes (Guo *et al.* 2002a,b), and others.

The present study tests the above mentioned hypothesis by examining the presence and character of Fos-like immunoreactivity evoked by repeated mechanical nasopharyngeal stimulation in non-paralyzed cats, thus mapping all brainstem regions involved in the generation of the AR. As documented below many brainstem structures specifically activated during the AR exhibited an increased level of Fos-like immuno-reactivity, compared with control breathing.

Methods

*General proce*dure

Animal care was in agreement with the Animal Welfare Guidelines of the Comenius University. Experiments were performed on 11 adult cats of either sex weighing 2.9-3.2 kg (mean body weight 3.02±0.32 kg). The animals were divided into two groups: a control group of non-stimulated cats with quiet breathing (n=5), and a group of stimulated cats with repeated provocation of the AR (n=6). The surgical procedure was the same for all animals. Cats were anesthetized with pentobarbitone

(Vetbutal, Biowet, Poland) by an initial intraperitoneal dose of 35-40 mg/kg. After cannulation of the trachea and the right femoral vein and artery, the surgical level of anesthesia was maintained by a repetitive dose of Vetbutal 1-2 mg/kg/h given intravenously. Local skin and muscular infiltrations by mesocain were performed in both groups at the sites of surgical interventions to minimize any induction of Fos reactivity related to stimulation of pain receptors. Histological processing was restricted to animals that had maintained systolic/diastolic values of blood pressure within the range 17-24/12-17 (kPa). Rectal temperature was maintained between 37-38 °C using a servo-controlled heating lamp.

Recording and mechanical stimulation

In both groups of animals the following parameters were monitored: arterial blood pressure (BP) by an electromanometer (Tesla LDP 186), the airflow (V) and tidal volume (V_T) of air by a pneumotachograph (Commet LBL 50), and the end-tidal CO_2 ($ETCO_2$) by a capnograph (Novametrix). From the records the mean values of breathing rate, blood pressure and $ETCO_2$ were calculated. Series of aspiration reflexes were evoked by gentle repetitive mechanical stimulation of the nasopharyngeal mucosa with an elastic, nylon fiber (diameter 0.2-0.35 mm). The nylon fiber was once introduced through the right or left nostril into the nasopharynx, where it remained during the whole period of stimulation. A sequence of ARs was evoked during each second inspiratory phase of rhythmic breathing. The ARs were elicited at least 10 min after the administration of any additional dose of the anesthetic.

Perfusion and fixation

After completion of surgery either with or without reflex stimulation, all cats had the same survival times (2 hours). Animals were then deeply anesthetized with Vetbutal (additional dose 10 mg/kg i.v.). In turn a medial thoracothomy was performed. Cats were perfused transcardially with a 2500 ml bolus of 0.9 % saline containing heparin (1000 U/100 ml), followed by a fixative solution of 1500 ml of 4 % paraformaldehyde in 0.1 M phosphate buffer (PBS) at pH 7.4. Both groups of animals (control and stimulated) were perfused within 210±30 min, after the induction of anesthesia, in order to maximize the Fos accumulation within the stimulated neurons (Herdegen *et al.* 1991, Orendáčová *et al.* 2000).

Immunohistochemical tissue procedure

Immediately after perfusion, the brainstem was removed from the skull and postfixed in the same fixative solution for 6 h. Tissues were then cryoprotected in solutions containing 10, 20, and 30 % sucrose at 4 °C during a period of 48-72 h. Transversal frozen tissue sections (40 μm) were prepared using a cryostat and collected in PBS (0.9 % NaCl in 0.1 M phosphate buffer, pH 7.4). Immunohistochemistry was performed using standard procedures. In order to block peroxidase activity, free-floating sections were first incubated (30 min at room temperature) in PBS containing 0.3 % H_2O_2. The sections were then rinsed twice in PBS. To prevent non-specific binding sites, the sections were incubated for 3 h in 0.1 PBS containing 1 % bovine albumin, 3 % normal goat serum and 0.2 % Triton X-100. The sections were then incubated in polyclonal Fos rabbit antibody (C-fos Ab-5 PC 38, Calbiochem, diluted 1:7000) for 24 h at 4 °C. This primary antibody recognizes both Fos and Fos-related antigens. The sections were then rinsed twice in PBS and incubated for 2 h in biotinylated goat-anti rabbit antiserum (BA 100, Vector Laboratories, CA, diluted 1: 600 in PBS) and washed twice after incubation. Finally, the sections were incubated in an avidin-biotin-peroxidase complex (1:100 for one hour, Peroxidase Vectastain Elite ABC kit VC-PK 6100, Vector Laboratories, CA). After two washes in PBS and one in Tris-HCl saline, the sections were processed for using 0.05 % diaminobenzidine as a chromogene. Finally, after two washes in distilled water, the sections were mounted on gelatine-coated slides, then air-dried, dehydrated, cleared, and coverslipped in Canadian balsam. Adjacent sections were counterstained with Nissl staining to delineate the location of nervous structures.

Data analysis

Sections processed immunocytochemically for Fos-like proteins were drawn by camera lucida. For some brainstem structures, anatomical landmarks were established using adjacent counterstained sections. Criteria used to select specific sections for quantification included: 1) identification of the areas with nuclei or subnuclei of interest in the section; 2) the absence of artifacts in the area; 3) the same rostrocaudal level for sections from different animals. The graphic reconstructions of representative sections were performed according to the cytoarchitectonic atlas (Berman 1968). The subdivision of the nucleus of the solitary tract is referred according to Kalia and Mesulam (1980), and that of Berman (1968) and Petrovický (1980) for other brainstem structures. Every fifth brainstem section was used to identify and count Fos-like immunoreactive

neurons. Distribution and the number of immunoreactive cytoplasmatic granules were determined with an optical Leitz microscope and video-scanning system (CCD Philips) coupled to a Pentium III computer using software (Ellipse, ViDiTo), to evaluate the grain density. Automatic counts were based on the average density of Fos-positive cell nuclei relative to a given threshold. The appropriate threshold providing the best discrimination of the target cells from the background was set up from the first digitized image of the series. Hence, the same threshold was used for counting immunoreactive cells in the remaining digitized images, obtained from sections processed in parallel during immunocytochemistry (Gestreau *et al.* 1997). The changes in intensity of Fos immunostaining induced by ARs, representing the level of neuronal activation were not evaluated quantitatively for this paper. An increase in the number of immunoreactive neurons in stimulated cats compared to control animals indicates the level of "recruitment" of neurons provoked by ARs.

Statistical evaluation

The number of immunoreactive neurons in selected structures were averaged to obtain the mean count for each area (±S.E.M.) from either side of the brainstem. Individual data were collected for comparison between the right and left sides as well as between the stimulated and control cats. Computer software (Graphpad Prism) was used for statistical evaluation of the results. Analysis of variance (ANOVA) and Mann-Whitney test were used to determine the statistical significance of the difference. Differences were considered significant at $p<0.05$.

Results

Effects of nasopharyngeal stimulation on quiet breathing and blood pressure

The basal rate of breathing was similar in both groups of animals either stimulated (with AR) or the controls (sham operated, non-stimulated). After surgery, mean rate of quiet breathing was 17±1.3 (cycles. min^{-1}) with $ETCO_2$=5.3±0.2 % in stimulated cats compared to that of control animals 15.4±3.3 ($p>0.05$), with $ETCO_2$=5.2±0.5 % ($p>0.05$), respectively. A significant decrease in $ETCO_2$ was found only in a group of stimulated animals at the end of stimulation ($ETCO_2$=4.5±0.2 %, $p<0.05$) and before perfusion ($ETCO_2$=4.6±0.3 %, $p<0.05$), compared to the same animals after surgery. Repetitive mechanical stimulation of the nasopharyngeal mucosa regularly elicited a series of typical aspiration reflexes (Fig. 1). The average number of provoked aspiration reflexes was 450±30 during a 30 min period of stimulation), i.e. 15±1 per min. The respiratory rate returned to basal levels within a short delay (<10 s) following a series of ARs.

