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## Distribution and Number of Intracardiac Ganglion Cells in the Rat

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### Abstract

The purpose of this study was to reevaluate the distribution and the number of intracardiac ganglion cells in the rat. Hearts were excised from adult male Wistar rats. The distribution of the ganglia was mapped using thick, frozen sections of rat heart histochemically stained for acetylcholinesterase. The ganglion cells were divided into several groups with the largest group located dorsal to the inferior interatrial septum. The disector method was used to estimate the number of neurons in seven micron thick paraffin sections. The number of neurons in the rat heart was  $3629 \pm 337$  (mean  $\pm$  S.E.,  $n=3$ ).

**Key words:** disector method, heart, postganglionic neuron, stereology

### INTRODUCTION

Heart function is tightly regulated by the sympathetic and parasympathetic nervous systems. These sympathetic and parasympathetic nerves control the heart directly via axon terminals originating in neurons in the extracardiac ganglia and indirectly by influencing neurons in the intracardiac ganglia. The intracardiac ganglia are especially important because they are capable of maintaining normal heart function even after being completely denervated, as in the case of heart transplant patients (Ardell, 1994).

The distribution of the intracardiac ganglia in the heart has been studied extensively in several mammalian species, including the rat (King & Coakley, 1958; Calaresu & St. Louis, 1967; Burkholder *et al.*, 1992). There has been only one study that examined the number of neurons in the intracardiac ganglia of the rat (Pardini *et al.*, 1987) and that study used the traditional Abercrombie method (Abercrombie, 1946). Recently, a stereological method, the disector method, has been devised. Because counting particles with the

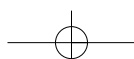
disector method is not biased by particle size and shape, a total reevaluation of neuron numbers estimated by the Abercrombie method seems to be necessary (Coggeshall, 1992). Therefore, the objective of this study was to estimate the number of neurons in the intracardiac ganglia of the rat using the disector method.

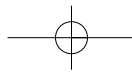
### MATERIALS AND METHODS

Two series of experiments were performed to examine the intracardiac ganglia of adult male Wistar rats, which weighed 200-330 g. In the first series, thick sections of frozen heart tissue were processed for acetylcholinesterase histochemistry to examine the distribution of the intracardiac ganglia in the heart. In the second series, thin paraffin sections were made of the heart tissue to count cardiac ganglion neurons with the disector method.

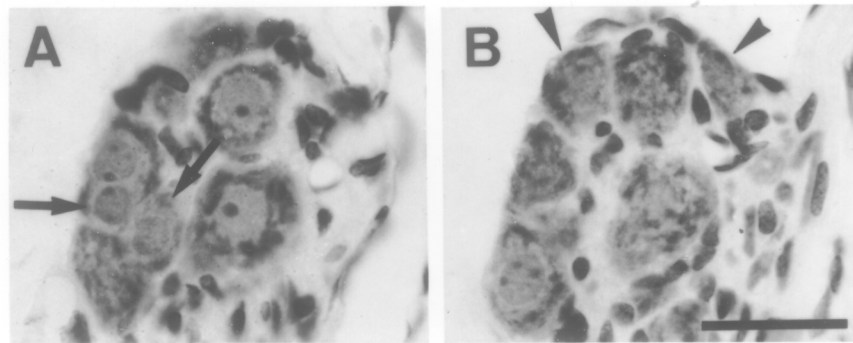
#### Acetylcholinesterase staining

Seven rats were anesthetized with sodium pentobarbital (50 mg/Kg i.p.). Their hearts were perfused through a cannula inserted into the descending aorta. The perfusate consisted





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*Figure 1.* Photomicrograph of adjacent sections of a small intracardiac ganglion of a rat, H & E stained. Note the two neurons with round nuclei in A (arrows) are missing in B. Conversely, the nuclei of the two neurons marked with arrowheads in B are missing in A. Section A was the disector, section B the look-up section. Thus, the two neurons marked with arrows in A are the “tops”.

of heparinized saline followed by a fixative containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.3). The heart and the great vessels and attached trachea and esophagus were excised and postfixed in the same fixatives for 1 to 2 days. These tissues were embedded in gelatin and immersed *en bloc* in the same fixative, with 20% sucrose added, for an additional 3 to 5 days. Serial, 100- $\mu$ m thick, frozen sections were cut on a sliding microtome. They were processed for acetylcholinesterase histochemistry by the method of Woolf & Butcher (1981). These sections were then mounted on subbed slides. After dehydration, clearing and coverslipped, the sections were inspected under a compound microscope and photographed.

