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Distribution of compound potentials recorded from the surface of isolated stellate ganglia following stimulation of postganglionic branches, in rats and cats

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Abstract. The experiments were performed on 9 cat and 18 rat isolated stellate ganglia. Rats and cats were anesthetized with alpha-glucochloralose or urethane, respectively. The ganglia, isolated with their branches, were transferred to a recording chamber and constantly superfused with artificial extracellular fluid bubbled with 95% O_2 and 5% CO_2. Branches of the ganglion were one by one placed in suction electrodes and stimulated. Antidromic evoked potentials were systematically recorded from numerous points on the ganglion surface. The area under the curve of the negative wave of each recorded potential was considered proportional to the number of neurons located in the vicinity of the recording electrode, projecting to the stimulated nerve. We have found that: (1) cardiac sympathetic neurons are located in the lower, caudal half of the ganglia; (2) vertebral sympathetic neurons occupy the cranial, upper half of the ganglia; (3) neurons with axons in the ansae are positioned near the point of exit of the respective ansa from the ganglion; (4) localization of neurons projecting to the same branches is very similar on both sides - right and left; (5) this localization is also similar in rats compared to cats.

Key words: stellate ganglion, cat, rat, isolated ganglia, antidromic stimulation, ganglionic branches, postganglionic sympathetic neurons, compound potentials

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INTRODUCTION

Postganglionic sympathetic neurons of the stellate ganglia innervate vital autonomic effectors located in the heart (Armour and Hopkins 1981, Brandys et al. 1984, Shih et al. 1985, Szulczyk and Szulczyk 1987, Kamosińska et al. 1989, Kamosińska et al. 1991, Bałkowiec and Szulczyk 1992b, Gootman et al. 1992), airways (Bałkowiec and Szulczyk 1995), forelimb (Langley 1891a,b 1893) diaphragm (Bałkowiec and Szulczyk 1992a, Bałkowiec et al. 1993, Bałkowiec and Szulczyk 1993) and other organs. Their dysfunction may provoke hyperhydrosis, causalgia, vascular disorders of upper limb, the long Q-T syndrome (Drott et al. 1993) and cardiac ischaemia (Wattervik et al. 1995), these assumptions based on beneficial effects of stellate ganglion sympathetic neurons ablation in the above illnesses (Erickson et al. 1993).

Selective mechanical destruction (Drott et al. 1993) or chemical blockade (Erickson et al. 1993) of fragments of the thoracic sympathetic chain and ganglia has recently gained more clinical significance as a therapeutic method due to the introduction of magnetic resonance (Hogan et al. 1992) or computer tomography guided (Erickson et al. 1993) transthoracic endoscopy. Therefore, the knowledge of the places where the distinctive functional groups of postganglionic sympathetic thoracic neurons are located may be of practical significance.

To assess the location of postganglionic neurons in the stellate ganglia, the following points should be considered. Firstly, it must be established, whether neurons with axons located in a single sympathetic nerve occupy a defined part of the ganglion or are more or less randomly distributed within the stellate ganglion. In this respect, Bowers and Zigmond (1979), testing the superior cervical ganglion in rats and Fateev et al. (1995), testing the stellate ganglion in cats, concluded that the somata of postganglionic sympathetic neurons are grouped near the point of exit of the sympathetic nerve containing their axons. Secondly, it is of interest, whether neurons which innervate a single autonomic effector are located randomly or in a defined position within the stellate ganglion. Finally, considering possible application of the knowledge of cardiac sympathetic neurons localization within the stellate ganglia to the treatment of specific diseases of the heart, it is of interest, whether there are significant interspecies differences in this respect.

The aim of the present study was to map the location of postganglionic sympathetic neurons with axons in cardiac nerves, vertebral nerves as well as ansae superior and inferior, in the stellate ganglia of two animal species - rats and cats. For that purpose, we employed the method of electrical stimulation of the stellate ganglion branches and systematic recording of antidromically evoked potentials from the ganglion surface *in vitro*.

METHODS

Experiments were performed on the stellate ganglia isolated from 9 cats (2.0-4.0 kg) and 18 Wistar rats (250-350 g). After initial induction of anesthesia with ether, 80 mg/kg alfa-glucochloralose or 1,000 mg/kg urethane was injected intraperitoneally - in cats and rats, respectively. A heating pad maintained the animals body temperature at a constant 37.5 ± 0.2°C. The animals were artificially ventilated and a positive end-expiratory pressure was applied.

The stellate ganglia and their branches were approached retropleurally, after removing the vertebral ends of ribs 1-3, and isolated (Szulczyk and Szulczyk 1987). After isolation, whole ganglia, together with their branches, were submerged in a custom made perfusion chamber and had their surrounding connective tissue sheath mechanically removed. The chamber was continuously perfused with artificial extracellular fluid containing (values in mmol/l): NaCl 117; KCl 4.7; NaH_2PO_4 1.2; $CaCl_2$ 2.5; $MgCl_2$ 1.2; $NaHCO_3$ 25, glucose 11.5; equilibrated with 95% O_2 and 5% CO_2. Fluid flow rate was around 3 ml/min and pH 7.4. Experiments were performed at room temperature (20-23°C). The partial gas pressures in the fluid - pO_2 and pCO_2 were kept in the respective ranges of 400-450 mmHg and 35-40 mmHg.

The branches of every ganglion were one by one placed in a suction stimulating electrode. In rats, sometimes more than one cardiac nerve was present. If possible, an attempt was made to simultaneously place all cardiac nerves in the suction electrode, as they were usually located very close to each other (see results). In the case of cardiac nerves emerging from cat stellate ganglia, we usually placed one thick branch in the suction electrode. The branch bifurcated farther on its way into one nerve to the heart (known as the cardiac nerve) and the other to the vagus nerve (Szulczyk and Szulczyk 1987, Kamosińska et al. 1989, Bałkowiec and Szulczyk 1992b). It has been found that the branch from stellate ganglion to the vagus contains a majority of postganglionic sympathetic cardiac fibers in cats (Bałkowiec and Szulczyk 1992b).

