BRAIN OF THE BUFFALO (*Bubalus Bubalis*) IN STEREOTAXIC COORDINATES

KIRAN SINGH, KHUB SINGH*, B. K. SONI** AND S. K. MANCHANDA

Department of Physiology, All-India Institute of Medical Sciences, New Delhi-16

and

Department of Physiology and Pharmacology, College of Veterinary Medicine, G. B. Pant University of Agriculture and Technology, Pant Nagar, District Nainital (U.P.)

Summary: A stereotaxic atlas of the brain of buffalo calves, weighing 75 to 90 kg and ranging in age from 8 to 9 months has been prepared. The three zero planes used were (i) the horizontal plane (Ho) lying parallel and 30 mm above the plane passing through external auditory meati and infraorbital margin, (ii) the frontal plane (Fo) passing through interaural line and perpendicular to the horizontal zero plane and (iii) the sagittal plane passing through midline of the brain at right angle to the other two zero planes. The point of intersection of three zero planes has served as the reference zero

The atlas consists of drawings of transverse sections through the brain at 2 mm intervals from 14 mm caudal to 56 mm rostral to the frontal zero plane.

Key Words : stereotaxic atlas ruminant brain stereotaxic coordinates buffalo (bubalus bubalis) brain.

INTRODUCTION

Neurophysiological investigations have been greatly facilitated by the application of stereotaxic techniques first introduced by Horsley and Clarke in 1908 (5). The method has been used in many animal species and in spite of many modifications principally remains the same. The absence of a readily available stereotaxic atlas for electrode implantation in the brain of Indian buffalo has prompted the preparatation of this stereotaxic atlas. Primarily it was required for our investigations on the central nervous regulation of the rumino-reticular motility in the buffalo (9). In this communication the stereotaxic coordinates of the buffalo brain are presented for use in the investigations which require stereotaxic intervention of the brain.

MATERIALS AND METHODS

8-9 months old buffalo calves within the weight range of 75-90 *Kg* were chosen for the preparation of this stereotaxic atlas.

System of coordinates. The cranial landmarks on the buffalo skull were used to establish the three zero reference planes which permitted convenient and accurate orientation of the animal's head in the stereotaxic apparatus (8).

*Division of Physiology and Pharmacology, Indian Veterinary Research Institute, Izatnagar, U.P., India.
**Deputy Director-General (Animal Sciences), Indian Council of Agricultural Research, Krishi Bhavan, New Delhi-1.

The *interaural line*, connecting the external auditory meatus, passes through the axis for rotation of the skull. The eye bars are aligned with the infraorbital margin. The central lines of the eye bars and the aural bars are in the same horizontal plane (Fig. 1). The horizontal zero plane (Ho) lies parallel to and 30 *mm* above the plane passing through interaural line and infraorbital margin (Fig. 2).

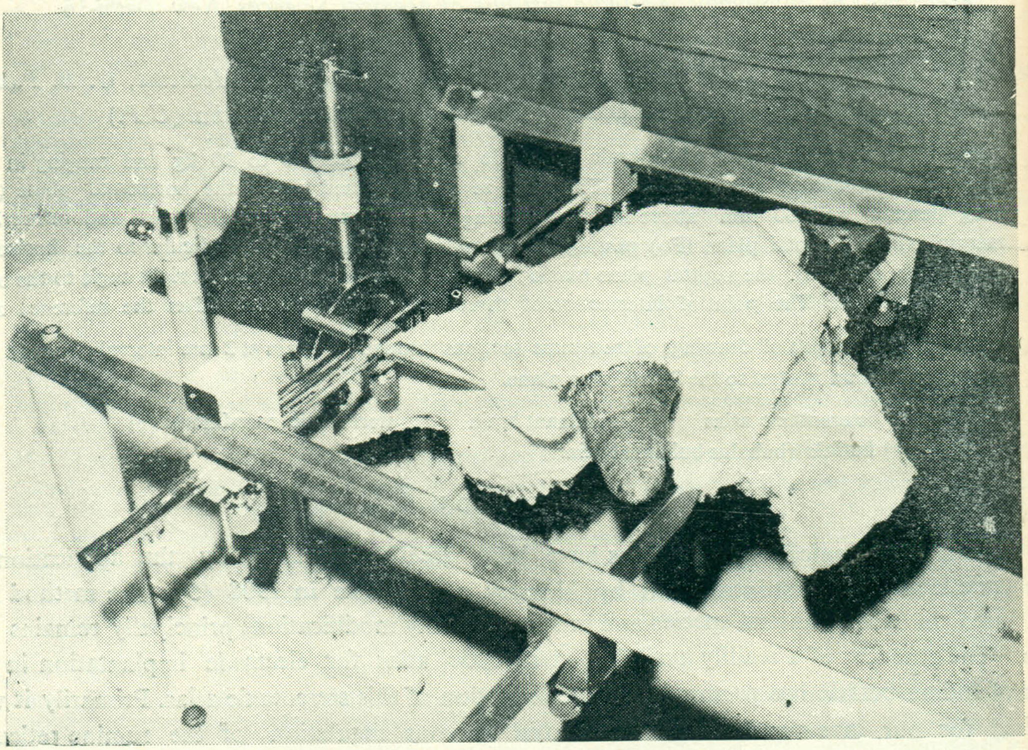

Fig. 1: A buffalo skull shown in position in the stereotaxic instrument. The aural bars, located in posterior slots, are inserted in the external auditory meati; the eye bars located in anterior slots are placed on the infraorbital margin.

The frontal (or vertical) zero plane passes through the interaural line and is perpendicular to the horizontal plane. *The lateral (or midsagittal) zero plane* passes through the midline of the brain dividing it into two symmetrical halves and serves as the zero plane for designating the location of the intracerebral point to be in the left or right halves of the brain ('L' or 'R'). The point of intersection of the three zero planes has been termed as "reference zero". All positions are thus expressed in relation to this (reference) zero. Horizontal positions (H) are expressed in plus (+) when above the zero, and in minus (—) when below this zero. Frontal positions (F) have been expressed as A when anterior and as P when posterior to this zero. For example a position labelled $P_5R_6H_{+10}$ would be located 5 *mm* posterior 6 *mm* to the right and 10 *mm* above the reference zero.

