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The cytoarchitecture of the nucleus cuneiformis. A Nissl and Golgi study

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INTRODUCTION

The nucleus cuneiformis, a reticular nucleus of the midbrain extending ventrally to the colliculi in the dorsolateral part of the mesencephalic tegmentum, is a region of considerable functional interest. In fact, Shik, Severin & Orlovsky (1967) and later Grillner & Shik (1973), Steeves, Schmitt, Skovgaard & Jordan (1975, 1980), Mori, Shik & Yagodnitsyn (1977), Jordan, Pratt & Menzies (1979) and Grillner (1981) showed that it corresponds to the mesencephalic locomotor area, a region which physiological studies (Miller, Van der Burg & Van der Meché, 1975; Jordan *et al.* 1979; Mori, Nishimura & Aoki, 1980; Steeves & Jordan, 1984; Amemya & Yamaguchi, 1984; Jell, Elliott & Jordan, 1985) have shown to be of extreme importance in the initiation of locomotion.

The nucleus cuneiformis, which has been identified in guinea-pig (Castaldi, 1926), cat (Taber, 1961; Berman, 1968), rat (Beitz, 1982*a*), monkey (Chung *et al.* 1983) and man (Olszewski & Baxter, 1954) is connected with many areas of the CNS. In particular Edwards (1975) and Edwards & de Olmos (1976) studied the efferent connections of the nucleus which end in: thalamic and hypothalamic nuclei, nuclei with ascending projections (reticularis gigantocellularis, reticularis pontis caudalis, linearis intermedius, linearis rostralis, locus coeruleus, raphe dorsalis, superior colliculus and central gray matter), nuclei which project to the cerebellum (reticularis tegmenti ponti, griseum pontis, lateral reticular nucleus, inferior olive and locus coeruleus), reticulospinal nuclei (reticularis pontis caudalis, reticularis gigantocellularis and raphe magnus) and cranial nerve nuclei (facial and oculomotor). Other authors have confirmed the connections with the reticularis magnocellularis nucleus (Abols & Basbaum, 1981; Chung *et al.* 1983) and with the raphe magnus nucleus (Abols & Basbaum, 1981; Beitz, 1982*b*; Chung *et al.* 1983) and in addition Castiglioni, Gallaway & Coulter (1983) have described efferent connections to the spinal cord. Afferent connections to the nucleus cuneiformis arise from the spinal cord (Kerr, 1975; Menetrey, Chaouch, Binder & Besson, 1982) and from the gracile and cuneate nuclei (Björkeland & Boivie, 1984).

This nucleus has also been investigated from the biochemical point of view and it has been found to contain the following: enkephalins (Sar *et al.* 1978; Moss, Glazer & Basbaum, 1983), substance P (Ljungdahl, Hokfelt & Nilsson, 1978; Moss & Basbaum, 1983), neurotensin (Beitz, 1982*b*) and serotonin (Beitz, 1982*c*).

In a previous electron microscopical investigation (Gioia & Bianchi, 1987), the ultrastructural characteristics of the neurons, of the neuropil and particularly of the synaptic complex of the nucleus in cat (a species very often used in physiological research) have been described.

Given the functional importance of this nucleus, and as there is no other information in the literature regarding its cytoarchitecture, we wanted to complete our study using Nissl material to define the quantitative and morphological features of the nerve cell bodies, and Golgi material to identify the characteristics of the dendritic and axonal arborisation and the presence and frequency of spines, all these being factors of particular functional interest. In addition the study includes observations made in man, which permitted a comparative analysis with the data obtained in the cat to be made.

MATERIALS AND METHODS

The study was carried out on the midbrain of seven adult cats weighing from 2 to 3 kg and of two men aged 36 and 39. The cats were anaesthethised with sodium pentobarbitone (Nembutal) and perfused transcardially with 4% formaldehyde in 0·1 M phosphate buffer (pH 7·2). The human midbrains were fixed by immersion in 10% buffered formalin.

