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# The forebrain of the goat in stereotaxic coordinates

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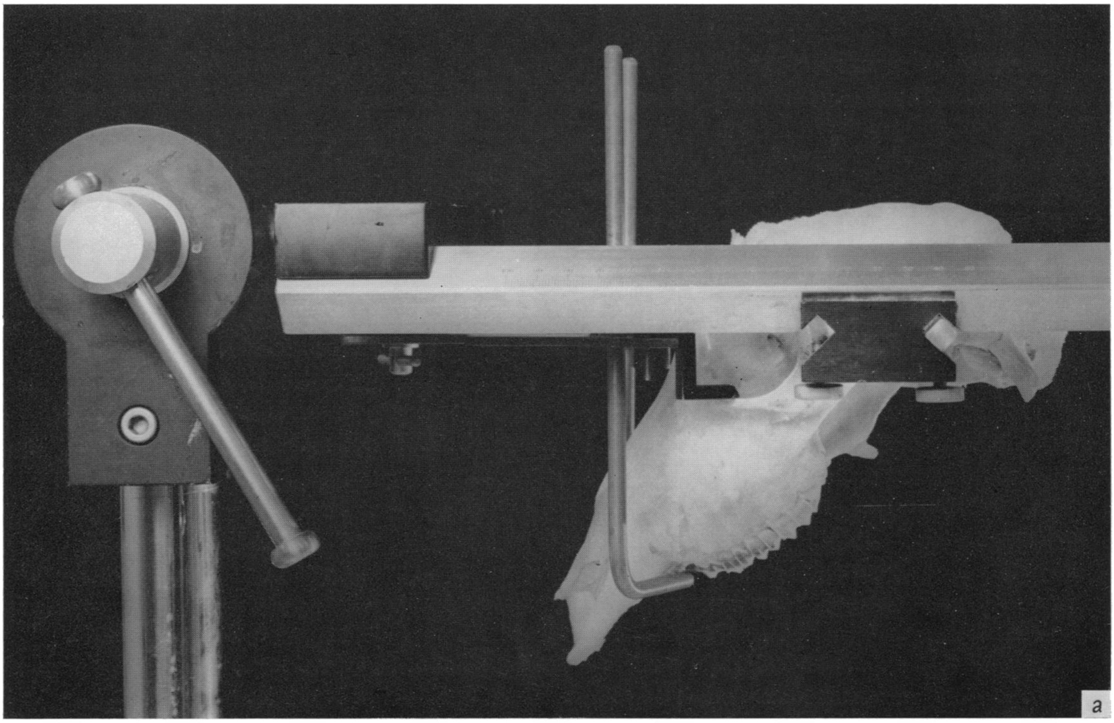
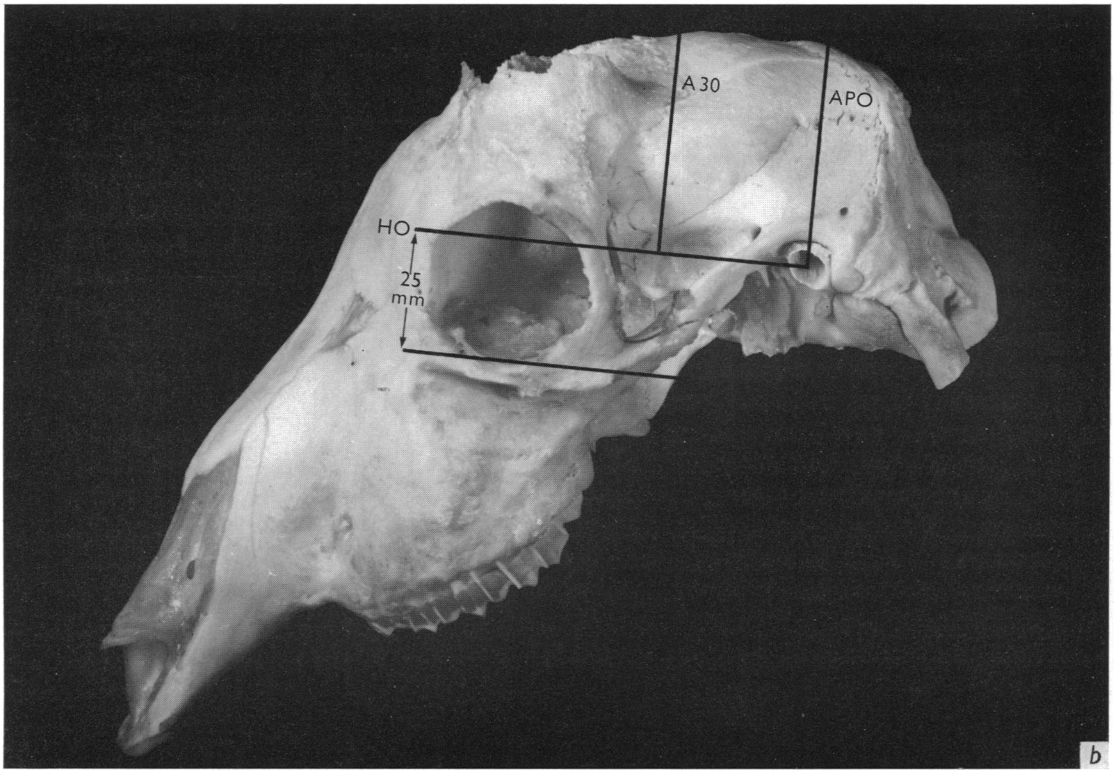
## INTRODUCTION

