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The morphology of Golgi-stained neurons in Lamina II of the rat spinal cord

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INTRODUCTION

Several recent studies have examined the morphology of neurons in the substantia gelatinosa (Lamina II) of the spinal cord, or spinal trigeminal nucleus in cat and primate (Gobel, 1975, 1978; Beal & Cooper, 1978; Bennett, Abdelmoumene, Hayashi & Dubner, 1980; Schoenen, 1982), using either the Golgi technique or the intracellular injection of horseradish peroxidase.

Gobel (1975, 1978) recognised two main cell types in the spinal trigeminal nucleus of the cat. Islet cells were found throughout the substantia gelatinosa and their dendrites and axons remained within this layer. Stalked cells were found in the superficial part and had medially directed spine-covered dendrites, and axons which entered the marginal layer. Bennett *et al.* (1980) found that, of 22 neurons injected with horseradish peroxidase in Lamina II of cat spinal cord, all but 2 could be classed as stalked or islet cells. Beal & Cooper (1978) made no attempt to classify Golgi-stained cells in the monkey and stressed the variability of the neurons in this region. In a Golgi study of human dorsal horn, Schoenen (1982) described islet cells and also filamentous and curly cells (which may correspond to stalked cells) but found that these made up only 60 % of the population in Lamina II. He classed the remainder as stellate cells which were situated in the ventral part of the lamina and had long dendrites extending along both rostrocaudal and dorsoventral axes and axons which entered the nucleus proprius.

The present investigation of Lamina II cells in rat spinal cord was carried out because there have been few reports of the morphology of rat substantia gelatinosa neurons (Woolf & Fitzgerald, 1983). Some of the cells described in the present report will subsequently be examined by electron microscopy in order to determine the extent to which cells of a particular class share ultrastructural features.

MATERIAL AND METHODS

The material used in the present study was obtained from six young male Albino Swiss rats weighing between 100 and 200 g. The animals were deeply anaesthetised with pentobarbitone and perfused through the left ventricle with a fixative containing 2 % glutaraldehyde and 2 % formaldehyde in 0·1 M phosphate buffer at pH 7·4 and 4 °C. The lumbar spinal cord was removed, divided into blocks 3–5 mm in length and stored in the same fixative overnight. The blocks were rinsed in buffer, postfixed in 2 % osmium tetroxide for five hours and then placed in 3 % potassium dichromate for three or four days followed by one or two days in 0·75 % silver nitrate. Sections 100–200 μm thick were cut by hand in the sagittal plane and gold-toned using the

method of Somogyi, Hodgson & Smith (1979). The gold-toned sections were rinsed in water, dehydrated in alcohol, cleared in propylene oxide and embedded in Spurr's resin between glass coverslips.

In the present material it was not always possible to identify the border between Laminae II and III, and a depth of 100 μm below the dorsal white matter was used as a guide to the ventral border of Lamina II. This corresponded to the Lamina II–III border when it was rendered visible by the osmium staining of myelinated axons in Lamina III and is in good agreement with the measurements of Ribeiro-da-Silva & Coimbra (1982). In the most medial part of the dorsal horn, the boundary between Lamina I and the dorsal columns is indistinct because of the presence of bundles of myelinated axons, and in the lateral one third, where the laminae curve ventrally, they are difficult to distinguish and the cells change their orientation. Sections from the middle region of the dorsal horn were therefore used. The first 100 satisfactorily impregnated cells lying less than 100 μm from the dorsal white matter but ventral to Lamina I were identified and drawn with the aid of a camera lucida at a final magnification of approximately $\times 600$.

RESULTS

For the purpose of analysis, Lamina II was divided into a dorsal part, extending from approximately 20 to 60 μm below the dorsal white matter, and a ventral part from 60 to 100 μm below it. Of the 100 cells in this study, 49 had cell bodies in the dorsal part and 51 in the ventral part. Thirty one of the cells were identified as islet cells and 32 as stalked cells as described by Gobel (1978).