Nissl material

Four cat brains were carefully removed and the midbrains isolated. The pieces were postfixed in Bouin's solution, embedded in paraffin and serially cut (section thickness: 20 μm) transversely with respect to their long axis. The following cell characteristics were considered: the nucleus, the basophilia of the cytoplasm, the shape and section area of the perikarya containing nuclei and nucleoli, taking the presence of these organelles as being indicative of the paracentrality of the neuronal cell body section. The measurements were carried out at a final enlargement of × 1080 on a projection screen and using an image analyser (MOP III Zeiss). The quantitative determinations were made on 207 nerve cell bodies by a computerised semi-automatic system.

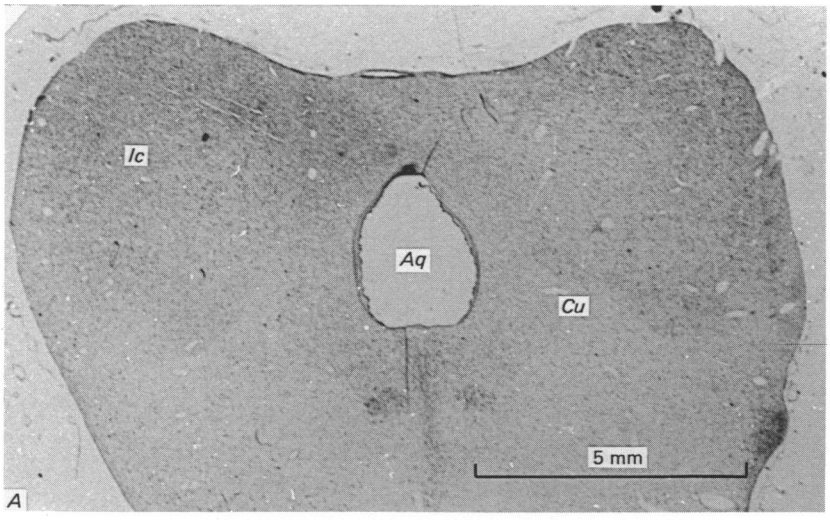
Golgi material

Three cat brains and the human brains were utilised for Golgi analysis. The midbrains were prepared according to the Golgi–Cox method with an impregnation period of 12–16 months. With this method, a complete heavy impregnation of the nucleus was obtained. The samples were embedded in celloidin and cut serially (section thickness: 120 μm). The cat midbrains were cut in transverse, sagittal and frontal planes with respect to the midbrain long axis. The human midbrains were cut transversely.

The nucleus was delimited using a Leitz microscope with a wall projector at a magnification of × 108 and the neurons were identified. Afterwards, the same neurons were drawn individually at × 1080. In all, drawings of 155 cat neurons and 80 human neurons were obtained. By means of an image analyser, the calibre and the length of the dendrites and axons (as far as they could be traced in the section) and the interspinous distances were measured.

RESULTS

In our material nucleus cuneiformis extends in a rostrocaudal direction for about 1200 μm in the cat and for 6000 μm in man. The nucleus begins in the caudal part of the midbrain at the level of the inferior colliculus (Fig. 1 *A*) extending as far as the rostral part of the pons. In the transverse plane its borders are well distinct thanks to the presence of passing fibres. In the dorsoventral direction it extends for 1370 μm in the cat and 1720 μm in man.



