# Sources of the motor and somatic sensory innervation of the trapezius muscle in the rat

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ABSTRACT: The study was carried out on nine sexually mature male rats of the Wistar breed weighing approximately 250 g each. Animals were anaesthetized with thiopental sodium injected intraperitoneally (30 mg/kg of body weight). The animals were then injected with Fast Blue tracer into the right trapezius muscle. After a survival period of five weeks the rats were transcardially perfused with buffered paraformaldehyde. The following tissue blocks were collected: spinal cord (cervical and thoracic part) with spinal ganglia and whole brain with medulla oblongata. The tissues collected were cut into 12  $\mu$ m-thick cryostat sections, which were viewed under a fluorescent microscope equipped with a filter block for FB. FB-positive (FB+) neurons were counted in every fourth section to avoid double analysis. After injections of the tracer to the right trapezius muscle FB+ neurons were found in many nuclei and ganglia. The labelled cells of the medulla oblongata nuclei were found in the bilateral vestibular nuclei including superior (SuVe), lateral (LVe), medial (MVe) and spinal (SpVe) vestibular nuclei and also in the dorsal raphe nucleus (DR) which is a single nucleus, but only in the ipsilateral ambiguous nucleus (Amb). FB+ perikarya were also found in the spinal cord, extending between the first cervical segment ( $C_1$ ) and the cranial half of the seventh spinal cervical segment ( $C_7$ ), in an ipsilateral area ventrolateraly with respect to the central canal, within the spinal nucleus of the accessory nerve (SAN). Retrograde labelled sensory neurons were found in the bilateral spinal ganglia (SPG-s), from the second cervical ganglion ( $C_7$ ) to the third thoracic ganglion ( $C_7$ ).

Keywords: trapezius muscle; innervations; nuclei; ganglia; retrograde tracing

The rat trapezius muscle consists of three distinct parts which play different but complementary roles in its function. The clavotrapezius passes from the external occipital protuberance to the posterior border of the clavicle. The acromiotrapezius passes from the spinous process of the vertebra  $(C_1 \text{ to } Th_4)$  to the acromion process and the rostral edge of the spine of the scapula. The spinotrapezius passes from the spinous process ( $Th_4$  to  $L_3$ ) to the medial end of the scapular spine. The trapezius is a major suspensory muscle of the shoulder girdle and joins the thoracic limb to the trunk. Moreover, the trapezius, in cooperation with other muscles like the sternocleidomastoid muscle, the omotransverse muscle and the broadest muscle of the back is responsible for coordinating the movements of the limb, trunk, head and neck; however, its most important function is the maintenance of balance

The trapezius muscle is generally regarded by anatomists as unusual with regard to its motor and sensory nerve supply. Nerves supplying this muscle reach their destination by different routes: the motor supply via the spinal accessory nerve and also via spinal nerves of the cervical and thoracic part of the spinal cord, and the sensory supply via the cervical plexus (Fitzgerald et al., 1982; Zhao et al., 2006; Pu et al., 2008).

Localization of the neurons supplying the trapezius muscle was established using two different methods: retrograde degeneration technique (Flieger, 1964, 1966, 1967) and retrograde tracing (Yan and Hitomi, 2006; Ullah et al., 2007; Yan et al., 2007). Tracing studies revealed neurons supplying the trapezius muscle in the spinal motor nucleus of cervical segments of the spinal cord. Krammer et al. (1987) used HRP-tracing and electrostimulation to show that neurons innervating the trapezius localized in the spinal segments of the cervical part of the motor spinal nucleus from  $C_2$  to  $C_7$ . In contrast to this data Kitamura and Sakai (1982) described motor neurons supplying the trapezius muscle as being present in the spinal motor nucleus segments  $C_2$  to  $C_5$  whereas Ullah et al. (2007) described trapezius-innervating neurons in the C<sub>2</sub> to C<sub>6</sub> spinal segments. The accessory nerve is known to consist of a spinal and cranial part which has its nucleus in the medulla oblongata - nucleus ambiguous. Until now there is no data in the literature regarding motor neurons supplying the trapezius muscle localised in the nucleus ambiguous, and therefore a study of this problem is of interest. In regard to sensory innervation of the trapezius muscle the body of evidence is very limited. From the paper by Tsukagoshi et al. (2006) it is known that sensory neurons supplying the trapezius muscle are localized in spinal SPG-s from C<sub>2</sub> to Th<sub>1</sub>. As mentioned previously, the trapezius muscle is involved in maintaining body balance. This function is regulated by vestibular nuclei located in the medulla oblongata; it is therefore of interest to determine whether any neurons have a neuronal connection with the trapezius muscle. The lack of comprehensive studies regarding innervation of the trapezius muscle in the rat and the many discrepancies between results described in previous papers dealing with this problem motivated us to elucidate this issue.

