A STEREOTAXIC ATLAS OF THE NEW ZEALAND RABBIT'S BRAIN

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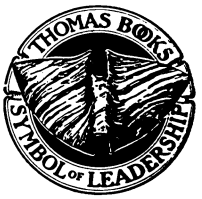

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Introduction

HORSLEY AND CLARKE'S invention of a stereotaxic instrument in 1908 (14) meant an introduction of a new three-dimensional way of locating intracranial structures. Their original idea relied on the assumption that there exists more or less constant relationship between intracerebral and skull structures in a particular animal species. Therefore, if one could select some prominent skull landmarks, preferably those less susceptible to individual variation of skull dimensions, as for example, meatus acusticus externus, margo infraorbitalis, and sutura sagittalis, one would be in a position to create a three-dimensional system of perpendicular basal planes defined by these landmarks. The location of any intracerebral structure could then be expressed in terms of its relationship to basal planes. According to these principles, Horsley and Clarke constructed the first stereotaxic instrument the main function of which was to orient the tools for intracranial intervention (an electrode etc.) into established basal planes. To achieve the correct orientation the head of the experimental subject was firmly fixed in the instrument in a manner securing identification of basal cranial planes with those of the apparatus. Favorable configuration of the chosen landmarks largely enhanced such fixation simply performed by insertion of a pair of horizontal ear bars into likewise oriented auditory meatus combined with a pair of infraorbital bracket bars pressing against infraorbital margins. In addition, such clear definition of fixation provided its good reproducibility within and between subjects.

Despite its progressive character, it was not until 1932 that the potential of the Horsley-Clarke method became fully understood due to the work of Ranson and his co-workers. These authors used the, until then, scarcely used stereotaxic technique for an extensive research of the cat diencephalon and the brain stem (15, 16), and the results achieved by this technique stimulated its further expansion and general acceptance in the research. Although the application of the method was initially restricted to the cat and the monkey for relative homogeneity of skull dimensions, the growth of brain research in the years that followed soon resulted in adaptation of stereotaxy to other animals, including birds. Of course, certain modifications had to be introduced in order to meet the requirements of new species, but basically the Horsley-Clarke concept of skull landmarks remained unchanged.

From recent articles reviewing the history and the development of the stereotaxic method (3, 7, 29), it became apparent that the rabbit, despite its popularity as an experimental subject, was not readily accepted into the family of animals to which the method was suited. The peculiar configuration of its auditory meatus—crucial landmarks and fixation points—was

one of the main reasons. While in most animals auditory meatus are oriented horizontally and thus allow an easy insertion of straight horizontal ear bars, in the rabbit their longitudinal axes show a substantial deviation from the horizontal plane. Consequently, it was practically impossible to use conventional biaural head fixation (and also the corresponding Horsley-Clarke system of basal planes) unless some modifications were introduced.

There have been several attempts to solve the problem by seeking different ways to fixate the head in specially designed instruments; for example, against zygomatic arcs and front incisors. Bregma and Lambda skull sutures then served as the landmarks for definition of the stereotaxic planes. Harris was probably the first to use this approach in the rabbit for the purpose of implanting electrodes into the hypothalamus (10). However, a stereotaxic atlas based on these principles did not appear until 1954, compiled by Sawyer, Everett, and Green (30), and was later followed by that of Fifková and Maršala (5). It might be pertinent to mention in association with the latter technique that zygomatic arcs-front incisors fixation requires certain experience to master it and that the accuracy in reaching intracerebral targets, using the Bregma-Lambda reference system, was found by the present authors to be satisfactory only within the forebrain.

An alternative solution was described by Monnier and Gangloff in 1961 (20). These authors took advantage of a streotaxic plate originally designed by Hess (13) for multiple electrode implantations in the conscious cat and adapted its dimensions to the rabbit's skull. Similarly, as in the previous method, skull sutures were taken as the basic landmarks. However, while such approach obviated the need for a stereotaxic head holder, it was not suited for those experiments requiring restraint of the experimental subject.