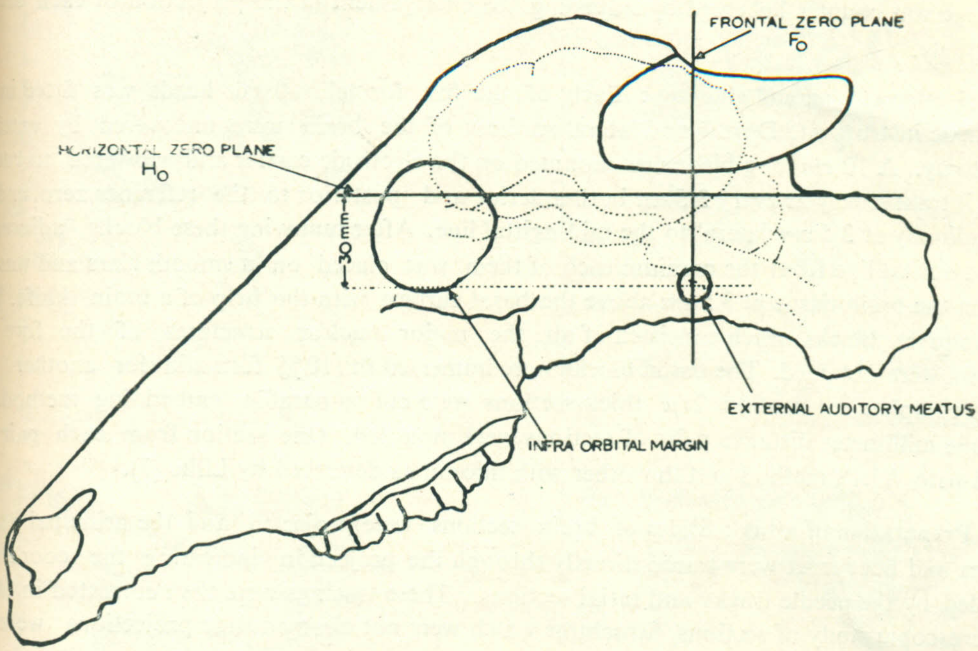

Fig. 2: Stereotaxic coordinates superimposed on a buffalo skull. The vertical interaural plane is the frontal reference point. The horizontal zero plane (H_0) interesects the frontal interaural plane, and is 30 mm above and parallel to the plane connecting auditory meati and the inferior orbital ridge. These planes are coincident with the fixation of skull in the head holder as shown in figure 1.

Formalin fixation of the brain: The heads of six buffalo calves used for the preparation of this atlas were fixed in the stereotaxic instrument in the standard position under nembutal anaesthesia. Four stainless steel needles were introduced into the brain at known parameters. The carotid artery and the jugular vein were secured on both sides. The animal was bled through the carotid artery of one side, and then was perfused through this artery of warm saline followed by perfusion with a fluid containing 10% formalin and 1% agar in normal saline. Perfusion was done under a pressure head of 200-250 *mm Hg*. Later all soft tissues and lower jaw were removed from the skull, and 4 or 5 holes of 3-4 *mm* diameter each were made in the cranium. The skull was then immersed in 10% formalin for at least one week.

Determination of shrinkage : To determine the shrinkage during histological processing initially serial sections of the brain of one animal were cut in the vertical planes of the needle tracks. The transverse as well as antero-posterior distances between the needle tracks were calculated by serial sections of the tissue and compared with the known distance, as calculated from the parameters at which these needles were initially introduced. A 16% shrinkage was found to have occurred which was counterbalanced by making serial sections of 21 μ thickness and considering them as 25μ thick in the subsequent brain sections. The lateral and vertical shrinkage was counter-balanced by adjusting the enlargement of the projection of each secti accordingly.

Section cutting and staining : Each of the five formalin fixed heads was fitted in t stereotaxic instrument. Dorsal and lateral surfaces of the brain were uncovered by extensi craniotomy. A 10 *cm* long blade was mounted on the electrode carrier and lowered to cut t brain (i) transversely at every 3.5 *cm* both anterior and posterior to the reference zero and (longitudinally at 3.5 *cm* lateral to the midsagittal line. After removing these blocks adjacent the midsagittal line from the cranium each of them was placed on a smooth glass and was c off from the brain tissue at 3.5 *cm* above the basal surface with the help of a brain knife. Th 3.5 *cm* square blocks which consisted of all the major nuclear structures of the fore a midbrain were obtained. The tissue blocks were immersed in 10% formalin for another fo days to complete the fixation. 21μ thick sections were cut by paraffin embedding method. A every one millimeter distance pairs of sections were mounted. One section from each pair wa stained with Wiel's method and the other with thionin as described by Lillie (7).

Preparation of atlas : Slides of brain sections were projected and the principal nucle outlines and fiber tract were traced directly through the projection indicating the coordinat as guided by the needle tracks and serial sections. These tracings were then corrected in deta by microscopic study of sections. Structures which were not clear on slide projections were als carefully filled in after the microscopic study.

For the identification of structures in the forebrain, the help of atlases prepared by Jasp and Ajmone-Marsan (6); Fifkova and Marsala (3) and Tindal *et al* (10) was taken. Similarl the structures in the midbrain were identified with the help of Truex and Carpenter (11) an Crosby *et al* (2). Breazile (1) and Gray and Goss (4) were consulted for the hind brain.

The location of major nuclei, fibre tracts and other structures of the brain has bee given in a total of 36 transverse sections taken at every two millimeters and extending fro P_{14} to A_{56} in the antero-posterior extent (figures 3-8).