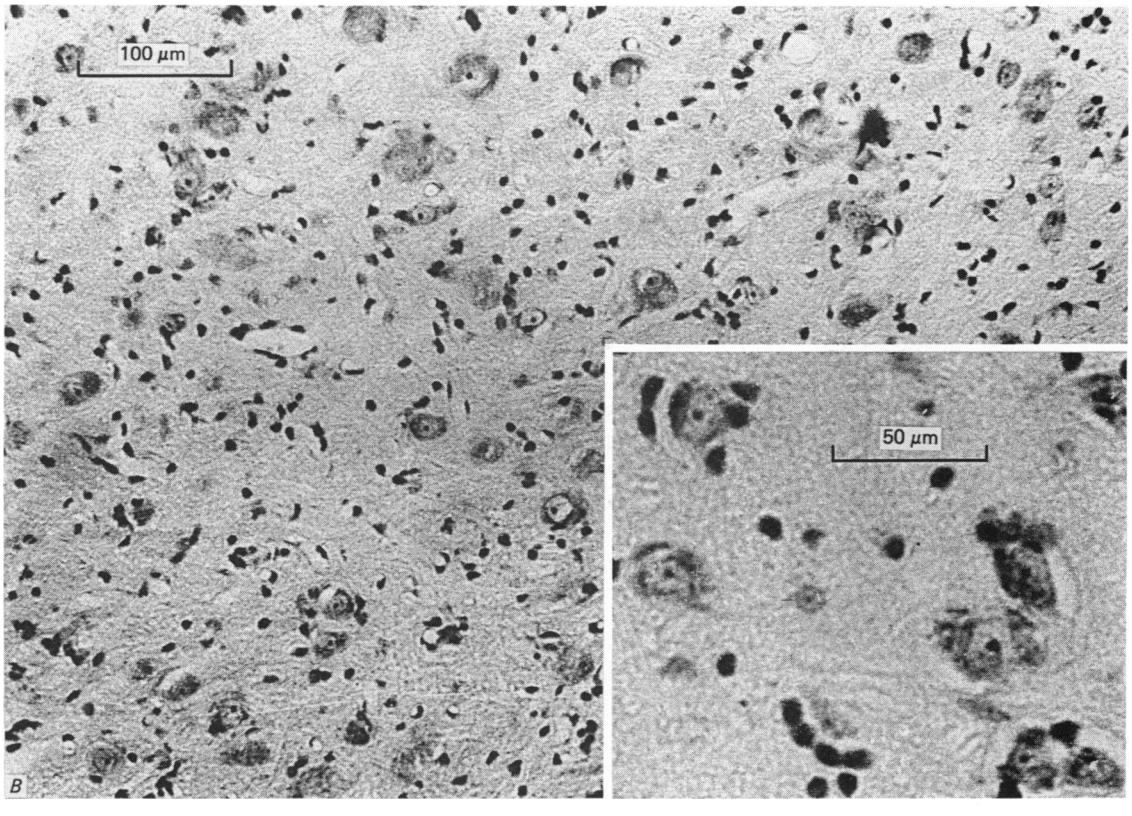


Fig. 1. (*A–B*). (*A*) Cresyl violet-stained transverse section through the caudal midbrain showing the location of the nucleus cuneiformis. *Cu*, nucleus cuneiformis; *Aq*, mesencephalic aqueduct; *Ic*, inferior colliculus. (*B*) Photomicrograph of the cat nucleus cuneiformis. In the inset a higher magnification. Cresyl violet.

Nissl material

The neurons (Fig. 1 *B*) which appear dispersed in a wide neuropil show round, oval or polygonal perikarya. Their cytoplasm contains moderately stained Nissl substance. The lightly stained nucleus, in which there is generally only one nucleolus, is large and occupies the central part of the neuronal cell body. The nuclear/cytoplasmatic ratio is always high. The perikarya are heterogeneous in size. There are cells with a section area of less than 100 μm^2 and others with an area of more than 450 μm^2. The average area is 238 μm^2 (S.D. 123·69; $n = 207$). The smallest cells are interspersed with the larger ones.

Golgi material (see Tables 1, 2)

In this material the neurons of the nucleus are fusiform or multipolar in shape. The multipolar cells (Figs. 2, 3) have polygonal soma from which 3–6 dendrites emerge. In man some neurons have up to 7 dendrites. The dendrites of the multipolar cells are on average 2·5 μm thick in the cat, while in man they are 3·5 μm thick. They run radially for 9–140 μm in the cat and for 110–230 μm in man.