So far, the solution reported by Chatelier and Buser (4) appears most attractive. The authors modified conventional horizontal ear bars according to the oblique angle of the rabbit's auditory meatus with the aim to facilitate their adequate insertion into the meatus for achievement desired rigid head fixation. Such modification, completed by an attachment for infraorbital fixation, permitted the adoption of classical landmarks with unaltered Horsley-Clarke basal planes defined as follows: the horizontal plane going through the middle of auditory meatus and infraorbital margins, the frontal plane intersecting perpendicularly the horizontal one in the center of the auditory orifices, and the sagittal plane being simply determined by sagittal suture. In addition, Chatelier and Buser technique circumvents the need for specially designed instruments, since any universal stereotaxic frame could be used, provided that the proper attachments are available.

We have adopted this approach because of the advantages it offers are simple and sufficiently accurate and because it provides more reliably reproducible head fixation than the methods mentioned above. In order to make full use of it we have prepared a corresponding stereotaxic atlas.

Materials and Methods

THIRTY-THREE one-year-old female New Zealand rabbits, weighing about 4.5 kg were used for preparation of the atlas. The animals, under urethane anesthesia (1.75 gm/kg), were biaurally mounted in the stereotaxic instrument (La Precision Cinematographique, Paris) provided with modified ear bars and the attachment for infraorbital fixation (for details see Chatelier and Buser [4]). Stainless steel needles were introduced into the brain bilaterally in frontal planes starting at A 0.0 mm and subsequently rostralwards at A 2.0, A 5.0, A 10.0, A 15.0, and A 20.0 mm (A 0.0 mm representing the middle of the auditory orifices—see Fig. 1) always 2 mm from the midline. The same procedure was repeated in the horizontal plane at H 2.0 mm and H 4.0 mm above H 0.0 mm on the left and at H 2.0 mm, H 4.0, and H 6.0 mm above H 0.0 mm on the right side (see Fig. 1). For

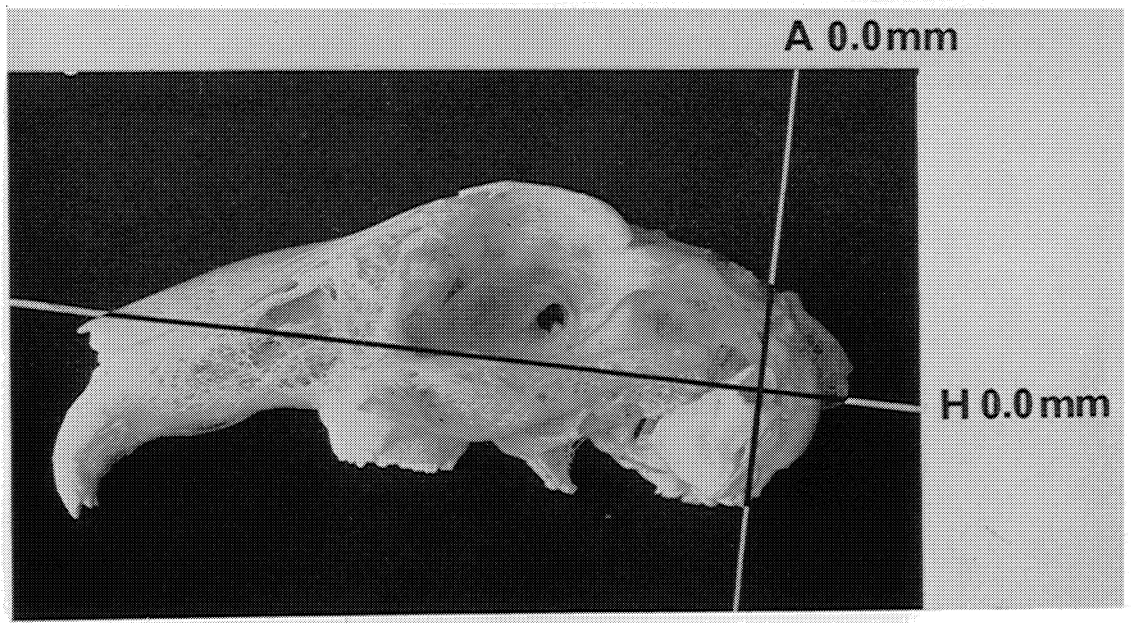


FIGURE 1. The lateral view of the rabbit's skull with marked horizontal (H 0.0 mm) and frontal (A 0.0 mm) Horsley-Clarke's basal planes.

the horizontal plane the needles were introduced 3 mm from the sagittal plane to avoid an interference of the horizontal tracks with those in the frontal planes. The main function of the tracks was to transfer stereotaxic planes intracranially and thus to facilitate later brain orientation.