Islet cells (Figs. 1, 2)

These were recognised by the distinctive rostrocaudal orientation of their dendritic trees, which could be very long (730 μm in one case). They were found throughout the whole thickness of Lamina II with 15 in the dorsal half and 16 in the ventral. Typical cells showed a recurrent branching pattern of dendrites which were often restricted to a narrow band within the lamina and had few dendritic spines (Fig. 1*b*). Terminal parts of dendrites sometimes exhibited bouton-like swellings. Impregnated axons began arborising in Lamina II but it was not possible to follow them to their terminations. As noted by Schoenen (1982) three types of islet cell could be recognised: those in which the soma lay dorsal to the major part of the dendritic tree (Fig. 1*a*); those in which both soma and dendritic tree lay in the same plane (Fig. 1*b*) and those with a soma ventral to most of the dendrites (Fig. 1*c*). Cells with a dorsal soma were usually situated in the dorsal part of Lamina II while the other types were more common in the ventral part of the lamina. A few islet cells had many dendritic spines (Fig. 2*a*) and several (11 of 31 cells) had dendrites which entered Lamina III. Some cells displayed features of both islet and stalked cells (Fig. 2*b*).

Stalked cells (Fig. 3)

A typical stalked cell (Fig. 3*a*) had a rounded soma in the dorsal part of Lamina II. Its branching dendrites passed ventrally with some spread along the rostrocaudal axis, giving the cell the shape of a cone with the soma situated dorsally at the apex. The dendrites were usually covered with spines and stalk-like branches. In several cases one or two dendrites left the rostral or caudal surfaces of the soma and travelled for some distance parallel to the dorsal border of the grey matter (Fig. 3*b*) or even

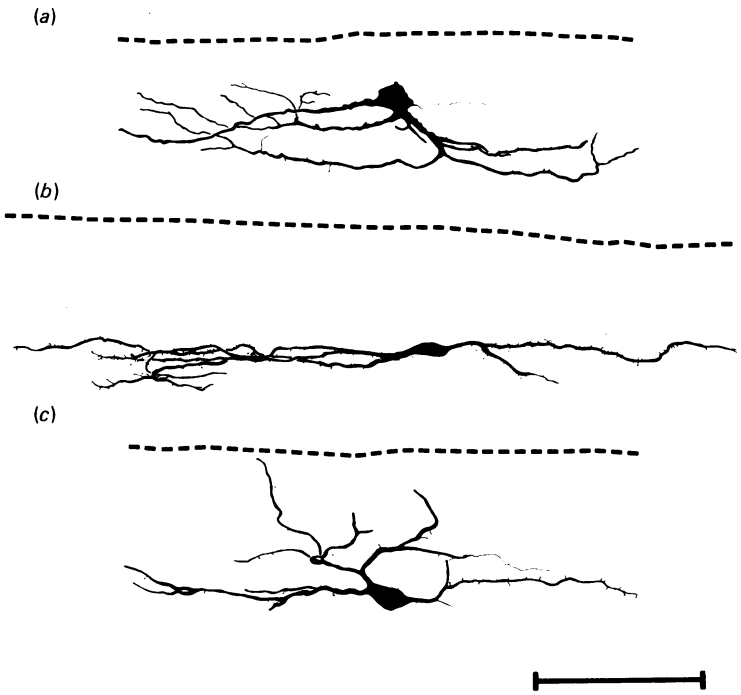

Fig. 1 (*a–c*). Camera lucida drawings of Golgi-stained islet cells in Lamina II. (*a*) An islet cell with its soma dorsal to the dendritic tree. (*b*) A cell with the soma at the same level as the dendrites. (*c*) An islet cell with a ventrally located soma. In this and subsequent Figures the broken line marks the dorsal limit of the dorsal horn. Scale bar, 100 μm.

