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Morpho-quantitative analysis of nuclear inclusions in periaqueductal grey matter neurons in the cat

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INTRODUCTION

The neurons of the periaqueductal grey matter constitute a heterogeneous cell population, characterised by neurons of small size, immersed in a dense neuropil. In a previous ultrastructural study (Gioia, Tredici & Bianchi, 1983), it was observed that the incidence of nuclear inclusions in periaqueductal neurons is much higher than in other mesencephalic regions (Tredici, Pizzini & Milanesi, 1976). It has been suggested that the frequency of nuclear inclusions is correlated with the intensity of cellular activity (Masurovsky, Benitez, Kim & Murray, 1970; Bouteille, Laval & Dupuy-Coin, 1974; Peters, Palay & Webster, 1976). In particular, the correlation between cyclic AMP and increase in the number of nuclear inclusions reported by Seïte, Leonetti, Luciani-Vuillet & Vio (1977) after injection of cyclic AMP analogues and theophylline is of particular interest. In nervous tissue, cyclic AMP is present in high concentrations (Rall, 1971; Drummond & Ma, 1973) and, in particular, can be determined in cerebrospinal fluid where it undergoes daily rhythmic variations in concentration (Perlow *et al.* 1977). Therefore, the possibility that nuclear inclusions may reflect an environmental microinfluence on neurons in close contact with the ventricular cavity may be considered.

Since the periaqueductal grey matter has a cylindrical arrangement around the cerebral aqueduct and therefore represents the mesencephalic nuclear structure nearest to the cerebrospinal fluid, a morphoquantitative study on the nuclear inclusions was carried out to determine whether a topographic distribution of nuclear inclusions exists which can be correlated with the position of the neurons in relation to the cerebral aqueduct.

MATERIALS AND METHODS

Four adult cats were used weighing between 2 and 3·5 kg. The animals were perfused through the abdominal aorta with 3 % glutaraldehyde in 0·24 M phosphate buffer, pH 7·2.

The midbrains, once isolated, were cut transversely along the long axis of the aqueduct into serial slices 1 mm thick, postfixed in 1 % osmium tetroxide in 0·18 M phosphate buffer and then embedded in Epon 812.

Sections, 1 μm thick, were obtained from rostral, central and caudal slices of the midbrain of each animal. Using the light microscope, the ring of periaqueductal grey matter was divided into two regions as follows: using a graduated eyepiece, the distance between the ependyma and the outer boundary of the periaqueductal grey matter was measured in the lateral, dorsal and ventral regions and the midpoints

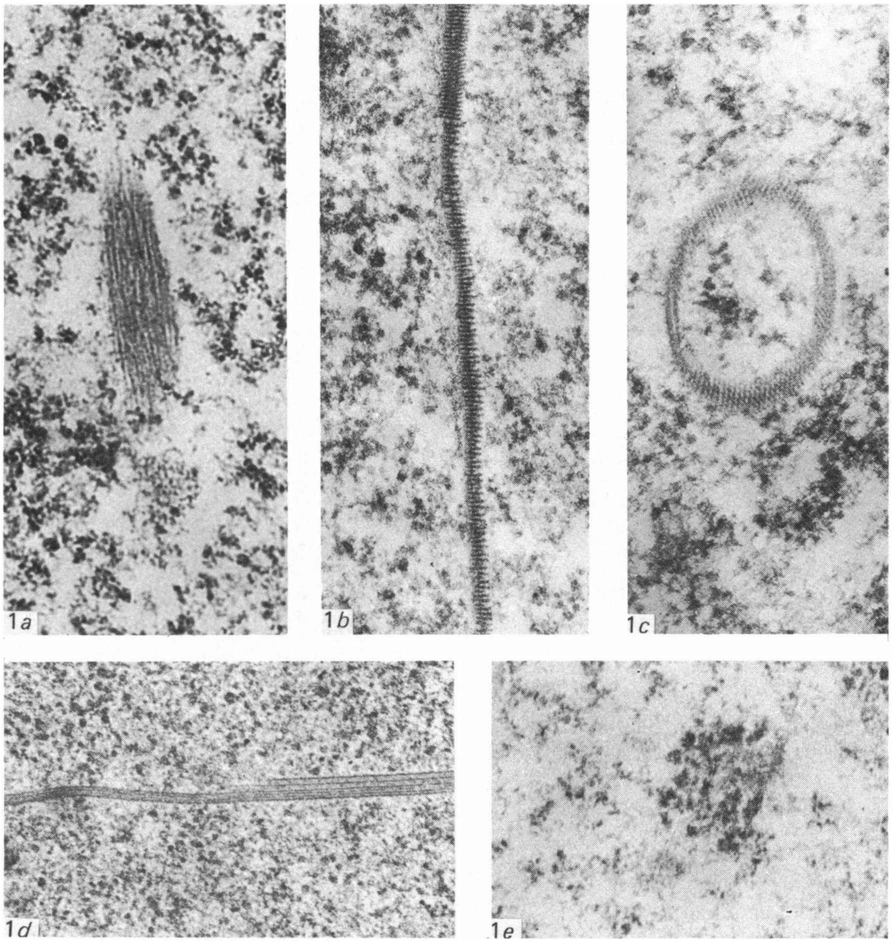

Fig. 1 (*a–e*). Electron micrographs of nuclear inclusions identified in neurons of the periaqueductal grey matter: (*a*) nuclear rod. ×30000; (*b, c*) paracrystalline inclusions. ×45000; (*d*) sheet-like inclusions with filaments arranged parallel to the longitudinal axis. ×42000; (*e*) perichromatin granule. ×30000.

between the inner and outer surfaces of the grey matter represented the boundary between the internal and external regions of the periaqueductal grey matter.

