Stereological estimation of dendritic coverage in the capybara SCG

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Abstract

This is a short overview on synaptic coverage of the dendritic tree in the superior cervical ganglion of capybara based on the application of the electron-immunocytochemistry of synaptophysin combined with stereological tools. It is shown how to estimate the surface coverage of dendrites by postsynaptic apposition zones and how to perform a model-based stereological estimate of the numbers and sizes of synaptophysin-labelled axo-dendritic synaptic disks.

Introduction

The functional importance of the superior cervical ganglion (SCG) including its complex arborisation in mammals is defined by the fact that this autonomic ganglion supplies sympathetic nerve fibres to the large cerebral vessels and iris and hence SCG is implicated in the neural control of cerebral blood flow as well as a vision [1-5].

Evidence suggests that there is a correlation between average size of central and autonomic neurons and body size and between total number of the neurons and body size [6-9]. As an example, a comparison of the SCGs of rat, capybara and horse revealed that the volume of SCG is 0.5 mm^3 in rats, 226 mm^3 in capybaras and 412 mm^3 in horses, while the total number of neurons per ganglion is 18,800, 1,520,000 and 3,390,000 and the number of neurons per cubic millimetre is 36,700, 7,000 and 8,250 in rats, capybaras and horses, respectively. Furthermore, the average neuronal size (measured as the area of the largest sectional profile of a neuron) is 358, 982 and 800 μm^2, and the percentage of volume occupied by neurons is 33, 21 and 17% in rats, capybaras and horses, respectively. When comparing the three species (average body weight: 200 g, 40 kg and 200 kg), most of the neuronal quantitative parameters change in line with the variation of body weight [10]. Our recent study also confirmed to some extent the presence of body size-related changes in structural parameters of the SCG in passing from rats to capybara to horses [11]. The above study also confirmed the earlier observations that SCG neurons of larger species display greater convergence, more complex arborisations

and a greater proportion of axo-dendritic synapses compared to axo-somatic ones [6,12,13].

Based on our recent study [11] the aim of the present article was to outline some numerical data related to axo-dendritic synapses in SCG of the biggest rodent – capybara, using immunocytochemistry of synaptophysin (SY), a glycoprotein of presynaptic vesicles, to mark functioning synapses [14] in SCG.

Materials and Methods

Data are based on the analysis of adult male capybaras (five ~2-year-old animals weighing on average ~ 56kg), which were the same animals used by Loesch et al. [11]. To identify SY-positive axon varicosities making synapses on dendrites a rabbit polyclonal antibody to SY (A010: DAKO, Glostrup, Denmark) was utilised using the pre-embedding electron-immunocytochemistry protocol as previously described [11]. Ultrathin (90 nm) sections of SCG were cut, contrasted with uranyl acetate and lead citrate and subsequently examined and photographed digitally using a CM-120 Philips TEM transmission electron microscope.

Design-based stereology at the TEM level.
The methodological details including a definition of a synapse can be found in articles by Mayhew [15,16]. Here it is shown how to obtain numerical data on the relative and absolute surface area of postsynaptic dendrite mem-

branes occupied by axo-dendritic synapses (Ssyn/Sdend). For this purpose, a test-line system was randomly superimposed onto systematic uniform random (SUR)-sampled TEM images (see Figure 1) taken at linear magnifications (i.e. x20,000 - 30,000). The total number of intersections between test lines and the membrane traces of dendrite profiles ($\sum I_{dend}$) and those between test lines and the postsynaptic density of the total apposition zone ($\sum I_{syn}$) were counted. Surface density was then estimated [15] as: $S_{syn}/S_{dend}:=\sum I_{syn}/\sum I_{dend}$.
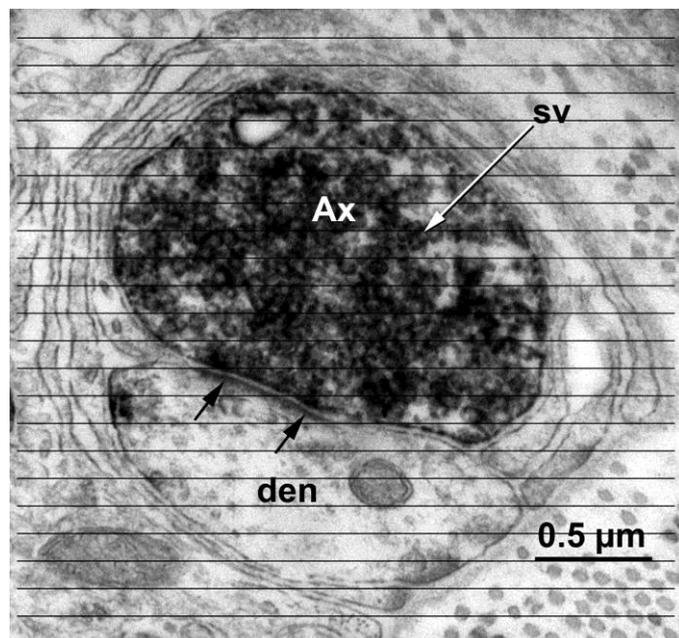

