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MORPHOLOGIC CHARACTERISATION OF THE INFERIOR OLIVARY COMPLEX IN THE CAMEL *(Camelus dromedarius)*

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ABSTRACT

The morphological structure of the inferior olivary complex in the single humped camel *(Camelus dromedarius)* was investigated in this paper. Serial sections through the whole rostro-caudal extent of the inferior olivary complex confirmed the configuration and interrelations of each compartment. The brain stems from 5 foetuses of 600-800 mm crown-to-rump length (CRL) and a newborn camel were removed, fixed in 10% formalin for 3 weeks, dehydrated and embedded in paraffin (2 cases) or/and celloidin (4 cases). The cytoarchitecture of inferior olivary complex was mapped in transverse serial sections, stained with toluidine blue or/and crystal violet. A descriptive nomenclature was adapted to a terminology that would imply analogy with other species. The inferior olivary complex in the camel consisted of 3 major nuclei and 4 small cell groups. The medial accessory olivary nucleus was the largest among the major nuclei; with its caudal half had a unique sickle-shaped configuration. In general the inferior olivary complex in the camel showed a phyletic homology with other mammals, i.e. the inferior olivary complex in the camel resembles that of other mammals very much, as well in its principal lines, as in many details.

Key words: *Camelus dromedarius*, CRL cytoarchitecture, inferior olivary complex, phyletic homology

The morphological characterisation of the inferior olivary complex (IOC) has been determined 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; Bukowska *et al*, 2002).

The mammalian IOC, is divided generally into medial and dorsal accessory olivary nuclei (MAO and DAO, respectively), and a principal olivary nucleus (PO), as well as 4 small cell groups representing, the dorsal cap of Kooy (DC), b nucleus, ventro-lateral outgrowth (VLO), and dorso-medial cell column (DMCC) (Kooy, 1916; Azizi and Woodward, 1987). In marsupials, the 3 major olivary nuclei can be detected but their relative positions are different (Martin *et al*, 1975; Watson and Herron, 1977). With ascent of the evolutionary scale, PO undergoes a progressive increase in relative size reaching its greatest development in man (Armstrong, 1974).

It is generally known that the IOC is the sole source of the climbing fibres that innervate Purkinje cells of the cerebellar cortex (Ramon, 1911; Szentagothai and Rajkovits, 1959; Desclin, 1974). A single neuron in the IOC projects with multiple climbing fibres to a single narrow longitudinal band-shaped area in the cerebellar cortex and, with its axon collaterals, to a small area in the cerebellar nuclei (Sugihara *et al*, 1999), thus defining functional compartmentalisation of the cerebellar system in the vermis and hemispheres (Sugihara *et al*, 2001) that is much finer than the A-D zonation of the olivocerebellar projection revealed by mass labeling (Groenewegen and Voogd, 1977). The compartmentalisation in the olivocerebellar and olivonuclear projections seems to reflect a fundamental principle of input-output circuitry of the cerebellar system.

Recently it is essential to elucidate the olivocerebellar and olivonuclear projections originating from each IOC region. However, it is very difficult to apply a tract tracing methods to large mammals such as the camel. The aim of this study is to describe the cytoarchitecture of the camel's IOC and to discuss its features in comparison with those in the IOC of the other mammals.

Materials and Methods

The brainstems from 5 foetuses of 600-800 mm crown-to-rump length (CRL) and a newborn camel

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were removed and fixed in 10% formalin for at least 3 weeks. The brainstems were dehydrated and embedded in paraffin (2 cases) or/and in 10 celloidin (4 cases). Serial sections were obtained at 50 mm thick from celloidin blocks and 10 mm thick from paraffin blocks, and were stained with toluidine blue or/and crystal violet. Series of line drawings of the IOC in the transverse plane were done. Each drawing represents the approximate shape of the nucleus at the every respective point (Fig 1).

The nuclei is described in topographical sequence that proceeds from the caudal pole toward the rostral pole of the IOC in the brain stem. Photos for the IOC were captured and cropped to an image processing application, to correct the brightness and contrast, and nothing else (Fig 2).