Subsequently, from the same slices, ultrathin sections were cut randomly in the external and internal regions, and stained with alcoholic uranyl acetate and lead citrate. A total of 140 nerve cell bodies located in the internal region and 160 in the external region of the periaqueductal grey matter was examined in which the nucleus and nucleolus were present in the plane of section.

Quantitative evaluation carried out on the electron micrographs estimated only the inclusions present in the plane of section. For this reason, the percentages reported do not indicate the true proportion but only the relative proportions of the frequency and of the different types of inclusions.

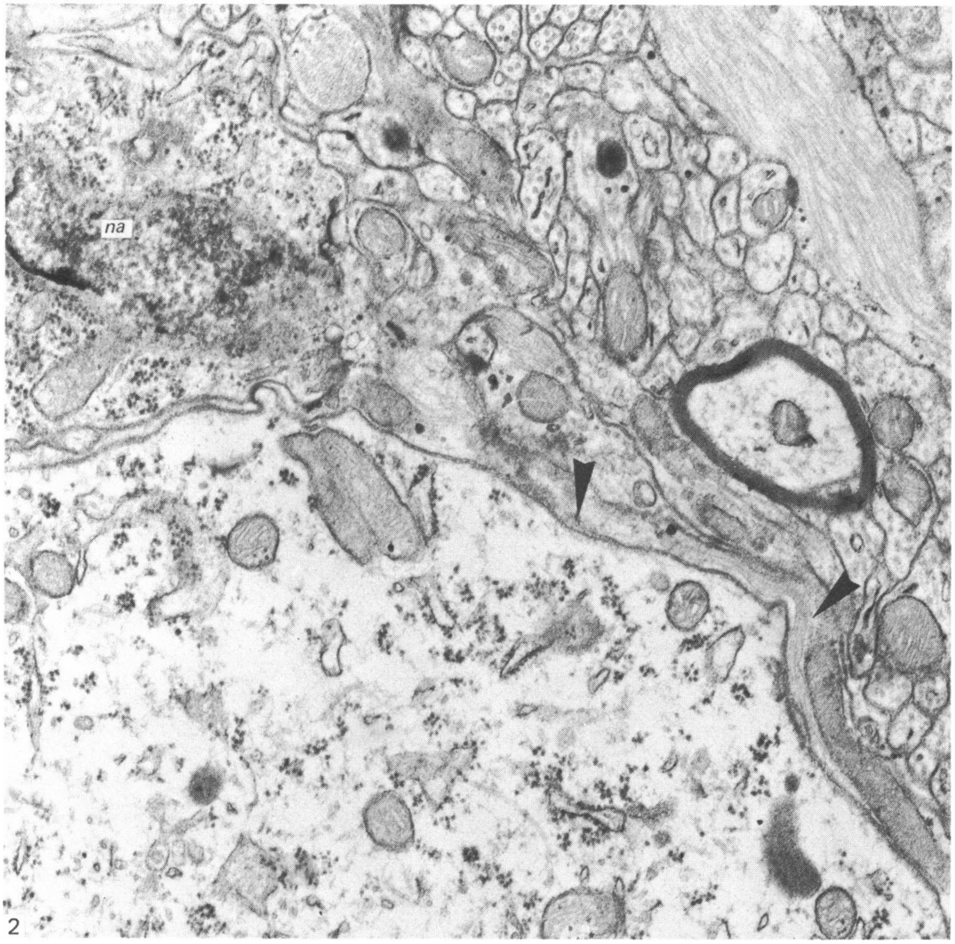

Fig. 2. Electron micrograph of a neuron situated in the internal region of the grey matter. The arrowheads indicate a glial fibrous process. *na*, nucleus of an astrocyte. × 30000.

RESULTS

Evaluation of the relative proportions of the different types of inclusion showed that 98 % were of a filamentous type, with the filaments assembled in two different patterns: as nuclear rods (45·8 %) and as sheet-like inclusions. Only 2 % of the nuclear inclusions were found to be of the perichromatin granule type.

In neurons containing perichromatin granules, the filamentous type of inclusion was always observed. The various types of nuclear inclusions are shown in Figure 1.

Quantitative evaluation yielded the following relative percentages. Of the neurons situated in the external area of the periaqueductal grey matter, 30 % had nuclear inclusions, while in the internal area, only 2 % of the nerve cells contained them. There was, therefore, a clear tendency for the inclusions to be localised in the outermost neurons. In the internal region of the periaqueductal grey matter, where the incidence of inclusions was extremely low, it could be observed that the glia was much more developed and formed a more extensive covering around each nerve cell body than in the external region.

