MATERIAL AND METHODS

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1. DEVELOPMENT AND GROWTH

Human

a. Foetus : Ten formalin fixed foetuses, one each from the following developmental stages were used to study the changes in the facial nucleus at the various stages.

C.R.length (cm)	3.5	7.0	10.5	15.5	19.0	23.0	30.0	35.5	40.5	46.0
Age (weeks)	8	12	14	16	18	20	24	28	32	36

b. Adult : Three adult formalin fixed brainstems collected within 6 hours of death were used to study the facial nucleus and its cytology.

Age	**20**	**20**	**25**
Sex	Male	Female	Male

Bonnet Monkey (*Macaca radiata*)

Five specimens which were perfused with buffered formalin transcardially were used for the study of growth changes in the facial nucleus.

Stage	**Foetus**	**Newborn**	**Adult**
Number of specimens	1	1	3

2. COURSE OF THE FACIAL NERVE in *Macaca radiata*

Ten adult formalin fixed bonnet monkeys already available in the department were used to dissect out the whole course of the facial nerve.

3. EXPERIMENTAL in *Macaca radiata*

(i) Neural tracer

A formalin fixed adult monkey brainstem was used for implantation of the lipophilic tracer D.i.I. (I-I' Dioctadecyl-3-3,3',3',3' tetramethindo carbocyanin perchlorate) in the facial nerve.

A live adult monkey was used for injection of an aqueous solution of D.i.l. into the facial nerve.

(ii) Nerve transection

Ten monkeys reared in standard cages, which had no facial deformities were used for facial nerve transection.

METHODS

Histological Study

Paraffin embedding : All the human and monkey material other than two human adult and one monkey adult brain stems were processed for standard paraffin embedding. Blocks were made and serially sectioned in the transverse plane at 20 microns. The serial sections from all the blocks except

one from an adult monkey were stained with cresyl fast violet and luxol fast blue (Kluver and Barrera, 1953) and were used to study the position, cytology and morphometry of the facial nucleus.

From the 20 micron serial sections made from one of the adult monkeys, five sets of sections, each containing 16 sections taken at regular intervals so as to cover the whole nucleus, were made. Each set was stained by one of the five methods as follows :- (1) CFV (Drury and Wallington, 1982), (2) CFV and LFB (Kluver and Barrera, 1953), (3) Silver (Guillary, Shirra and Webster, 1961) (4) LPH triple stain (Goto, 1987) and (5) Aldehyde fuchsin (Drury and Wallington, 1982). These sections were used to study the cytomorphology of the facial nucleus in detail.

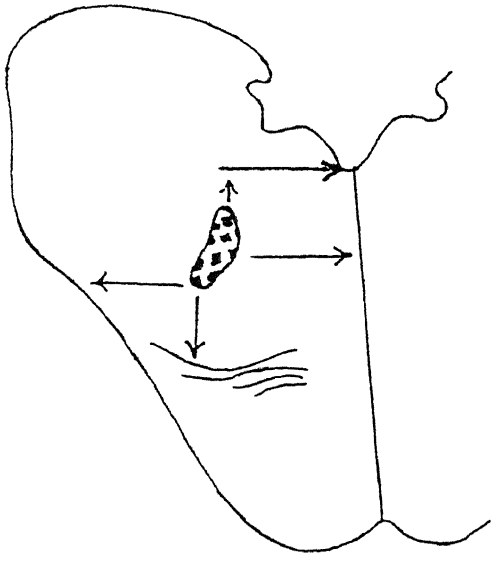
Rapid Golgi Method : Lower pons region of the two human 20 years old adults and that of an adult monkey were processed by the modified rapid Golgi method (Rao and Bijlani, 1983) and sectioned at 50 μ thickness to study the neuronal morphology and branching pattern of the fibres.