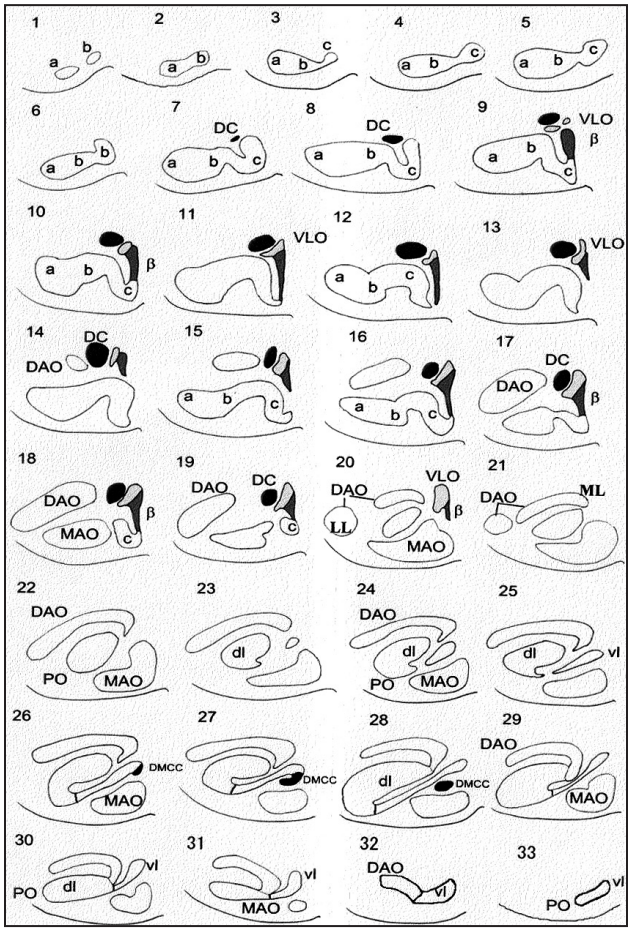

Fig 1. Line drawings of cross-section of the brainstem illustrating positions and relations among the nuclei of IOC in the camel. DAO; dorsal accessory olive, LL: lateral lamella of DAO, ML: medial lamella of DAO, MAO; medial accessory olive, PO; principal olive, a-c; groups a-c of the MAO, b; nucleus b, DC; dorsal cap, VLO; ventrolateral outgrowth, DMCC; dorsomedial cell column, dl; dorsal lamella of PO, vl; ventral lamella of PO.

Results

The IOC length was about 8 - 9 mm rostro-caudally and 3 major nuclei, MAO, DAO, and PO, as well as 4 small cell groups, were clearly detected in the camel IOC (Fig 1, 2).

MAO extended all over the total length of the IOC and disappeared a few sections before its rostral pole (Fig 1-31). At the caudal aspect of the MAO, three separate groups may be distinguished, labeled a, b, and c from laterally to medially (Fig 2-A and B). Groups a and b appeared at the most-caudal levels, forming the caudal pole of the IOC. More rostrally, group c developed from group b medially. These 3 groups increased gradually in size and group c bent ventro-medially, thus the MAO assumed the shape of a sickle (Fig 1-8). Nearly at the middle of IOC, a constriction separated group c from group b (Fig 1-18). Group c rapidly disappeared (Fig 1-20), while groups a and b continued more rostrally until they vanished (Fig 1-31). The cytoarchitectural boundaries of groups a, b and c were rather clear in the caudal half of the MAO but obscure in the rostral half.