#### Paraffin sections

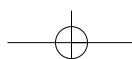
Sodium pentobarbital anesthesia was administered intraperitoneally to 6 additional rats which were then perfused through the descending aorta. The perfusate consisted of phosphate buffered saline containing 0.8% sodium chloride and 0.02% potassium chloride in 0.1M phosphate buffer (pH=7.3), followed by a fixative of 4% paraformaldehyde and 1.25% glutaraldehyde in the same buffer. The heart and its surrounding tissue were excised. After dehydration in an ascending

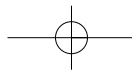
series of ethanol, the block was embedded in paraffin (mp. 60-64°C). Serial sections, 7  $\mu$ m thick, were cut coronally. The sections were stained with Mayer's Hematoxylin & Eosin (H & E). Particular attention was made to obtain sections of the rostral portion of the heart, because it is in this portion that most of the intracardiac ganglia lie. A typical series from one block was comprised of about 500 sections.

A Wild M32 stereomicroscope with a camera lucida was used to draw the contours of sections. A Vickers M41 photoplan light microscope, equipped with CCD camera and video graphic printer (Sony UP-870MD) was used to observe and record the details of the sections. A 40 $\times$  objective was used and the final magnification as calibrated against a stage micrometer was 316 $\times$  on the print paper.

#### The disector method

The disector method was used to estimate the number of the ganglion cells (Pakkenberg & Gunderson, 1989; Coggeshall, 1992; Pover *et al.*, 1993). Equispaced sections, 3 to 9 sections apart, were chosen as the disectors. The intersample distances were chosen so that between 100 to 200 “tops” would be counted in a large ganglion. The sections immediately adjacent to the disectors were used as the look-up sec-





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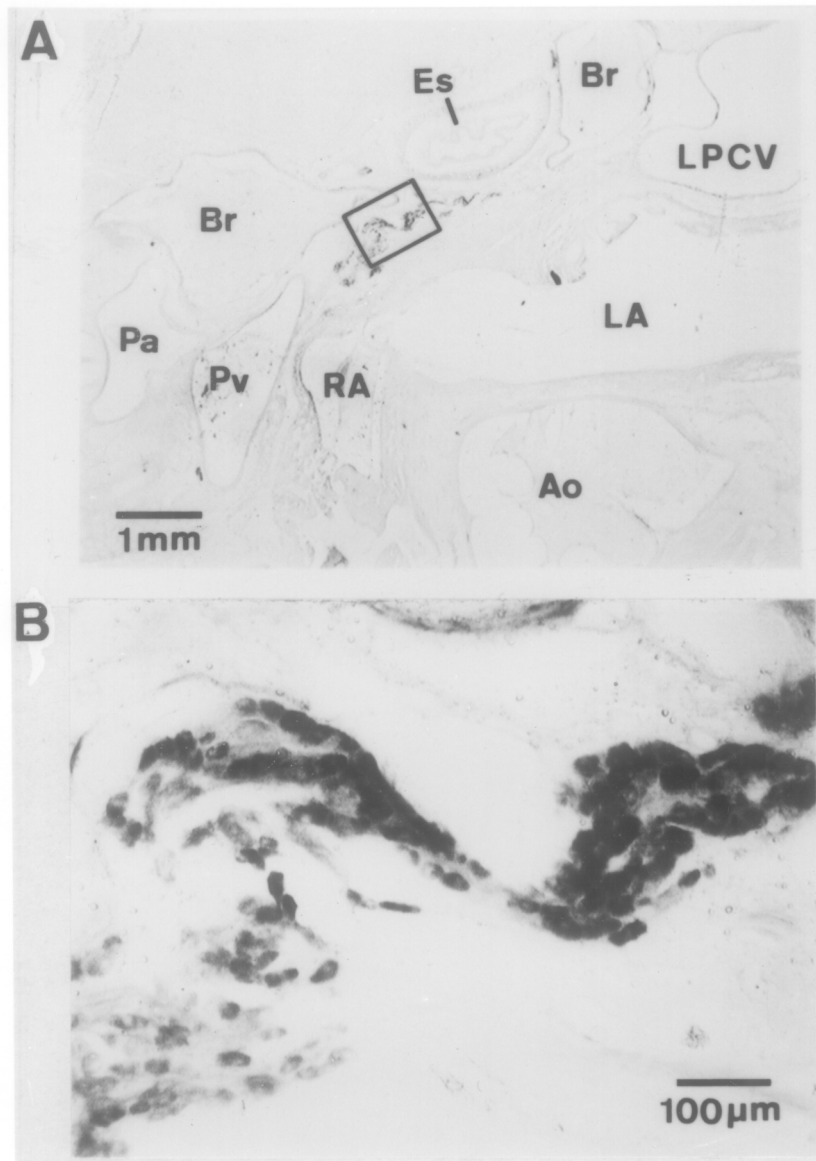


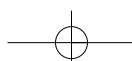
Figure 2. A. Photomicrograph of a portion of a coronal section of the rostral portion of the heart of a rat stained for acetylcholinesterase. B. Enlarged view of the boxed region in A. Note dark ganglion cells against a clear background.

