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Morphometric analysis of the neuronal numbers and densities of the inferior olivary complex in the donkey (*Equus asinus*)

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ABSTRACT

The morphometric interrelations between the compartments of the inferior olivary complex (IOC) in the donkey (*Equus asinus*) were ascertained by examining serial sections throughout the entire length of the IOC for both sides. Nissl-stained celloidin sections of four brainstems of donkeys were used. The IOC consisted of three major nuclei and four small cell groups. The total neuronal count in both sides of the IOC was 202,040 ± 8480 cells. The medial accessory olivary nucleus (MAO) had the largest relative area (46%) and the highest number of neurons (90,800 ± 7600). The dorsal accessory olivary nucleus (DAO) had the second largest relative area (33%), while the principal olivary nucleus (PO) had the lowest relative area (21%). However, the total neuron count in the PO was larger (60,840 ± 1840) than DAO (50,360 ± 4040). The average neuronal density was 2700 ± 400 cells/mm^3. The numerical values of the current study of the IOC in the donkey were similar to those of other mammals.

Introduction

The morphology of the inferior olivary complex (IOC) has been demonstrated in many mammals (Kooy, 1916; Kappers et al., 1960; Taber, 1961; Moatamed, 1966; Breazile, 1967; Schild, 1970; Bowman and King, 1973; Bowman and Sladek, 1973; Martin et al., 1975; Watson and Herron, 1977; Rutherford and Gwyn, 1980; Saigal et al., 1983; Azizi and Woodward, 1987; Tan et al., 1995; Bozhilova-Pasirova and Ovtscharoff, 2000; Bukowska et al., 2002; Rashed et al., 2007), including donkey (Rashed et al., 2006). However the neuronal number of the IOC in the donkey still unknown.

In the donkey the IOC is divided into three main nuclei: medial and dorsal accessory olivary nuclei (MAO and DAO, respectively) and a principal olivary nucleus (PO) and four small cell groups: the dorsal cap (DC), the ventro-lateral outgrowth (VLO), the nucleus β and the dorsal medial cell column (DMCC) (Rashed et al., 2006).

Generally, it is recognized that the IOC is the sole source of the climbing fibers (Szentagothai and Rajkovits, 1959; Desclin, 1974; Brodal et al., 1975; Freedman et al., 1977), and nearly all of the neurons in the IOC are projection neurons to the cerebellum (De Zeeuw et al., 1998). A single olivocerebellar fiber projects with multiple climbing fibers to a single narrow longitudinal band-shaped area in the cerebellar cortex and, with its collateral axons to a small area in the cerebellar nuclei (Sugihara et al., 1999). Each climbing fiber innervates a single Purkinje cell (Eccles et al., 1966). Each olivocerebellar fiber branches into about 4–7 (rat), to 14–17 (human) and to 16 (chicken) (Rashed et al., 2005). Thus we can estimate the number of Purkinje cells by direct counting or by the neuronal numbers of the IOC.

In a continuing study of the IOC in the donkey, we have tried to determine more detailed information about this nuclear complex by morphometrical observation of the neuronal number of each main nucleus.

Material and methods

Four donkeys (*Equus asinus*), 2–3 years old, were used in this study. The animals were anesthetized with an overdose of pentobarbital sodium, and then perfused with physiological saline followed by 10% formalin via the carotid artery. The brain stems were removed, post-fixed in formalin for 3 days or more, dehydrated using an ascending series of ethanol, and embedded in celloidin. The brain stem was serially cut in the transverse plane in 50 μm thick sections. Serial sections were stained with toluidine blue or cresyl violet using staining methods described in Bancroft and Stevens (1990).

Histology and procedure for neuron count

The sections were observed under a light microscope at a final magnification of 100× for cell counting. The IOC neurons were counted in every tenth section on both the right and left sides of

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Table 1
Numbers and densities of the IOC neurons in the donkey.

Major nuclei	MAO			DAO		PO		Total
Sub-nuclei	a, b and c	Nucleus b	DMCC		PO	DC	VLO	
Neuron number	83900	4970	1970	50360	53710	1970	5160	
Total ± SD	90,840 ± 7620			50,360 ± 4040		60,840 ± 1840		202,040 ± 8480
Percentage (%)	42	2	1	25	26.5	1	2.5	100
CV (%)		8.4		8		3		4
Relative area (%)		46		33		21		100
Neuronal density cells/mm^3 ± SD		2600 ± 600		1900 ± 140		3600 ± 500		2700 ± 400

SD, Standard deviation; Percentage (%), proportion of the neuronal number in the subnuclei to the total neuronal number of the IOC; CV, coefficient of variation; Relative area (%), the proportion of the area of the major nuclei to the total area of the IOC in a given number of sections.

each animal. All neurons in a given section were counted, whether or not a nucleolus could be identified. The total neuronal number of the IOC of the donkey was then calculated by the method of Escobar et al. (1968) as follows: the number of neurons (A) in each section counted was multiplied by half the number of sections not counted (B) between the section counted and the next counted section. By adding the products of AB/2 for all sections counted, the total number of neurons in the IOC of each specimen was obtained. The reliability of this method was checked previously by Rashed et al. (2005).