The head of each subject was then perfused with 10% formalin via a carotid artery; the animal was decapitated and the brain was left *in situ* in

formalin solution for ten days to complete fixation. Frozen sections of 100μ thickness were cut in the frontal plane and every fifth one was mounted, thus giving sections at 0.5 mm intervals throughout the brain. Cellular structures were stained with 0.1% thionine to visualize the Nissl substance, and main fiber systems were traced in 50μ thick sections stained by solochrome cyanin (23) countrastained with cresyl violet. To confirm anatomical reconstruction based on frontal sections, three brains were cut in the sagittal plane. Each slide was projected at the ten times calibrated magnification combined with higher power microscopic examination for morphological identification of structures, for orientation into the stereotaxic planes, and for illustrations. Final illustrations supplemented with corresponding photomicrographs were made in 0.5 mm intervals to represent the relevant part of the brain, starting at the frontal plane A 4.0 mm and going to A 20.0 mm. Sections preceding plane A 4.0 mm had to be sacrificed at the cost of correct brain orientation during cutting procedure.

To assess the degree of individual variation occurring in the population of thirty-three brains, the positions of four structures with different anteroposterior locations were examined in each brain, namely the oculomotor nucleus, the red nucleus, the anteroventral thalamic nucleus, and the anterior commissure. The stereotaxic coordinates within each brain having been determined, the mean values and the standard deviations (SD) of the coordinates were calculated and tabulated (Table I). As a reference for construction of the present atlas we took brains in which the stereotaxic coordinates of all named structures were found to approximate most closely the corresponding mean values.

Results

As INDICATED in Table I, the smallest SDs in the orocaudal direction were found between frontal planes A 10.0 mm and A 15.0 mm (approximately corresponding to the red nucleus and anteroventral thalamic nucleus respectively). The tendency for SDs to increase both anteriorly to the plane A 15.0 mm and posteriorly to the plane A 10.0 mm was probably due to the steeper angle of the brain stem against the horizontal plane. The greater heterogeneity in the horizontal plane (see Table I) was caused, in addition to the reasons given above, by the slight increase in flexibility of the electrode holder in this position. In the sagittal plane the accuracy was found to decrease with increasing distance from the midline. However, we feel it is important that for almost 60 per cent of the brains used in this study the maximum variation in the orocaudal direction was less than ± 0.5 mm.

TABLE I
INDIVIDUAL VARIATIONS OF BRAIN STRUCTURES

Structure	*Frontal plane* x *value* SD	*Horizontal plane* x *value* SD	*Sagittal plane* x *value* SD
Nucleus nervi oculomotorii	6.64 ± 0.98 mm	1.50 ± 1.36 mm	0*
Nucleus ruber	9.83 ± 0.73 mm	1.19 ± 1.30 mm	1.33 ± 0.15 mm
Nucleus anteroventralis thalami	14.16 ± 0.79 mm	7.33 ± 1.40 mm	2.06 ± 0.17 mm
Commissura anterior	18.25 ± 1.11 mm	5.78 ± 1.42 mm	0

SD: Standard deviation
* midline structures

Discussion

ONE-YEAR-OLD RABBITS come closer to an optimal age for stereotaxic purposes than did younger animals. This was revealed in the preliminary study performed on twenty subjects which were five months of age, since this group showed much greater heterogeneity of brain structure location than did one-year-old rabbits. Such results seem to reflect a pronounced individual difference in the rate of skull growth among young animals—a difference which tends to balance out later with increasing age. A similar situation has been reported in sheep (24). Nonetheless, the degree of individual variation found in the present study of one-year-old rabbits associated with adoption of the Horsley-Clarke system of basal planes using Chatelier and Buser technique of head fixation is thought to be still slightly greater than that in other species. This is probably due to the obliqueness of auditory meatus and their occasional asymmetry. Consequently, it is not always possible to obtain such a precise orientation of the head in the stereotaxic instrument as to form the required right angle between the auditory meatus and the sagittal plane.