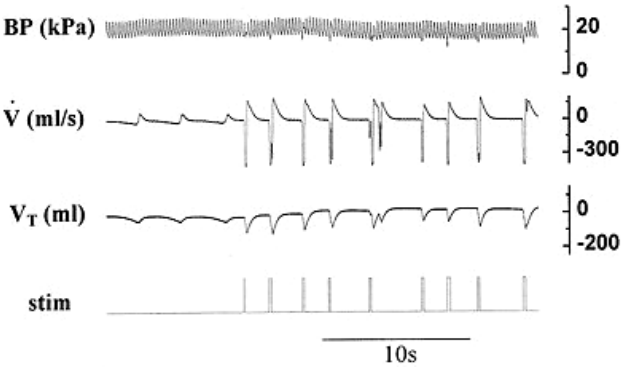

Fig. 1. Repetitive nasopharyngeal stimulation evokes a series of aspiration reflexes. BP - arterial blood pressure, V - airflow, V_T - tidal volume, stim - mechanical stimulation

The systolic/diastolic values of arterial BP oscillated within the range 17.3-24.0/12.2-17.0 kPa during the whole experiments. In stimulated cats after surgery, the mean values reached 19.4±1.1/14.9±1.3 kPa, compared to a group of control cats without stimulation (19.1±1.8/13.9±1.2 kPa, $p>0.05$).

Brainstem distribution of Fos-like immunoreactivity

Within the nuclei of immunoreactive neurons a Fos-like expression was detected as dark-brown staining of variable intensity. FLI was observed in both the stimulated and control animals, but to a different degree. All stimulated animals with series of provoked ARs exhibited marked increases in the number of immunolabeled nuclei as compared to the controls. Diagrammatic representation of FLI distribution within the medulla oblongata, pons Varoli, and mesencephalon, in both the control and stimulated cats is illustrated in Figure 2. Examples of immunostained neurons in particular structures at three above mentioned levels are shown on Figures 3 and 4 for both groups of animals. Table 1 summarizes the results of quantitative analysis of Fos-like immunoreactivity, indicating the average number of immunostained neurons in 8 critical brainstem areas with significant differences between the groups of control and stimulated cats.

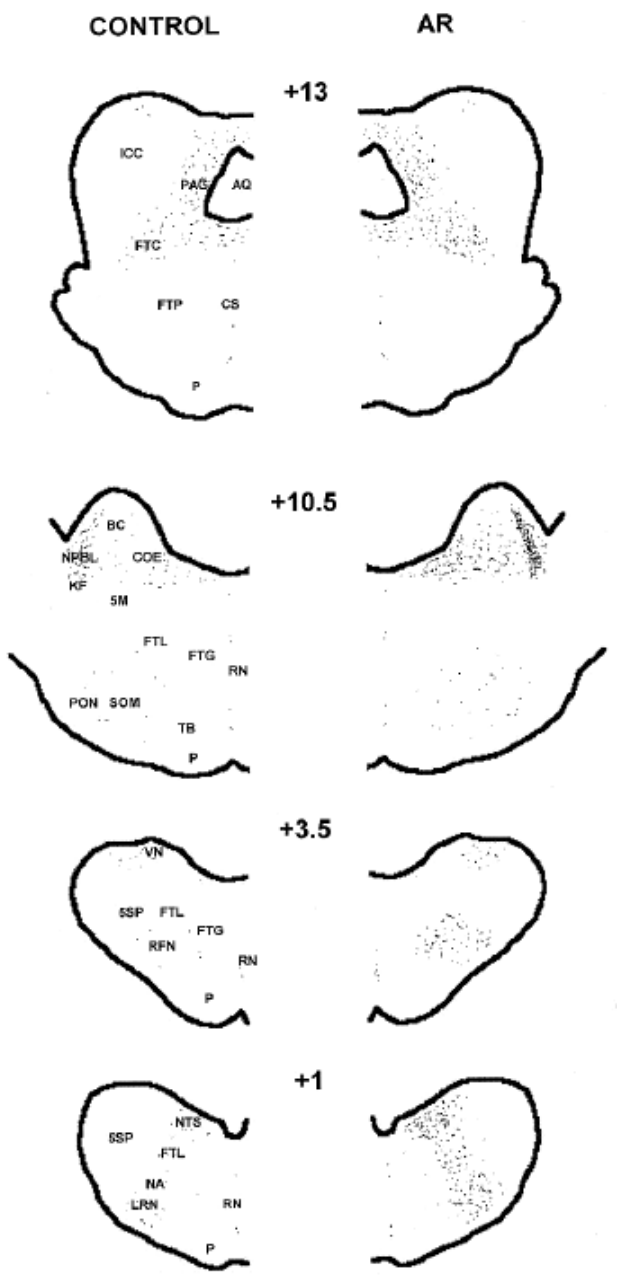

Fig. 2. Diagrammatic reconstruction of individual sections through the rostral medulla, the pons and the mesencephalon in separate transverse hemisections at four rostrocaudal brainstem levels (+13, 10.5, 3.5, and 1 mm rostral to the obex) in unstimulated cats (control) and in cats after repeated nasopharyngeal stimulation (AR). Black dots represent immunostained neurons as they appeared under low power of microscope. AQ - aqueduct, PAG - paraaqueductal gray, ICC - central nucleus of the inferior colliculus, FTC - central tegmental field of the mesencephalon, FTP - paralemniscal tegmental field, CS - superior central nucleus, P - pyramidal tract, COE - nucleus coeruleus, BC - brachium conjunctivum, NPBL - lateral parabrachial nucleus, KF - Kölliker-Fuse nucleus, 5M - motor trigeminal nucleus, FTL - pontine and medullary lateral tegmental fields, FTG - pontine and medullary gigantocellular tegmental fields, PON - preolivary nucleus, SOM - medial nucleus of the superior olivae, TB - trapezoid body, VN - vestibular nuclei, 5SP - spinal trigeminal nucleus, RFN - retrofacial nucleus, RN - raphe nuclei, NTS - solitarii tract nucleus, NA - ambigual nucleus, LRN - lateral reticular nucleus.

Immunostaining in control animals

In control (sham-operated, non-stimulated, n=5) cats FLI was scattered throughout the brainstem in sensory and motor areas related mostly to the respiratory, cardiac, vasomotor, vestibular and pain control. Basal FLI was quite similar on both sides of the transversal sections (as detected randomly by the software Ellipse). However, no Fos-like immunoreactive neurons were observed in the following regions: medullary hypoglossal nuclei, the area postrema, the dorsal vagal nucleus (DMV), the spinal trigeminal tract (5ST) and nucleus, in the subretrofacial nuclei, in the accesory olivae nuclei – medial (IOM), dorsal (IOD), inferior (IOP), in the cochlear anteroventral (CVA) and posteroventral (CVP) nuclei, within the brachium pontis (BP), in the trapezoid body (TB), in the tegmental reticular nuclei (TRC – reticular nuclei of brainstem tegmentum), in the external cuneate nucleus (CX), the ventral nucleus of the lateral lemniscus (LLV), and in the paralemniscal nuclei (tegmental field – FTP) of the mesencephalon.