Constant current supramaximal stimulation was applied from a stimulus isolation unit - Isolator 11 from Axon Instruments Inc. (0.2 ms pulse width, 0.8-1.2 mA pulse amplitude, once every 5 s). The compound action potentials evoked by electrical stimulation of each branch were recorded from the surface of the ganglion using a low resistance about 1 MΩ glass microelectrode filled with artificial extracellular fluid. The evoked responses were amplified (time constant of the amplifier - 0.3 s), averaged 20 times after digitization (10 kHz sampling rate) and displayed on the screen of a computer monitor. The obtained results were stored on disk and printed.

The following measurements were performed during and after each experiment:

1. The borders of the stellate ganglion as well as the points of exit of postganglionic nerves were measured with the recording electrode and from this data an outline of the ganglion was drawn. To accomplish that, the tip of microelectrode was moved along the border of the ganglion using a micromanipulator (adapted Transvertex) and the tip position was read from the micromanipulator scale.
2. The compound potential recordings were performed systematically from the surface of the ganglia. The distance between consecutive recording points in both cranio-caudal and medio-lateral directions was 100 or 200 μm. Examples of several such recordings performed along one medio-lateral line from the surface of a rat stellate ganglion are shown in Fig. 1C.
3. The area of the compound evoked potential in each point (negativity above the noise line; Fig. 1C, shaded area) was considered a measure of the response magnitude. It was assessed using a specially designed computer program, which displayed the averaged compound potentials as well as allowed for calculation of their areas.
4. The magnitudes of responses recorded following stimulation of each sympathetic branch were expressed as percentages of the maximum response. Every recording point was marked on the previously made outline of the ganglion and a value representing the response from that point was assigned to it.
5. The point of maximum response (100%) was then marked on the outline of the ganglion as well as the lines connecting points of 50% and 10% of maximum response (Figs. 1A, B, 3 and 4). The data graphically represented in the above way were considered by us maps of the evoked potential magnitudes.

The data in Figures 2A, C and 5A, C are expressed as Means ± SE and were analyzed using ANOVA and Duncans test. Because the data were expressed as percentages, they were transformed using the arc sin function prior to ANOVA. $P<0.05$ was considered significant.

RESULTS

Rats

Maps of the distributions of evoked potentials were constructed for left (10 rats) and right (3 rats) vertebral nerves and left (10 rats) and right (2 rats) cardiac nerves.

In 5 cases on the left side (e. g. Fig. 1A) and two cases on the right side (e.g. Fig. 1B) it was possible to construct maps of evoked potentials from both, cardiac and vertebral nerves in one ganglion. In the remaining experiments maps were constructed either for the cardiac or vertebral nerve alone.

In rats, one (Fig. 1A) or more (Fig. 1B) cardiac nerves emerged from the stellate ganglion. In 5 experiments all cardiac nerves present - one (e. g. Fig. 1A) or more (e. g. Fig. 1 B) were placed in the suction electrodes simultaneously. In the remaining experiments only one nerve from a larger number present (usually 2) was stimulated.

The largest compound potentials evoked by electrical stimulation of the vertebral nerve were recorded from the upper half of the ganglion on both sides (Fig. 1A and B).

Locations within the ganglion, from which the largest potentials could be recorded, showed a remarkable similarity on the left and the right side. This enabled us to present the results obtained from the ganglia on both sides collectively, when expressed in a quantitative way (Fig. 2). In the cranio-caudal, longitudinal orientation, along the line splitting the ganglion in half (Fig. 2B, line x), the responses were maximal at its cranial end and gradually decreased toward the center of the ganglion (Fig. 2A). In the mediolateral, transverse, dimension of the ganglion, the responses tended to be larger medially, when recorded along lines "a" and "b" (from Fig. 2B), as demonstrated in Fig. 2Ca and b.

Responses evoked by the cardiac nerve stimulation were present mostly in the lower half of the ganglion. The responses recorded along the central cranio-caudal line "x" from Fig. 2B tended to be largest in the center of the caudal half of the ganglion and diminished towards its middle and the caudal pole (Fig. 2A). The size of responses recorded along the central medio-lateral line (line b in Fig. 2B) tended to be larger in the lateral