COMMENTS

Since no other stereotaxic atlas exists for the brain of buffalo, it is not possible to comp are the present atlas with other atlases. Reference zero lies in the rostral half of the cerebellu just anterior to the rostral end of nucleus dentatus. The general orientation of most of th brain structures is approximately the same as that in other animals.

This stereotaxic atlas prepared for young buffaloes (weighing 75 to 90 *kg*) has been trie for placement of electrodes in various brain regions in acute experiments. Electrode position determined by the atlas as first approximation have not been always found to be on exac locations when confirmed by calculations made from serial sections. Instead variations upt 1.5 *mm* in all the three directions have been observed. Various factors which may be contribu ting to the problem of exact localization of electrodes could be: the unusual electrode length

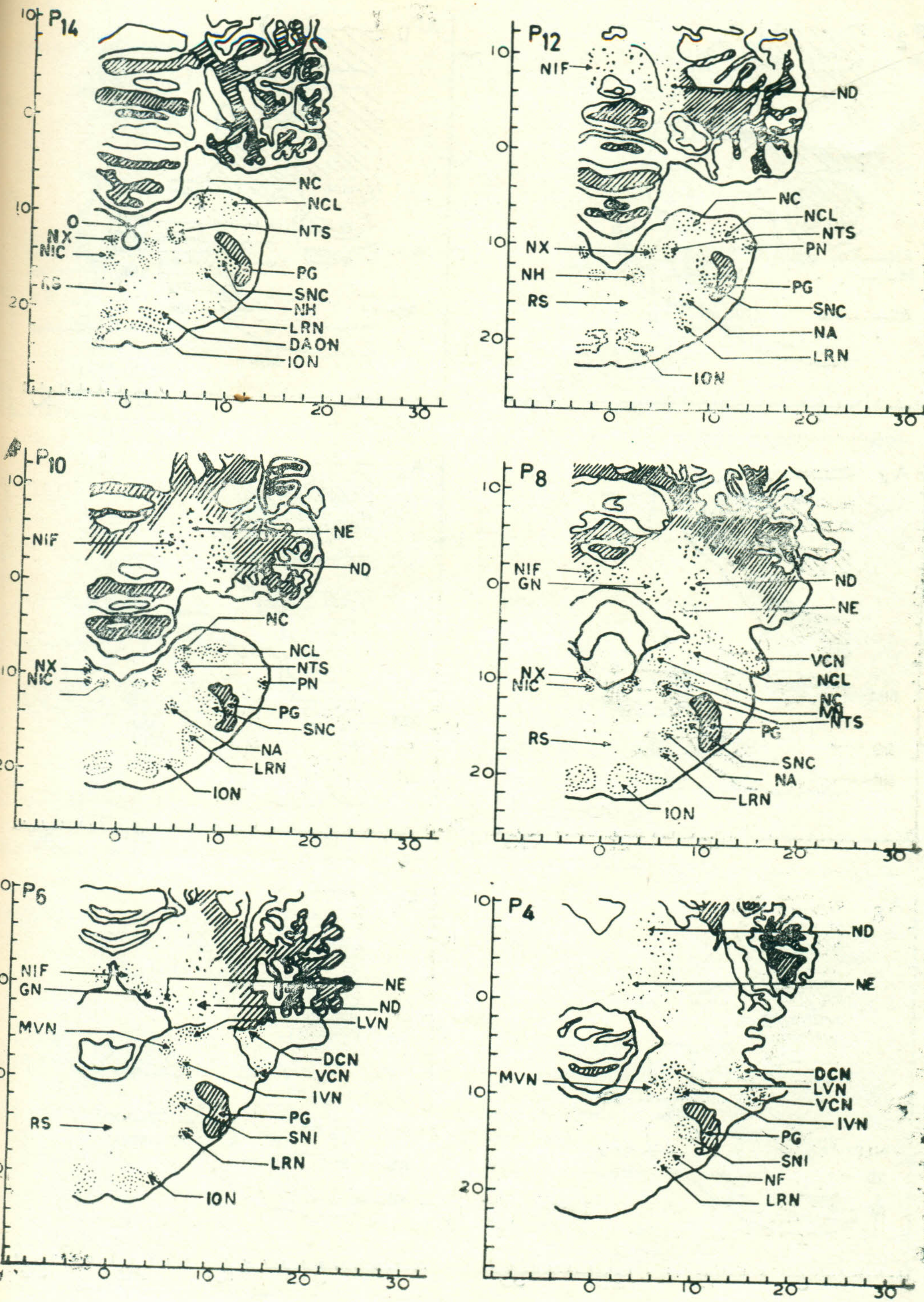
P14
O
NX
NIC
RS
NC
NCL
NTS
PG
SNC
NH
LRN
DAON
ION
P12
NIF
ND
NC
NCL
NTS
PN
NX
NH
RS
PG
SNC
NA
LRN
ION
P10
NIF
NE
ND
NC
NCL
NTS
PN
NX
NIC
PG
SNC
NA
LRN
ION
P8
NIF
GN
ND
NE
VCN
NCL
NX
NIC
NC
MN
NTS
PG
RS
SNC
NA
LRN
ION
P6
NIF
GN
NE
ND
LVN
MVN
DCN
VCN
IVN
PG
SNI
RS
LRN
ION
P4
ND
NE
DCN
LVN
MVN
VCN
IVN
PG
SNI
NF
LRN

Fig. 3

April 1972
Ind. J. Physiol. Pharmac.