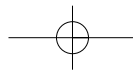
**Abbreviations:** Ao: aorta; Br: bronchi; Es: esophagus; LA: left atrium; LV: left ventricle; LPCV: left precaval vein; Pa: pulmonary artery; Pv: pulmonary vein; RA: right atrium; RV: right ventricle; SCV: superior vena cava.

tions. For each disector and its look-up sections, all neurons were examined under a compound microscope and on the video printout. Only those neurons whose nuclei appeared in a disector section but not in the look-up section were counted as "tops" (figure 1). The gan-

glion area ( $a_{dis}$ ) in each section was measured on a digitizer (Sigmascan, Jandel). The thickness of the sections ( $h$ ) averaged  $7 \mu\text{m}$ . The volume ( $V_{dis}$ ) of the intracardiac ganglia within the disector was calculated as:

$$V_{dis} = a_{dis} \times h$$





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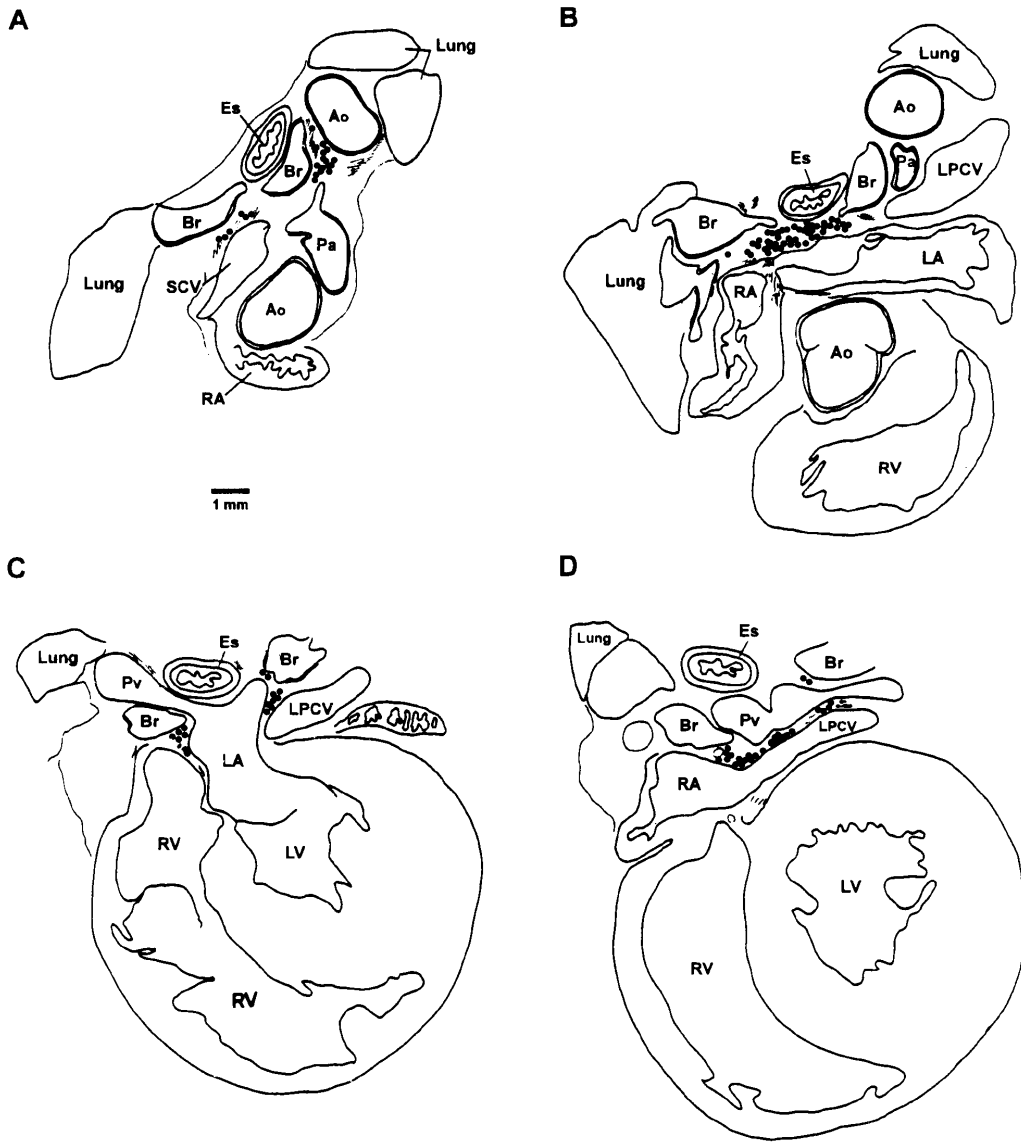