### MATERIAL AND METHODS

This study was carried out on nine sexually mature male rats of the Wistar breed weighing approximately 250 g each. The animals were housed and treated in accordance with the rules approved by the local Ethics Commission (confirming the principles of Laboratory Animal Care, NIH publication No. 86-23, revised in 1985).

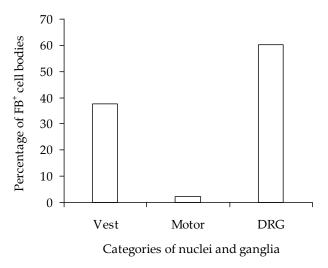
The animals were anaesthetized with thiopental sodium intraperitoneal injection (30 mg/kg of body weight). The animals were then injected with Fast Blue tracer (5% suspension of FB in distilled

water) into the right acromotrapezius and spinotrapezius muscles. Tracer was administered in five injections of 2 µl each. After a survival period of five weeks the rats were deeply anaesthetized (following the same procedure as described above) and transcardially perfused with 0.2-0.3 l of 4% ice-cold buffered paraformaldehyde (pH 7.4). No traces of contamination with FB were found in the surrounding tissues, muscles and skin, in the neighbourhood of the places of the tracer injections. The following tissue blocks were collected; namely, spinal cord (cervical and thoracic part) with spinal ganglia and whole brain with medulla oblongata. The tissue blocks were then postfixed by immersion in the same fixative for 30 min, rinsed with phosphate buffer (pH 7.4) and transferred to and stored in 30% buffered sucrose solution (pH 7.4) until further processing. Brain sample blocks were cut out about 7 mm caudally from the bregma, comprising the medulla oblongata with the pons and caudal half of the midbrain. The spinal ganglia, spinal cords and brain specimens were cut in the transverse (tissues from six animals) and dorsal (tissues from three animals) plane into 12 µm-thick cryostat sections. The series obtained were viewed under a fluorescent microscope equipped with a filter block for FB. FB<sup>+</sup> neurons were counted in every fourth section to avoid double analysis. For mathematical analysis numerical data received from five animals were used. Localization of the nuclei was achieved by comparison of the sections with images from the rat brain atlas of Paxinos and Watson (1998).

#### **RESULTS**

### Distribution and frequency of labelled cells

After tracer injections to the right trapezius muscle FB<sup>+</sup> neurons were found in numerous nuclei and ganglia. The labelled cells of the medulla oblongata nuclei were found in the bilateral vestibular nuclei including SuVe, LVe, MVe and SpVe vestibular nuclei (Vest) and also in DR, but only in the ipsilateral Amb. FB<sup>+</sup> perikarya were observed also in the spinal cord in an ipsilateral area ventrolaterally with respect to the central canal within the spinal nucleus of the accessory nerve (SAN). Sensory innervation of the trapezius muscle was accomplished by numerous neurons localized in SPG-s of the spinal nerves.