In the medulla oblongata, basal FLI was detected in quietly breathing cats bilaterally throughout the rostrocaudal axis concentrated mostly at two levels: 1-1.5 mm and 3-4 mm rostrally to the obex. Slightly below and above these levels, FLI was not consistent. FLI mostly affected the respiratory neurons of the dorsal (DRG) and ventral respiratory groups (VRG) and the adjacent reticular formation. At the level 1-1.5 mm rostrally to the obex, a few Fos-labeled neurons were distributed within the nucleus tractus solitarii (NTS), mainly within the dorsal (dNTS), interstitial (iNTS) and ventrolateral (vlNTS) subnuclei, where an average number of immunoreactive neurons in particular areas after hemisection (AGN/H) was 14±2. Similar density of FLI was detected at the same level in an area including the nucleus ambiguus (NA), nucl. paraambiguus (NPA) and partly the lateral reticular nucleus (LRN) and the alaminar part of the spinal trigeminal nucleus (5SP) with AGN/H 13±6 (Figs 2 and 3a, Table 1). FLI was also seen in the parvocellular reticular formation (Berman's lateral tegmental field – FTL) and in the medial part of 5SP with AGN/H 8±2 (Fig. 2, Table 1). At the medullary level 3-4 mm rostral to obex, discrete FLI labeling was detected in an area including the parvocellular reticular formation, ncl ambiguus and paraambiguus (FTL, NA, NPA) and the retrofacial nucleus (RF), with AGN/H 5±2. Weak Fos-like immunoreactivity was also detected in the medulla below the obex (at the level approximately 1-3 mm) mostly confined to the commissural nuclei and the

adjacent lateral tegmental field (AGN/H 2±1). At the level of the rostral medulla (approx. 5-5.5 mm) and the ponto-medullary junction (approx. 6 mm) scattered immunoreactivity was also observed in neurons of the raphe nuclei (ncl. raphealis magnus and parvus), with AGN/H 4±2 (Table1).

At the pontine level, the highest basal density of immunoreactive neurons was found in its rostral, dorsolateral part (approx. 9.5-11.0 mm), bilaterally. This region corresponds functionally to the "pontine respiratory group" neurons, also contributing to the cardiorespiratory integration. In particular, an intense bilateral distribution of FLI was detected in the lateral regions of parabrachial nucleus (NPBL), the Kölliker-Fuse (KF) nucleus (AGN/H 83±9) (Figs 2 and 4a, Table 1). A smaller number of immunostained neurons was detected in an area including the medial part of the brachium conjunctivum (BC) and the nucleus coeruleus (COE) with AGN/H 25±5 (Figs 2 and 4a, Table 1). Above and below this pontine level, the number of labeled neurons rapidly decreased. However, at the level of the middle and the lower pons (approx. 8 and 6.5 mm, respectively) a higher number of immunoreactive neurons was found in the medial and superior vestibular nuclei (VN) (AGN/H 10±3), in the medullary raphe nuclei (AGN/H 7±1), and within a larger area (including the lateral parts of the gigantocellular nucleus, the paragigantocellular nucleus (vlFTG), the preolivary nucleus and the medial nucleus of the superior olivae (AGN/H 10±3). (Table 1) Within the mesencephalon, at the level of inferior colliculi (approx. 12-13.0 mm rostral to the obex), more intense FLI was detected bilaterally in the subcuneiform nucleus (ncl. subcuneiformis) of the central tegmental field (FTC) where AGN/H was 62±8 and partly also around the periaqueductal gray (PAG), with AGN/H 87±15 (Figs 2 and 4a, Table 1). Slightly below and above this level, the FLI was not consistent.

Immunostaining in stimulated animals

In stimulated cats (with the aspiration reflex, n=6), dense Fos-immunoreactivity was detected bilaterally throughout the brainstem. At the medulla oblongata stimulation-related FLI (sFLI) neurons extended from –3 mm to +4.0 mm rostral to the obex, including both the dorsal and ventral respiratory groups. In the pons sFLI neurons were restricted mostly to NPBL, KF, BC and COE. In the mesencephalon they were found mainly in the FTC. In general, no sFLI neurons (or non-significant increase in the number of Fos-labeled neurons, compared to control brainstem nuclei), were observed: in the medullary hypoglossal nuclei, the area postrema, the DMV, 5ST, in the subretrofacial nuclei, the accesory inferior olivae nuclei (IOM, IOD, IOP), in the CVA, CVP, the brachium pontis, in the TB, TRC, CX and LLV, as well as in the paralemniscal tegmental field (FTP) of the mesencephalon. Figures 3b and 4b illustrate an increased density and distribution of sFLI within particular structures of the brainstem.

In the dorsal and intermedio-lateral medulla oblongata (a level approx. 1 mm rostral to the obex), a significantly increased number of sFLI neurons was detected in the ncl. tractus solitarii (dorsal, interstitial and ventrolateral subnuclei) with AGN/H 123±15, $p<0.01$, also in a dorsolateral part of the lateral reticular nuclei-FTL, and in the medial part of 5SP (AGN/H 42±8, $p<0.01$), compared to the number of labeled neurons at those structures in control animals (Figs 2 and 3b, Table 1).

In the ventral medulla oblongata, numerous sFLI neurons were distributed bilaterally along a column extending from the caudal parts of lateral reticular formation, e.g. ncl. reticularis ventralis or FTL, through the NA to LRN (from –3 mm caudal to +4 mm rostral to the obex). Compared to control animals, at the level approx. –3 to –1 mm below the obex, the sFLI neurons were detected mostly in the lateral reticular formation (FTL), and in the commissural subnuclei of NTS, whereas at the level approx. +1 mm rostral to the obex, the sFLI neurons were most prominent in a larger area including the medial part of FTL, the NA, NPA and LRN (AGN/H 66±15, $p<0.02$). At the level approx. +3.5 mm rostral to the obex the sFLI was seen mainly in the ncl. paragigantocellularis (vl.FTG), in the lateral reticular formation (FTL), 5SP and RFN (AGN/H 61±11, $p<0.02$). Similarly, a significantly higher number of labeled sFLI neurons compared to control, was observed at the rostral medulla oblongata (level +5 to +5.5 mm) within the raphe nuclei (ncl. raphealis magnus et parvus) with AGN/H 23±7 ($p<0.02$) (Figs 2 and 3b, Table 1).

Within the dorsolateral pons (compared to the control FLI), compact clusters of sFLI neurons were seen bilaterally, in extension about 8-10.5 mm rostral to the obex. In particular, at the level of approx. 10.5 mm, a significant number of sFLI neurons was found mostly in the NPBL (subnucleus lateralis of parabrachial ncl.) and KF nuclei (AGN/H 266±50, $p<0.05$), as well as in the medial border of BC and the COE (AGN/H 74±9, $p<0.01$). A significant number of sFLI neurons was found