Figure 3. Distribution of ganglion cells in the heart and adjacent tissues of a rat. A, B, C and D are representative sections of the heart from rostral to caudal levels. For abbreviations see legend for Figure 2.

The number of neurons per mm<sup>3</sup> ( $N_v$ ) is:

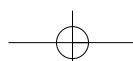
$$N_v = \frac{\sum Q^-}{\sum V_{dis}} \times 10^9$$

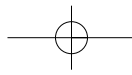
$$N_v = \frac{10^9}{7} \frac{\sum Q^-}{\sum a_{dis}}$$

where  $Q^-$  is the number of the tops in each area counted. The number of neurons in the

ganglion was obtained by multiplying  $N_v$  by total volume.

The number of neurons in small ganglia was counted manually. The total number of intracardiac ganglion cells in an individual rat was obtained by summing the number of neurons in all intracardiac ganglia.





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### RESULTS

#### Distribution

Intracardiac ganglion cells were identified by their round or ovoid shape and their large clear nucleus with 1 to 3 nucleoli (Figure 1). In sections stained for acetylcholinesterase, ganglion cells, nerve fibers and terminals stood out clearly as dark brown objects against a white background (Figure 2). In H & E stained sections, many supportive cells could be seen interposed between the ganglion cells (Figure 1).

There were many ganglia in the heart tissue and their sizes varied greatly. Most of them were found in the fat tissues between the great vessels. Three large groups of ganglia were consistently found closely associated with the left atrium. The largest of the three groups (group 1) was located rostrally outside the junction of the left and right atria (Figure 3B). Slightly caudal, at the level where the pulmonary veins joined the left atrium, there were two groups of ganglia, one on each side of the juncture of the veins and the atrium (groups 2 and 3, Figure 3C). More rostrally, there were ganglia located between the ascending aorta and the root of the pulmonary artery, between the vena cava, pulmonary artery and bronchi, and on the left side of the pulmonary artery between the bronchi and the descending aorta (Figure 3A).

In some rats, all these ganglia were separate entities, such that there were one or two chains of ganglia extending from the great vessels to the atrial-ventricular junction. No ganglion was found in ventricular tissue. In other rats, groups of ganglia were joined here and there. For example, in some animals, group 1 joined rostrally with the rostral groups of ganglia and caudally with either group 2 or group 3 to form a large ganglion. In this case, the chain of ganglion might be Y-shaped.

#### Neuron size and number

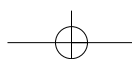
The size of 110 ganglion cells were measured. The major and minor diameters were

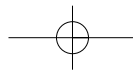
$18.6 \pm 0.4 \mu\text{m}$  and  $13.0 \pm 0.3 \mu\text{m}$ , respectively (Figure 4). The number of neurons in large ganglia was estimated using the disector method. Complete sampling of all ganglia in three rats yielded counts of 3642, 4206 and 3039 neurons. The average was  $3629 \pm 337$  (mean  $\pm$  S.E.).

### DISCUSSION

There have been many papers describing the distribution of ganglion cells in the mammalian heart. King & Coakley (1958) summarized the distribution of intracardiac ganglion cells in 19 species, including the rat. More recently, this distribution has been studied by Pardini *et al.* (1987) and Burkholder *et al.* (1992). Seven clusters were found by King & Coakley (1958), 4 by Pardini *et al.* (1987) and Burkholder *et al.* (1992) found another 4. Our data agrees well with the results by Pardini *et al.* (1987). Our group 1 corresponds to their group in the region of the superior interatrial septum. Our group 2 corresponds to their group posterior to the inferior interatrial septum and right atrium. Our group 3 corresponds to their group posterior to the left atrium. Their smallest group, the one between the superior vena cava and aorta, can be also seen in our Figure 3A. Essentially, the data in all papers agree well for the larger groups of ganglia but with differences in minor details. The variations described in the present paper may account for these minor differences. The rat has comparative smaller and less extensive intracardiac ganglia than most other mammals (King & Coakley 1958).