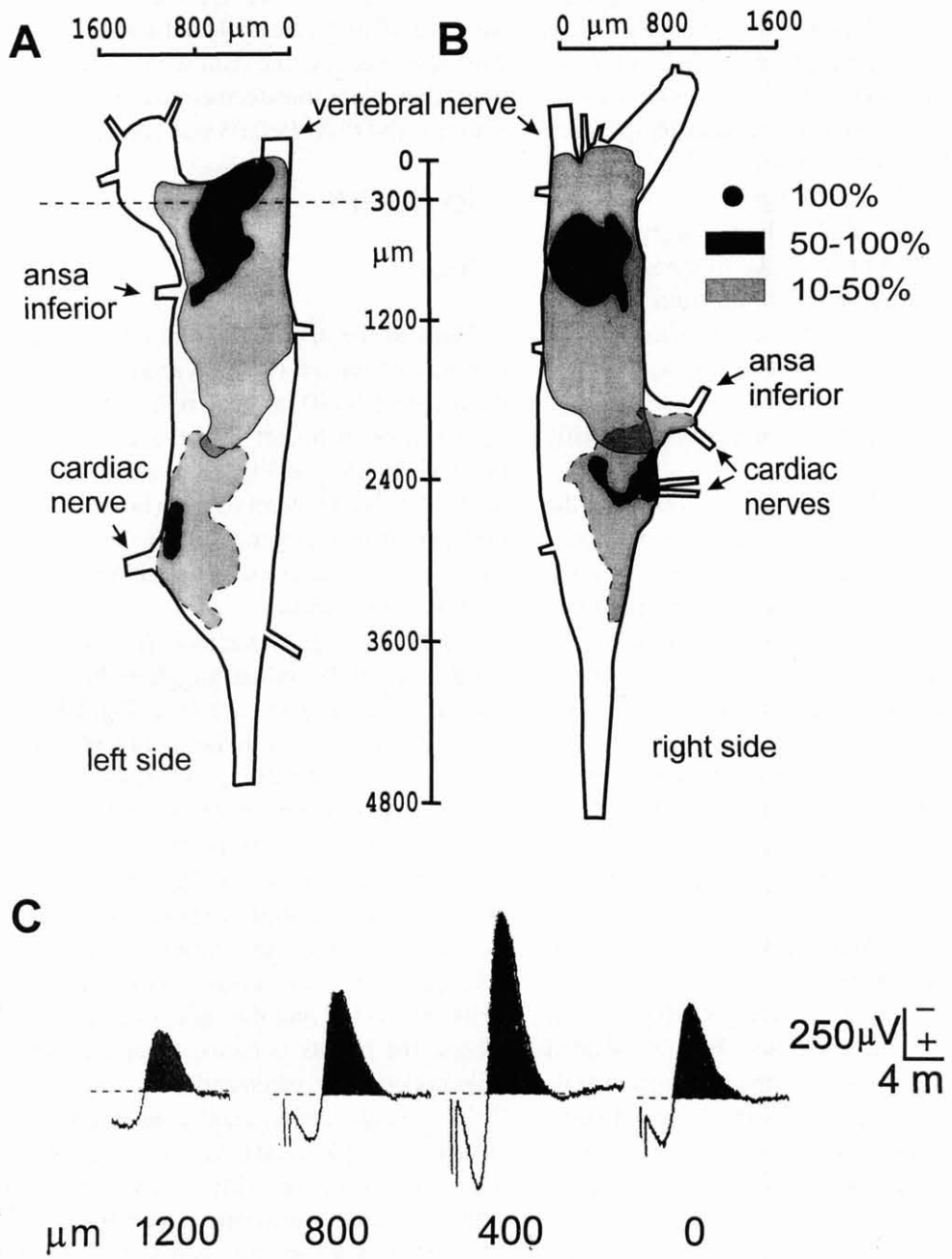

Fig. 1. Diagrams of the left (A) and right (B) rat stellate ganglia with maps of the sizes of responses evoked by electrical stimulation of vertebral (delimited by continuous lines) and cardiac (delimited by broken lines) nerve(s). The maximum response (100%) obtained by stimulation of the respective nerve is marked by the black dot. The areas from which the recorded responses were between 100% and 50%, and between 50% and 10% - as indicated in the Figure. In this particular example there was only one cardiac nerve present on the left side. On the right, 3 cardiac nerves were present and all 3 were simultaneously placed in the stimulating suction electrode. The longitudinal and mediolateral sizes of the ganglia in micrometers are indicated in the Figure. C, examples of the averaged potentials evoked by electrical stimulation of the vertebral nerve (0.2 ms; 0.9 mA once every 5 s, averaged 20 times). The potentials were recorded along the indicated horizontal line marked in A, at the level of 300 µm from the rostral end of the left ganglion. The medio-lateral positions of recording points (placed along the broken line in A) are marked below each original evoked response. Negativity is upward from the broken zero lines indicated in the Figure. The area of negativity is shaded. Vertical and horizontal calibrations were 250 µV and 4 ms, respectively.