Procedure for neuronal density

The neuronal density was obtained by projecting the microscopic sections onto a video micrometer (VM-29; Olympus, Tokyo, Japan) at a final magnification 90×. The relative areas of each nucleus and small cell group of the IOC were measured in every tenth section on the left side (two cases) and on the right side (one case). The number of neurons per square millimeter in each counted section was obtained, and then the number of neurons per cubic millimeter was obtained by multiplying the mean number of neurons/mm^2 by the Escobar coefficient derived from the following formula:

$$\text{Escobar coefficient} = \frac{1000}{\text{section thickness} \times 2^*}$$

2^* is the neuronal count for one section is equal to the neuronal count for two successive sections (Escobar et al., 1968). In this study the Escobar coefficient = 1000/(50 × 2) = 10.

Micrographs of the nuclei of the IOC were taken at different levels and cropped to an image processing application; only the brightness and contrast were corrected.

Results

Histological findings

The IOC in donkey was divided into three main nuclei: medial and dorsal accessory olivary nuclei (MAO and DAO, respectively) and a principal olivary nucleus (PO) and four small cell groups: the dorsal cap (DC), the ventro-lateral outgrowth (VLO), the nucleus β and the dorsal medial cell column (DMCC).

Neuron number, size and density in the IOC

The neuron numbers in the IOC of the donkey were estimated (Table 1). The differences between the neuron numbers in the right and left side of the IOC were non-significant. Among the three major nuclei, the MAO had the largest cell number (90,800 ± 7600 cells). Although the relative area of the PO was smaller than that of the DAO the former had the second largest neuron number (Table 1). Over 500 cells at different levels of each nucleus in the IOC were measured. The average neuron size (represented by diameter) was 25 μm (Fig. 1). The average neuron density of the IOC of the donkey was calculated to be 2700 ± 400 with its highest value in the PO and its lowest value in the DAO (Table 1).

Discussion

The total numbers of the IOC neurons have been estimated at about 909,000, 1,025,000 or 1,060,000 in humans (Moatamed, 1966; Escobar et al., 1968; Futami and Okamoto, 1968), 27,000 in the vampire bat (Escobar et al., 1968), 140,000 or 150,000 in the cat (Escobar et al., 1968; Mlonyeni, 1973), 49,000 or 57,000 in the rat (Delhaye-Bouchaud et al., 1985; Schild, 1970) and 211,000 in the water buffalo (Rashed et al., 2007), respectively. In the present study, the IOC in the donkey contained 202,000 cells. Thus the IOC neurons in the donkey showed morphometrical similarities to that of the water buffalo.

The MAO is the largest nucleus of the IOC in most mammals studied except for humans in which the PO is the largest nucleus (Moatamed, 1966; Armstrong, 1974; Azizi and Woodward, 1987). Previous studies showed that the MAO, DAO and PO contain neurons at proportions of 10%, 4% and 86%, respectively in humans (Moatamed, 1966), 49%, 24% and 27% or 46%, 25% and 29% in the rat (Delhaye-Bouchaud et al., 1985; Schild, 1970), 47%, 26% and 27% in the water buffalo (Rashed et al., 2007), and 45%, 25% and 30% in this study. Therefore, the IOC in the donkey was similar to that of rat and water buffalo in the proportions of its three major nuclei.

Estimates have been made for the packing density of cell within the IOC. It is estimated as 65,000 cells/mm^3 in the vampire bat and in carp (Escobar et al., 1968; Bozhilova-Pasirova and Ovtscharoff, 2000), 44,000 cells/mm^3 in the rat (Escobar et al., 1968; Schild, 1970), 28,000 cells/mm^3 in the pigeon (Bozhilova-Pasirova and Ovtscharoff, 2000), 23,000 cells/mm^3 in the ground squirrel (Bozhilova-Pasirova and Ovtscharoff, 2000), 8000–15,000 cells/mm^3 in the cat (Escobar et al., 1968; Bozhilova-Pasirova and Ovtscharoff, 2000), 5000–15,000 cells/mm^3 in humans (Escobar et al., 1968; Bozhilova-Pasirova and Ovtscharoff, 2000) and 3000 cells/mm^3 in the water buffalo (Rashed et al., 2007). The neuron density in the IOC of the donkey was 2700 ± 400 cells/mm^3. The previous and current studies showed that the neuron densities correlate inversely with the body weight. Since the donkey is heavier than human, but lighter than water buffalo, the neuron density of the donkey IOC may be lower than that of human and higher than that of water buffalo. Actually the neuron densities in the IOC of the donkey and water buffalo were nearly the same. This may be attributed to the decreased neuron density in the DAO of the donkey, which affected the average neuron density for the three major nuclei.