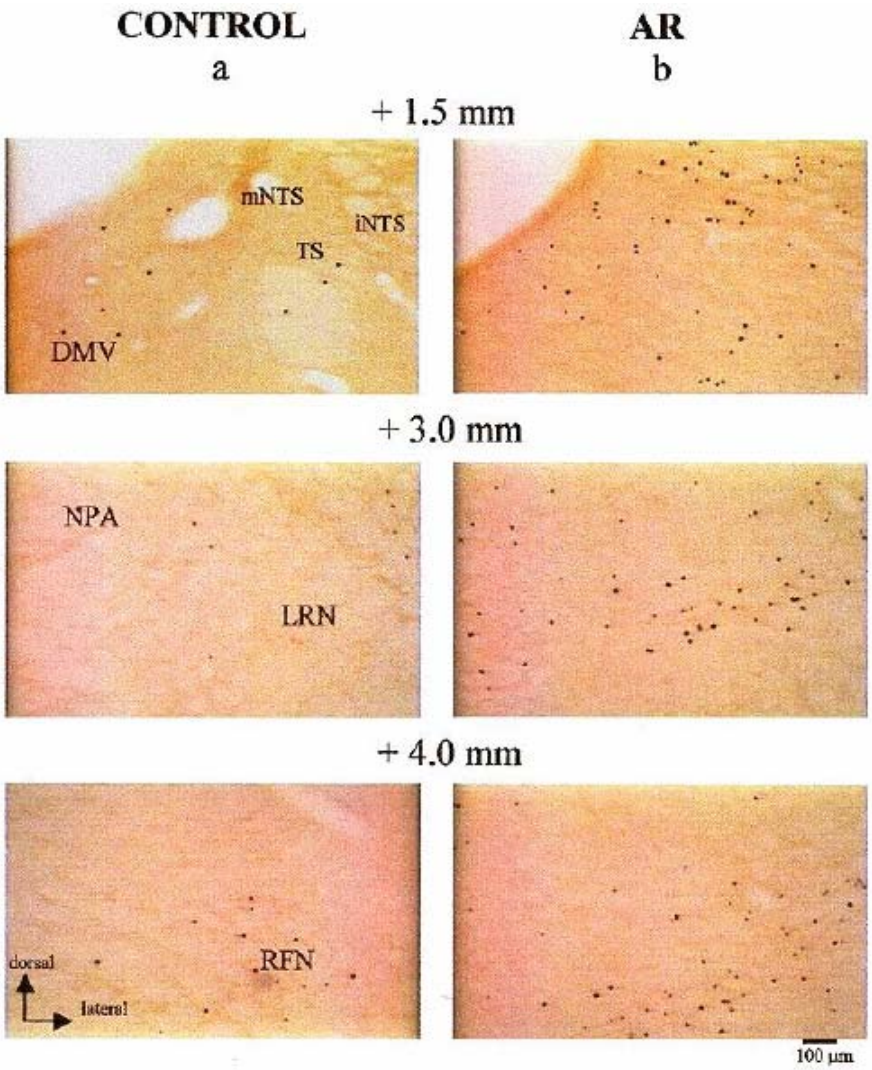

Fig. 3. Computer assisted samples of c-Fos immunoreactive neurons (dark dots), taken from the transversal sections at the medullary levels (+1.5, 3.0, 4.0 mm rostral to the obex) in group of sham operated, unstimulated cats (CONTROL a) and in group of cats after repeated nasopharyngeal stimulation (AR b). For explanation see the text (Results). Abbreviations: mNTS, iNTS - medial and interstitial subnuclei of the solitary tract nucleus, respectively. TS - solitary tract, DMV - dorsal motor nucleus of the vagus, NPA - the paraambigual nucleus, LRN - lateral reticular nucleus, RFN - retrofacial nucleus. Arrows indicate dorsal and lateral directions. Scale bar 100 µm.

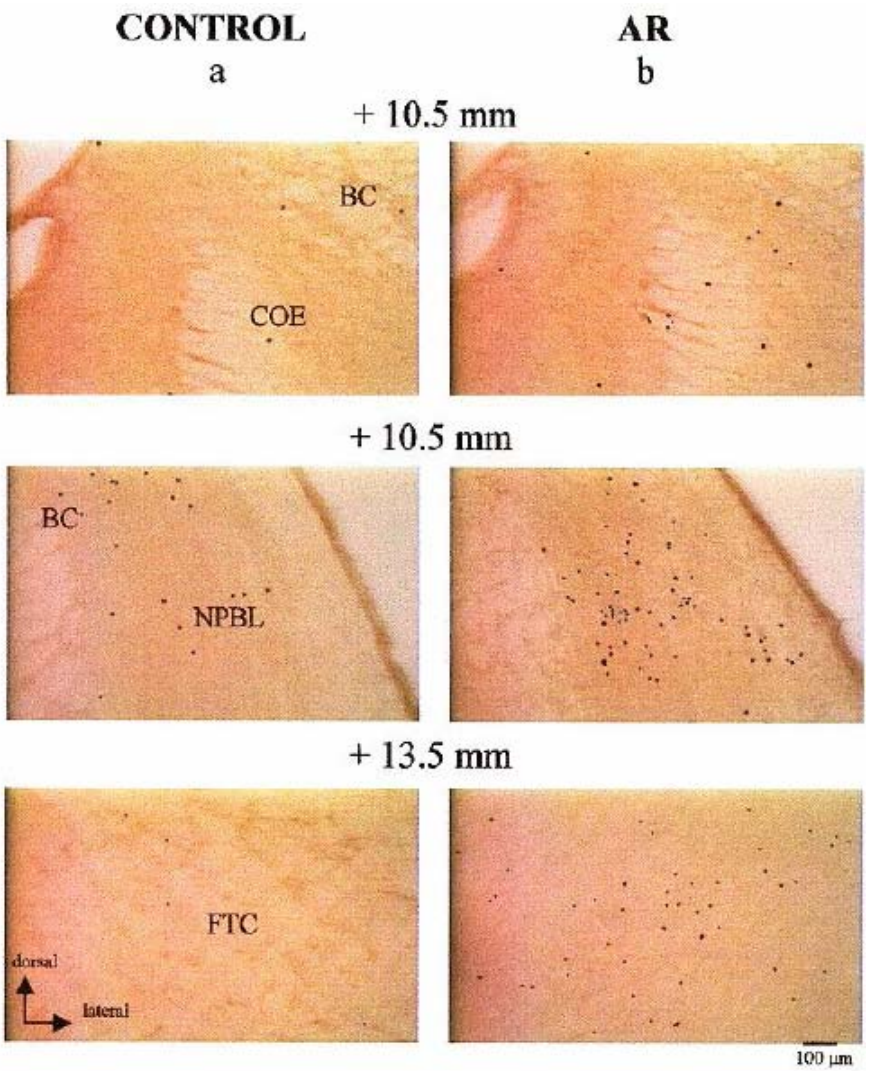

Fig. 4. Computer assisted examples of c-Fos immunoreactive neurons (dark dots), taken from the transversal sections at the pontine (+10.5 mm) and the mesencephalic (+13.5 mm) levels, respectively, in a group of sham operated, unstimulated cats (CONTROL a) and in group of cats after nasopharyngeal stimulation (AR b). For detailed explanation see the text (Results). For abbreviations of the pontine and midbrain nuclei see Fig. 2. Other symbols as in Fig. 3

at the level approx. +10 mm above the obex, particularly within the BC and COE (AGN/H 102±23, $p<0.01$), at the area including the lateral and ventrolateral reticular formation (ncl. parvocellularis, ventral parts of ncl. gigantocellularis and ncl. paragigantocellularis – FTL, vl. FTG) and the accessory inferior oliva (SOM) with AGN/H 31±4, $p<0.02$, as well as within the raphe nuclei (AGN/H 15±2, $p<0.02$). Around and above the ponto-medullary border, a scattered but significantly higher number of sFLI neurons was detected in the medial and lateral vestibular nuclei, and in the 5SP (AGN/H 23±3, $p<0.05$) compared to control (Fig. 2, Table 1).

The highest density of sFLI within the mesencephalon was seen at the level between superior and inferior colliculi (the level of 12-16 mm rostral to the obex). However, a higher number of sFLI neurons was found predominantly in the central tegmental field of the mesencephalon (area ventralis tegmenti Tsai) (AGN/H 98±9, $p<0.05$) compared to control (Fig. 2, Table 1). Only moderate and non-significant increase in the number of sFLI neurons was detected in the ventrolateral PAG (AGN/H 136±15, $p>0.05$).