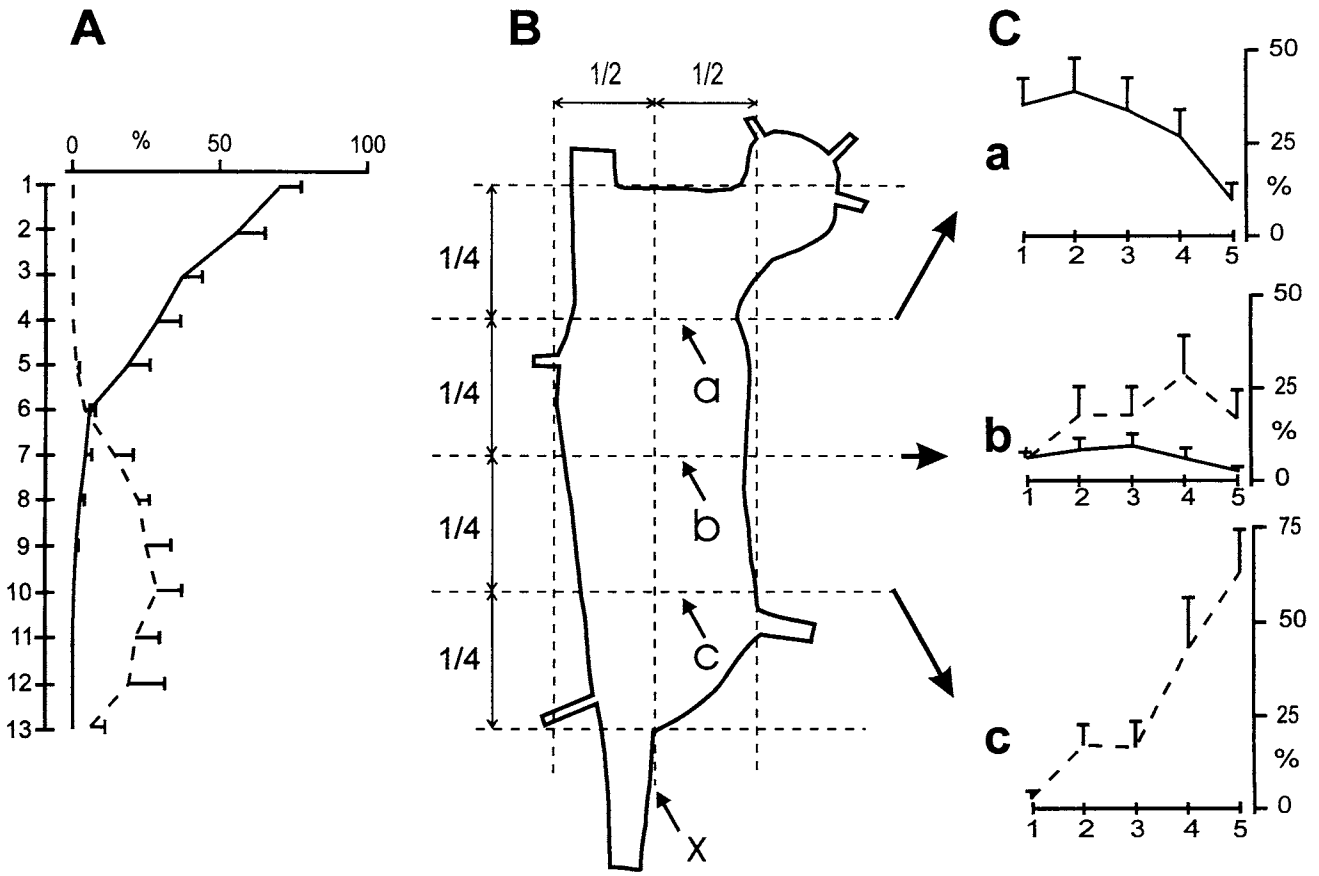

Fig. 2. Charts of the magnitudes of responses evoked by the stimulation of vertebral (continuous lines, $n = 11$) or cardiac (broken lines, $n = 8$) nerves in rats. A, the potentials were recorded in 13 points at equal intervals along the cranio-caudal line in the middle of the ganglion (line x from B). The size of the responses evoked by cardiac or vertebral nerve stimulation was expressed as a percentage of the maximum response obtained during stimulation of the respective nerve in each experiment. ANOVA for vertebral nerves $F(12,132) = 27.44$, $P<0.001$. The responses measured in points from 1 to 5 were significantly larger than those in points from 6 to 13 (Duncan's test $P<0.05$). ANOVA for cardiac nerves $F(12,96) = 7.25$, $P<0.001$. The responses measured in points from 8 to 12 were significantly larger than in points from 1 to 7 and point 13 ($P<0.05$). B, x- the cranio-caudal line along which the responses demonstrated in A were recorded; a, b, c, medio-lateral lines along which the responses demonstrated in C were recorded. C, the potentials were recorded in 5 points at equal intervals, along the medio-lateral lines a, b and c (from B). The size of responses evoked by cardiac (broken lines, $n = 8$) or vertebral (continuous lines, $n=11$) nerve stimulation was expressed as a percentage of the maximum response for each respective nerve. Points 1 and 5 were located at the medial and lateral border of the ganglion, respectively. Ca, ANOVA $F(4,44) = 3.57$, $P<0.05$. Measurements in points 1, 2 and 3 were significantly larger than in points 4 and 5 (Duncan's test, $P<0.05$). At this level only the responses evoked from vertebral nerve were present. Cb, ANOVA for vertebral nerves $F(4,44) = 0.91$, $P>0.05$. ANOVA for cardiac nerves $F(4,32) = 1.18$, $P>0.05$. In neither case the sizes of responses differed significantly across the ganglion. Cc, ANOVA $F(4,32) = 6.18$, $P<0.01$. Measurements in points 2 and 3 were significantly larger than in point 1. Points 4 and 5 were significantly larger than 1, 2 and 3 ($P<0.05$, Duncan's test). At this level only the responses evoked by cardiac nerve stimulation were present. In A and C the results were obtained from 11 experiments for vertebral nerve and for 8 experiments for cardiac nerve. The magnitudes of the evoked responses (expressed as a percentage of the maximum) were averaged and are demonstrated as Means ± SE. The data obtained from the right and left stellate ganglia are given collectively.

part of the ganglion. The responses recorded along the line "c" from Fig. 2B were clearly largest in the lateral part of the ganglion (Fig. 2Cc). No responses were ever recorded along the line a from Fig. 2B.

Relatively small responses evoked from vertebral and cardiac nerves could be recorded in overlapping areas near the center of the ganglion (Figs. 1, 2A and 2Cb).