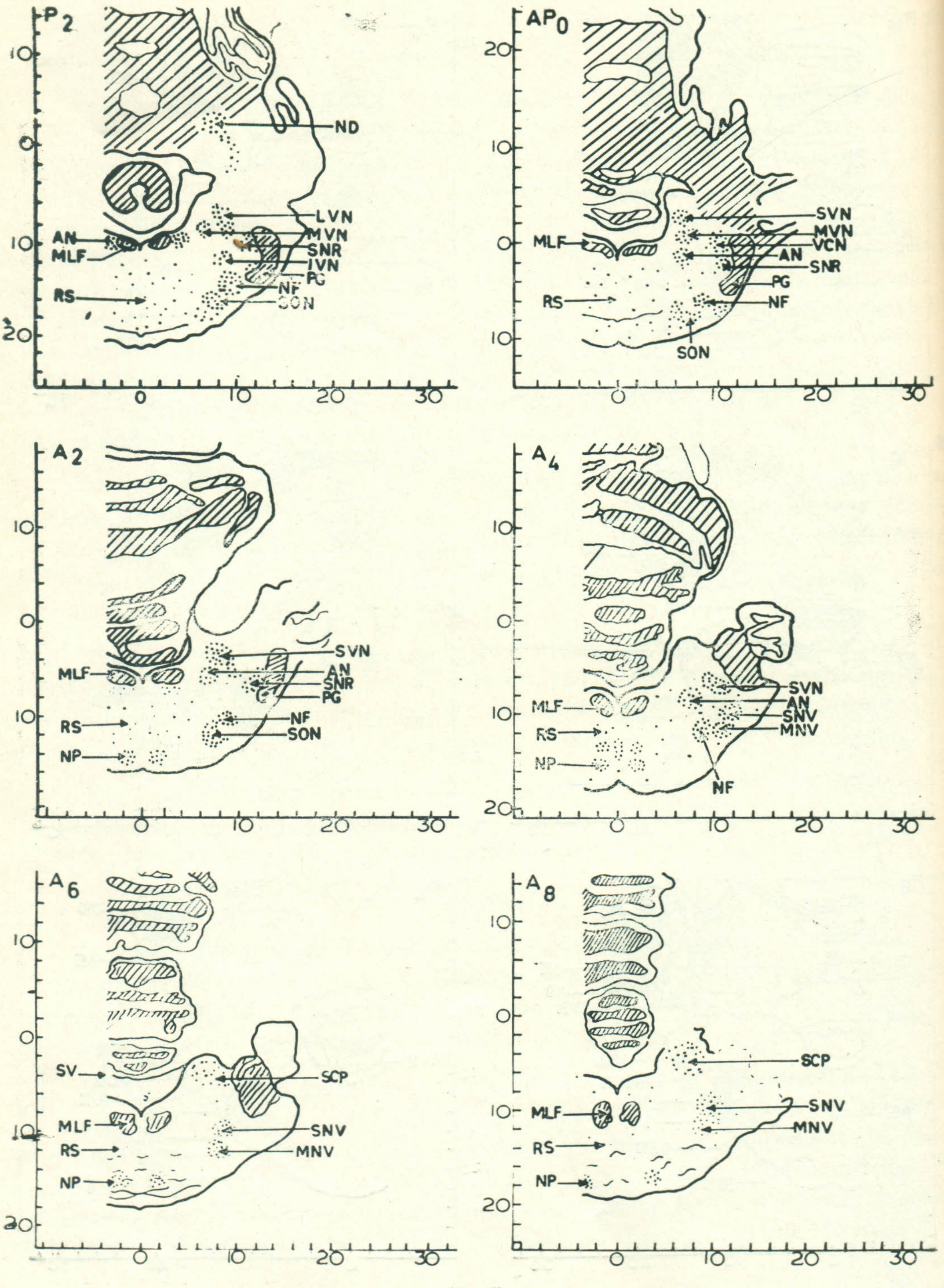

Fig. 4

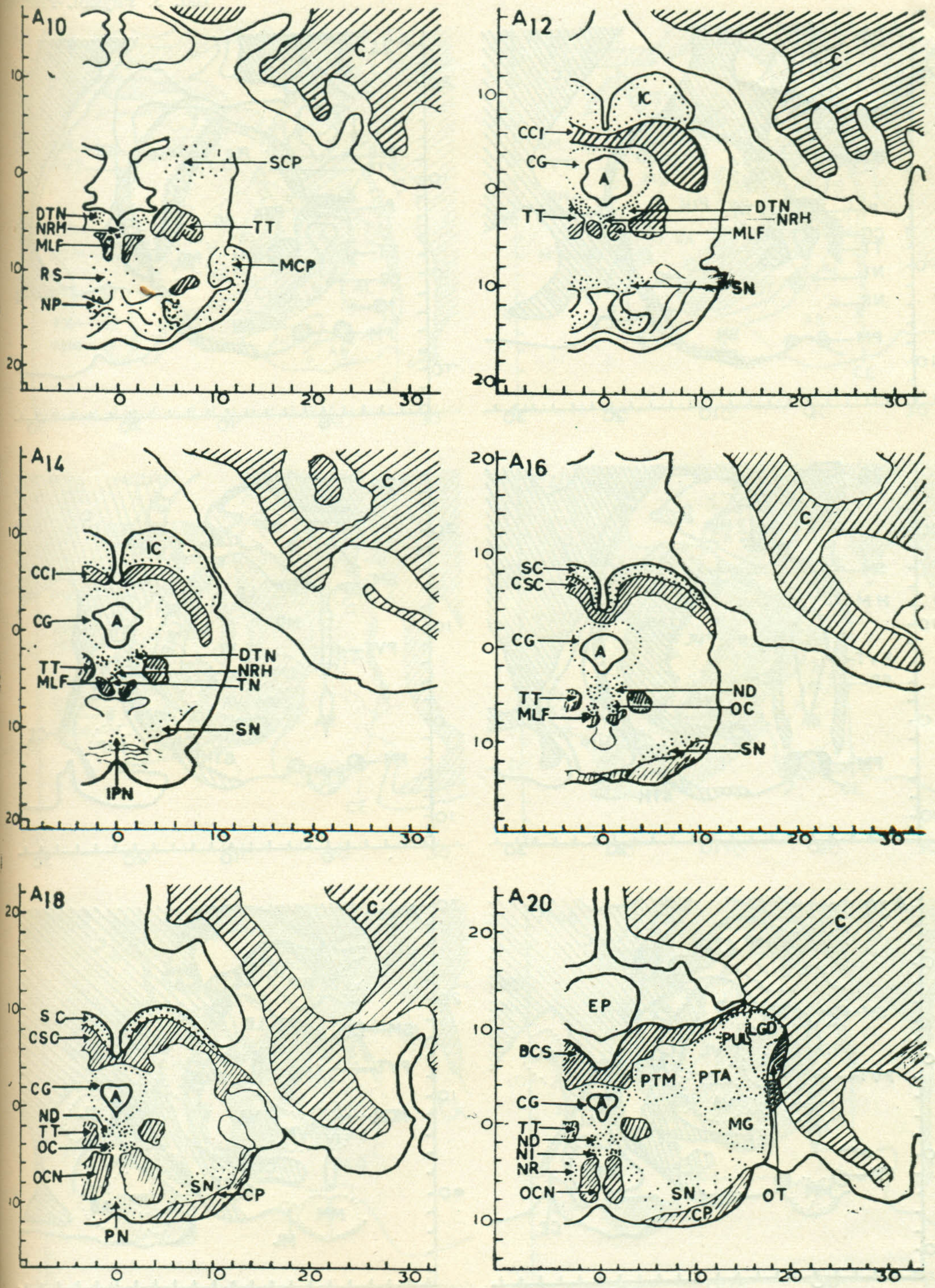
A10
C
SCP
DTN
NRH
MLF
TT
RS
MCP
NP
A12
C
IC
CCI
CG
A
DTN
TT
NRH
MLF
SN
A14
C
IC
CCI
CG
A
DTN
TT
NRH
MLF
TN
SN
IPN
A16
C
SC
CSC
CG
A
ND
TT
OC
MLF
SN
A18
C
SC
CSC
CG
A
ND
TT
OC
OCN
SN
CP
PN
A20
C
EP
LGD
PUL
BCS
PTM
PTA
CG
A
MG
TT
ND
NI
NR
OCN
SN
CP
OT