Figure 1. Capybara SCG immunolabelled for SY. The general principle of the quantitative method at TEM level for assessing the relative surface area of dendrites occupied by axo-dendritic synapses. An axon profile (Ax) positive for SY ("black" immunoprecipitate) makes synaptic contact with a dendrite (den). In dendrite, note the presence of post-synaptic density specialisation (short arrows), while small synaptic vesicles (sv) of ~50 nm diameter dominate in the SY-positive axon. The intersections between test lines and the linear membrane traces of the synaptic density specialisation and dendrite are used to calculate the fractional area of dendrite occupied by synapses. Note that the relative surface area of the dendrite occupied by the axo-dendritic synapse: S_{syn}/S_{dend} seen in this image is 3/18 = 0.1666 = 16.66%.

Approximate numbers of synapses per neuron and per ganglion: N_{syn}/N_{all} and N_{syn}

We obtained rough estimates of the numbers of synapses using a model-based approach treating the synaptic apposition sites as flat circular disks of uniform diameter. The

approach produces approximations because axo-dendritic synapses do not conform to this simplistic model. Indirect estimates were obtained at several steps. First, we estimated the surface density of dendrite membranes in ganglion volume S_{dend}/V_{scg}.

To do this, the test-line system was randomly superimposed onto SUR-sampled TEM images in order to count intersections between test lines and the membrane traces of dendrite profiles and the test points falling on the ganglion. Subsequently, the total surface area of dendrites in a given SCG was then calculated as:
$S_{dend}:= V_{scg}.S_{dend}/V_{scg}$
where V_{scg} is the volume of the corresponding ganglion.

Next, the absolute surface of axo-dendritic synapses in the whole ganglion, S_{syn}, was calculated by multiplying S_{dend} by S_{syn}/S_{dend} for each individual ganglion.

We then obtained estimates of the mean area of a synaptic disk by treating apposition zones as a monodisperse set of flat circular disks [11,15]. The mean trace length of sectioned disks, L_{syn}, was used to calculate mean disk surface area by the formula:
$A_{syn}:=(4/\pi).(L_{syn})^{2}$.

From the total surface area of all synapses, and the mean area of a synaptic disk, the total number of synapses per ganglion was estimated as:
$N_{syn}:=S_{syn}/A_{syn}$
and the number per neuron as: N_{syn}/N_{all}.

Results

As we previously reported [11] axon profiles including the axon varicosities making synaptic contacts on dendrites were positive for SY in capybara SCG (Figure 1). Postsynaptic specializations were visible, while the presynaptic specializations were poorly defined due to obscuring effects of immunoprecipitate. The axon varicosities synapsing on dendrites showed small spherical agranular vesicles (of ~ 50 nm) but a few larger granular vesicles (of ~ 70-100 nm) were also present. Immunoprecipitate decorated the vesicle membrane and the core in granular vesicles. SY-positive axo-dendritic synapses were asymmetrical. SY-negative axo-dendritic synapses were also seen.

The relative surface of dendrites occupied by synaptic apposition zones was 0.19 (0.01), the mean disk areas of 0.474 μm^2 (0.13), the total number of axo-dendritic synapses per ganglion averaged 56,390 million (0.36), and the number of axo-dendritic synapses per neuron was 20,370 (0.38).

Discussion and Conclusion

In the present article we showed how to assess quantitatively the axon-dendritic synapses in capybara SCG. The focus was on synapse size and the size (%) of dendrite occupied by synaptic axon profiles by using a combination of electron-immunohistochemical labelling for synaptophysin and applying a design-based stereological methodology. Based on an allometric analysis, which took into account the SCG volume, we have previously predicted some 34.1 billion axo-dendritic synapses in capybara SCG against the actual finding of 56.4 billion synapses [11]. Consequently, the SCG volume alone is not the principal determinant of synapse number when compared animals of different sizes i.e. rat, capybara and horse [11]. However, the above-cited allometric data confirmed that SCG volume is an excellent predictor of neuron number but a poor predictor of synapse number per ganglion, at least for the larger mammals [11]. We have also reported that numbers of axo-dendritic synapses per neuron were roughly 48-fold larger in capybaras as i.e. compared to 10.3 million of the synapses present in rat SCG. Here we stress that the complexity of axo-dendritic arrangements in SCG is relevant to the ganglion function.

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