In the cat the primary dendrites give off 0·5–1·5 bifurcations; in man the secondary ramification is wider, the primary dendrites giving off up to 2·5 bifurcations. In both species the dendrites are spiny. The spines have varicose features and occur with interspinous distances that are not constant, but always less than 20 μm. The axon, 2–4·5 μm thick in both species, generally emerges from the soma and can be traced in the section for 90–140 μm in the cat and for 45–170 μm in man. Axonal collaterals were not observed. Axons projecting to the periaqueductal grey matter and to the inferior colliculus were seen. The multipolar cells constitute 68% of the impregnated cells in cat and 78% in man.

The fusiform cells (Figs. 4, 5) have one or two dendrites which emerge from the poles of the elongated soma and may dichotomise into two secondary branches that generally end after 130–250 μm in the cat. In man the secondary dendrites are usually more numerous than in the cat. The dendrites of the fusiform cells are spiny, but less so than those of the multipolar cells. The interspinous distance is constant, being 20 μm in the cat and 22 μm in man. The axon, about the same thickness in the cat and in man, could generally be seen emerging from the soma. Collaterals were not observed and only a few axons projecting outside the nucleus were seen. In the cat the fusiform cells constitute 32% of the impregnated cells and in man 22%. In both species the multipolar neurons appear to have a soma larger than that of the fusiform cells. A preferential location for the two neuronal types could not be identified.

DISCUSSION

Our observations, which show that the neurons of the nucleus cuneiformis have a small or medium sized nerve cell body endowed with a large nucleus and with a light basophilic cytoplasm, support and correspond with the ultrastructural findings described by Gioia & Bianchi (1987). In particular the light cytoplasmic basophilia corresponds to a dispersed arrangement of the cisternae of the granular endoplasmic reticulum. According to the characteristics of the neuronal arborisation, two different neuron types can be identified – fusiform cells with only one or two dendrites and multipolar cells with a wide primary and secondary arborisation. These two different cell types appear to be randomly intermingled in the nucleus without a preferential localisation. As the multipolar cells have dendrites and axons which often spread

Table 1. *Characteristics and frequency of the nucleus cuneiformis neurons in the cat*

	Dendrites					Axon		
	Number	Bifurcations (N°/dendrite)	Length (μm)	Diameter (μm)	Interspinous distance (μm)	Length (μm)	Diameter (μm)	Frequency (%)
Fusiform neurons	1–2	1–2	90–150	2·5–5	20	100–120	2–3	32
Multipolar neurons	3–5	0·5–1·5	90–140	1·8–4·6	10–18	90–140	2–4·5	68

Table 2. *Characteristics and frequency of the nucleus cuneiformis neurons in man*

	Dendrites					Axon		
	Number	Bifurcations (N°/dendrite)	Length (μm)	Diameter (μm)	Interspinous distance (μm)	Length (μm)	Diameter (μm)	Frequency (%)
Fusiform neurons	1–2	1–3	130–250	2·7–4·6	22	90–150	2·7–4·6	22
Multipolar neurons	3–7	0·5–2·5	110–230	2·6–4·6	12–19	45–170	2–4·5	78