But despite these facts, one is still safe to conclude that the accuracy of the Horsley-Clarke method when applied to the one-year-old rabbits is adequate and generally comparable with such achieved in other species. Moreover, the possibility for substantial reduction of error exists, particularly in the horizontal plane, if recording of electrical activity from penetrated structures is simultaneously used.

Nomenclature

THE ANATOMICAL DESCRIPTION of the brain structures is based on certain published morphological studies of the rabbit's central nervous system. These include Winkler and Potter's neuroanatomical atlas of the rabbit brain (35), cytoarchitectonic description of the hypothalamus (8), cortex (27), telencephalon (36), pretectal region (18), nontectal part of the mesencephalon (6), a study on the structure and relations of the limbic cortex with anterior thalamus (27), the projection of fibers from amygdaloid nuclei (2), tactile projection in the thalamus (26), and recent work on tectothalamic connections in the rabbit (32). The terminology set out in *Nomina Anatomica* (Paris, 1967) was adhered to whenever possible except for those few structures described exclusively in the rabbit where reference has been given.

Abbreviations

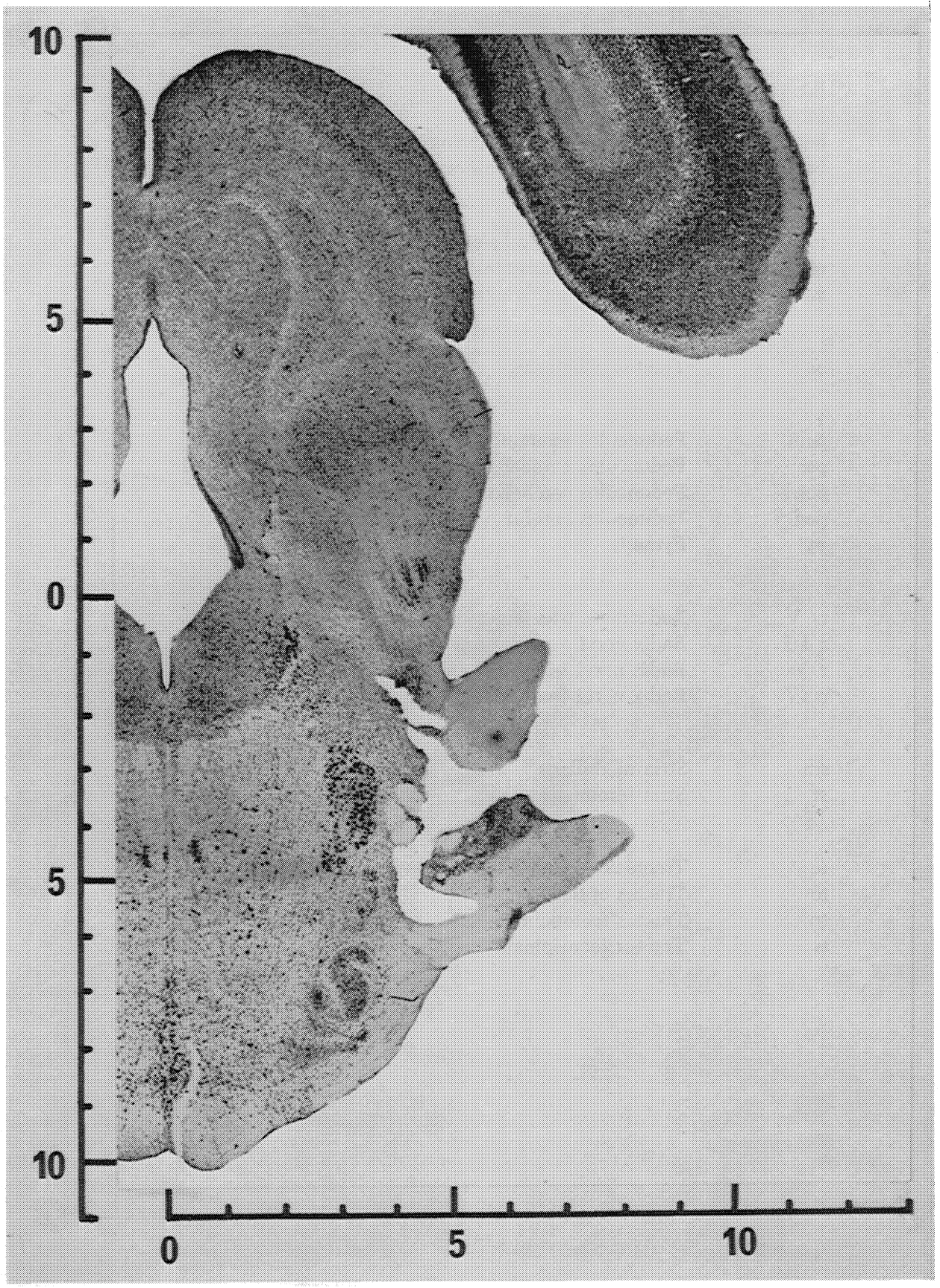
AA	Area amygdaloid anterior
AB	Nucleus amygdaloid basalis
ABL	Nucleus amygdaloid basalis pars lateralis
ABM	Nucleus amygdaloid basalis pars medialis
AC	Nucleus amygdaloid corticalis
ACC	Nucleus accombens
ACE	Nucleus amygdaloid centralis
AD	Nucleus anterodorsalis thalami
ALA	Nucleus amygdaloid lateralis pars anterior
ALP	Nucleus amygdaloid lateralis pars posterior
AM	Nucleus anteromedialis thalami
AME	Nucleus amygdaloid medialis
ASL	Area septalis lateralis
ASY	Aqueductus Sylvii
AV	Nucleus anteroventralis thalami
BCI	Nucleus brachii colliculi inferioris
CA	Nucleus commissurae anterioris
CD	Nucleus caudatus
CEM	Nucleus centralis medialis
CF	Campi Foreli
CHD	Nucleus commissurae hippocampi dorsalis
CHM	Nucleus tracti corticohabenularis medialis
CI	Colliculus inferior
CL	Nucleus centralis lateralis (19) (Nucleus angularis [26])
CLA	Claustrum
CM	Centrum medianum
CP	Nuclei commissurae posterioris
CPY	Cortex pyriformis
CSU	Nucleus centralis superior
D	Nucleus Darkschewitsch
DBB	Nucleus tracti diagonalis telencephali (Broca)
EW	Nucleus Edinger-Westphal
FHI	Fissura hippocampi
FR	Formatio reticularis
FRH	Fissura rhinalis