Graph 1. Percentage of FB<sup>+</sup> cell bodies in different categories of the nuclei and ganglia among all FB<sup>+</sup> counted

SuVe LVe MVe SpVe DR

Vestibular nuclei

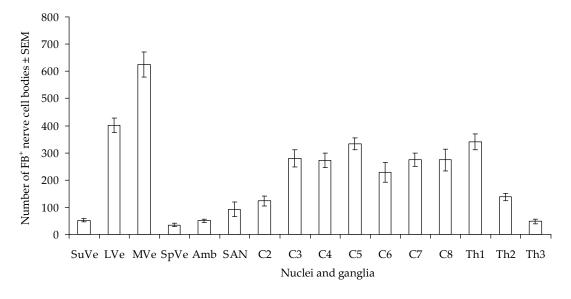
Graph 2. Percentage of FB<sup>+</sup> cell bodies in vestibular nuclei among all FB<sup>+</sup> vestibular neurones counted

The vestibular nuclei contained 37.52% of all retrograde traced neurons counted in all animals (Graph 1).

FB<sup>+</sup> neurons present in SuVe constituted 3.69% of all vestibular neurons (Graph 2). The mean number of SuVe FB-positive perikarya was  $83.4 \pm 5.17$  per animal (mean  $\pm$  SEM, all the numerical values regarding the mean number of labelled cells cited in the text are expressed as means  $\pm$  SEM). The mean number of SuVe perikarya located in the ipsilateral nucleus was 52.0  $\pm$  6.73 which constituted 61.59  $\pm$  5.07% of all FB<sup>+</sup> SuVe neurons, whereas in contralateral SuVe the mean number of FB<sup>+</sup> neurons was 31.40  $\pm$  3.42 which constituted 38.41  $\pm$  5.07% of perikarya.

The neurons observed were round or oval and approximately 30  $\mu$ m in diameter (Figure 1).

Traced neuronal somata in LVe constituted 31.14% of all vestibular neurons (Graph 2). In LVe the mean number of FB-positive perikarya was 704.4  $\pm$  36.21 per animal. The mean number of LVe perikarya located in the ipsilateral nucleus was 402  $\pm$  26.77 (Graph 3), which constituted 57.18  $\pm$  3.11% of all FB+ LVe neurons, whereas in contralateral LVe the mean number of FB+ neurons was 302.4  $\pm$  28.97 (Graph 4) which consisted of 42.82  $\pm$  3.11% of perikarya. The neurons observed were spindle-shaped, with a longitudinal axis of approximately 40  $\mu m$  and a short axis of approximately 20  $\mu m$  (Figure 2).



Graph 3. Number of FB<sup>+</sup> nerve cell bodies in ipsilateral vestibular and motor nuclei and dorsal root ganglia

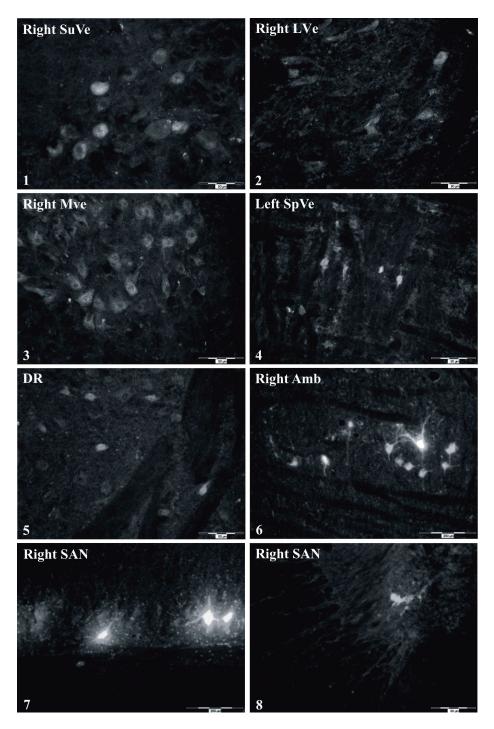
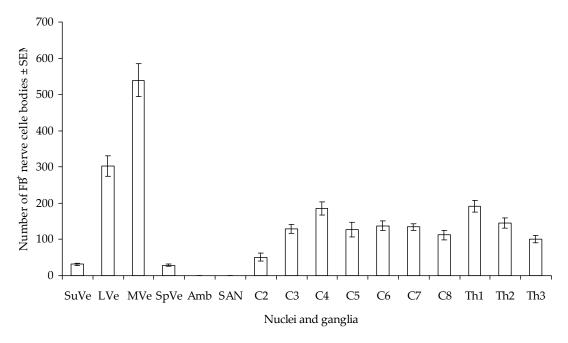