Table 1. Unilateral FLI counts in brainstem structures in a group of the control and stimulated cats, respectively. The eight labeled brainstem areas, with the average group number of immunoreactive neurons in particular area/hemisection (AGN/H), and significant differences between the control and stimulated cats, are shown. For abbreviations of brainstem nuclei see Fig. 2 and the text.

Brainstem level (mm)	Labeled areas	Number of neurons AGN/H		Significance
		control	AR	p
	Medulla Oblongata			
-3 till -1	Commissural nuclei of NTS and the FTL	2 ± 1	22 ± 3	<0.01
+1-1.5	dNTS, iNTS and vlNTS subnuclei	14 ± 2	123 ± 15	<0.01
	FTL and the medial part of 5SP	8 ± 2	42 ± 8	<0.02
	LRN, NA, NPA	13 ± 7	66 ± 15	<0.02
+3.5-4	vlFTG, FTL, 5SP			
	NA, NPA, RFN	5 ± 2	61 ± 11	<0.02
+5.5-6	Raphe nuclei	4 ± 2	23 ± 7	<0.02
	Ponto-medullary junction and Pons Varoli			
+6.5-8	medial and lateral vestibular nucl.			
	and dorsal part of 5SP	10 ± 3	23 ± 3	<0.05
+9-10	COE and BC	10 ± 3	102 ± 9	<0.01
	vlFTG, FTL, PON, SOM	10 ± 2	31 ± 4	<0.02
	Raphe nuclei	7 ± 1	15 ± 2	<0.02
+10.5	NPBL, KF	83 ± 9	266 ± 50	<0.05
	BC, BCM, NPBM, COE	25 ± 5	74 ± 9	<0.01
	Mesencephalon			
+13-14	FTC (area ventralis tegmenti Tsai)	62 ± 8	98 ± 9	<0.05
	PAG	87 ± 15	136 ± 15	>0.05

Discussion

This is the first study identifying brainstem neurons exhibiting Fos-like immunoreactivity after the aspiration reflex in spontaneously breathing cats. Repetitive mechanical stimulation of the rapidly adapting receptors in the nasopharyngeal mucous membrane of cats elicited a series of frequent and repeated aspiration reflexes. Our study is based upon a comparison of Fos-like immunoreactive neurons between control and stimulated cats, while keeping the same experimental conditions (type and level of anesthesia, surgical preparation, body temperature, delay between stimulation and sacrifice, parallel histochemical processing, etc.) in both groups of animals. Thus, we believe that a stable level of pentobarbitone anesthesia, the mean systemic blood pressure and a constant body temperature during the experiments, with minimal interventions, and a frequent use of local anesthetic had prevented undesirable Fos-like immunoreactivity in the brainstem neurons both in control and stimulated cats. Although Fos expression is generally acknowledged to be a reliable marker of neuronal activation, it may not necessarily express nuclear FLI under all circumstances. This experience is typical mainly for the pools of motoneurons engaged in a particular motor act, even when they are strongly activated (Hunt *et al.* 1987, Dragunow and Faull 1989, Gestreau *et al.* 1997, Berquin *et al.* 2000). This was also demonstrated in our study, because the facial, vagal or hypoglossal motoneurons did not exhibit marked FLI in spite of their participation in the AR. Furthermore, it is still not clear whether inhibitory or subthreshold excitatory synaptic events can be reflected in the distribution of Fos neurons. Thus, we can not exclude the possibility that our results may slightly underestimate the number of real neurons actually engaged in the aspiration reflex.

The brainstem contains both spontaneously active respiratory and recruited respiration-related neurons extending from the level of medulla (the dorsal and ventral respiratory groups, the rapheal, medial and lateral systems of the reticular formation) to the pons (the pontine respiratory group) spreading further to a variety of suprapontine structures (including the mesencephalon, thalamus, hypothalamus, and the somatosensory cortex). In contrast to the cough, sneeze and the expiration reflexes, pools of exclusively inspiratory or inspiration-related neurons were proposed to be important for production of the aspiration reflex (Batsel and Lines 1973, Jakuš *et al.* 1985, du Pont 1987, Fung *et al.* 1994). However, Fung *et al.* (1995) also reported a recruitment of non-respiratory neurons in the medullary lateral tegmental field following nasopharyngeal stimulation in the cat. Hence, the lateral tegmental field of the medulla, between the NTS and the nucleus ambiguus was established as a critical region for fictive gasping (St. John 1990, 1996) and the fictive gasp-like AR in decerebrate, paralyzed and artificially ventilated cats (Fung *et al.* 1994). However, the second- and higher order neurons composing the central arc of the AR are completely unknown. Another difficulty results from a limited data about the projections and functions of the brainstem reticular nuclei, which seem to be critically involved in a variety of reflex behaviors. Since the nomenclature of the reticular nuclei is not uniform (especially between anatomists and physiologists) in this study we described the FLI in labeled reticular neurons according to the stereotaxic atlas of Berman (1968), using his terminology, Kalia and Mesulam (1980), Petrovický (1980) and Pavlásek and Petrovický (1994) for particular structures.

In the control group of non-stimulated, quietly breathing cats (without the AR), very low density of FLI was found in a variety of brainstem areas. A dense and more intense Fos labeling was detected in the rostral dorsolateral pons (in the NPBL and KF nuclei), and in the PAG and FTC of the mesencephalon. These supramedullary regions in the cat are known to be involved mostly in integration of cardiorespiratory and vasomotor control (Guo *et al.* 2002a), as well as in processing of pain inputs (Hoskin *et al.* 2001). Also, at the level of medulla oblongata, scattered clusters of FLI neurons were found in the nuclei associated with respiratory and cardiovasomotor control. However, in contrast to reports of a massive control FLI detected in both the vestibular nuclei and the spinal trigeminal nucleus in decerebrate and paralyzed cats prior to fictive vomiting (Miller and Ruggiero 1994) or fictive laryngeal coughing (Gestreau *et al.* 1997), only a few FLI neurons were seen in those structures in our control group of cats. This possibly indicates a substantial reduction of nociceptive stimuli under our experimental conditions.

Involvement of medullary structures

In the group of cats with AR, an intense nuclear *c-fos* gene expression was observed in longitudinal columns extending from the caudal medulla oblongata, through the dorsolateral pons up to the intermediolateral

part of the mid-mesencephalon. In the dorsal medulla of our cats dense clusters of sFLI neurons were detected within the NTS complex. Compared to control conditions, a significant number of sFLI neurons was found predominantly in the commissural, dorsal, interstitial and ventrolateral subnuclei of NTS. As revealed by antidromic mapping in cats, the sensory input from the pharyngeal rapidly-adapting receptors (RAR) may terminate at these nuclei (Jordan and Spyer 1986, Jordan 1997, Taylor *et al.* 1999). This sensory input (believed to trigger the AR), is conveyed predominantly by the pharyngeal branch of the glossopharyngeal (IX) nerve (Tomori 1979). As shown later (Jordan 2001), despite some convergence of inputs from a variety of peripheral receptors, it seems to be some functional organization within the NTS. While the glossopharyngeal afferents of RAR terminate mainly in the rostral two thirds of the NTS, the vagal afferents from other airway regions, pulmonary or laryngeal receptors target mainly on the caudal two thirds of the NTS, overlapping the level of the obex. Hence, it was revealed that bilateral terminals of RAR afferents are densely concentrated in the commissural and interstitial nuclei and to a lesser extent within the dorsal and ventrolateral subnuclei of the NTS (Jordan 2001). This finding corroborates our present results, indicating that the Fos-positive neurons within the above mentioned NTS subnuclei can be held as second-order neurons of the aspiration reflex in cats. However, the naso- and oropharyngeal regions possess multiple sensory innervation (i.e. in addition to the pharyngeal branch of the IX nerve, the sensory information from the nasopharyngeal RAR may also be carried out *via* the trigeminal, olfactory and intermediofacial nerves) (Tomori 1979). Therefore, it is possible that a minority of other neurons (e.g. those in the spinal trigeminal nucleus or the facial nucleus) with increased Fos labeling, may also co-participate in the AR. The neural activity from these second order neurons is believed to spread bilaterally to various nuclei in the medulla oblongata (Batsel and Lines 1973), to the pons Varoli and the mesencephalon (Jakuš *et al.* 2000) also reaching the thalamic and the somatosensory cortical areas (Tomori *et al.* 2000). Our Fos-like experiments validate this idea, that the second order NTS neurons may transmit the information bilaterally, to the medial and ventral parts of the medulla through the commissural nuclei, spreading farther both in ascending and descending manner through the FTG, FTL, ambigual and para-ambigual regions, the paragigantocellular and the retrofacial nuclei, localized in the ventral and ventrolateral medulla. This view is supported by the present finding of an intense bilateral sFLI labeling in all the above mentioned areas in cats with the AR. Since the glossopharyngeal afferents do not project directly to the ventrolateral medulla or to the pontine and mesencephalic reticular formations, it is reasonable to suppose that Fos-positive neurons overlapping these regions are synapticaly activated and may correspond to the higher-order neurons of the aspiration reflex.