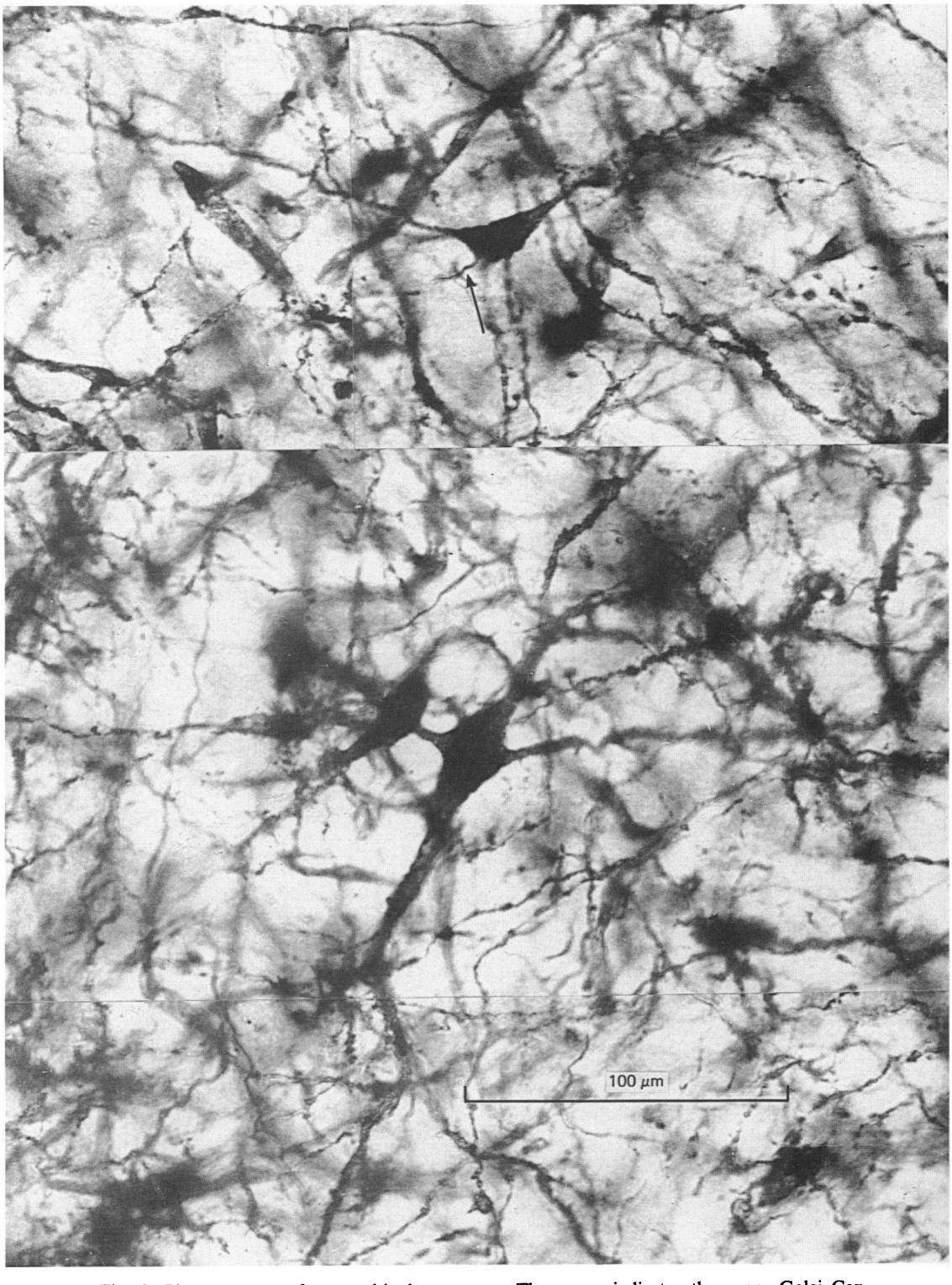


Fig. 2. Photomontage of cat multipolar neurons. The arrow indicates the axon. Golgi–Cox preparation.