Figure 1. FB labeled neurons in right superior vestibular nucleus. Dorsal section through the medulla oblongata Figure 2. FB labeled neurons in right lateral vestibular nucleus. Dorsal section through the medulla oblongata Figure 3. Numerous FB labeled neurons in right medial vestibular nucleus. Dorsal section through the medulla oblongata Figure 4. FB labeled neurons in left spinal vestibular nucleus. Dorsal section through the medulla oblongata Figure 5. FB labeled neurons in dorsal raphe nucleus. Dorsal section through the medulla oblongata Figure 6. Intensively FB labeled neurons in right ambiguous nucleus. Transverse section through the medulla oblongata Figure 7. Intensively FB labeled neurons in right spinal accessory nucleus. Note the large amount of FB in the surrounding of the traced neurons and the very numerous FB-labeled glia cells (small blue dots). Dorsal section through the spinal cord

Figure 8. FB labeled neuron in right spinal accessory nucleus. Micrograph showing typical localization of traced neurons in ventral horn of the spinal cord



Graph 4. Number of FB<sup>+</sup> nerve cell bodies in contralateral vestibular and motor nuclei and dorsal root ganglia

MVe FB-labelled neurons constituted 51.46 % of all FB<sup>+</sup> vestibular neurons (Graph 2). In MVe the mean number of FB-positive perikarya was  $1162.0 \pm 49.9$  per animal. The mean number of MVe perikarya located in the ipsilateral nucleus was  $624.0 \pm 47.1$  (Graph 3), which constituted  $53.66 \pm 3.31\%$  of all FB<sup>+</sup> MVe neurons, whereas in contralateral MVe the mean number of FB<sup>+</sup> neurons was  $539.4 \pm 45.2$  (Graph 4) which constituted  $46.34 \pm 3.31\%$  of neurons. The observed neurons were triangular or spindle-shaped, with a long axis of approximately 40  $\mu$ m and a short axis of approximately 20  $\mu$ m (Figure 3).

SpVe tracer-containing neuronal somata constituted 2.82% of all FB labelled vestibular neurons (Graph 2). In SpVe the mean number of FB-positive perikarya was  $63.80 \pm 9.11$  per animal. The mean number of SpVe perikarya located in the ipsilateral

nucleus was  $34.60 \pm 5.57$ , which constituted  $53.81 \pm 2.37\%$  of all FB<sup>+</sup> SpVe neurons, whereas in the contralateral SpVe the mean number of FB<sup>+</sup> neurons was  $29.20 \pm 3.80$ , which constituted  $46.19 \pm 2.37\%$  of all FB<sup>+</sup> SpVe neurons. The neurons observed were spindle-shaped, with a long axis approximately  $20-25~\mu m$  (Figure 4).

DR tracer-containing neuronal somata constituted 10.89% of all FB<sup>+</sup> vestibular neurons (Graph 2). In DR, which is an unpaired nucleus, the mean number of FB-positive perikarya was  $246.4 \pm 26.32$  (Graph 5). The neurons observed were oval or spindle-shaped, with a long axis of approximately  $20 \ \mu m$  (Figure 5).

The motor nuclei contained 2.36% of all retrogradely traced neurons counted in all animals (Graph 1).