In the ventral medulla of stimulated cats, the sFLI significantly overlapped the VRG neurons affecting predominantly its rostral part above the obex, which is associated with the NPA, NA and RFN. All these nuclei contain pools of respiratory neurons, distributing the central respiratory motor drive to both the thoracoabdominal "pump" and the upper airway "valve" muscles with respiratory-related functions. As reported before (Batsel and Lines 1973, Jakuš *et al.* 1985), the rostral VRG inspiratory neurons (including both interneurons and premotoneurons), fired during the AR with an extremely high frequency reaching sometimes even 400 imp/s, i.e. a value much higher than in severe hypoxia or in coughing (Nail *et al.* 1979, Jodkowski *et al.* 1989, Beňačka and Tomori 1995). Such vigorous activation of the rostral VRG neurons may explain the appearance of intense sFLI within the ventral medullary regions of our cats with the repeatedly provoked AR. From the present study it cannot be established whether pharyngeal, laryngeal or other motoneurons form a part of the rostral VRG neurons with sFLI. A significant increase of sFLI labeling was also found in cats with the AR within the caudal and rostral ventrolateral medulla including the medial and lateral vestibular nuclei around the pontomedullary junction. It may result from the transient episodes of hypertension during nasopharyngeal stimulation, since the activation of both the cardiac and vasomotor sympathetic efferents in the cat (resulting in tachycardia, vasoconstriction, and hypertension) are typical accompanying signs of the AR (Tomori and Widdicombe 1969, Tomori 1979). A similar increase of Fos-expression within the vestibular nuclei was also referred to cardiac sympathoexcitatory reflexes in the cat (Guo *et al.* 2002b).

Involvement of pontine and mesencephalic structures

In the pons many sFLI neurons were detected mainly in the NPBL and the KF nuclei of stimulated cats. These nuclei are referred to as a part of the pontine

respiratory group (PRG), which promotes inspiratory terminations (Bianchi *et al.* 1995). The PRG may modulate breathing *via* multiple topographically organized connections with the spinal cord, brainstem and forebrain structures (Maršala *et al.* 2002). The high density of labeling within the dorsolateral pons in our cats with provoked AR could be explained by direct projections from the commissural nuclei to the PRG (Otake *et al.* 1992), the ponto-mesencephalic rapheal neurons to the pontine reticular nuclei (Petrovický 1980), and/or by dense projections from VRG neurons to the dorsolateral pontine nuclei (Smith *et al.* 1989). The massive Fos labeling found in cats with the AR, at the NPBL, KF and to a lesser extent in the COE, BC, LTF and vlFTG, fits the most recent experimental finding that kainic acid lesions of the pontine parabrachial area and the adjacent reticular formation in cats regularly reduced the strength and partly also the elicitability of the AR (Poliaček *et al.* 2004). Nevertheless, the high density of Fos labeling found in the parabrachial neurons during the aspiration reflex was unexpected and has not yet been documented. Thus, it is highly probable that the PRG neurons might be involved in modulation of the AR in cats, similarly as was proposed in coughing (Jakuš *et al.* 1987, Gestreau *et al.* 1997, Poliaček *et al.* 2004) and in the expiration reflex (Korpáš and Jakuš 2000). It is interesting, that the pontine sFLI distribution during the AR resembles the pattern of Fos immunoreactivity occurring in the pons during fictive laryngeal coughing (Gestreau *et al.* 1997), but it differs from the patterns induced in sneezing (Wallois *et al.* 1995), swallowing (Amirali *et al.* 2001) or in the cardiac sympatoexcitatory reflexes (Guo *et al.* 2002a), suggesting a specific involvement of the pontine regions in modulation of both the laryngeal cough and aspiration reflex.

In the mesencephalon the most enhanced Fos labeling was seen bilaterally at the level of the mid-mesencephalon, within the intermediolateral part of the central tegmental field and partly around the ventrolateral periaqueductal gray. The organization of these midbrain areas is very complex, and among many different functions they seem to be involved in the modulation of the central respiratory and cardiac sympathetic drives during both defense reactions, changes in the arousal state, in vocalization, etc. These effects are mediated mainly by projections from the aforementioned Fos labeled areas to the ventrolateral medulla and the NTS (Chen and Aston-Jones 1996, Hudson and Lumb 1996, Holstege and Kuypers 1997), as well as from the periaqueductal gray to the lateral reticular nuclei in the cat (Roste *et al.* 1985). However, the finding of large Fos labeling in the central tegment field of the mid-mesencephalon was expected, since it had been reported in former experiments on cats with midcollicular decerebration that the AR disappeared after kainic acid lesion to the medullary FTL (Fung *et al.* 1994), nevertheless in our experiment in cats without decerebration, the AR surprisingly persisted even after the lesion of the FTL and disappeared only after successive midcollicular decerebration (Jakuš *et al.* 2000). Hence, we believe that the sFLI positive neurons in the mid-mesencephalon (together with those in the dorsolateral pons and the dorsal and ventral medulla oblongata, including the magno- and parvicellular reticular nuclei) may form a substantial part of a long loop in the basic brainstem coordination of the aspiration reflex in cats.

It is of interest that in stimulated cats with the AR, massive sFLI was detected in the ventrolateral part of the FTG, the ventromedial part of the FTL, and in the LRN. These parts of the medial and lateral medullary reticular formations are usually not associated with the respiratory central pattern generator (CPG). The somata of these reticular neurons create a high density of reticulo-nuclear and reticulo-reticular connections with a variety of nuclei in the medulla, pons and mesencephalon (Scheibel 1984), which may mediate various effects of the AR on many functions of the body. However, their morphological and functional connections with the proposed second and higher order neurons of the aspiration reflex remains unknown. Nevertheless, as reported previously (Brodal 1957, Pavlásek and Petrovický 1994), the medioventral part of the rostral medullary reticular formation, being mostly Fos labeled in our cats, may transmit the received information mainly in the descendent direction (i.e. from the suprapontine, pontine and the rostral medullary areas to the medullary VRG and the spinal phrenic motoneurons – Maršala *et al.* 2002). On the other hand, the ventrolateral part of the FTG and the laterodorsal part of the reticular formation, being also involved in Fos labeling, mediate signals mainly in the ascending direction (i.e. from the airway receptors and the medullary NTS input neurons to the raphe, pontine, and midbrain neurons). Recent studies have suggested an involvement of the medullary FTG and FTL in generation of swallowing (Amirali *et al.* 2001). Regular involvement of the FTL neurons was also reported in the sympathetic and baroreceptor control

