Acta Neuropathol (2006) 112:647–649
DOI 10.1007/s00401-006-0139-8

CORRESPONDENCE

Presence of binucleate neurons in the spinal cord of young and senile rats

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Received: 2 August 2006 / Revised: 24 August 2006 / Accepted: 24 August 2006 / Published online: 22 September 2006

Abstract The presence of binucleate cells constitutes a normal feature of some animal tissues but is rare in the normal brain and has not been documented in the spinal cord. We assessed different segments of the rat spinal cord in order to determine the frequency and distribution of binucleate neurons in this structure as well as the impact of aging on this neuronal population. Young (4–5 months) and senile (32 months) female Sprague–Dawley rats were used. Sections from cervical, thoracic and lumbar segments were histochemically and immunohistochemically (NeuN) stained and the frequency and distribution of binucleate neurons was determined by manual counting. The frequency of binucleate neurons in all of the analysed segments was comparable between young and senile animals. Binucleate neurons were particularly frequent in the C5 and C6 segments. The overall distribution of binucleate neurons in the different laminae assessed was, Lm-III = 19%; Lm-VI = 17%; Lm-VII = 39%; LmVIII = 8%; Lm-IX = 11%; Lm-X = 6%, and was comparable between young and senile rats. We conclude that binucleate neurons occur as a normal feature of the rat spinal cord and that their frequency and distribution does not change with aging.

Keywords Spinal cord · Binucleate neurons · Cell counting · Morphology · Aging

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During a systematic study of the spinal cord of young and aging rats we observed the presence of binucleate neurons in the grey matter, a phenomenon that has not been previously documented. We also quantified binucleate neurons in different segments of the rat spinal cord and compared the frequency of this type of cells in young and senile animals. The results are reported here.

Five young (4–5 months) and four senile (32 months) female Sprague–Dawley rats were used according to previously approved protocols (IACUC approval date 04/01/06). Animals were intracardially perfused with a 10% buffered formalin solution and the spinal cord dissected and processed as previously described [3]. Paraffin embedded blocks from the cervical (C1–C8), thoracic (T1, T4, T6) and lumbar (L2) segments were processed. Fifteen-micrometer sections

were histochemically stained with the cresyl fast violet technique for morphologic identification and morphometric analysis. From each block, five slices, 100 μm apart, were analysed. Some sections from young animals were immunohistochemically stained with mouse anti-neuron-specific nuclear protein (NeuN) mAb (Chemicon International, USA) and the DAB-peroxidase system as previously described [4].

Images of spinal cord sections were captured and processed as previously described [4]. The grey matter region of each block was grossly divided into ten laminas (Lm) according to the scheme previously reported [3].

A systematic screening of the analysed segments revealed the presence of binucleate neurons in all assessed segments of both young and senile rats (Fig. 1). The number of binucleate neurons found was comparable between young and senile animals (data not shown). The highest number of binucleate neurons was found at the C5 and C6 segments. In the young animals the highest frequency of binucleate neurons was observed in the thoracic region ($\overline{\times}$ ± SEM; 1.14 ± 0.41%), although no statistical differences were observed among segments (cervical segments 0.59 ± 0.23%; lumbar segment 0.50 ± 0.27%). Percentages refer to the total number of neurons.

The overall distribution of binucleate neurons in the different laminae (Lm) of the grey matter showed a clear prevalence in the Lm-VII (Lm-III = 19%; Lm-VI = 17%; Lm-VII = 39%; LmVIII = 8%; Lm-IX = 11%; Lm-X = 6%) considering all of the analysed segments.

The presence of binucleate cells constitutes a normal feature of some animal tissues. Thus, some trophoblastic cells of the ruminant's placenta are binucleated [1]. In the mouse liver, the occurrence of hepatocytes possessing two nuclei is a common finding [2]. In the central nervous system (CNS), Ramón y Cajal [5] described binucleate neurons in ganglia of the sympathetic system. Neurons with the same characteristics were described in the human brain stem, predominantly in the pons, next to the thalamus, and to a lesser extent, in the mid-brain and medulla [6]. The present results add the spinal cord to the CNS structures where binucleate neurons occur in healthy individuals.