GLD	Corpus geniculatum laterale dorsale
GLV	Corpus geniculatum laterale ventrale
GM	Corpus geniculatum mediale
GP	Globus pallidus
GYD	Gyrus dentatus hippocampi
HA	Area hypothalamica anterior
HD	Area hypothalamica dorsalis
HDM	Nucleus dorsomedialis hypothalami
HID	Hippocampus dorsalis
HIV	Hippocampus ventralis
HL	Nucleus habenulae lateralis
HM	Nucleus habenulae medialis
HP	Area hypothalamica posterior
HVM	Nucleus ventromedialis hypothalami
HYL	Area hypothalamica lateralis
IFLM	Nucleus interstitialis fasciculi longitudinalis medialis
IMD	Nucleus intermediodorsalis
INF	Nucleus infundibularis
IP	Nucleus interpeduncularis
IST	Nucleus interstitialis striae terminalis
IV	Nucleus interventralis
LD	Nucleus lateralis pars dorsalis
LL	Nucleus lemnisci lateralis
LP	Nucleus lateralis pars posterior
MA	Nucleus mammillaris anterior
MD	Nucleus medialis dorsalis thalami
MP	Nucleus mammillaris posterior
MG	Nucleus preopticus magnocellularis (36)
ML	Nucleus mammillaris lateralis
MML	Nucleus mammillaris medialis pars lateralis
MMM	Nucleus mammillaris medialis pars medialis
MV	Nucleus medialis ventralis (25)
NPOM	Nucleus preopticus medialis
N III	Nucleus nervi oculomotorii
N IV	Nucleus nervi trochlearis
N VM	Nucleus nervi trigemini pars motorica
N VS	Nucleus nervi trigemini pars sensorica
OL	Nucleus tracti olfactorii
OS	Nucleus olivaris superior
PAV	Nucleus paraventricularis thalami anterior
PC	Nucleus paracentralis
PCS	Nucleus pedunculi cerebellaris superior