Location of the nucleus

The mean distance of the facial nucleus from (a) the midline of the pons, (b) the lowest point on the floor of the fourth ventricle, (c) the lateral border of pons and (d) the transverse fibres seen ventrally, were measured from the transverse sections (Fig.1) and tabulated.

Figure - 1

LOCATION OF FACIAL NUCLEUS



Location from:

→ Midline

↓ Transverse fibres

← Lateral border

↑ Lowest point on the floor of ventricle

Position of facial nucleus in relation to the abducent and spinal nucleus of trigeminal were observed (Fig.2). Diagrammatic reconstructions of the facial nucleus in the human and monkey foetuses, and the new born and adult monkeys were made according to the method used by Jacobs (1971). These were used to study its development in human and monkey foetuses and its postnatal growth changes in the monkey.

Morphometry

Morphometric analysis of the human foetal material and all normal monkey material was done. The neuronal number, volume and surface area per unit volume at the cranial, middle and caudal levels of the facial nucleus in the human foetus were calculated using standard stereological formulae (Weibel, 1966; Elias, 1967). In the case of monkey material the same parameters were investigated for the subdivisions in the nucleus.

Using a test grid (reticule) superimposed repeatedly and randomly over the neurons of facial nucleus at 400X magnification (Fig.3) the following quantities were estimated (Weibel and Gomez, 1962).

1. Number of neurons per unit test volume (Nv)

$$Nv = \frac{N}{A\,(D+t)} \qquad (number/mm^3)$$

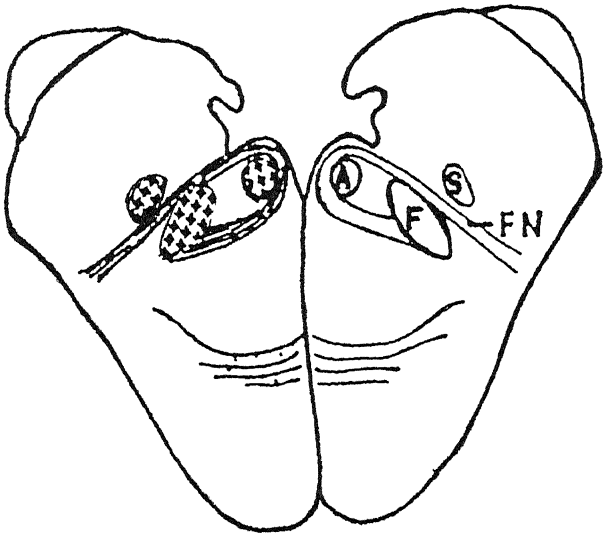
N = Number of neurons

A = Test Area

D = Average diameter of neurons

t = Thickness of section.

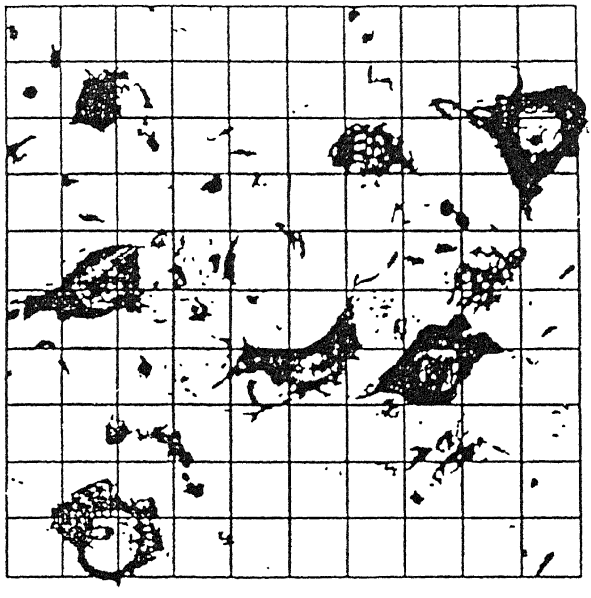
Figure - 2



Diagrammatic representation of the position of the facial nerve(F.N) and nucleus(F)in relation to abducent nucleus(A) and spinal nucleus of trigeminal(S)

Figure - 3

Reticule Superimposed over the neurons of facial Nucleus.