The goat has been used for many years in this Department for the study of the endocrine control of the mammary gland, and, in particular, the role of the pituitary hormones. In order to investigate the neuroendocrine mechanisms involved in mammary growth and milk secretion in the goat, a stereotaxic technique is essential for the accurate insertion of electrodes, or cannulae bearing steroids or drugs, into specific brain structures. Since, as far as is known, no atlas exists for the goat brain, it was necessary to construct an atlas of stereotaxic coordinates for the brain of the adult goat to facilitate our studies.

## MATERIALS AND METHODS

Nine female British Saanen goats from the herd maintained in this Department, weighing 54–77 kg and ranging in age from 1 yr 9 mo. to 4 yr 5 mo., were used for determination and confirmation of stereotaxic coordinates. Each animal was killed by intravenous injection of pentobarbitone sodium B.P., the carotid arteries were cannulated, the external jugular veins lanced and the neck severed in the mid-cervical region. The head was perfused with 3 l of 0·9 % NaCl solution, followed by 3 l of 10 % formalin, after which a portion of the occipital region of the skull was removed. The head was then mounted in a stereotaxic instrument (made by Libero Bonetti, Bologna, Italy). The position of the skull in relation to the instrument is shown in Fig. 1*a*. Graduated ear bars, of square cross-section, and which taper to a blunt point, were inserted in the external auditory meati, with the head held below the stereotaxic frame. The head was then raised to allow the ear bars to be located in their slots in the frame, after which they were clamped in position with their tips equidistant from each side of it. The front of the head was supported by two vertical rods, of circular cross-section, and bent at right-angles at their lower ends. These short horizontal ends of the rods were inserted at the sides of the mouth to rest against the hard palate and the jaw was bound to them with cotton bandage. The rods themselves were clamped to a horizontal plate at the front of the instrument, whose position could be adjusted forwards or backwards. This plate also carried two angled eye bars, shaped like a capital letter Z which has been straightened out so that the two horizontal arms are at right angles to the vertical arm. The angled eye bars were mounted by their top extremities to the plate and were free to swing from side to side. The tips of their lower extremities were exactly 25 mm below the horizontal plane passing through the meeting-point of the ear bars. The jaw was either raised or lowered by sliding the rods which were bound to it up or down, until the lower tips of the two-angled eye bars rested on the lower margins of the orbits (see Fig. 1*a*).


a


A 30
APO
HO
25
mm
b

This system of supporting the head was originally devised for the sheep by Professor F. R. Bell, Royal Veterinary College, London (personal communication).