The DAO in the camel consisted of two lamellae; lateral and medial (LL and ML, respectively). The LL appeared medially as a round cell mass before the middle of the IOC, lateral to the DC and dorsal to the MAO (Fig 1-14). LL of DAO increased in size and shifted laterally to take its permanent position (Fig 1-18). ML of DAO appeared

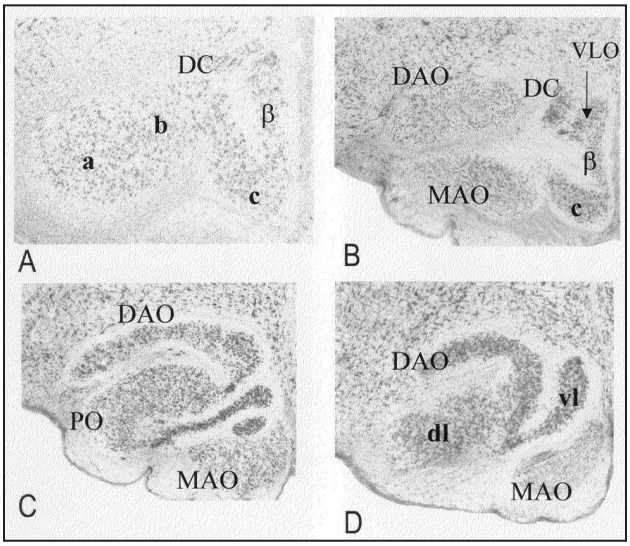

Fig 2. Light micrographs of transverse section of the IOC. (A) Light micrograph illustrating the sickle-shaped MAO. (B) Light micrograph illustrating the division of the MAO. (C) Light micrograph illustrating the three major nuclei of the IOC. (D) Light micrograph IOC in the camel before its rostral pole. **A)** corresponds to Fig 1-8. **B)** corresponds to Fig 1- 18. **C)** corresponds to Fig 1- 28. **D)** corresponds to Fig 1-30

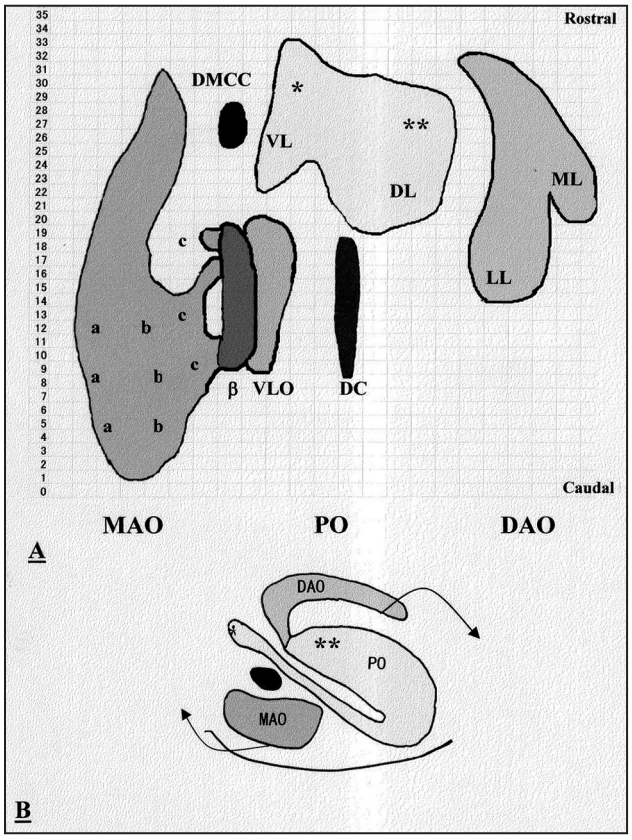

Fig 3. A) and **B)** Summarising diagram of the imagined unfolded IOC. See fig 1 for abbreviations.