PED	Nucleus paraventricularis hypothalami dorsalis
PEP	Nucleus preopticus paraventricularis
PF	Nucleus parafascicularis
PL	Nucleus pontis lateralis
PM	Nucleus pontis medialis
POL	Area preoptica lateralis
POM	Area preoptica medialis
PO+PLV	Posterior thalamic complex + pulvinar
PP	Nucleus preopticus principalis
PRM	Nucleus premammillaris
PT	Nucleus paratenialis
PTA	Nucleus pretectalis anterior
PTM	Nucleus pretectalis medialis
PTP	Nucleus pretectalis posterior
PU	Putamen
PV	Nucleus paraventricularis hypothalami
PVP	Nucleus paraventricularis thalami posterior
R	Nucleus ruber
RD	Nucleus raphae tegmenti dorsalis
RE	Nucleus reuniens
RET	Nucleus reticularis thalami
RH	Nucleus rhomboides
RT	Nucleus reticularis tegmenti Bechterew
RV	Nucleus raphae tegmenti ventralis
SCH	Nucleus suprachiasmaticus
SEF	Nucleus septalis fimbrialis
SG	Nucleus supragenículatum
SGC	Substantia grisea centralis
SGM	Stratum griseum medium colliculi superioris
SGP	Stratum griseum profundum colliculi superioris
SGPT	Nucleus supragenículatum pretectalis (32)
SGS	Stratum griseum superfitiale colloculi superioris
SH	Nucleus tracti septohabenularis
SL	Nucleus septalis lateralis
SM	Nucleus septalis medialis
SMA	Nucleus supramammillaris
SN	Substantia nigra
SO	Nucleus supraopticus
ST	Nucleus striae terminalis
STH	Nucleus subthalamicus
TBC	Area tuberis cinerei
TD	Nucleus tegmentalis dorsalis
TGV	Area tegmentalis ventralis Tsai
TO	Nucleus tracti optici
TP	Nucleus tegmentalis profundus

TR	Nucleus corporis trapezoides
TRI	Nucleus triangularis
V	Ventriculus
VA	Nucleus ventralis anterior thalami
VFLM	Nucleus ventralis fasciculi longitudinalis medialis
VL	Nucleus ventralis lateralis thalami
VM	Nucleus ventralis medialis thalami
VP	Nucleus ventralis posterior thalami
ZI	Zona incerta
ZIC	Zona incerta pars caudalis
ac	Tractus accusticus centralis
bci	Brachium colliculi inferioris
bcs	Brachium colliculi superioris
bp	Brachium pontis
ca	Commissura anterior
cala	Commissura anterior limbus anterior
calp	Commissura anterior limbus posterior
cc	Corpus callosum
ccs	Commissura colliculi superioris
cex	Capsula externa
cfx	Columna fornicis
chd	Commissura hippocampi dorsalis
chv	Commissura hippocampi ventralis
cho	Chiasma fasciculi optici
ci	Capsula interna
cin	Cingulum
cp	Commissura posterior
ct	Corpus trapezoides
dbb	Fasciculus diagonalis telencephali Broca
dct	Deccussatio corporis trapezoides
dpcs	Decussatio pedunculorum cerebellaris superior
drs	Decussatio tracti rubrospinalis
dts	Decussatio tracti tectospinalis
fi	Fimbria hippocampi
flm	Fasciculus longitudinalis medialis
fr	Fasciculus retroflexus
fpd	Fasciculus predorsalis
fsc	Fasciculus subcallosus

hia	Hippocampus pars anterior
ll	Lemniscus lateralis
lm	Lemniscus medialis
lmex	Lamina medullaris externa
lmi	Lamina medullaris interna
mat	Tractus mammillothalamicus
mfb	Fasciculus medialis telencephali
mt	Tractus mammillotegmentalis
ol	Tractus olfactorius
pcm	Pedunculus mammillaris
pcs	Pedunculus cerebellaris superior
pcsd	Pedunculus cerebellaris superior pars descendens
ped	Pedunculus cerebri
py	Pyramis
rs V	Radix nervi trigemini pars spinalis
r III	Radix nervi oculomotorii
r V	Radix nervi trigemini
r IV	Radix nervi trochlearis
sm	Stria medullaris
st	Stria terminalis
tc	Tractus tegmentalis centralis
to	Tractus opticus
tp	Tractus tectoponticus
ts	Tractus tectospinalis