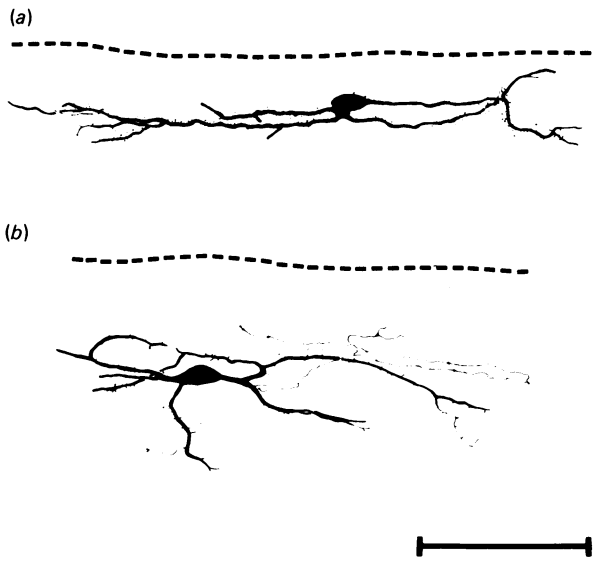

Fig. 2 (*a–b*). Two islet cells showing atypical features. (*a*) An islet cell with many dendritic spines. (*b*) An islet cell with typical recurrent dendrites arising from rostral and caudal poles of the soma, and an unusual ventrally directed dendrite. The axon begins branching in Lamina II. Scale bar, 100 μm.

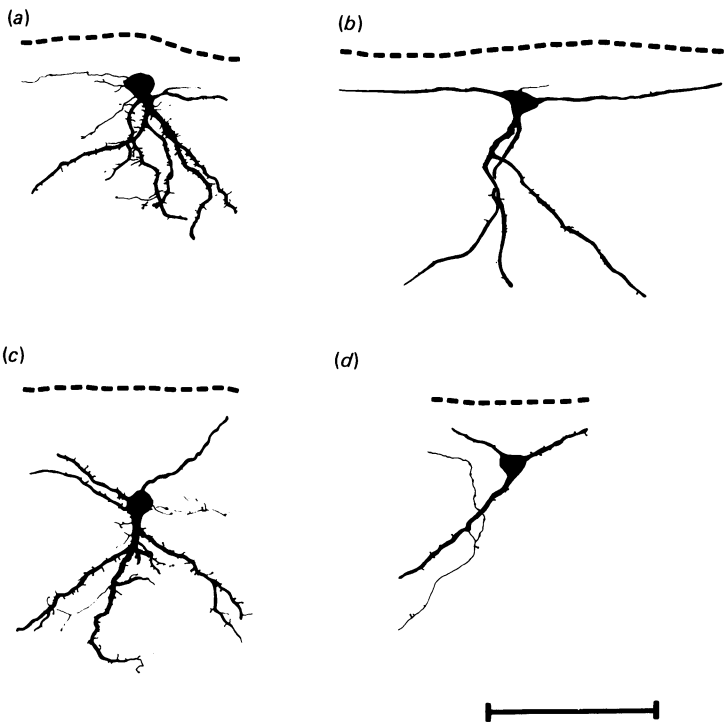

Fig. 3 (*a–d*). Four stalked cells. (*a*) A typical stalked cell with a dorsal soma in the outer part of Lamina II and ventrally directed spine-covered dendrites. (*b*) A cell with dendrites leaving the rostral and caudal poles of the soma and passing parallel to the dorsal border of the dorsal horn. (*c*) A stalked cell similar to that shown in (*a*) but lying 68 μm below the white matter in the ventral half of Lamina II. (*d*) A stalked cell with one ventral dendrite and an axon which gives a branch to Lamina III as well as one which passes in a dorsal direction. Scale bar, 100 μm.

passed obliquely dorsally (Fig. 3*c*). These dendrites often had few spines. When axons of stalked cells were stained they either gave rise to boutons in Lamina II at the level of the cell body (Fig. 3*a*, *c*) or else passed dorsally towards Lamina I. They were often rapidly lost from the section.

As with islet cells many stalked cells showed atypical features. Nine of the 32 cells had cell bodies in the ventral half of the lamina (Fig. 3*c*), while some cells had few spines on some or all of their ventral dendrites (Fig. 3*b*, *d*). The axon of the cell in Figure 3*d* gave a branch which passed ventrally into Lamina III.