Taking the vertical interaural plane as the anterior–posterior zero reference point (APO) and the horizontal interaural plane as horizontal zero (HO) (see Fig. 1*b*), holes were drilled in the skull 10, 20 and 30 mm rostral to APO, and a length of 24 s.w.g. stainless steel tubing was lowered vertically to HO at each of these positions. A length of 18 s.w.g. stainless steel tubing was inserted horizontally in the sagittal plane at HO, entering the brain at the junction of cerebellum and medulla oblongata. Each of the vertical tubes was held in position by a drop of dental acrylic placed at the top of the drill hole in the skull, while the horizontal tube had sufficient of its length within the brain to be self-supporting. The head was taken out of the stereotaxic instrument, the remainder of the neck and the lower jaw were removed, and some of the skull chipped away with rongeurs to allow free access for the fixative. The head was then immersed in 10% formalin for 2 weeks until the brain had hardened. The remainder of the skull was then removed, save for a small plate of bone immediately surrounding the vertical steel tubes, and the pituitary stalk was severed as low down as possible. After removal of the dura mater, the plate of bone with the vertical tubes attached was withdrawn vertically 20–25 mm. The brain was lined up on the bench so that the 18 s.w.g. tube was horizontal and the 24 s.w.g. tubes were vertical. Since the length of the horizontal tube was known, it was possible to withdraw it caudally until its rostral end lay caudal to A 30, and the brain was then cut transversely in the vertical stereotaxic plane just rostral to A 30. The horizontal tube was then withdrawn completely and a second transverse cut made just caudal to APO. After immersion in 20% ethanol for 24 h to minimize crystallization during the cutting process, the brain was mounted on the block of a sledge microtome, using the projecting 24 s.w.g. tubes to assist in levelling the brain accurately, after which they were withdrawn completely. The brain was then frozen, and serial sections 80 $\mu$m thick were cut by a modification of the dry-ice method of Marshall (1940). In agreement with a previous study (Tindal, 1965), it was found that the frozen-section technique causes only trivial shrinkage of brain tissue. Six brains were cut in the transverse plane, and pairs of sections were saved every 0·5 mm. One of each pair was stained for cellular structures with toluidine blue; the other was stained for myelinated fibres using the Weil technique, or in the more recent work the solochrome cyanin method (Page, 1965).

After studying all the material and the position and spacing of marker tracks, one brain was selected to provide the final planes for the atlas. Slides of brain tissue were projected in a photographic enlarger so that the image was exactly four times the size of the original. Tracings were made of the outlines of sections and of major

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Fig. 1. (*a*) A goat's skull shown in position in the stereotaxic instrument. The ear bars, located in the posterior pair of slots, are inserted in the external auditory meati, the jaw rods rest on the hard palate to support the front of the head, and the angled eye bars rest on the lower margins of the orbits. (*b*) Stereotaxic coordinates superimposed on a goat's skull. The vertical interaural plane is the anterior-posterior reference point for coordinates (APO) and the horizontal zero plane (HO) intersects the interaural point and passes forward 25 mm above the lower margin of the orbit. This plane is coincident with the lower edge of the graduated stereotaxic frame in the upper photograph.

structures for the transverse stereotaxic planes A2 to A30. Fine detail was added later after microscopic study of the histological sections from this and the other five brains, since individual nuclear structures often happened to be stained more clearly in one particular specimen than in the others, presumably due to slight variations in the degree of differentiation during staining. Sagittal reconstructions were made from this series of histological sections of the mid-sagittal plane and at 3 mm lateral to this plane.

In addition to the six brains cut in the transverse plane, serial sections 80 $\mu$m thick were cut from one brain in the sagittal plane by the dry-ice method, and served as a useful guide when making the sagittal reconstructions. The two remaining brains were dissected out of the skull with the sella turcica attached; each was trimmed to a block of tissue comprising diencephalon and sella turcica, and then immersed in decalcifying fluid for 10 d. Serial sections 80 $\mu$m thick were cut from one of them in the sagittal plane by the dry-ice method, while the other was embedded in low melting-point ester-wax and serial sections 20 $\mu$m thick were cut in the sagittal plane. These two brains were studied to determine both the outline of the stalk-median eminence region and the shape of the pituitary gland and its position relative to the base of the brain.