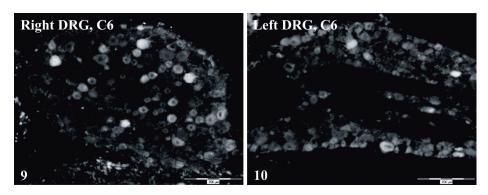
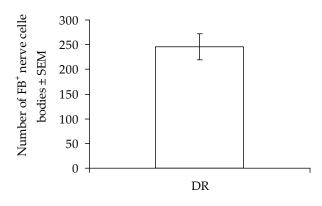
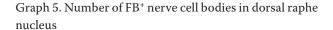


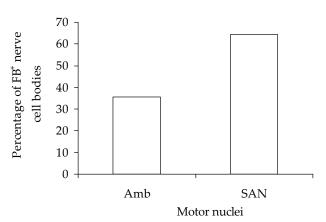
Figure 9. FB labeled neurons in right sixth spinal ganglion Figure 10. FB labeled neurons in left sixth spinal ganglion





Amb tracer-containing neuronal somata constituted 35.53% of all traced motoneurons (Graph 6). In Amb the mean number of FB-positive perikarya was 50.6  $\pm$  5.96 per animal (Graph 3). All the FB+ neurons were localized in the ipsilateral nucleus. The neurons observed were multipolar and with a relatively large diameter ranging from 40 to 80  $\mu m$  (Figure 6).

 ${
m FB}^+$  perikarya were also found in the spinal medulla, extending between the  ${
m C}_1$  and the cranial half of the  ${
m C}_7$  spinal cervical segments.  ${
m FB}^+$  neurons were located in an ipsilateral area ventrolaterally with respect to the central canal. This localization corresponds to the localization of the spinal accessory nerve nucleus. SAN tracer containing neuronal somata constituted 64.47% of all traced motoneurons (Graph 6). The mean number of SAN labelled motoneurons was 91.8  $\pm$  26.18 per animal (Graph 3). All the  ${
m FB}^+$  neurons were localized in

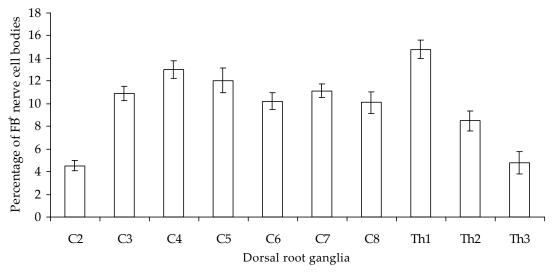


Graph 6. Percentage of FB<sup>+</sup> cell bodies in motor nuclei among all FB<sup>+</sup> motor neurones counted

the ipsilateral nucleus. The neurons observed were multipolar with a relatively large diameter ranging from 60 to 80  $\mu$ m (Figures 7, 8).

The sensory ganglia contained 60.12 % of all retrogradely traced neurons counted in all animals (Graph 1).

Retrograde labelled sensory neurons were found in the bilateral SPG-s, from the second cervical ganglion ( $C_2$ ) to the third thoracic ganglion ( $Th_3$ ). The mean number of SPG perikarya located in the ipsilateral ganglia was 230.9  $\pm$  15.14 per ganglion (Graph 3), which constituted 63.52  $\pm$  1.18% of FB+ SPG neurons, whereas in the contralateral SPG the mean number of FB+ neurons was 131  $\pm$  6.78 (Graph 4) per ganglion which constituted 36.48  $\pm$  1.18% of neurons. The highest numbers of FB+ neurons were observed in SPG-s from the second cervical to the eighth cervical ganglion, in both ipsi- and contralateral ganglia. Tracer containing



Graph 7. Percentage of FB+ cell bodies in bilateral DRG-s among all FB+ sensory neurones counted

neuronal somata present in  $C_2$  constituted 4.51  $\pm$  0.47%, in  $C_3$  10.92  $\pm$  0.63%, in  $C_4$  13.01  $\pm$  0.75%, in  $C_5$  12.05  $\pm$  1.1%, in  $C_6$  10.21  $\pm$  0.73%, in  $C_7$  11.14  $\pm$  0.58%, in  $C_8$  10.11  $\pm$  0.94%, in Th $_1$  14.78  $\pm$  0.81%, in Th $_2$  8.48  $\pm$  0.89%, and in Th $_3$  4.80  $\pm$  0.98% of all traced sensory neurons (Graph 7). The neurons observed were round or sometimes oval, with a relatively large diameter ranging from 25–80  $\mu m$  (Figure 9, 10).