Fig. 5

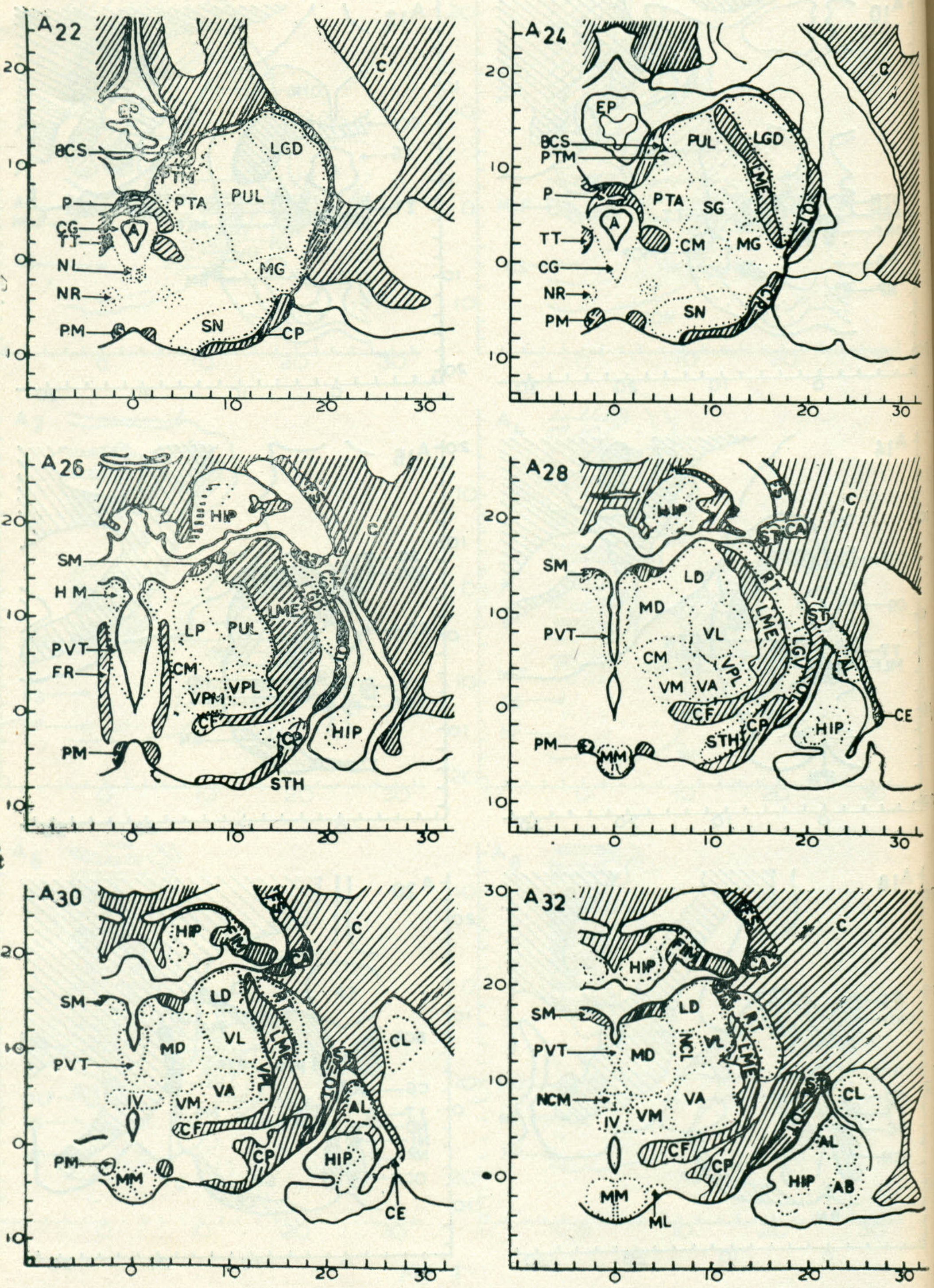
A22
C
EP
BCS
PTM
LGD
PTA
PUL
P
CG
A
TT
NI
MG
NR
SN
PM
CP
A24
C
EP
BCS
PTM
PUL
LGD
LME
P
PTA
SG
A
TT
CM
MG
CG
NR
CP
PM
SN
A26
HIP
C
SM
HM
LP
PUL
LME
PVT
FR
CM
VPM
VPL
CF
HIP
PM
STH
A28
HIP
C
CA
SM
LD
RT
MD
LME
PVT
VL
CM
LGV
VPL
VM
VA
CE
CF
HIP
PM
MM
STH
CP
A30
HIP
C
CA
SM
LD
RT
VL
LME
CL
PVT
MD
VPL
IV
VM
VA
AL
CF
CP
HIP
PM
MM
CE
A32
C
HIP
CA
SM
LD
RT
PVT
MD
NCL
VL
LME
NCM
VA
CL
IV
VM
AL
CF
CP
HIP
AB
MM
ML