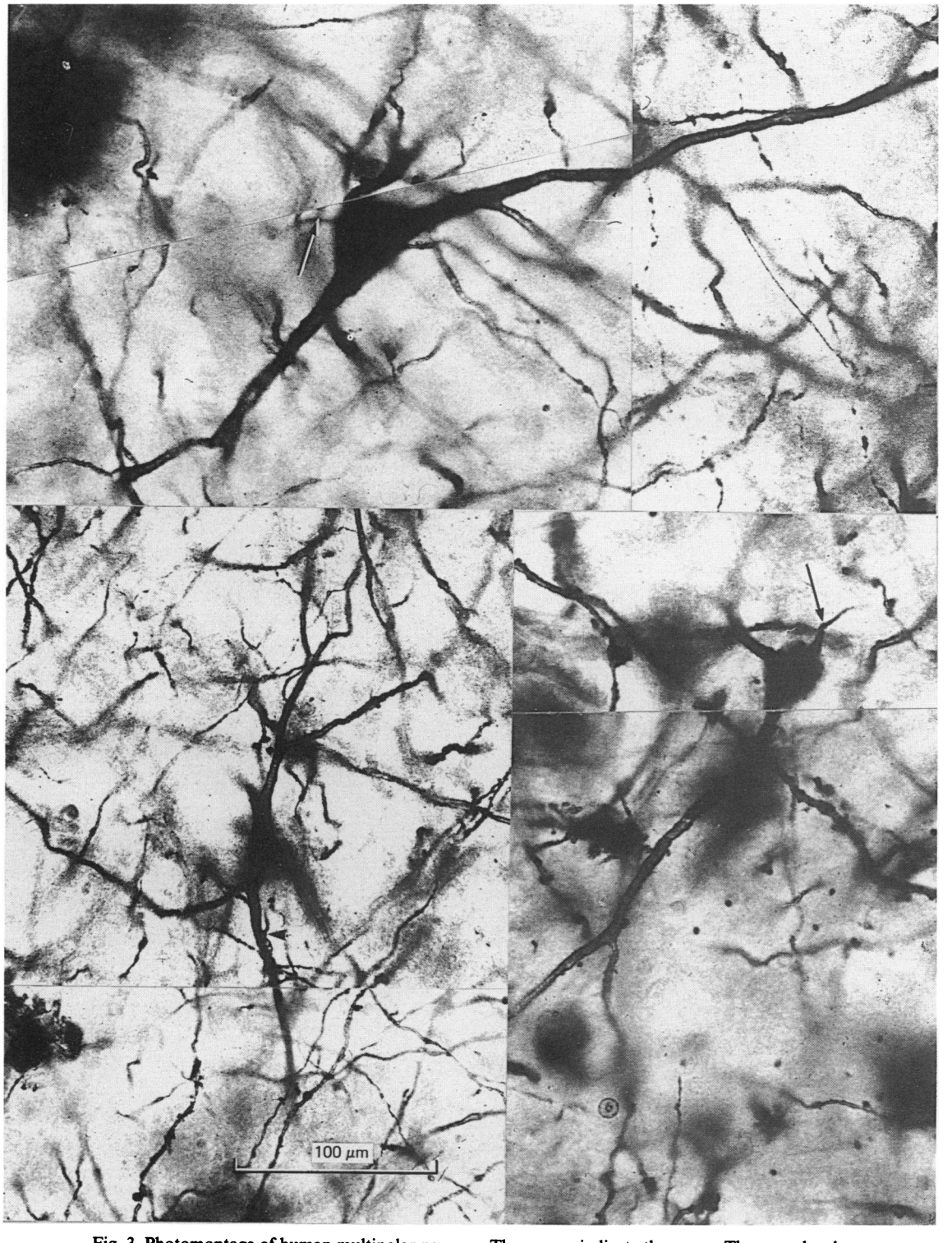


Fig. 3. Photomontage of human multipolar neurons. The arrows indicate the axons. The arrowhead indicates a dendrite on which an axon ends with a terminal button. Golgi–Cox preparation.

Fig. 4. Photomontage of cat fusiform neurons. Golgi–Cox preparation.