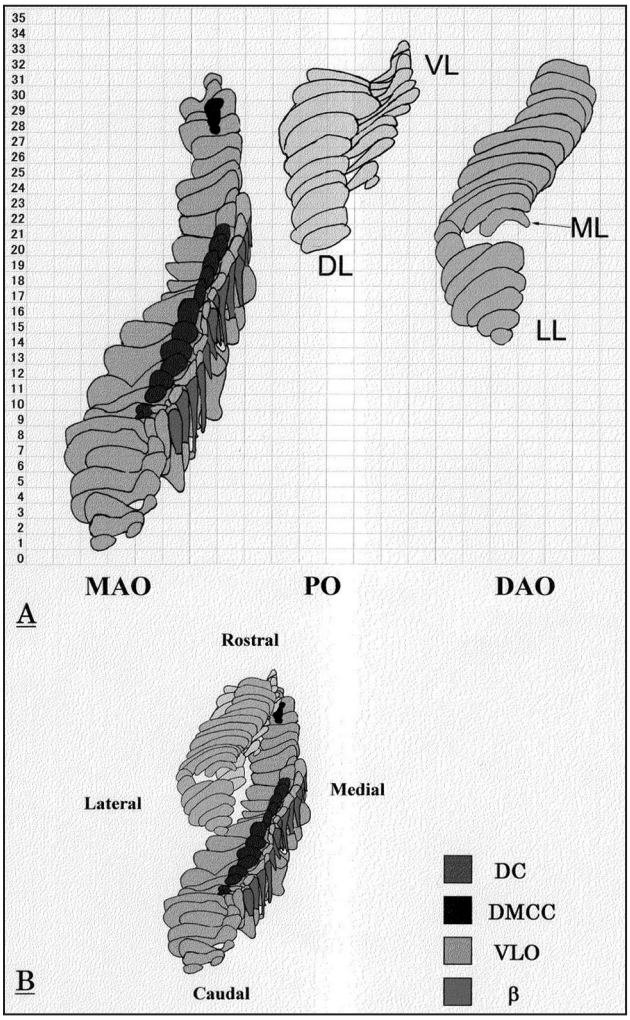

Fig 4. A) and **B)** Three-dimensional Model of MAO, PO, and DAO in the camel IOC.

in the rostral half of IOC (Fig 1-20) and rapidly had a connection with the lateral lamella of DAO, further rostrally the DAO extends as an horizontal sheet of cells and then it curved ventro-laterally to connect with the dorsal lamella of PO (Fig 1-28) followed by a connection with the ventral lamella of PO and then it disappeared just before the rostral pole of the IOC.

The PO in the camel consisted of two lamellae; dorsal lamella (DL) and ventral lamella (VL). The DL was the first lamella appeared as a group of cells between the ML of DAO and MAO (Fig 1-20). The DL of PO was larger than the VL. The VL of PO appeared more rostrally and its medial end continued with DMCC. The two lamellae form a U-shape with its hilus opened dorso-medially. More rostrally, the DL was the first to become shorter, while the VL appeared to be present at the same time with the DAO until the rostral pole.

Four small cell groups represented the dorsal cap of Kooy (DC), b nucleus, ventrolateral outgrowth (VLO) and the dorsomedial cell column (DMCC). The DC appeared as a small mass of cells dorsal to the group C (Fig 1-7), and then increased in size and extended rostrally as a separate entity to a level after the middle of the IOC (Fig 1-19). The b nucleus appeared as a dorsal extension form the group c (Fig 1-9), which rapidly continued dorsally with the VLO (Fig 1-10). The b nucleus separated from group c and shifted dorsally (Fig 1-12). More rostrally, the nucleus b shifted ventrally, rejoined the subgroup c once again (Fig 1-16) and continued rostrally with group c until the group c disappeared. The b nucleus was never separated from the VLO until it disappeared around the middle of the IOC (Fig 1-21). The VLO, appeared as an isolated two cell masses, then coalesced and continued ventrally with the nucleus b (Fig 1-10). More rostrally, the VLO increased in size and pushed the DC laterally. The VLO disappeared at the same level with the b nucleus (Fig 1-21). The DMCC appeared nearly at the beginning of the rostral one-fourth of the IOC as a part of the medial side of the ventral lamella of PO (Fig 1-26). Slightly rostrally, DMCC appeared as a separate entity of cell cluster between the ventral lamella of the PO and the MAO and then disappeared (Fig 1-28).