Fig. 6

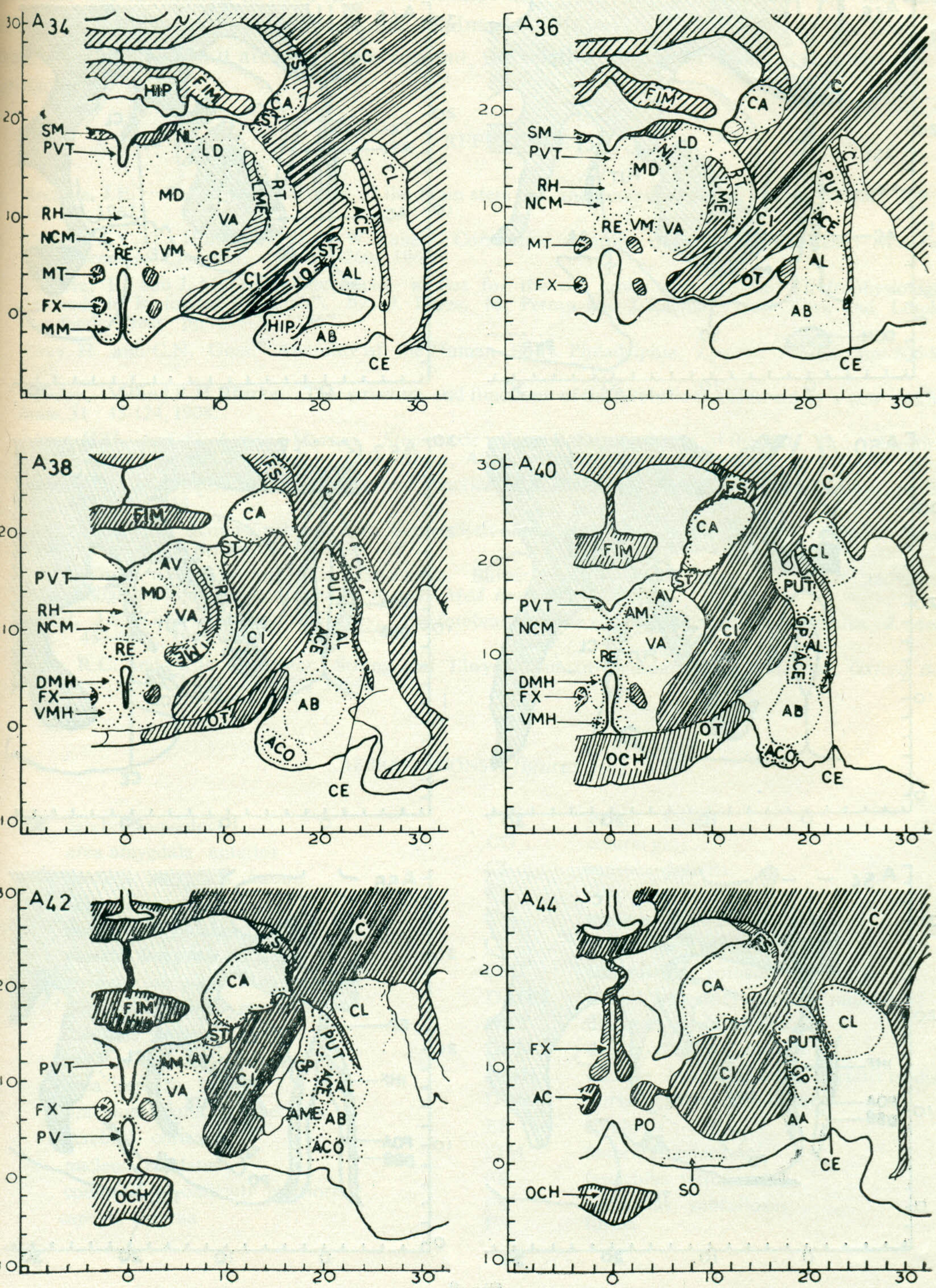
A34
C
FS
FIM
HIP
CA
ST
SM
PVT
NL
LD
MD
RT
LME
VA
RH
NCM
RE
VM
CF
CI
ST
OT
ACE
CL
AL
MT
FX
MM
HIP
AB
CE
A36
FIM
CA
C
SM
PVT
NL
LD
MD
RT
LME
CL
PUT
ACE
RH
NCM
RE
VM
VA
CI
MT
FX
OT
AL
AB
CE
A38
FS
C
FIM
CA
ST
PVT
AV
MD
RT
VA
CL
PUT
ACE
AL
RH
NCM
RE
LME
CI
DMH
FX
VMH
OT
AB
ACO
CE
A40
FS
C
FIM
CA
CL
ST
PUT
PVT
AV
AM
NCM
VA
CI
GP
AL
ACE
RE
DMH
FX
VMH
AB
OT
OCH
ACO
CE
A42
C
CA
FIM
CL
ST
PUT
PVT
AM
AV
VA
CI
GP
ACE
AL
FX
AME
AB
PV
ACO
OCH
A44
C
FS
CA
CL
PUT
CI
GP
FX
AC
PO
AA
CE
SO
OCH
0
10
20
30