(Gebber and Barman 1985), in the vestibulo-sympathetic reflexes (Yates *et al.* 1995), and in many reflex motor behaviors, e.g. gasping (St. John 1990), the "gasp-like" aspiration reflex (Fung *et al.* 1994, 1995), in vomiting (Miller and Ruggiero 1994), sneezing (Wallois *et al.* 1995), as well as in fictive laryngeal coughing (Gestreau *et al.* 1997). All these reflex motor behaviors involve the contraction of respiratory-related muscles. Hence, in addition to these reflexes, the activation of FTG and FTL reticular neurons during the AR (as detected by our Fos immunohistochemistry) also seems to be necessary for the basic production/modulation of the motor programs associated with the aspiration reflex. However, the involvement of the respiratory CPG itself in the generation of the AR it is not excluded, since we also detected intense sFLI along the column of respiratory nuclei within the rostral ventrolateral medulla, known as a morphological basis of the respiratory CPG (Feldman 1986). Similar involvement of the respiratory CPG in the generation of the tracheobronchial cough (Shannon *et al.* 1998, 2000) and the expiration reflex (Baekey *et al.* 2001) was recently suggested in cats.

Although the neural substrate underlying the coordination of the AR remains unclear, this Fos study demonstrates that nasopharyngeal stimulation evokes a selective FLI in several brainstem areas, suggesting a crucial role of these structures in coordination of various functional components of the AR. This FLI overlapped the dorsal and ventral medullary areas and the dorsolateral pontine regions (containing the pools of respiratory or respiratory-related neurons), but also involved a variety of non-respiratory reticular neurons. They are localized in the lateral part of the FTG, the medial part of the FTL, the medullary LRN and in the pontine vlFTG, FTL, as well as in the central tegmental region of the mesencephalon. Present data corroborate our former idea that the aspiration reflex is coordinated by a long loop of the control circuit rather than by a unique well-defined "center".

Acknowledgements

This study was supported by grant No. 1/8153/01 (VEGA) from the Grant Agency for Science of the Slovak Republic. We thank to Prof. MUDr. P. Petrovický, DSc. for valuable comments to the results.

References

AMIRALI A, TSAI G, SCHRADER N, WEISY D, SANDERS I: Mapping of brain stem neuronal circuitry active during swallowing. *Ann Otol Rhinol Laryngol* **110**: 502-513, 2001.

BAEKEY DM, MORRIS KF, GESTREAU C, LI Z, LINDSEY BG, SHANNON R: Medullary respiratory neurones and control of laryngeal motoneurones during fictive eupnoea and cough in the cat. *J Physiol Lond* **534**: 565-581, 2001.

BATSEL HJ, LINES AJ: Bulbar respiratory neurons participating in the sniff reflex in the cat. *Exp Neurol* **39**: 469-481, 1973.

BEŇAČKA R, TOMORI Z: The sniff-like aspiration reflex evoked by electrical stimulation of the nasopharynx. *Respir Physiol* **102**: 163-174, 1995.

BERMAN AL: *The Brainstem of the Cat. A Cytoarchitectonic Atlas with Stereotaxic Coordinates*. The University of Wisconsin Press, Madison, 1968, 175 p.

BERQUIN P, BODINEAU L, GROS N, LARNICOL N: Brainstem and hypothalamic areas involved in respiratory chemoreflexes: a Fos study in adult rats. *Brain Res* **857**: 30-40, 2000.

BIANCHI AL, DENAVIT-SAUBIÉ M, CHAMPAGNAT J: Central control of breathing in mammals: neuronal circuitry, membrane properties, and neurotransmitters. *Physiol Rev* **75**: 1-46, 1995.

BRODAL A: *The Reticular Formation of the Brain Stem. Anatomical Aspects and Functional Correlations.* Oliver and Boyd, Edinburgh, 1957, 87 p.

CHEN G, ASTON-JONES G: Extensive projections from the midbrain periaqueductal gray to the caudal ventrolateral medulla: a retrograde and anterograde tracing study in the rat. *Neuroscience* **71**: 443-459, 1996.

DAMPNEY RA, LI YW, HIROOKA Y, POTTS P, POLSON JW: Use of c-fos functional mapping to identify the central baroreceptor reflex pathway: advantages and limitations. *Clin Exp Hypertens* **17**: 197-208, 1995.

DRAGUNOW M, FAULL R: The use of c-fos as a metabolic marker in neuronal pathway tracing. *J Neurosci Methods* **29**: 261-265, 1989.

DU PONT JS: Firing patterns of bulbar respiratory neurons during sniffing in the conscious, non-paralysed rabbit. *Brain Res* **414**: 163-168, 1987.

FELDMAN JL: Neurophysiology of breathing in mammals. In: *Handbook of Physiology. The Neurons System. IV. Intrinsic Regulatory Systems of the Brain.* FE BLOOM (ed), American Physiological Society, Bethesda, MD, 1986, pp 463-524.

FUNG ML, ST. JOHN WM, TOMORI Z: Reflex recruitment of medullary gasping mechanisms in eupnoea by pharyngeal stimulations in cats. *J Physiol Lond* **475**: 519-529, 1994.

FUNG ML, TOMORI Z, ST. JOHN WM: Medullary neuronal activities in gasping induced by pharyngeal stimulation and hypoxia. *Respir Physiol* **100**: 195-202, 1995.

GEBBER GL, BARMAN SM: Lateral tegmental field neurons of cat medulla: a potential source of basal sympathetic nerve discharge. *J Neurophysiol* **54**: 1498-1512, 1985.

GESTREAU CH, BIANCHI AL, GRÉLOT L: Differential brainstem fos-like immunoreactivity following laryngeal-induced coughing and its reduction by codeine. *J Neurosci* **17**: 9340-9352, 1997.

GUO ZL, LI P, LONGHURST JC: Central pathways in the pons and midbrain involved in cardiac sympathoexcitatory reflexes in cats. *Neuroscience* **113**: 435-447, 2002a.

GUO ZL, LAI HC, LONGHURST JC: Medullary pathways involved in cardiac sympathoexcitatory reflexes in the cat. *Brain Res* **925**: 55-66, 2002b.

HERDEGEN T, KOVARY K, LEAH J, BRAVO R: Specific temporal and spatial distribution of JUN, FOS and KROX-24 proteins in spinal neurons following noxious transsynaptic stimulation. *J Comp Neurol* **313**: 178-191, 1991.

HOLSTEGE G, KUYPERS HGJM: Propriobulbar fibre connections to the trigeminal, facial and hypoglossal motor nuclei. I. An anterograde degeneration study in the cat. *Brain* **100**: 239-242, 1997.

HOSKIN KL, BULMER DC, LASALANDRA M, JONKMAN A, GOADSBY PJ: Fos expression in the midbrain periaqueductal grey after trigeminovascular stimulation. *J Anat* **198**: 29-35, 2001.

HUDSON PM, LUMB BM: *Neuron*s in the midbrain periaqueductal gray send collateral projections to nucleus raphe magnus and the rostral ventrolateral medulla in the rat. *Brain Res* **733**: 138-141, 1996.

HUNT PS, PINI A, EVAN G: Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. *Nature* **328**: 632-634, 1987.

JAKUŠ J, TOMORI Z, STRÁNSKY A: Activity of bulbar respiratory neurons during cough and other respiratory tract reflexes in cats. *Physiol Bohemoslov* **34**: 127-136, 1985.

JAKUŠ J, TOMORI Z, BOŠEĽOVÁ Ľ, NAGYOVÁ B, KUBINEC V: Respiration and airway reflexes after transversal brain stem lesions in cats. *Physiol Bohemoslov* **36**: 329-340, 1987.