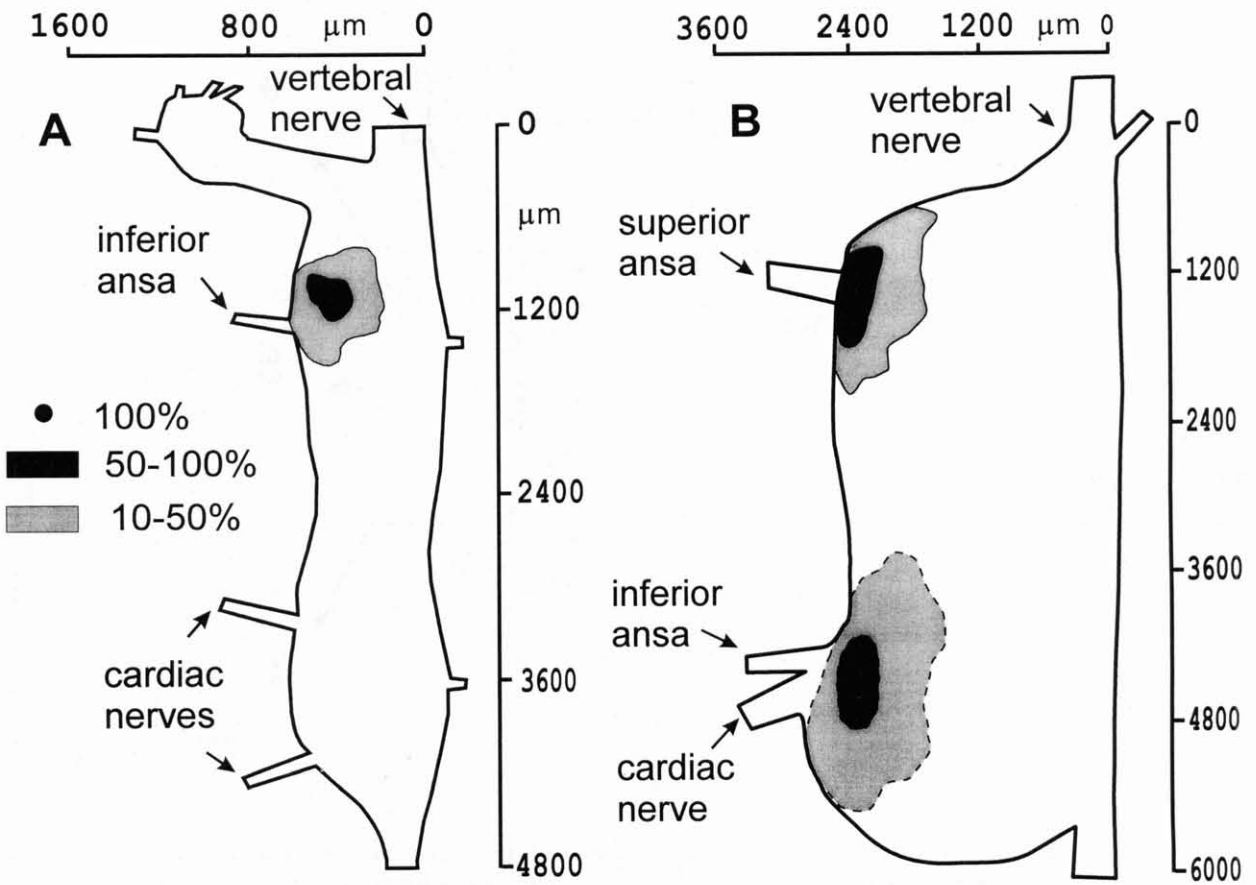

Fig. 3. Diagrams of a rat (A) and a cat (B) left stellate ganglia with the maps of different response sizes, evoked by electrical stimulation of the ansa superior (continuous lines) and ansa inferior (broken lines). Other descriptions as in Fig. 1.

The potentials evoked by electrical stimulation of the inferior ansa in rats were recorded in 2 experiments on the right and in 2 cases on the left side. The evoked responses centered around the point of exit of this nerve from the ganglion. One example of maps constructed for these responses is demonstrated in Fig. 3A.

Cats

In cats, maps of potentials evoked by the right (6 cases) and left (2 cases) vertebral nerve stimulations and right (3 cases) and left (2 cases) cardiac nerve stimulations were constructed. In both experiments on the left (Fig. 4A) and in 2 experiments on the right side (e. g. Fig. 4B) the maps were constructed for potentials evoked by consecutive electrical stimulation of vertebral and cardiac nerves of the same ganglion. In the remaining experiments either the cardiac or vertebral nerve was stimulated.

The potentials evoked from the vertebral nerve could be recorded mostly in the rostral half of the ganglion (Fig. 4), while those evoked from the cardiac nerve - from the caudal half of the ganglion.

The size of potentials evoked by electrical stimulation of the vertebral nerve and recorded along the central line "x" from Fig. 5B was largest near the cranial end of the ganglion and gradually diminished towards the ganglion center. The evoked responses recorded along lines "a" and "b" from Fig. 5B tended to be greater toward the medial side of the ganglion (Fig. 5Ca and Cb, respectively).

The areas of responses recorded along the line "x" from Fig. 5B following stimulation of the cardiac nerve

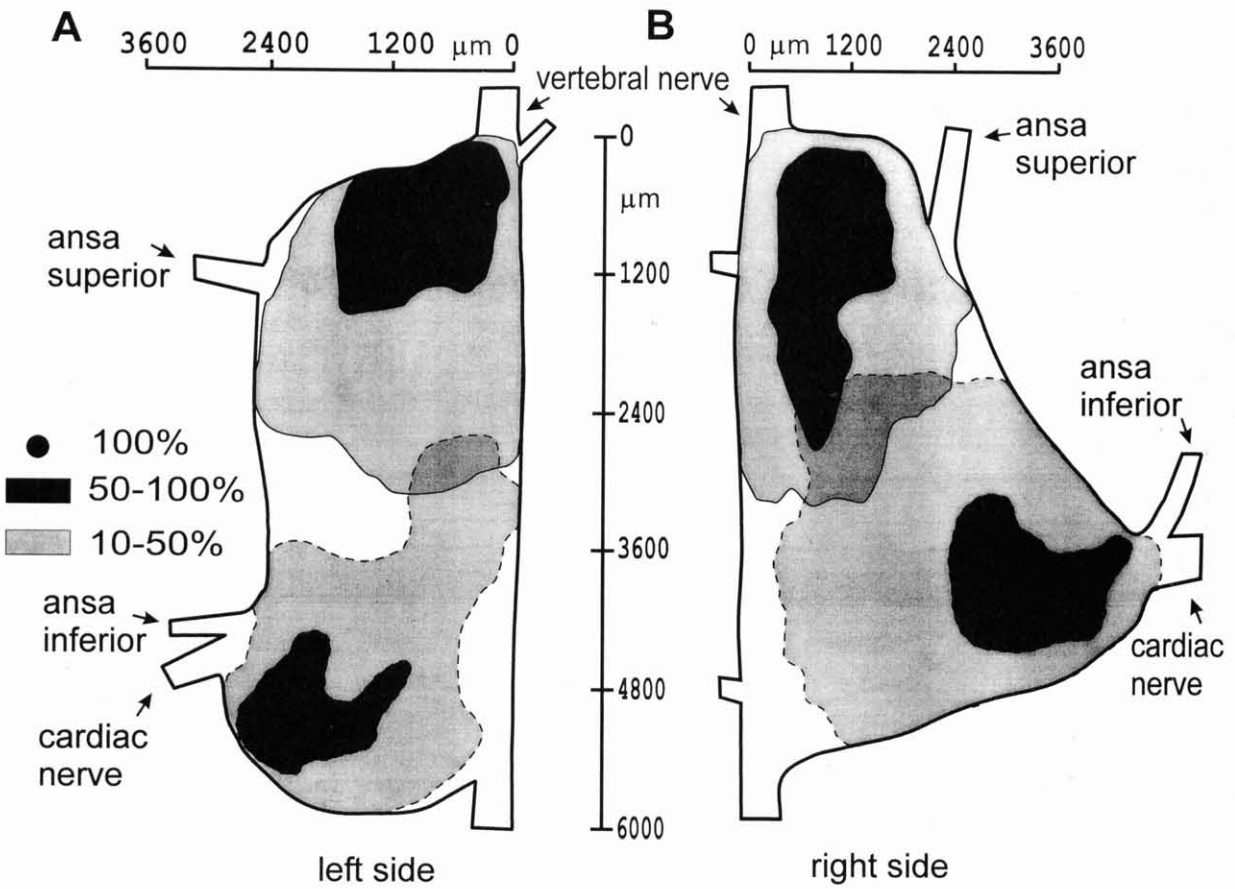

Fig. 4. Diagrams of left (A) and right (B) cat stellate ganglia with maps of different response sizes obtained by electrical stimulation of a vertebral (continuous lines) and cardiac (broken lines) nerve. Other descriptions as in Fig. 1.