The IOC was imagined unfolded in one plane (Brodal *et al,* 1975) by pulling its compartments apart in the latero-medial direction (Fig 3- A,B). The 3 dimensional construction of each nucleus was made using the transverse serial sections (Fig 4- A,B).

Discussion

The mammalian IOC is divided generally into 3 major nuclei and 4 small cell groups (Kooy, 1916; Azizi and Woodward, 1987). The general conformation of IOC of the camel as described in this study did as well.

In most mammals except for the primates, the MAO is the largest of the 3 main nuclei of the IOC (Moatamed, 1966; Armstrong, 1974; Azizi and Woodward, 1987). According to Ruigrok and Voogd (2000), the medial accessory olive (MAO) is usually divided into rostral and caudal halves in the rat. At the caudal aspect of the MAO 3 separate groups may be distinguished, labeled a, b, and c from laterally to medially (Whitworth and Haines, 1986). At the caudal most levels, b subnucleus is located medially but, upon advancing rostralward, it takes up a more dorsal position relative to group c (Azizi and Woodward, 1987). In the camel, MAO resembled that of other mammals but a, b and c groups took the shape of a sickle, which are different from other mammals, which take the form of an horizontal lamina. b nucleus in camel appeared as the dorsomedial angle of the sickle- shaped MAO. Slightly rostrally, nucleus b was continued dorsally with VLO and ventrally with group c.

According to Voogd and Ruigrok (2004), the DMCC with its bilateral and branching projections (Sugihara *et al*, 1999) to the lateral P3+ and the P4+ bands of the caudal vermis (Voogd *et al*, 1996) should be considered as a separate subnucleus of the IOC. In most mammals, the DMCC appeared as isolated cell cluster dorsal to MAO (Taber, 1961; Breazile, 1967; Bowman and Sladek, 1973; Tan *et al*, 1995; Bukowska *et al,* 2002). According to Azizi and Woodward (1987), the DMCC in the rat starts as isolated cell cluster and more rostrally, (DMCC) it fuses with the medial part of VL of PO (Gwyn *et al*, 1977; Azizi and Woodward, 1987). While In pig, the DMCC is much reduced and has no continuity with other cell groups (Breazile, 1967). The DMCC in the camel in this study appeared initially continuous with the VL of PO, further rostrally, detached from VL; this course of DMCC is much similar to (that) the DMCC of rhesus monkey (Bowman and Sladek, 1973).

The DAO is generally the smallest nucleus of the IOC and consists of one lamella, except for the rat, where it consists of two lamellae joined laterally (Schild, 1970; Delhaye-Bouchaud *et al*, 1985; Azizi and Woodward, 1987). The DAO in pig, goat, and horse start medially then moves laterally then medially (Kooy, 1916). The medial side of the DAO is continuous with the dorsal lamella of PO, and more rostrally becomes continuous with the ventral lamella of the PO (Kooy, 1916; Brodal *et al*, 1975). In the pig, this configuration is reversed (Breazile, 1967), and in sheep the DAO has no connection with the PO (Saigal *et al,* 1983). The DAO in the camel IOC consisted of 2 lamellae; lateral lamella (LL) which started more caudally than the medial lamella (ML) and then the two lamellae united together forming one lamella, which arched over the PO and MAO. After the level 22 (Fig 1) the DAO resembles that of other mammals.