Although the binucleate neurons observed in the spinal cord of our rats appeared structurally well preserved and therefore presumably healthy, it is not clear whether they serve any specific function. In our study, the constancy in the number and distribution of binucleate spinal cord neurons in very old rats suggests that these cells were not a pathological component. Otherwise one could expect that their number would be modified in senile animals.

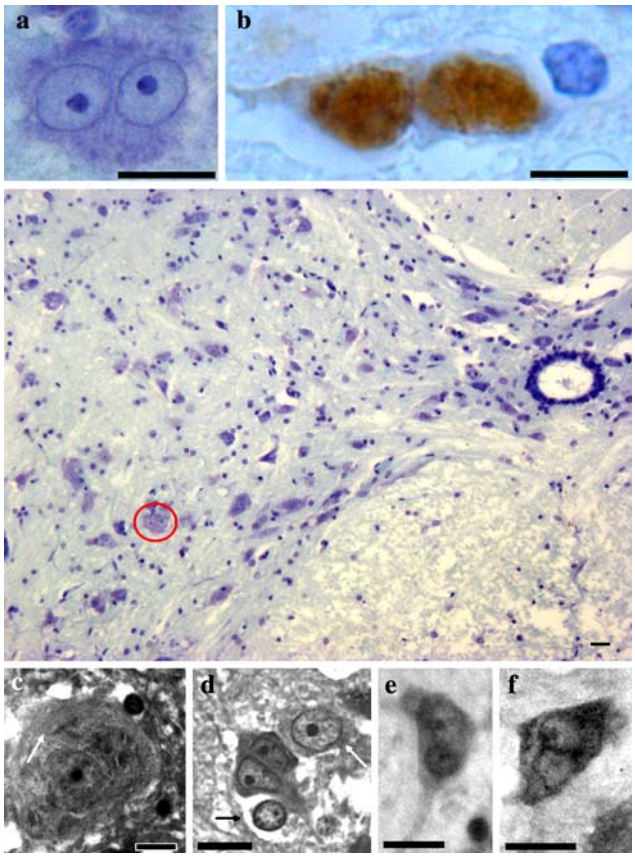

Fig. 1 Binucleate neurons in the spinal cord of young and senile rats. The *main panel* shows a binucleate neuron (*encircled*) in the grey matter of the C5 segment of a young rat. Panel **a** shows a higher magnification of the encircled neuron with the two nuclei in the same section plane. Panel **b** shows a binucleate neuron immunostained for NeuN; it was found in Lm-VII of the C5 segment of a young rat in close contact with a negative glial cell. *Lower panels* show four binucleate neurons present in different spinal cord segments of a senile rat. Notice that the morphology of the young and senile binucleate neurons is comparable except for the presence of lipofuscin in some of the senile cells (*arrow* in **c**). Panel **c** shows a neuron found in Lm-IX of the T6 segment. Panel **d** shows a neuron found in the Lm-VII of the C6 segment. The binucleate neuron is in close contact with a glial cell (*black arrow*) and another neuron (*white arrow*). The neuron shown in **e** was found in the Lm-VII of the C5 segment. Panel **f** shows a binucleate neuron found in the Lm-VII of the C4 segment. Cresyl violet staining. *Bars* = 10 μm

We conclude that binucleate neurons occur in the rat spinal cord and that their frequency neither decreases nor increases with aging. They do not seem to represent a pathologic feature although their physiologic role, if any, is not clear at this stage.

Acknowledgements Our gratitude to Mrs. Rosa Villegas and Maria Guadalupe Guidi for their technical assistance. ELP, CGB, EJG and RGG are Research Career Members of CONICET (Argentinean National Scientific Research Council). Financial support was provided in part by a grant from Agencia Nacional de Promoción Científica (ANPCyT) (PICT-12544).

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