were largest in the caudal end of the ganglion and decreased toward the ganglion center (Fig. 5A). The potentials evoked from cardiac nerves, recorded along lines "b" and "c" from Fig. 5B, tended to be greater in the lateral part of the ganglion (Fig. 5Cb and Cc, respectively).

As in rats, the overlapping evoked responses from both nerves could be detected in similar places, close to the center of the ganglion (Figs. 4 and 5A).

The superior ansa in cats was stimulated in two cases on the right and in one case on the left side. The inferior ansa was stimulated 5 times on the right and once on the left side. In all cases, relatively large potentials could be detected only near the point of exit of the respective branch from the ganglion, as demonstrated in Fig. 3B.

DISCUSSION

The localization and size of the compound potentials recorded from the stellate ganglion surface in rats and cats was assessed in this study. The potentials were evoked by stimulation of vertebral and cardiac nerves as well as of the superior and inferior ansae.

The recorded compound potentials could originate from three main sources, considering our experimental conditions:

- antidromically activated somata of postganglionic neurons,
- postsynaptically activated postganglionic sympathetic neurons,
- antidromically activated axons of postganglionic neurons.

We infer, that antidromically activated somata of postganglionic neurons were the primary source of the compound potentials we recorded. Below we present experimental data supporting this inference.

It is known, that cardiac sympathetic nerves contain afferent sensory fibers, which innervate mainly the cardiopulmonary area (Malliani et al. 1973, Maksymowicz