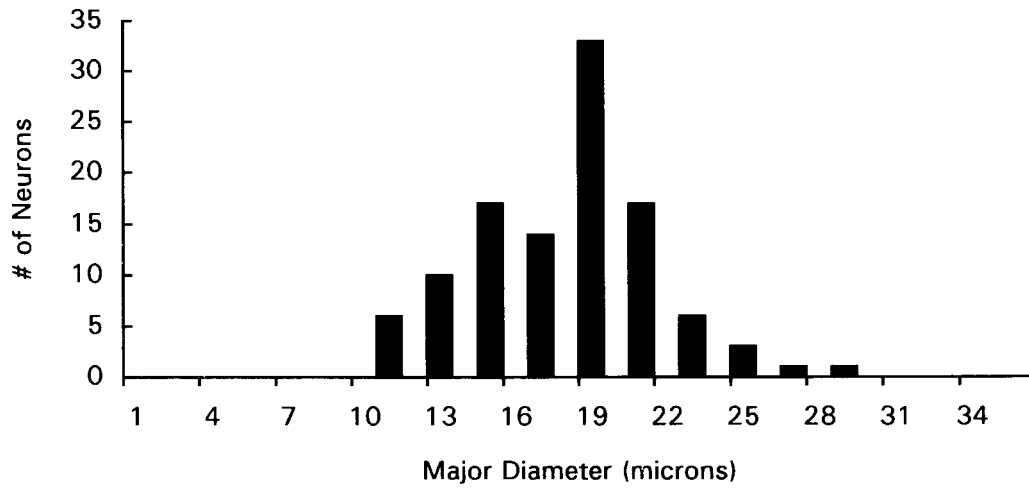
Our numerical data, of  $3629 \pm 337$  intracardiac neurons per rat, agrees very well with the result of  $3980 \pm 444$  obtained by Pardini *et al.* (1987). Taking individual variation into account, this difference is not statistically significant. Therefore, there appears to be no difference in the results obtained by the Abercrombie and disector methods in estimating the number of rat intracardiac ganglion cells. It is interesting to note that the number of intracardiac ganglion cells in the cat has





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**A**



**B**

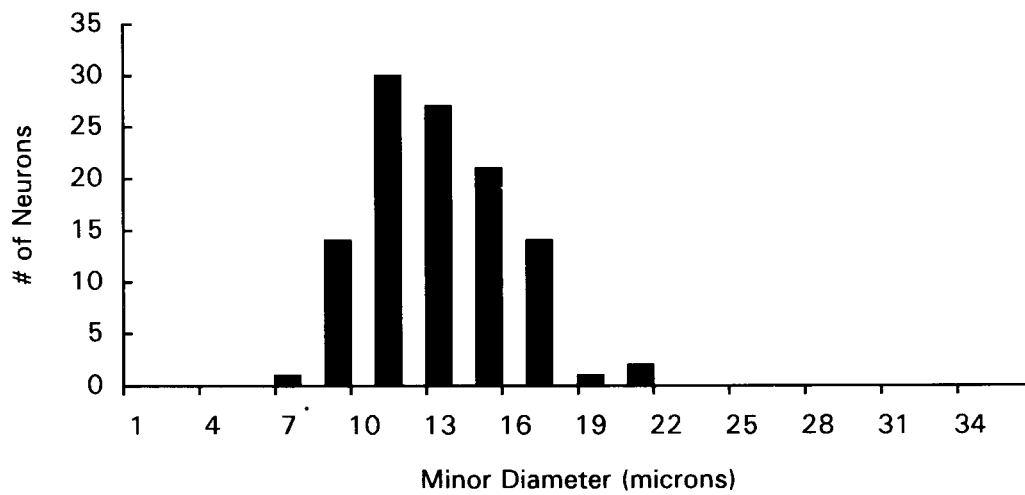
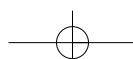
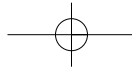


Figure 4. Histograms of the size of 110 intracardiac ganglion cells measured across the major axis (A) and the minor axis (B).





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been estimated to be 8,405 by Calaresu & St. Louis (1967). The smaller number of ganglion cells in the rat corresponds with its smaller and less extensive distribution of intracardiac ganglia.

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