## **DISCUSSION**

In the present study neurons innervating the trapezius muscle were localised in the medulla oblongata, spinal medulla and spinal ganglia. Sensory innervation of studied muscle was accomplished by neurons located within the ipsi- and contralateral spinal ganglia extending from the C<sub>2</sub> to Th<sub>3</sub> spinal medulla segments. In the available literature there is no data regarding this problem in the rat except the paper by Tsukagoshi et al. (2006). It was found that sensory neurons supplying the trapezius muscle were located in SPG-s extending from C<sub>2</sub> to Th, (Tsukagoshi et al., 2006). Unfortunately, only ipsilateral ganglia were studied. In the above-mentioned paper (which was devoted to the distribution of the vanilloid receptor in sensory neurons supplying skin and muscles) there is no data regarding the number of FB+ cells in particular ganglia, only the total number of neurons per animal is mentioned and equals 1599.7. In our studies the average number of FB+ SPG neurons equals 2309 per animal in ipsilateral ganglia. Such a difference may be the result of the larger amount of tracer used in our study (10 µl of 5% FB suspension in our studies versus 5 µl 2% FB suspension in the study of Tsukagoshi et al., 2006) or longer post-surgery survival period. In the experiments of Tsukagoshi et al. (2006) animals were sacrificed seven days after tracer injection, whereas in our studies ganglia were collected five weeks after surgery. In the available literature there are no data regarding contralaterally located sensory neurons innervating trapezius muscle, but it has been reported in papers regarding the smooth retractor penis muscle of the pig (Panu et al., 2003), and the striated bulbospongiosus muscle of the pig (Botti et al., 2009). In studies by Ishii (1989) no HPR neurons were found in contralateral ganglia. The observed presence of  $FB^+$  neurons (total number per animal = 1315.4) in contralateral SPG-s may be the result of the existence of contralateral projections of the afferent nerves from dorsal spinal nerve branches, but this hypothesis needs to be evaluated by further studies. Such a possibility of bilateral sensory projections was already postulated by Smith (1986). In this paper, it was described that contralaterally projecting sensory neurons had axons in subdivisions of the thoracic nerves that supply skin adjacent to the body midlines. Infiltration of the contralateral trapezius muscle by FB injected into the studied muscle should be also taken into consideration, but it is unlikely because, no traces of contamination with FB were found in the surrounding tissues. It is also known from electrophysiological experiments performed in humans (Alexander and Harrison, 2002) that sensory neuron endings reach the motoneurons located in ipsi- and contra-lateral anterior horns of the spinal cord. If the SPG sensory neurons are able to absorb tracer released by motoneurons into the intercellular space, the presence of FB<sup>+</sup> perikarya in contralateral ganglia can be explained by this phenomenon. Such a possibility will be discussed in detail in a further part of the discussion regarding vestibular nuclei.