JAKUŠ J, STRÁNSKY A, POLIAČEK I, BARÁNI H, BOŠEĽOVÁ Ľ: Kainic acid lesion to the lateral tegmental field of medulla. Effects on cough, expiration and aspiration reflexes in anesthetized cats. *Physiol Res* **49**: 387-398, 2000.

JODKOWSKI JS, GUTRIE RD, CAMERON WE: The activity pattern of phrenic motoneurons during the aspiration reflex: an intracellular study. *Brain Res* **505**: 187-194, 1989.

JORDAN D: Central nervous control of the airways. In: *Central Nervous Control of Autonomic Function*. D JORDAN (ed), Harwood Academic Publishers, Chur, 1997, pp 63-107.

JORDAN D: Central nervous pathways and control of the airways. *Respir Physiol* **125**: 67- 81, 2001.

JORDAN D, SPYER KM: Brainstem integration of cardiovascular and pulmonary afferent activity. *Prog Brain Res* **67**: 295-314, 1986.

KALIA M, MESULAM MM: Brain stem projections of sensory and motor components of the vagus complex in cat. II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. *J Comp Neurol* **193**: 467-508, 1980.

KORPÁŠ J, JAKUŠ J: The expiration reflex from the vocal folds. *Acta Physiol Hung* **87**: 201-215, 2000.

MARŠALA J, LUKÁČOVÁ N, ČIŽKOVÁ D, KAFKA J, KATSUBE N, KUCHÁROVÁ K, MARŠALA M: The case for the bulbospinal respiratory nitric oxide synthase-immunoreactive pathway in the dog. *Exp Neurol* **177**: 115-132, 2002.

MILLER AD, RUGGIERO D: Emetic reflex arc revealed by expression of the immediate-early gene s-Fos in the cat. *J Neurosci* **14**: 871-888, 1994.

NAIL BS, STERLING GM, WIDDICOMBE JG: Patterns of spontaneous and reflexly-induced activity in phrenic and intercostal motoneurons. *Exp Brain Res* **15**: 318-332, 1979.

OTAKE K, EZURE K, LIPSKI J, WONG SHE RB: Projections from the commissural subnucleus of the nucleus of the solitary tract: an anterograde tracing study in the cat. *J Comp Neurol* **324**: 365-378, 1992.

ORENDÁČOVÁ J, MARŠALA M, ŠULLA I, KAFKA J, JALČ P, ČÍŽKOVÁ D, TAIRA Y, MARŠALA J: Incipient cauda equina syndrome as a model of somatovisceral pain in dogs: spinal cord structures involved as revealed by the expression of c-fos and NADPH diaphorase activity. *Neuroscience* **95**: 543-557, 2000.

PAVLÁSEK J, PETROVICKÝ P: *The Reticular Formation and Reticulospinal System*. Veda, Bratislava, 1994, 224 p.

PETROVICKÝ P: *Reticular Formation and Its Raphe System I. Cytoarchitectonics with Comparative Aspects*. Acta Univ. Carolinae Med, Monographia XLIX, Prague, 1980, 117 p.

POLIAČEK I: *Electrophysiological Analysis of Neuronal Mechanisms Underlying the Motor Control of the Larynx* (in Slovak). PhD Thesis, Comenius University, Faculty of Medicine, Martin, 2000, 158 p.

POLIAČEK I, STRÁNSKY A, JAKUŠ J, BARÁNI H, TOMORI Z, HALAŠOVÁ E: Activity of laryngeal abductor and adductor muscles during cough, expiration and aspiration reflexes in cats. *Physiol Res* **52**: 749-762, 2003.

POLIAČEK I, JAKUŠ J, STRÁNSKY A, BARÁNI H, HALAŠOVÁ E, TOMORI Z: Cough, expiration and aspiration reflexes following kainic acid lesions to the pontine respiratory group in anesthetized cats. *Physiol Res* **53**: 155-163, 2004.

ROSTE LS, DIETRICHS E, WALBERG F: A projection from the the periaqueductal grey to the lateral reticular nucleus in the cat. *Anat Embryol (Berlin)* **172**: 339-343, 1985.

SCHEIBEL AB: The brain stem reticular core and sensory function. In: *Handbook of Physiology*. *The Nervous System, Sensory Processes.* American Physiological Society, Bethesda, MD, 1984, pp 213-256.

SHANNON R, BAEKEY DM, MORRIS KF, LINDSEY BG: Ventrolateral medulla respiratory network and a model of cough motor pattern generation. *J Appl Physiol* **84**: 2020-2035, 1998.

SHANNON R, BAEKEY DM, MORRIS KF, LI Z, LINDSEY BG: Functional connectivity among ventrolateral medullary respiratory neurons and responses during fictive cough in the cat. *J Physio Londl* **525**: 207-224, 2000.

SMITH JC, MORRISON DE, ELLENBERGER HH, OTTO MR, FELDMAN JL: Brainstem projections to the major respiratory neuron populations in the medulla of the cat. *J Comp Neurol* **281**: 69-96, 1989.

STRÁNSKY A, SZEREDA-PRZESTASZEWSKA M, WIDDICOMBE JG, TOMORI Z: The activity of laryngeal motoneurons and laryngeal resistance during defensive reflexes from the respiratory tract. *Physiol Bohemoslov* **25**: 471, 1976.

ST. JOHN WM: Neurogenesis, control, and functional significance of gasping. *J Appl Physiol* **68**: 1305-1315, 1990.

ST. JOHN WM: Medullary regions for neurogenesis of gasping: noeud vital or noeuds vitals. *J Appl Physiol* **81**: 1865-1877, 1996.

TAYLOR EW, JORDAN D, COOTE JH: Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiol Rev* **79**: 855-916, 1999.

TOMORI Z: Pleural, tracheal and abdominal pressure variations in defensive and pathologic reflexes of the respiratory tract. *Physiol Bohemoslov* **14**: 382, 1965.

TOMORI Z: "Aspiration reflex": a respiratory reaction extremely resistant to suppression. *Experientia* **26**: 664, 1970.

TOMORI Z: The sniff-like aspiration reflex, In: *Cough and Other Respiratory Reflexes*. KORPÁŠ J, TOMORI Z (eds). Karger, Basel, 1979, pp 224-250.

TOMORI Z, WIDDICOMBE JG: Muscular, bronchomotor and cardiovascular reflexes elicited by the mechanical stimulation of the respiratory tract. *J Physiol London* **200**: 25-49, 1969.

TOMORI Z, BEŇAČKA R, DONIČ V: Mechanisms and clinicophysiological implications of the sniff- and gasp-like aspiration reflex. *Respir Physiol* **114**: 83-98, 1998.

TOMORI Z, BEŇAČKA R, DONIČ V, JAKUŠ J: Contribution of upper airway reflexes to apnoea reversal, arousal, and resuscitation. *Monaldi Arch Chest Dis* **55**: 398-403, 2000.

WALLOIS F, GROS F, MASMOUDI K, LARNICOL N: C-Fos like immunoreactivity in the cat brainstem evoked by sneeze-inducing air puff stimulation of the nasal mucosa. *Brain Res* **687**: 143-154, 1995.

YATES BJ, BALABAN CD, MILLER AD, ENDO K, YAMAGUCHI Y: Vestibular inputs to the lateral tegmental field of the cat: potential role in autonomic control. *Brain Res* **689**: 197-206, 1995.

Reprint requests

Prof. Dr. Med. J. Jakuš, DSc., Department of Biophysics, Comenius University, Jessenius Faculty of Medicine, Malá Hora 4, 037 54 Martin, Slovak Republic, e-mail jakus@fmed.uniba.sk