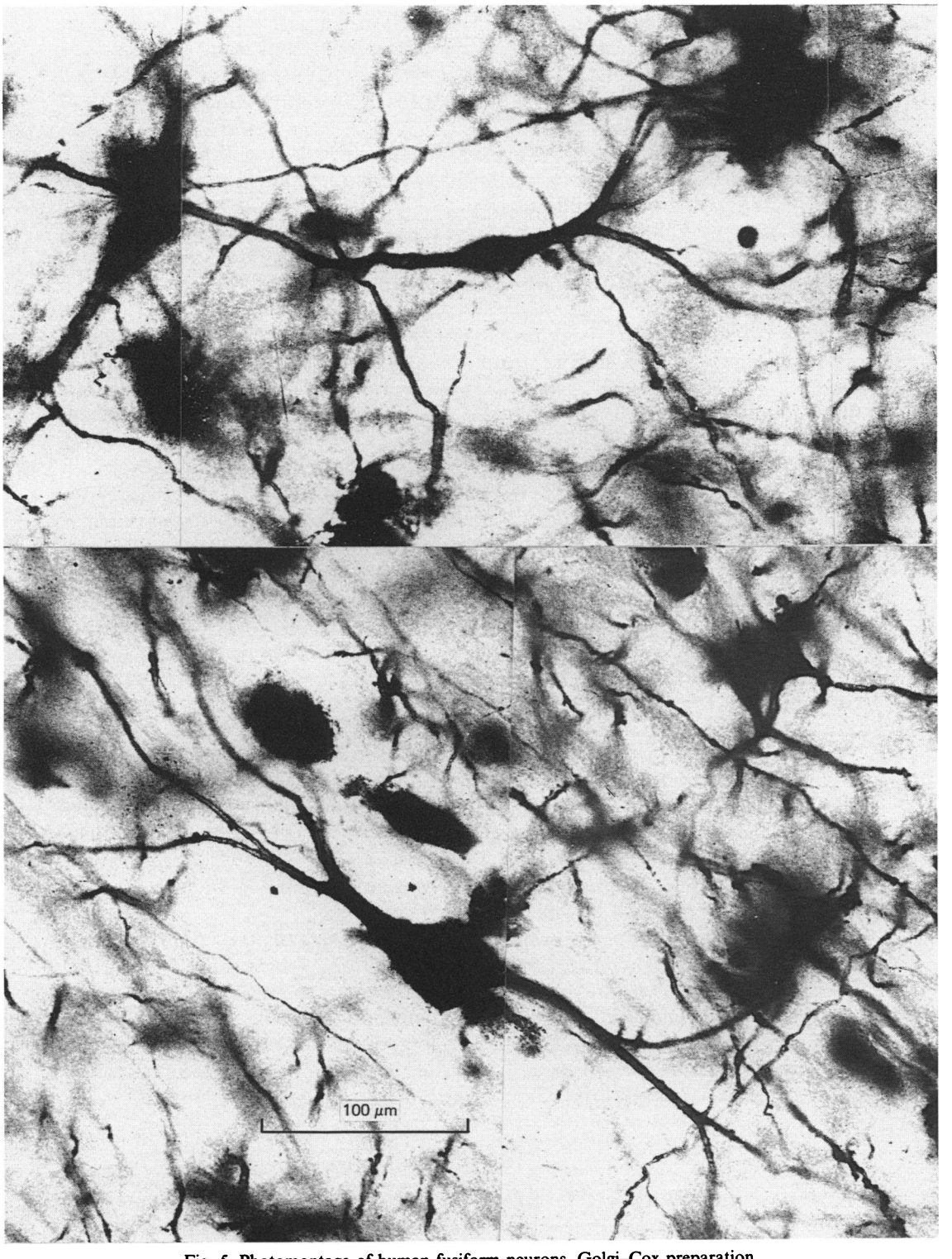


Fig. 5. Photomontage of human fusiform neurons. Golgi–Cox preparation.

outside the nucleus cuneiformis, it is possible that they may have a projective role. The fusiform cells, however, which have an arborisation that almost always ends inside the nucleus, can be considered as elements having a mainly interneuronal function. As multipolar cells constitute the majority of the neuronal population, it is possible that this cytoarchitectural finding may be the basis for the manifold functional activities attributed to the nucleus (see below).