The PO has the same shape in all mammals (Kooy, 1916). The PO consists of dorsal and ventral lamellae, which are joined at their lateral bend. Moreover, the ventrolateral outgrowth (VLO) and the dorsal cap of Kooy (DC) are usually considered as a caudal continuation of the PO (Brodal and Kawamura, 1980; Ruigrok and Cella, 1995; Ruigrok and Voogd, 2000) another related cell group related to the PO specific for rodents (Whitworth and Haines, 1986) is the dorsomedial cell group (DM) (Azizi and Woodward, 1987) this cell group appears as a dorsomedial continuation of the ventral lamella of the PO. It is intercalated between the DMCC and the medial-most part of the DAO at the caudal regions and remains directly medial to the DAO at more rostral levels. The PO is the smallest nucleus in the rodent IOC and the largest in the human IOC (Moatamed, 1968; Armstrong, 1974; Azizi and Woodward, 1987). In primates, the PO is well developed with many folds (Kappers *et al*, 1960; Bowman and Sladek, 1973). Numerical studies show that the MAO, DAO and PO contain neurons at proportions of 10, 3 and 86, respectively, in humans (Moatamed, 1966) and 49%, 24 and 27, in rat (Schild, 1970) or 46, 25, 33 (Delhaye-Bouchaud *et al*, 1985). The camel PO had no flexure and consisted of two lamellae; dorsal lamella, which was larger than the ventral lamella (VL) (Fig 2-C). Generally, the PO of the camel was similar to many mammals in its relative area and configuration (Kooy, 1916; Taber, 1961; Breazile, 1967; Schild, 1970; Gwyn *et al*, 1977; Rutherford and Gwyn, 1980; Azizi & Woodward, 1987; Bukowska *et al*, 2002). The dorsomedial cell group (DM) was not detected in the camel.

According to Azizi and Woodward (1987), in the rat a dorsal extension develops from group c and then separates to form the DC, while the

remaining dorsal extension of group c comprises the nucleus b. In the rat, cat, rhesus monkey and rabbit, the DC elongates ventrolaterally at rostral levels and becomes the VLO (Bowman and Sladek, 1973; Brodal *et al*, 1975; Flumerfelt and Hrycyshyn, 1986; Azizi and Woodward, 1987; Bukowska *et al*, 2002). Moreover, the DC in the pig forms a connection between the MAO and the ventral lamella of PO (Breazile, 1967). Together, with the rostral DC (rDC), the VLO is responsible for the generation of the vertical compensatory eye movements (Leonard *et al*, 1988; Graf *et al*, 1988), whereas the caudal DC (cDC) is responsible for the horizontal movements. In the camel, the DC appeared as an isolated cell cluster over the MAO and never had a connection with any other nuclei. The VLO in the camel IOC appeared caudally as an isolated small cell clusters which coalesce and joined the b nucleus.

The cerebellar cortex is classically and functionally divided into the vermis, paravermis (intermediate zone), and lateral hemispheres (Voogd and Glickstein, 1998; Voogd and Ruigrok, 2004). These 3 broad zones are subdivided into 7 longitudinal sub-zones, each of (its) which have its particular climbing fibres afferent from subnuclei of the IOC. Vermal zones A and X receives mainly projection from the caudal MAO (group a-c) and the vermal zone B from the caudal DAO. The paravermal zones C_1, C_2, and C_3 are innervated by the rostral main part of the MAO and the rostral DAO respectively, and the hemisphere from the PO. The D zones of the hemispheres receive climbing fibres from the PO. The DC and VLO innervate the nodulus and flocculus of the vestibulo-cerebellum; the group b and the DMCC project into the nodulus and the uvula (Flumerfelt and Hrycyshyn, 1986; Azizi and Woodward, 1987).

The basic topography of the olivary projection to the CN in the rat resembles the organisation observed in the cat based on retrograde (Dietrichs and Walberg, 1989) and anterograde tracer studies (Courville, 1975; Groenewegen and Voogd, 1977; Groenewegen *et al*, 1979; Ruigrok and Voogd, 2000). It was shown that the caudal half of the MAO is connected to the fastigial nucleus, which is the equivalent of the MCN in the rat. The PO was shown to project to the dentate nucleus (LCN) and the rostral halves of both accessory olives were connected to the interposed nuclei.

Conclusion

The IOC in the camel resembles very much that of other mammals. The IOC of the camel, horse, goat, cat, rodent and rabbit showed a phyletic continuity within these species (Butler and Hodos, 2005). The olivocerebellar and olivonuclear projections of camel IOC are similar to that of the aforementioned species.

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