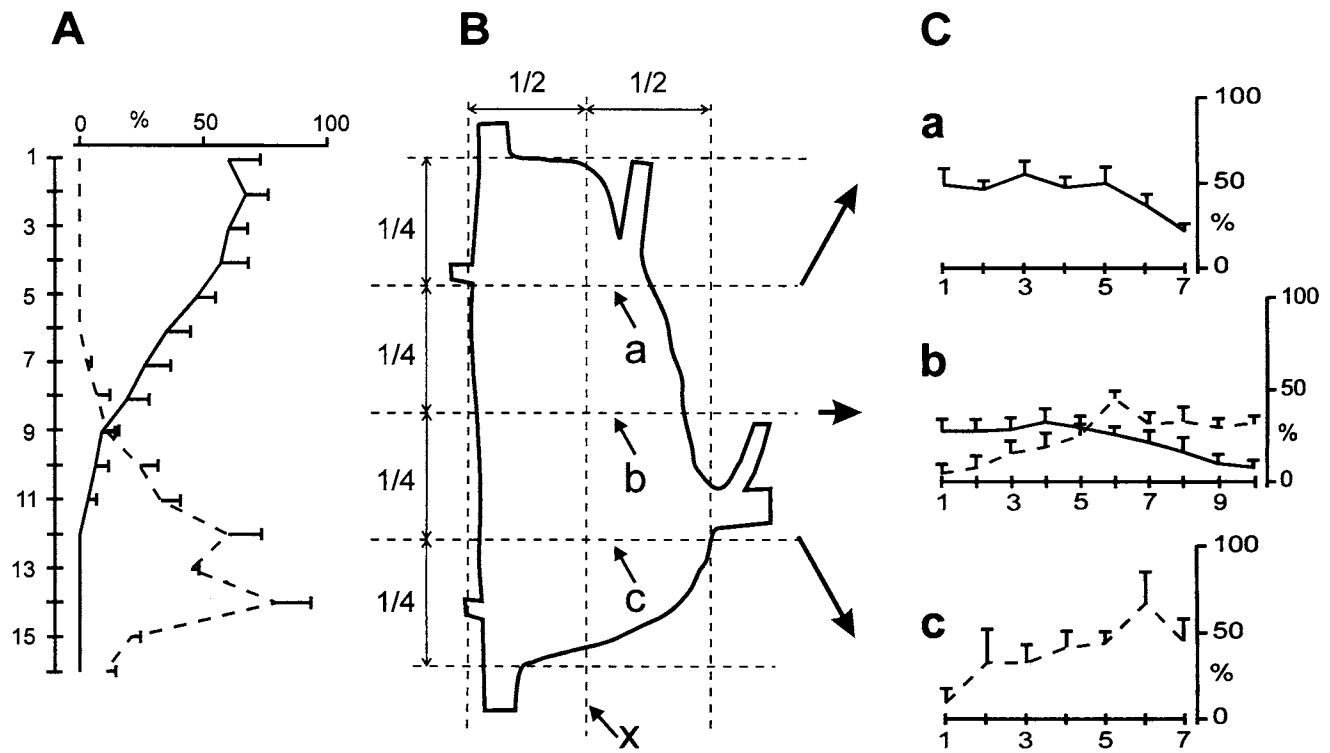

Fig. 5. A, the potentials were recorded in 16 points at equal intervals along the cranio-caudal line in the middle of the ganglion (line "x" from B). The size of the responses evoked by cardiac (broken lines, $n = 4$) or vertebral (continuous lines, $n = 8$) nerve stimulation was expressed as a percentage of the maximum response obtained during stimulation of the respective nerve in each experiment. ANOVA for vertebral nerves $F(15,120) = 15.5$, $P<0.001$. Measurements in points from 1 to 5 were significantly larger than measurements in points from 7 to 16 (Duncan's test, $P<0.05$). ANOVA for cardiac nerves $F(15,60) = 7.74$, $P<0.001$. Responses from points 12, 13 and 14 were significantly larger than those from remaining points. B, description as in Fig. 2B. C, the potentials were recorded in 7 points (a, c) and 10 points (b) at equal distance, along medio-lateral lines "a", "b" and "c" (from B). The size of responses evoked by cardiac (broken lines, $n = 4$) or vertebral (continuous lines, $n = 8$) nerve stimulation was expressed as a percentage of the maximum response for each respective nerve. The first recording point was located at the medial border of the ganglion and the last - near the lateral border. Ca, ANOVA $F(6,48) = 4.36$, $P<0.01$. Measurements in points from 1 to 5 were significantly larger than in points 6 and 7 ($P<0.05$, Duncan's test). At this level only the responses evoked by vertebral nerves were present. Cb, for vertebral nerves ANOVA $F(9,72) = 3.17$, $P<0.01$. Measurements in points 9 and 10 were not significantly different ($P>0.05$) and were significantly lower than the responses obtained in other points ($P<0.05$, Duncan's test). For cardiac nerves ANOVA $F(9,36) = 2.44$, $P<0.01$. Measurements in points 1 and 2 were significantly lower than measurements in other points ($P<0.05$, Duncan's test). Cc, ANOVA $F(6,24) = 4.14$, $P<0.01$. The measurement in point 1 was significantly lower than those obtained in points from 4 to 7 ($P<0.05$). Additionally, the response in point 6 was significantly larger than other measurements ($P<0.05$, Duncan's test). At this level only the responses from cardiac nerve were present. In A and C the results were obtained from 8 experiments for the vertebral nerve and for 4 experiments for the cardiac nerve. The data from right and left stellate ganglia were presented collectively.

and Szulczyk 1983, Nowicki and Szulczyk 1986). Activation of these fibers may postsynaptically activate stellate ganglion neurons in animals with disrupted preganglionic input. It has been found that electrical stimulation of sympathetic afferents activates 31% of stellate ganglion sympathetic neurons, while 5% of these neurons are activated following direct stimulation of cardiopulmonary visceral receptors (Bosnjak and Kampine 1982). On the other hand, in isolated rat stellate ganglia, electrical stimulation of cardiac sympathetic branches activated postsynaptically only 16% of stellate ganglion sympathetic neurons (Mo et al. 1994).

Additionally, sympathetic stellate ganglion neurons were labeled transsynaptically, by the injection of a marker (WGA-HRP) into thoracic dorsal root ganglia, following the assumption that visceral thoracic sympathetic

afferents have synaptic terminals on stellate ganglion neurons (Quigg et al. 1990).

Although postsynaptic activation of postganglionic sympathetic neurons by sensory visceral afferents seems unquestionable, their participation in the generation of compound potentials recorded under our experimental conditions may be negligible. The following points support this assumption:

1. Electrical stimulation of afferents from a single sympathetic branch of stellate ganglion isolated from spinal input evokes a postsynaptic response of around 10-25 μV amplitude in a companion branch. This response is blocked by hexamethonium (Szulczyk and Wilk 1985, Szulczyk and Wilk - unpublished data). The amplitude of the response recorded from the same sympathetic axons, under the same experimental conditions, but following electrical stimulation of preganglionic T_3 white ramus, was in the range of 1 to 5 mV (Szulczyk and Szulczyk 1987). Therefore, postsynaptic activation of sympathetic stellate ganglion neurons is 100-500 more effective in case of white ramus stimulation, than in the case of stimulating afferents of a postganglionic branch. It is believed that the same postganglionic neurons that respond to the white ramus stimulation are also activated antidromically, even to a greater degree, after electrical stimulation of postganglionic nerves.
2. It has been found, that postganglionic sympathetic neurons, which receive synaptic input from visceral afferents are located mainly along the caudal medial border of the stellate ganglion in the guinea pig (Quigg et al. 1990). However, the compound potentials recorded in our study were distributed mostly in the lateral and caudal parts of the stellate ganglia.
3. If the stimulation of visceral afferents significantly participates in the activation of postganglionic sympathetic neurons, such response should also be present, when preganglionic input to cardiac sympathetic neurons is abolished following ganglionic blockade. Many authors, including ourselves, have found that application of hexamethonium bromide is sufficient to block the response in postganglionic sympathetic nerves evoked by a single stimulus or short trains of electrical stimuli delivered to preganglionic white rami (Volle and Hancock 1970, Hoffmeister et al. 1978, Szulczyk and Szulczyk 1987). This point is valid only if nicotinic synapses are not involved in mediation of ganglionic reflexes, though it has been established in dogs, that hexamethonium may block the ganglionic reflexes either partially (Armour 1983) or totally (Bosnjak et al. 1982).
4. As shown above, hexamethonium is believed to at least partially abolish ganglionic reflexes. We have found, however, that hexamethonium does not visibly affect compound potentials recorded in our studies under the described experimental conditions in rats (Kukuła, Szulczyk and Urbanowicz - unpublished data).

To summarize these considerations, there is a substantial evidence, that compound potentials recorded in our study were of an antidromic and not postsynaptic nature.

The next question arising, is whether the compound potentials recorded in the presented study reflect antidromic activation of axons or of the neuronal somata (somatodendritic tree). A closer inspection of Figures reveals, that areas where the nerves exited ganglia were usually sources of compound potentials of a magnitude of 10% of maximum response or less. It may of course be expected, that these are the points of the ganglion, where axons are most closely packed. Farther toward the center of the ganglion, postganglionic axons must be dispersed among cell bodies to a much greater degree. This is why we suggest, that axonal action potentials did not significantly contribute to the generation of the antidromic compound potential in our study.