It is interesting to note how the cytoarchitecture (i.e. the two types of neurons, having about the same incidence and size characteristics) is similar in the two species studied. This is particularly significant given that in man and the cat, which have a very different somatic size, the neurons have nerve cell bodies of similar sizes and processes of similar thickness. In the two species the main difference seems to be the length of the processes and the width of the arborisation, both of which are considerably greater in man.

In both cat and man the dendrites have spines, which at the ultrastructural level have sometimes been seen making synaptic contacts with axonal terminals (Gioia & Bianchi, 1987). This data may give support to the hypothesis that dendritic spines are a means of increasing the dendritic surface area for the reception of axonal terminals and as the dendrites of multipolar cells are richer in spines than those of the fusiform cells, it is possible to argue that the former cells, besides having a probable projective role, are the cells which are most involved in the afferent inputs to the nucleus.

The light microscopical observations confirm the similarity already found (Gioia & Bianchi, 1987) at the ultrastructural level between the nucleus cuneiformis and adjoining periaqueductal grey matter, both of these regions being ascribed to the reticular formation of the midbrain. In both the nucleus itself and the periaqueductal grey matter (Hamilton, 1973; Laemle, 1979; Liu & Hamilton, 1980; Mantyh, 1982; Gioia, Tredici & Bianchi, 1983, 1985; Gioia, Bianchi & Tredici, 1984; Beitz, 1985; Beitz & Shephard, 1985) the neurons are located in a wide neuropil, are small or medium sized, have a high nuclear–cytoplasmic ratio, have fusiform or multipolar arborisation characteristics and have spiny dendrites.

On the other hand the data confirm the lesser degree of complexity of the organisation of the neuropil and the synaptic apparatus of the nucleus (Gioia & Bianchi, 1987) compared to that of the periaqueductal grey matter (Gioia *et al.* 1983; Bianchi & Gioia, 1984). Moreover, in the latter, various authors (Liu & Hamilton, 1980; Laemle, 1979; Mantyh, 1982; Gioia *et al.* 1985; Beitz & Shephard, 1985) have identified within the two groups of fusiform and multipolar neurons, neuronal types with different characteristics and possible functional activities.

This investigation, together with the previous ultrastructural study (Gioia & Bianchi, 1987) adds to the knowledge of the cytoarchitecture of the nucleus cuneiformis, thus providing an anatomical substratum for the numerous physiological investigations which have pointed out the functional importance of the nucleus as an element of the mesencephalic locomotor area and also as a region which is involved in cortical activation (Steriade, Ropert, Kitsikis & Oakson, 1980) and in responding to sensory stimuli, viz. visual, somatic and auditory (Bell, Sierra, Buendia & Segundo, 1964).

SUMMARY

This investigation attempts to clarify the cytoarchitectural organisation of the neurons of the nucleus cuneiformis, a reticular nucleus of the midbrain particularly involved in locomotor activities. The study was carried out on the cat and man, in Nissl and Golgi material.

The nerve cell bodies, which are small or medium sized, have a light basophilic cytoplasm and a large light nucleus usually containing one nucleolus. In Golgi material multipolar and fusiform cells can be identified. Multipolar cells, which form the majority of the neural population, have 3–7 primary spiny dendrites and an axon which often projects outside the nucleus. Fusiform cells have one or two primary dendrites endowed with spines, which are, however, less numerous than those of multipolar neurons. The axons generally end inside the nucleus. The main difference between man and the cat seems to be in the length and width of the neuronal arborisation, which are considerably greater in the former species.

The characteristics of the two neuronal types suggest a projective function of the multipolar elements, but an interneuronal activity of the fusiform ones. The data support the similarity already found at the ultrastructural level between the nucleus cuneiformis and the periaqueductal grey matter, but on the other hand confirm the lesser degree of cytoarchitectural complexity of the nucleus.

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