In the present study the average neuron size (represented by diameter) was 25 μm. This reflects the fact that the olivary neurons in the donkey are within the animal range (Armstrong, 1974). The

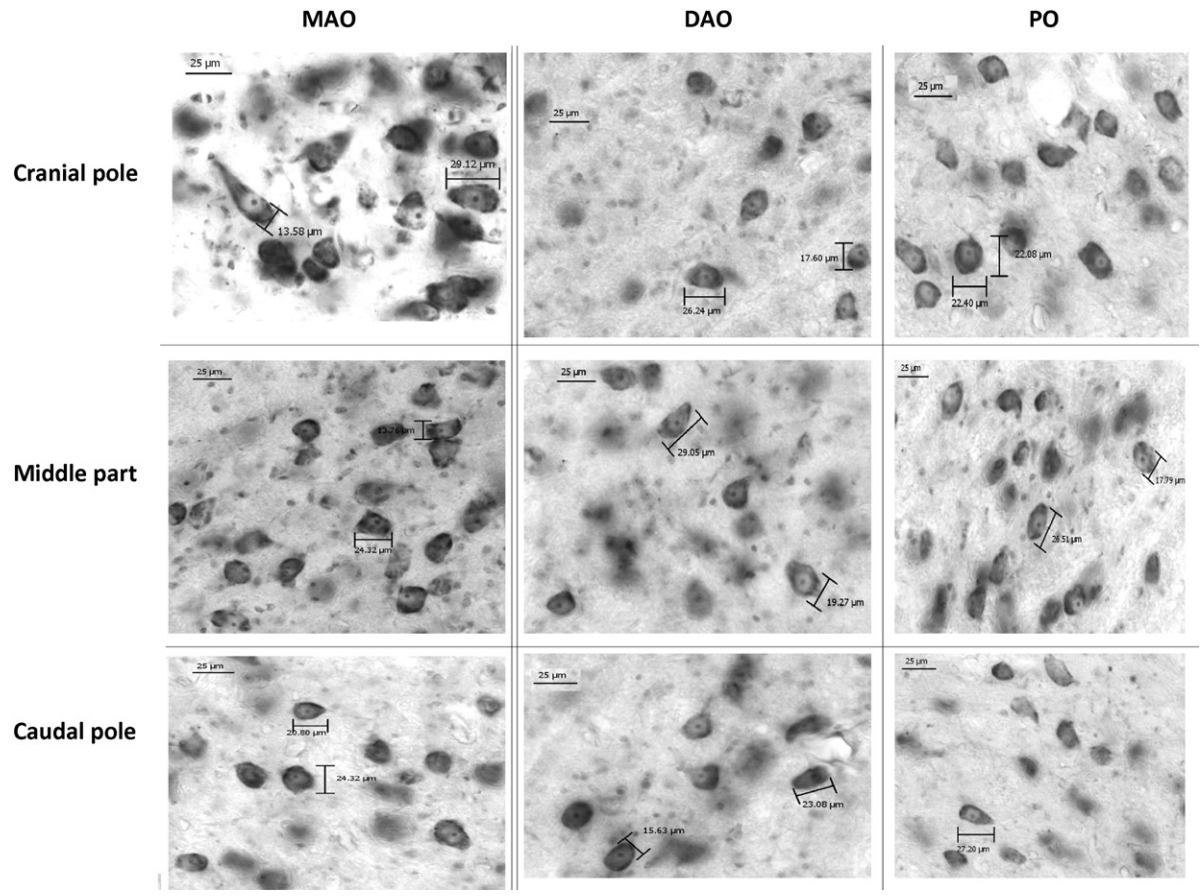

Fig. 1. Photomicrographs of the inferior olivary complex at different levels. Abbreviations: MAO, Medial accessory olive, DAO, dorsal accessory olive, PO, principal olive.

study of the neuron size in the donkey did not reveal any significant regional differences. This contrasts with the finding that human PO cells were about twice as large as DAO or MAO cells (Moatamed, 1966).

It is worth noting that the cerebellum can be divided functionally into a medial sector called the paleocerebellum and a larger lateral sector called the neocerebellum and a narrow strip of protruding tissue along the midline called the vermis (Ghez and Fahn, 1985). The paleocerebellum functions mainly to fine-tune body and limb movements, while the neocerebellum is thought to be involved in planning movement that is about to occur and evaluating sensory information for action (Ghez and Fahn, 1985) and probably in a number of purely cognitive functions as well (Timmann and Daum, 2007). The MAO and DAO projects to the paleocerebellum while the PO projects to the neocerebellum (Groenewegen et al., 1979).

From previous reports and the present study we conclude that the neocerebellum of higher primates functions better than other mammals as a cognitive and learning machine. This conclusion may be reflected in the high neuronal number in the PO in humans when compared with other mammals (Moatamed, 1966).

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