The principle on which we based our study is, that the size of compound potential recorded from a particular point on the ganglion surface is proportional to the number and density of antidromically activated neurons located near the recording electrode. It has been shown before, that this method of recording of the magnitudes of negativity reflects the dispersion and number of neurons generating it (Bernhard et al. 1953, Lindblom and Ottoson 1953). Indeed, it has since been confirmed that localization of field potentials recorded in the spinal cord following antidromic stimulation of motoneurons innervating the gastrocnemius and the soleus muscles (van Buren and Frank 1965) closely reflects the localization of HRP (horseradish peroxidase) labeled neurons after injecting HRP into respective nerves (Burke et al. 1977). As will be discussed later, electrophysiological data obtained in our study resemble rather well the morphological results obtained in another study performed for similar reasons (Fateev et al. 1995).

Cardiac postganglionic sympathetic neurons

It seems that stellate ganglion cardiac sympathetic neurons in rats are the main source of sympathetic innervation of the heart. Sympathetic cardiac branches have been traced macroscopically from the stellate gan-

glia to the heart (Yasunaga and Nosaka 1979). Surgical or chemical destruction of the rat stellate ganglion decreases norepinephrine content in all heart regions to negligible levels (Pardini et al. 1990). The application of a neuronal tracer to cardiac sympathetic nerves (Pardini et al. 1989, Mo et al. 1994) or to the heart tissue (Shih et al. 1985) in rats, labels neurons almost exclusively confined to the stellate ganglia.

Similar observations have been made in cats. Ninety six percent of single sympathetic axons isolated from thoracic visceral nerves possess similar resting and reflex properties, implying their functional homogeneity and suggesting that they innervate the heart (Bałkowiec and Szulczyk 1992b). In cats, application of horseradish peroxidase to the inferior cardiac nerve (Kuo et al. 1984) or to the heart tissue itself (Shih et al. 1985) marks neurons located almost exclusively in the stellate ganglia.

Such observations indicate that sympathetic cardiac neurons are almost exclusively located within the stellate ganglia, at least in cats and rats. On the other hand, cardiac sympathetic nerves certainly contain axons innervating other effectors inside the thorax, apart from the heart.

The results presented in this study show that neurons with axons in cardiac nerves are located in the caudal and lateral part of stellate ganglia, both in cats and rats. These results are in agreement with morphological data. Application of HRP to the inferior cardiac nerves in cats (Kuo et al. 1984) and cobalt chloride in rats (Mo et al. 1994) labeled neurons in areas adhering to the point of exit of cardiac nerves, which roughly resembles our data.

Considering other species, it has been found that the application of horseradish peroxidase to the cardiac tissue (Hopkins and Armour 1984) or cardiac sympathetic nerves (Armour and Hopkins 1981) in dogs marks neurons in the cranial end of the ganglion. In dogs (Brandys et al. 1984) and swines (Gootman et al. 1992), a discrete, space limited electrical stimulation of the stellate ganglion evoked cardiovascular responses only when applied to the cranial end of the ganglion. These results indicate an interspecies difference in the localization of cardiac neurons in the ganglia of rats and cats, as compared to dogs and swines. Claes et al. (unpublished data from Drott et al. 1993) found a decrease of symptoms of the long Q-T syndrome after ablation of the caudal part of the stellate ganglion, as well as of the second and third thoracic ganglia. It is believed that the long Q-T syndrome is provoked by the overactivity of postganglionic cardiac neurons on the left side. Thus, this evidence suggests that a significant number of cardiac postganglionic sympathetic neurons in humans are found in the caudal part of the stellate ganglion.

Localization of vertebral sympathetic neurons

Vertebral nerve contains pilomotor, sudomotor and vasomotor sympathetic fibers which are directed to the autonomic effectors located in the forepaw, neck and thorax (Langley 1891ab, 1893, Bałkowiec and Szulczyk 1992a, Bałkowiec and Szulczyk 1993, Bałkowiec et al. 1993). Neurons labeled following application of horseradish peroxidase to the vertebral nerve in cats, were located in the upper pole of the stellate ganglion, near the point of exit of the nerve from the ganglion (Fateev et al. 1995). Our results suggest that vertebral postganglionic sympathetic neurons occupy upper half of the ganglion, both in rats and cats. The maximum density of neurons was not necessarily registered very close to the point of exit of the vertebral nerve, but was frequently shifted toward the center of the upper half of the ganglion (for example, Figs. 1B and 4B).

Localization of sympathetic neurons with axons in the ansae

In rats, the superior ansa is usually absent. The middle cervical ganglion is more or less incorporated into the stellate ganglion and forms the cervico-stellate ganglion complex (Yasunaga and Nosaka 1979). Both morphological results, and the data demonstrated in this paper indicate that postganglionic neurons projecting to the inferior ansa in rats, as well as ansa superior and inferior in cats, are located close to the exit of these branches from the stellate ganglion. The results support earlier data, that some of the fibers in the ansae and the cervical sympathetic trunk are of postganglionic nature and that their somata are located in the stellate ganglia (Kamosińska et al. 1991, Mo et al. 1994).

Areas, from which no evoked responses were recorded

We have never found the evoked responses in the lowermost and the extreme medial parts of the ganglion. Most likely, those are the areas, where the postganglionic neurons with axons in gray rami T1 - T4 are located. These rami were not stimulated in our study. Fateev et al. (1995) in cats and Mo et al. (1994) in rats,

after application of horseradish peroxidase to the gray rami T1 and T2, labeled some neurons in the extreme medial part of the ganglion. The only difference between ours and their study was, that the neurons they labeled concentrated near the point of exit of the gray rami from the ganglion. Our "empty" area was located medially, but largely in the caudal part of the ganglion. It is possible, that in the morphological studies, the tracer did not reach more distant neurons located in the caudal part of the ganglion.

The obtained results indicate:

1. Sympathetic neurons with axons in the vertebral nerve occupy the upper (rostral) part of the stellate ganglion, while those with axons in the cardiac nerve(s) - the lower (caudal) part of the ganglion.
2. There was some overlap registered in the localization of vertebral and cardiac sympathetic neurons in the central part of the stellate ganglion.
3. Neurons with axons in the ansae were located close to the exit of these nerves from the ganglion.
4. The localization of sympathetic neurons with axons projecting to the same branches was very similar on both sides, right and left.
5. The localization of sympathetic neurons projecting to the same branches was also remarkably similar in rats as compared to cats.

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