Other cells

A total of 37 neurons could not be classified as either stalked or islet cells. Although they showed a wide variation in shape, it was possible to subdivide 33 of them into three groups largely on the basis of dendritic spread.

The first group consisted of 10 cells with spindle-shaped somata and large dendritic trees which ranged from 300–450 μm along the rostrocaudal axis and 100–220 μm along the dorsoventral axis. Eight of these cells had somata in the ventral half of the lamina and all had dendrites which extended throughout Lamina II and into Lamina III (Fig. 4*a*). Most of the cells had few dendritic spines, although in a few cases some of the dendrites had moderate numbers. Axons were seen in seven cases

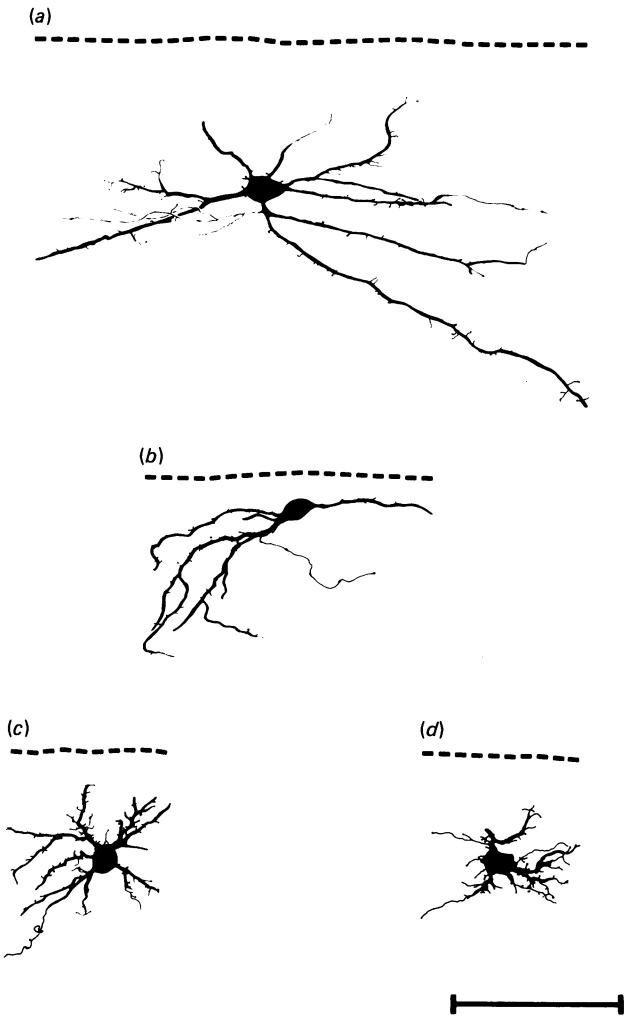

Fig. 4(*a–d*). Four cells which do not belong to either stalked or islet classes. (*a*) A cell with a large dendritic tree extending into Lamina III and an axon which begins branching at the level of the Lamina II–III border. (*b*) A cell with an intermediate dendritic spread. The dendrites are moderately spiny and just reach Lamina III. (*c*) A cell with a small dendritic tree. The dendrites, which are confined to Lamina II, are covered in short and long necked spines. The axon can be followed into Lamina III before it leaves the section. (*d*) Another small cell. The soma and the short primary and secondary dendrites give rise to spines and also to longer 'dendritic appendages'. Scale bar, 100 μm.

and usually passed initially ventral to the soma. Five gave rise to boutons in the ventral part of Lamina II or in Lamina III.

Another group of eight cells had slightly smaller dendritic trees. These cells also had fusiform cell bodies all but one of which were located in the ventral part of Lamina II. The dendrites, which were usually moderately spiny (Fig. 4*b*) extended for 160–250 μm along the long axis and 80–125 μm along the dorsoventral axis. Most of their dendritic trees lay in Lamina II, but all showed some spread into the dorsal part of Lamina III. Axons were found in four cases: three of these gave rise to boutons in Lamina II (Fig. 4*b*) and one passed ventrally.