Fig. 3. Electron micrograph of a neuron situated in the internal area of the PAG. The arrows indicate the laminar structures that emanate from astrocytic processes. *g*, glia. × 30000.

Two types of gliocytes were clearly identifiable. The first type was formed by astrocytes with numerous fibrous processes from which laminar structures often emanated (Fig. 2). These laminae were frequently piled on top of one another, arranging themselves on the plasma membrane of the nerve cell body, which was thus separated from surrounding structures (Fig. 3). The second type was formed by gliocytes which, for rather long stretches, were directly in contact with the nerve cell body itself, thus behaving like satellite cells (Fig. 4).

DISCUSSION

Nuclear inclusions have variable distributions in different regions of both the central and the peripheral nervous systems. They are quite common in some brainstem nuclei, in the cerebral cortex and in sympathetic ganglia, while in other parts, such as the hippocampus, the thalamus or the spinal ganglia, they are quite rare.

In the periaqueductal grey matter, nuclear inclusions are very numerous with characteristics common to the inclusions of the other mesencephalic nuclei such as the oculomotor nucleus and the red nucleus (Pizzini, Tredici & Petruccioli-Pizzini, 1980). Perichromatin granules have only occasionally been observed, and so their significance in this context can be disregarded.

Quantitative evaluation of the distribution of the inclusions shows them to be very frequent with a distinct lack of homogeneity in their distribution; nuclear inclusions

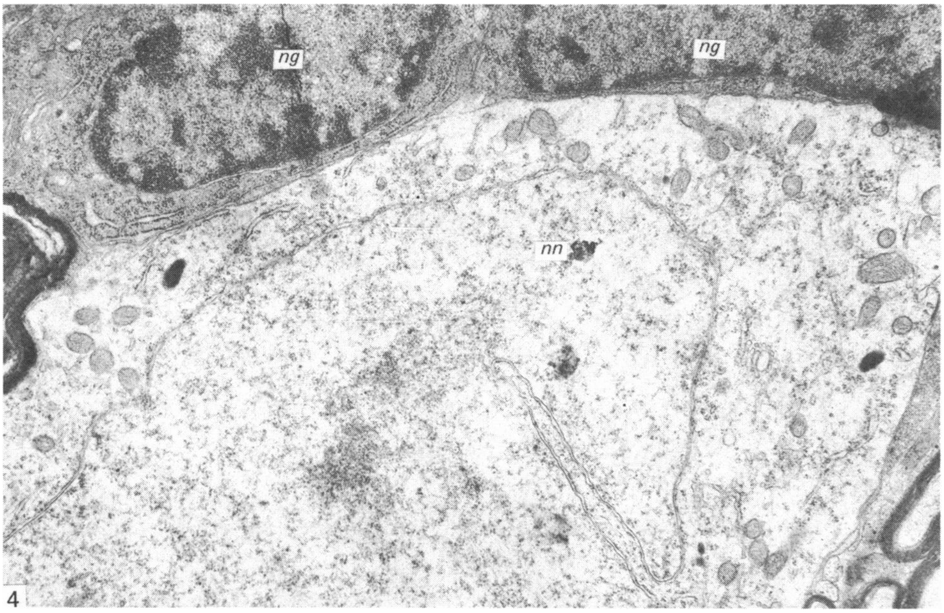

Fig. 4. Electron micrograph of two gliocytes directly in contact with a nerve cell body, behaving like satellite cells. *ng*, nucleus of the gliocyte; *nn*, nucleus of the neuron. × 12000.

are much more frequent in neurons located in the external region of the periaqueductal grey matter, i.e. in the area furthest from the aqueduct.

The present observations, therefore, do not seem to support the hypothesis put forward earlier that there is a correlation between the frequency of the inclusions and the proximity of the neurons to the central aqueduct. On the contrary, the inclusions are much less numerous in the zone nearest to the central aqueduct. This indicates that there is a negative correlation between the frequency of inclusions and their distance from the aqueduct. Furthermore, in the internal area, where the frequency of the inclusions is lower, the glial component is most highly developed. These findings suggest that the incidence of the nuclear inclusions may be influenced not only by the intensity of cell activity but also by the neuron/glia relationship.

SUMMARY

The morpho-quantitative analysis carried out in the neuronal population of the periaqueductal grey matter of the cat has shown that nuclear inclusions are mainly of the filamentous type and that they are distributed predominantly in the external region, i.e. in the part of the periaqueductal grey matter situated furthest from the cerebral aqueduct, where 30 % of the cells contain nuclear inclusions. In the internal region, i.e. in the part nearest the subependymal zone, only 2 % of the neurons have nuclear inclusions.

The glia in the internal region is more abundant and surrounds each nerve cell body while in the external zone of the periaqueductal grey matter it is scanty and does not delimit the neuronal soma. This difference suggests that there may be a relationship between the incidence of nuclear inclusions and the neuron/glia ratio.

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