Motor innervation of the trapezius muscle has been studied in many species including the rat (Gottschall et al., 1980; Kitamura and Sakai, 1982; Matesz and Szekely, 1983; Brichta et al., 1987; Ullah et al., 2007), rabbit (Ullah and Salman, 1986), cat (Rapoport, 1978; Satomi et al., 1985; Liinamaa et al., 1997), sheep (Clavenzani et al., 1994), primates (Augustine and White, 1986; Ueyama et al., 1990) and human (Routal and Pal, 2000). This innervation is accomplished mainly by the accessory nerve, but there are some papers reporting partial innervation of this muscle by motor fibres from spinal nerves (Zhao et al., 2006; Yan et al., 2007; Pu et al., 2008). Neuronal tracing studies performed on the rat showed tracer labelled neurons in the ventral horn of the spinal cord. Neurons traced with cholera toxin-conjugated horseradish peroxidase were found in the C<sub>2</sub> to C<sub>4</sub> spinal segments (Hayakawa et al., 2002). In other studies traced neurons were found in the  $C_2$  to  $C_6$  spinal segments (Ullah et al., 2007). This localization corresponds to the localization of the spinal accessory nucleus. Kitamura and Sakai (1982) and Matesz and Szekely (1983) located the SAN in the rat. Kitamura and Sakai (1982) found them in the upper six cervical segments of the spinal cord, forming three longitudinal columns, whereas Matesz and Szekely (1983) located them in the caudal part of the medulla oblongata

and upper six cervical segments of the spinal cord, also forming three columns. In studies by Ullah et al. (2007) the SAN was found to be located in the caudal part (caudal 0.9-1.2 mm) of the medulla oblongata, the whole lengths of cervical spinal cord segments from  $C_1$  to  $C_5$  and rostral fourth of  $C_6$ . In the caudal part of the medulla oblongata, the SAN was represented by a group of perikarya of motor neurons lying directly ventrolateral to the pyramidal fibers that were passing dorsolaterally after their decussation. In the spinal cord, the motor neuronal somata of the SAN were located in the dorsomedial and central columns at C<sub>1</sub>, in the dorsomedial, central and ventrolateral columns at  $C_2$  and in the ventrolateral column only at  $C_3$ ,  $C_4$ ,  $C_5$  and rostral quarter of  $C_6$ .

Many studies have reported that neurons in the medial column innervate the sternomastoid and the cleidomastoid muscles and the neurons in the lateral column innervate the trapezius muscle and a portion of the cleidomastoid muscle in the cat (Rapoport, 1978; Satomi et al., 1985; Liinamaa et al., 1997), rabbit (Ullah and Salman, 1986) and rat (Gottschall et al., 1980; Kitamura and Sakai, 1982; Matesz and Szekely, 1983; Brichta et al., 1987). Kitamura and Sakai (1982) reported localization of the SAN trapezius perikarya in C<sub>2</sub> (caudal half),  $C_3$  to  $C_5$  and  $C_6$  (rostral half) spinal segments. The perikarya of motor neurons supplying the trapezius muscle were located in the ventrolateral column in the caudal three-quarters of C<sub>2</sub> only, the whole lengths of  $C_3$ ,  $C_4$  and  $C_5$  and in the rostral quarter of C<sub>6</sub> (Ullah et al., 2007). In our studies FB<sup>+</sup> perikarya of SAN were found in the spinal cord, extending between C<sub>1</sub> and the cranial half of C<sub>7</sub> spinal cervical segments. The slightly broader extent of SAN shown in our paper can be explained by the much longer exposure of the animals used in our studies to the tracer. There is a controversy among scientists regarding the extent of the SAN in various animal species. The most cranial extent was described in the caudal part of the medulla oblongata and upper cervical segments of the spinal cord in sheep (Flieger, 1964), horse (Flieger, 1966), cow (Flieger, 1967), rat (Matesz and Szekely, 1983; Ullah et al., 2007), rabbit (Ullah and Salman, 1986), baboon (Augustine and White, 1986), japanese monkey (Ueyama et al., 1990), and human (Routal and Pal, 2000). In our studies no traced neurons were found in the caudal part of the medulla oblongata, in contrast to previous findings. On the other hand other researchers studying this problem in human foetuses (Pearson, 1938), rabbits (Romanes, 1941), cats (Holomanova et al., 1972) dogs (Ruminska-Kowalska et al., 1976), rats (Kitamura and Sakai, 1982), cats (Satomi et al., (1985), monkeys (Jenny et al., 1988), sheep (Romanes, 1940; Clavenzani et al., 1994) and felines (Liinamaa et al., 1997) found the SAN to be located in the cervical segments of the spinal cord only, which is in accordance with our results. Surprisingly, in our studies a group of FB<sup>+</sup> neurons was found within the cranial nucleus of the accessory nerve (nucleus ambiguous). In the available literature there are no reports of the trapezius innervating neurons located in Amb. On the other hand, in all described experiments survival time after tracer injection was no longer than a week. In our experiment this time was much longer (five weeks) and perhaps this was long enough for saturation of the Amb neurons with FB. The accessory nerve has two kinds of rootlets, namely spinal and cranial. Cranial rootlets join the vagus nerve and are involved in the innervation of the musculature of the head viscera (Lee et al., 1992; Hayakawa et al., 1999; Pascual-Font et al., 2006), but it is also possible that some of these fibres may join the spinal accessory nerve and may be involved in the innervation of the trapezius muscle. In our studies numerous FB+ "trapezius" neurons were found within the vestibular nuclei. This observation has no confirmation in papers written by other scientists, but it is known that brainstem-located neuronal centres are involved in an indirect manner in innervation of the muscles (Lois et al., 2009). In this paper, describing trans-neuronal tracing of the diaphragm, the presence of perikarya infected with pseudorabies virus was found within many nuclei; namely, pontine and medullary respiratory groups, the medial and lateral medullary reticular formation, the region immediately ventral to the spinal trigeminal nucleus, raphe pallidus and obscurus and the vestibular nuclei. Another paper describing trans-neuronal tracing done with the same tracer injected into the masseter muscle in the rat reported the presence of the traced neurons in all, ipsi- and contra-lateral vestibular nuclei (Giaconi et al., 2006), which is in accordance with our observations. Such a configuration can be explained in two ways. First - least probably vestibular neurons have a direct monosynaptic connection with the trapezius muscle. Secondly the vestibular neurons can absorb FB released from traced trapezius motoneurons localized in the ventral horns of the spinal cord. Such a possibility was