The third group consisted of 15 cells with small compact dendritic trees. Cell bodies in this group could be round (Fig. 4*c*), polygonal (Fig. 4*d*) or fusiform and in all but three cases were in the ventral part of the lamina. The dendritic territory was usually limited to Lamina II and in most cases varied from 100–200 μm along the rostrocaudal axis. All the cells had dendritic spines, often in large numbers (Fig. 4*c*) and in many cases dendritic appendages (Beal & Cooper, 1978) were present on cell bodies or dendrites (Fig. 4*d*). Primary and secondary dendrites were often short and terminated in these appendages (Fig. 4*d*). An axon could only be identified with certainty on three cells and in each case passed ventrally and gave rise to boutons in Lamina III (Fig. 4*c*).

There were four cells all in the dorsal part of Lamina II with irregular dendritic trees, which did not fit into any of these classes. Two of the cells had one primary dendrite which travelled along the mediolateral axis and another which passed rostrally or caudally.

DISCUSSION

Gobel (1975, 1978) originally described islet and stalked cells in the substantia gelatinosa, but recognised other minor cell types. However, other workers (Bennett *et al.* 1980) have stressed the importance of these two cell types almost to the exclusion of all others. The present study has shown that, although many islet and stalked cells are found in Lamina II of the rat spinal cord, over one third of the cells observed in the whole lamina and half of the cells in its ventral part did not belong to either of these classes.

In the human spinal cord Schoenen (1982) found that islet cells constituted 30 % of the stained neurons in Lamina II, which is in good agreement with the results presented here. He did not define stalked cells as a group, but his filamentous and curly cells both had dendrites with many stalks or spines and an axon which entered Lamina I. Many of these cells (which made up 30 % of the population in Lamina II) may therefore have corresponded to stalked cells.

Most of the cells in the three remaining groups described here had dendritic trees whose major extent was along the long axis of the spinal cord, but with moderate spread along the dorsoventral axis. Many of these cells had dendrites which ran obliquely for much of their length. It is possible that they form part of a single group with a range of dendritic spread and a tendency for larger cells to have simpler dendritic trees with fewer spines.

The stellate cells described by Schoenen (1982) are similar to the large cells seen here. Both had cell bodies in the ventral part of the lamina and long dendrites with irregular spines which extended into Lamina III. Stellate cells had axons which ramified in Lamina III and IV, while some gave collaterals to Lamina II. The axons of the large cells described here gave rise to boutons in ventral Lamina II and in Lamina III. The II–III border cells of Gobel (1978) show some resemblance to Schoenen's stellate cells but had dendrites arising from rostral and caudal poles of the soma and bearing more spines. They might therefore correspond to the medium sized cells reported here although these had simpler dendritic trees than those illustrated by Gobel (1978). No cells of the small spiny variety reported here were described by Gobel or Schoenen, but these cells do resemble some of those found by Beal & Cooper (1978) in the monkey (their Fig. 8, cell A2 and Fig. 9, cell A1).

One problem which arises in attempts to classify neurons in Lamina II is the presence of cells which show features of more than one class. For example the cell

illustrated in Figure 2*b* had recurrent dendrites – a feature of islet cells, but a ventrally directed dendrite arising from the soma, which is more typical of stalked cells. An ultrastructural study of Golgi-stained Lamina II neurons should help to distinguish between cells in different classes and provide direct evidence about the synaptic connections of particular neurons in the substantia gelatinosa.

SUMMARY

Golgi-stained neurons in Lamina II of the rat spinal cord were examined by light microscopy. Stalked and islet cells similar to those seen in other species were found. Stalked cells were present in large numbers in the dorsal part of the lamina where they made up nearly half the population of stained cells. Islet cells were found throughout the lamina and constituted about one third of the total population. In the ventral part of the lamina half of the stained cells did not fall into either category, but could be divided into groups on the basis of dendritic spread. The axons of many of these cells either remained in Lamina II or passed ventrally into Lamina III. Some of these cells may correspond to the stellate or the II–III border cells which have been seen in human spinal cord and cat medulla respectively.

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