Fig. 7

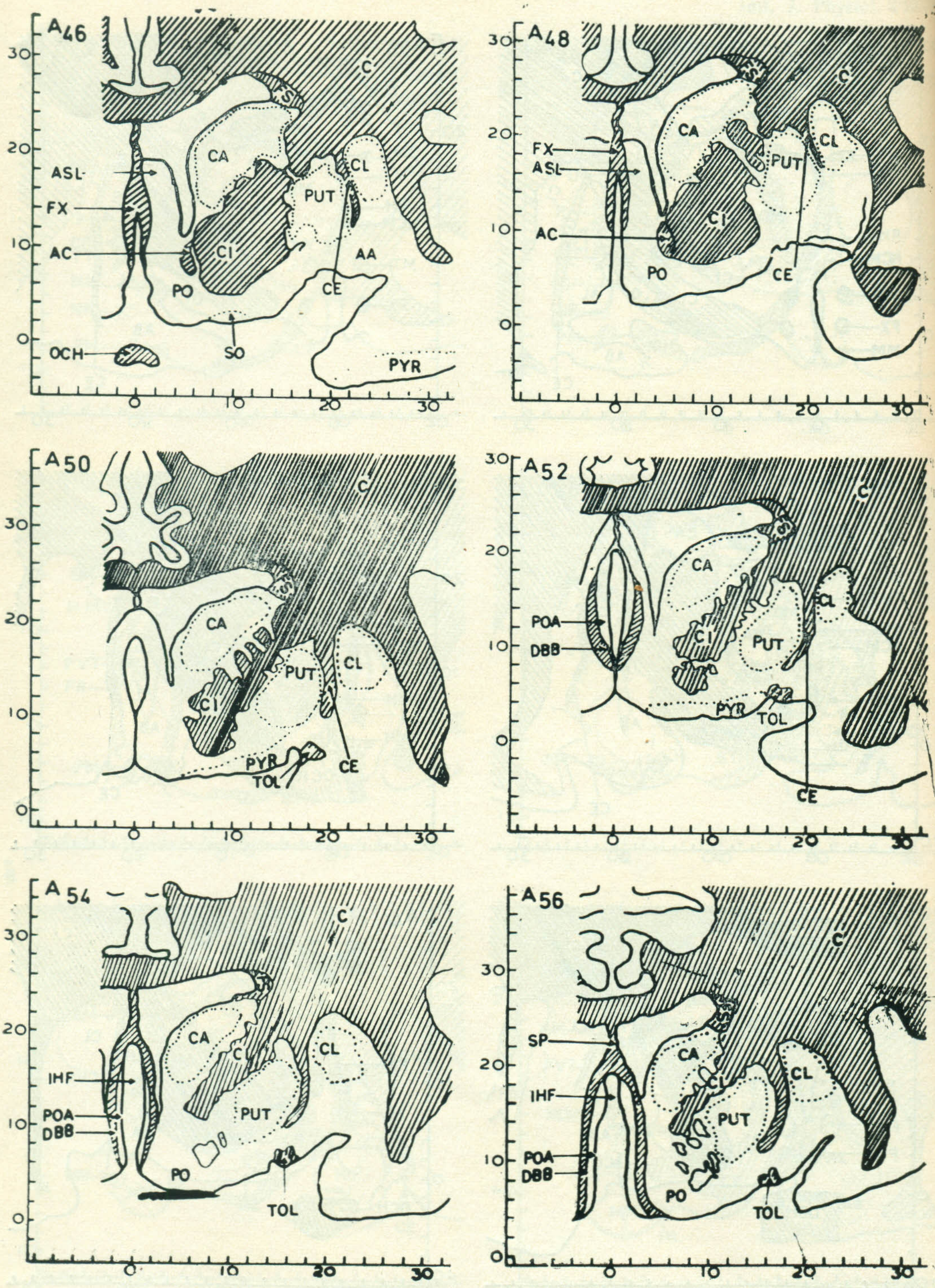
A46
C
FS
CA
ASL
FX
AC
CI
PUT
CL
AA
CE
PO
SO
OCH
PYR
A48
C
FS
CA
FX
ASL
AC
CI
PUT
CL
CE
PO
A50
C
FS
CA
CI
PUT
CL
PYR
TOL
CE
A52
C
FS
CA
POA
DBB
CI
PUT
CL
PYR
TOL
CE
A54
C
CA
C
CL
PUT
IHF
POA
DBB
PO
TOL
A56
C
FS
SP
CA
CL
IHF
PUT
POA
DBB
PO
TOL
0
10
20
30

Fig. 8

the errors due to fixation of head in the stereotaxic instrument and possibly also the individual variability of the intracerebral or cranial landmarks. However, if the size of the head and brain structures within it are taken into account, the relative degree of accuracy seems to be satisfactory.