already reported by Wigston and Kennedy (1987) in an experiment performed with diamino yellow. This study, performed in the axolotl showed a significant increase in the number of diamino yellow-labeled motoneurons 2-3 months following tracer administration to a peripheral nerve, as compared to 9–11 days. The authors suggested that the observed increase may be explained by transfer between axons within nerves en route to the spinal cord or a direct transfer of diamino yellow to adjacent neurons and glia within the spinal cord. Such a possibility may explain the presence of traced neurons within the vestibular nuclei observed in our studies. The tracer released from spinal motoneurons may be transported into vestibular nuclei via the vestibulospinal tract. The vestibulospinal tract derives mainly from the cells of LVe, of the same and the opposite side. The other vestibular nuclei also contribute fibres. Their terminals and collaterals end either directly or indirectly among the motor cells of the ventral column, so, such a connection perfectly fits the above mentioned hypothesis. Also, the intensity of labeling may confirm this possibility because motoneurons were intensively stained, whereas vestibular neurons were weakly stained, as they can absorb the tracer released from primary labeled motoneurons and the amount of the available tracer is relatively small. If so, FB may act as a transneuronal tracer, especially in long term experiments.

In the present studies the sources of sensory and motor innervation of the rat trapezius muscle were described. Surprisingly, additionally to ipsilateral SPG neurons, sensory neurons localised in the contralateral SPG were found. In respect to the motor innervation, ambiguous neurons innervating the trapezius muscle were described for the first time. Also numerous FB<sup>+</sup> perikarya were found within vestibular nuclei. Such an observation in regard to the trapezius muscle and FB used as a tracer is also described for the first time. Neuronal connections between the different kinds of neurons involved in directly and indirectly in innervation of the trapezius muscle, as proposed and discussed in this paper, can be very important for complex coordination of movement and general postural control.

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