2. Volume of neurons per unit test volume (Vv)

$$Vv = \frac{Pi}{Pt} \quad (mm^3/mm^3)$$

Pi = Average point counts

Pt = Total number of test points

3. Surface area of neurons per unit test volume (Sv)

$$Sv = \frac{2\,P}{L} \quad (mm^2/mm^3)$$

P = Average intercept counts

L = Total length of test lines

= 22 x 0.25 mm

The neuronal diameter was measured [(maximum diameter + minimum diameter)/2] and the mean neuronal diameter was calculated. A standard ocular micrometer was used to measure the linear measurements.

Analysis of Variance

Analysis of variance was applied to study the morphometric results. When some differences were found, Duncan's Multiple Range test was applied and their levels of significance were calculated (Duncan, 1959; Kotz and Johnson, 1982).

EXPERIMENTAL (MONKEY)

USE OF DiI TO LOCATE THE FACIAL NUCLEUS (Wadhwa, Hayaran and Bijlani, 1991; Bodnarenko, Jayarasasingam and Chalupa, 1995)

Tracing the facial nucleus

a) In a formalin fixed monkey brainstem, crystals of DiI were implanted in the facial nerve stump at the ventrolateral border of pons and the brainstem was immersed in the fixative for 5 months. A photograph of a transverse section through the brinstem made through a level showing the facial nucleus was taken.

b) In a live adult monkey, the main trunk of facial nerve was surgically exposed after its exit from the stylomastoid foramen. The main trunk of the nerve was transected and the fluorescent tracer DiI, dissolved in normal saline was carefully injected into its proximal stump. 5 days after injection of the tracer, the animal was transcardially perfused with paraformaldehyde and the brainstem was removed and kept in the fixative for one month. The brainstem was then frozen and cut transversely at 75 microns using a cryostat. These sections were examined through a Nickon labophot microscope using rhodamine fluorescent filter, and photographs were taken.

Facial nerve course (Monkey)

The intracranial course of the facial nerve through the petrous part of temporal bone was carefully dissected and traced back as far as its entry into

brainstem. From the face the nerve was traced through the piecemealed parotid gland to the stylomastoid foramen. The posterior auricular nerve was also traced (Andrew and Kamakshi, 1993). The facial muscles were identified and the nerve supply to each muscle was dissected out and diagrammatically represented.

Nerve Transection : Surgical

The adult monkeys were anaesthetized with intraperitoneal injection of pentathal sodium, 28 mgs/Kg body weight. A posterior auricular incision was made and the facial nerve was traced back to the stylomastoid foramen and also distally upto the parotid gland. 1 cm of the nerve before it entered the parotid was removed carefully. Table-I shows the details of the surgery done.

Table : I

No.	Facial nerve transection level	Side and nature of surgery	Sacrificed on post operative day	Number of monkeys
1.	Upper trunk	Unilateral-right	2	1
2.	Lower trunk	Unilateral-right	4	1
3.	Main trunk	Bilateral	9	1
4.	Main trunk	Unilateral-right	14	1
5.	Main trunk right and upper trunk left	Bilateral	21	1
6.	Main trunk	Unilateral-right	28	1
7.	Main trunk	Unilateral-right	42	1
8.	Main trunk	Unilateral-right	60	1
9.	Main trunk	Unilateral-right	120	1
10.	Main trunk	Unilateral-right	180	1

After the facial nerve transection the parotid fascia and the skin were sutured. The animals were treated with antibiotics and analgesics post operatively.

Behavioural changes and the state of paralysis at various post operative periods were assessed, including an evaluation of the effect on some of the muscles, in the case of the monkey which was allowed to survive for 180 days.

The three normal adult monkeys were used as controls.