REFERENCES

1. Breazile, J.E. The cytoarchitecture of the brain stem of the domestic pig. *J. Comp. Neurol.*, **129** : 169-187, 1967.
2. Crosby, E.C., T. Humphrey and E.W. Lauer. Correlative Anatomy of the Nervous System. *New York, The Macmillan Company*, pp 221-260, 1962.
3. Fifkova, E. and J. Marsala. Stereotaxic atlases for the cat, rabbit and rat. In "Electrophysiological Methods in Biological Research". by J. Bures, M. Petran and J. Zachar. New York and London, Academic Press pp 653-731, 1967.
4. Gray, H. and C.N. Goss. Anatomy of the Human Body, Philadelphia, *Lea and Febiger*, pp 806-869, 1966.
5. Horsley, V. and R.H. Clarke. The structure and functions of the cerebellum examined by a new method. *Brain*, **31** : 45-124, 1908.
6. Jasper, H.H. and C. Ajmone-Marsan. Stereotaxic atlases B. Diencephalon of the cat. In : "Electirical Stimulation of the Brain" edited by D.E. Sheer, Austin, *University of Texas Press*, pp 203-231, 1961.
7. Lillie, R.D. Histopathologic Technique and Practical Histochemistry, New York *Blackiston Co.*, pp 328-332, 142-143, 1954
8. Manchanda, S.K., Kiran Singh and B.K. Soni. Stereotaxic apparatus for the buffalo (*Bubalus bubalis*) brain. *Physiol. and Behav.*, **6**: 727-728, 1971.
9. Singh Kiran, B.K. Soni and S.K. Manchanda. Bulbar control of the rumino-retricular movements in the Indian buffalo (*Bubalus bubalis*). *Indian J. Med. Res.*, **59** : 1886-1892, 1971.
10. Tindal, J.S., G.S. Knags and A. Turvey. The forebrain of the goat in stereotaxic coordinates. *J. Anat.*, **103**: 457-469, 1968.
11. Truex, R.C. and M.B. Carpenter. Strong and Elwyn's Human Neuroanatomy, Calcutta. *Oxford Book Company*. pp. 251-438, 539-554, 1964.

ABBREVIATIONS (*figures* 3-8)

A	cerebral aqueduct
AA	area amygdala anterior
AB	nucleus amygdala basalis
AC	commissura anterior
ACE	nucleus amygdala centralis
ACO	nucleus amygdala corticalis
AL	nucleus amygdala lateralis
AM	nucleus anterior medialis
AN	abducens nucleus
AV	nucleus anterior ventralis
ASL	area septalis lateralis
BCS	brachium colliculi superioris
C	cerebral cortex
CA	nucleus caudatus
CCI	commissura colliculi inferioris
CE	capsula externa
CF	campi Foreli
CG	central gray
CI	capsula interna
CL	claustrum
CM	nucleus centrum medianum
CP	pedunculus cerebri
CSC	commissura colliculi superioris
DAON	dorsal accessory olivary nucleus
DBB	diagonal band of Broca
DCN	dorsal cochlear nucleus
DMH	nucleus hypothalamicus dorsalis medialis
DTN	dorsal tegmental nucleus
EP	epiphysis
FIM	fimbria hippocampi
FR	fasciculus retroflexus
FS	fasciculus subcallosus
FX	fornix

GN	globose nucleus
GP	globus pallidus
HIP	hippocampus
HM	nucleus habenula lateralis
IC	colliculi inferioris
IHF	interhemispheric fissure
ION	inferior olivary nucleus
IPN	interpednucular nucleus
IV	nucleus interventralis
IVN	inferior vestibular nucleus
LD	nucleus lateralis dorsalis
LGD	nucleus corpus geniculatum lateralis dorsalis
LGV	nucleus corpus geniculatum lateralis
LME	lamina medullaris externa
LP	nucleus lateralis posterior
LRN	lateral reticular nucleus
LVN	lateral vestibular nucleus
MCP	middle cerebellar peduncle
MD	nucleus medialis dorsalis
MG	nucleus corpus geniculatum medialis
ML	corpus mamillaris lateralis
MLF	medial longitudinal fasciculus
MM	corpus mamillaris medialis
MNV	motor nucleus of trigeminal nerve
MT	fasciculus mamillothalamicus
MVN	medial vestibular nucleus
NA	nucleus ambiguus
NC	nucleus cuneatus
NCL	nucleus cuneatus lateralis
NCM	nucleus centralis medialis
ND	nucleus of Darkschewitch
NE	nucleus emboliformis
NF	nucleus facialis
NIF	nucleus fastigii
NH	nucleus hypoglossus
NI	nucleus interstitialis
NIC	nucleus intercalatus
NL	nucleus centralis lateralis
NP	pontine nucleus
NR	nucleus ruber
NRH	nucleus of raphae
NTS	nucleus and tractus solitarius
NX	dorsal motor nucleus of vagus
O	obex
OC	oculomotor nucleus (nucleus oculomotorius)
OCH	chiasma opticum
OCN	nerve oculomotorius
OT	tractus opticus
P	commisura posterior
PG	tract of trigeminal nerve
PM	pedunculus corpus mamillaris
PN	pontobulbar nucleus
PO	area preoptica
POA	paraolfactory area
PTA	nucleus pretectalis anteriar
PTM	nucleus pretectalia medialis
PUL	pulvinar
PUT	putamen
PV	nucleus paraventricularis hypothalami
PVT	nucleus paraventricularis thalami
PYR	cortex pyriformis
RE	nucleus reuniens
RH	nucleus rhomboideus
RS	substantia reticularis
RT	nucleus reticularis thalami
SC	colliculus superior
SCP	superior cerebellar peduncle
SG	nucleus suprageniculatus
SM	stria medullaris thalami
SN	substantia nigra
SNC	subnucleus caudalis
SNI	subnucleus interpolaris
SNR	subnucleus rostralis
SNV	chief sensory nucleus of trigeminal nerve
SO	nucleus supraopticus
SON	superior olivary nucleus
SP	septum pellucidum
ST	stria terminalis
STH	nucleus subthalamicus
SV	superior vellum
SVN	superior vestibular nucleus
TN	nucleus trochlearis
TOL	tractus olfactorius lateralis
TT	tractus tegmentalis centralis
VA	nucleus ventralis anterior
VCN	ventral cochlear nucleus
VL	nucleus ventralis lateralis
VM	nucleus ventralis medialis
VMH	nucleus hypothalamicus ventralis medialis
VPL	nucleus ventralis posterior lateralis
VPM	nucleus ventralis posterior medialis