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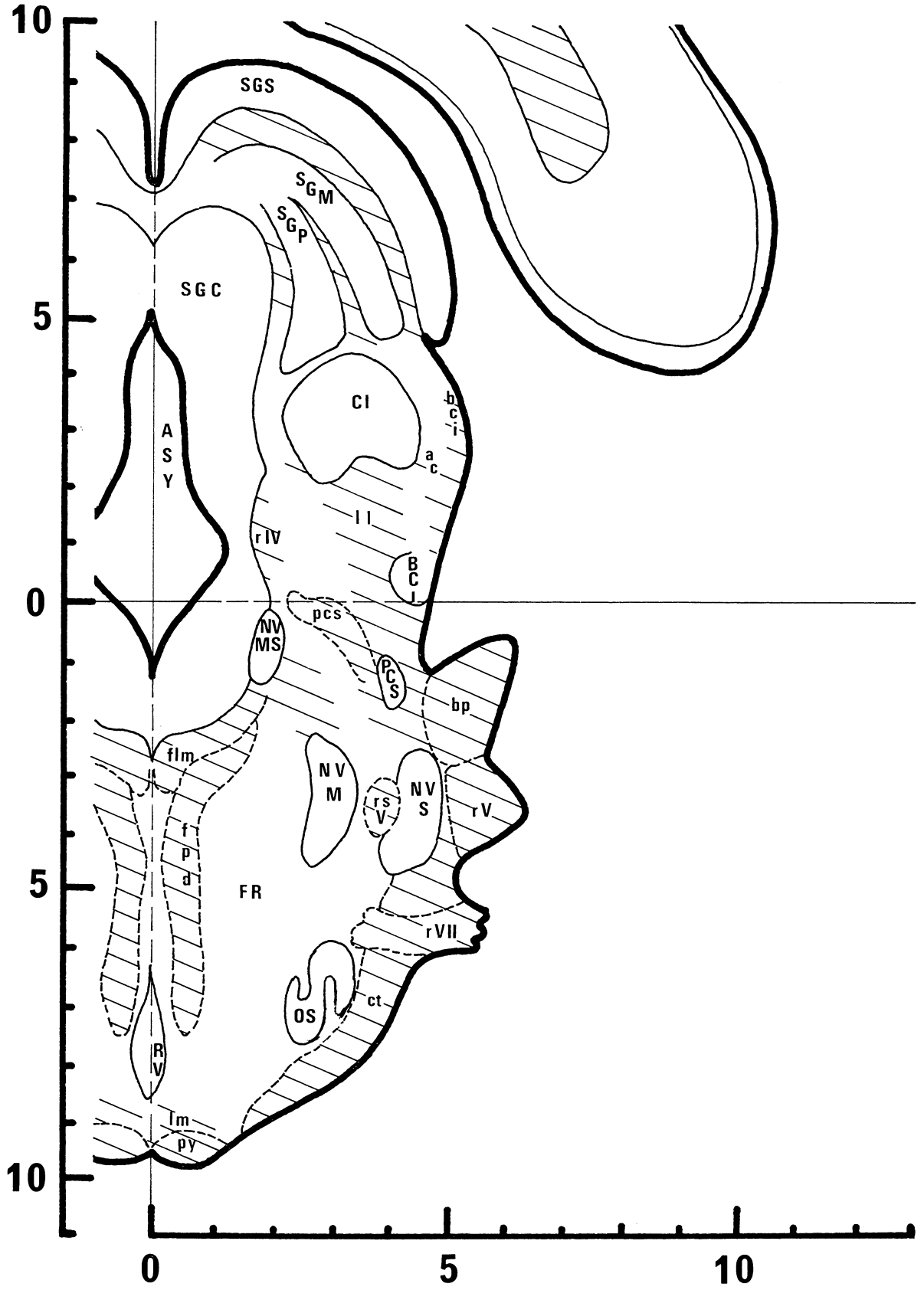
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