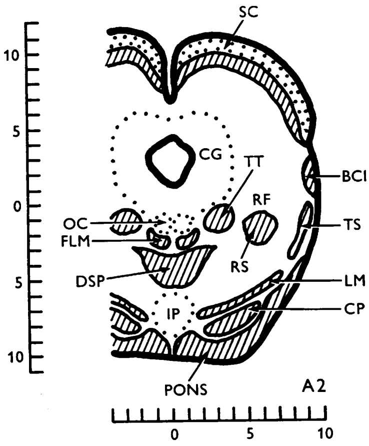
Literature consulted for identification of brain structures was as follows: Solnitzky (1938), Arai (1939), Rose (1942), Jasper & Ajmone-Marsan (1961), Welento (1964) and Richard (1967) for the diencephalon, Fukuchi (1952) for the amygdala, and Lim, Liu & Moffitt (1960), Chomiak (1963) and Adrianov & Mering (1964) for the mesencephalon.

## RESULTS

Transverse stereotaxic planes passing rostrally at 1 mm intervals from anterior 2 mm to anterior 30 mm appear in Figs. 2–30. Sagittal reconstructions in the mid-sagittal plane and at 3 mm lateral to the mid-line showing the outlines of major structures and fibre tracts appear in Figs. 31 and 32 respectively. Comparison of the transverse stereotaxic planes of the brain chosen for the atlas with the planes of the five other brains cut in the transverse plane showed that variations in anterior–posterior coordinates between brains were surprisingly small. One brain varied from the reference brain between 0·5 and 1·5 mm at different levels, one varied by 1 mm uniformly throughout all planes, two agreed to within 0·5 mm, and one brain was an exact match. Measurements of the preserved brains before histological processing indicated that there was some variation in total brain size between animals; thus the maximum width of the brain varied between 61 and 63 mm, while the length of the cerebral hemispheres, measured between occipital and frontal poles, varied between 69 and 75 mm. These variations could be accounted for primarily by differences in the extent of the cerebral cortex. Moreover, the variations in length appeared to concern those parts of the brain rostral and caudal to the portion chosen for the atlas. The 2 mm variation in width means that the stereotaxic reference within a given transverse plane may, in some animals, bear an inherent (i.e. as opposed to an experimental) error of approximately 1 mm for cortical structures. The position of structures in the brainstem with reference to the stereotaxic coordinates was remark-

## ABBREVIATIONS

| | |
|---|---|
| AA | area amygdala anterior |
| AB | nucleus amygdala basalis |
| AC | commissura anterior |
| ACE | nucleus amygdala centralis |
| ACO | nucleus amygdala corticalis |
| AD | nucleus anterior dorsalis |
| AHA | area hypothalamica anterior |
| AL | nucleus amygdala lateralis |
| AM | nucleus anterior medialis |
| AME | nucleus amygdala medialis |
| ASL | area septalis lateralis |
| ASM | area septalis medialis |
| AV | nucleus anterior ventralis |
| BCI | brachium colliculi inferioris |
| BCS | brachium colliculi superioris |
| CA | nucleus caudatus |
| CC | corpus callosum |
| CE | capsula externa |
| CF | campi Foreli |
| CG | substantia grisea centralis |
| CI | capsula interna |
| CL | claustrum |
| CM | nucleus centrum medianum |
| CP | pedunculus cerebri |
| CSC | commissura colliculi superioris |
| DMH | nucleus hypothalamicus dorsalis medialis |
| DS | decussatio supramamillaris |
| DSP | decussatio pedunculorum cerebellarium superiorum |
| DT | decussatio tegmenti |
| EP | epiphysis |
| FIM | fimbria hippocampi |
| FLM | fasciculus longitudinalis medialis |
| FR | fasciculus retroflexus |
| FS | fasciculus subcallosus |
| FX | fornix |
| GP | globus pallidus |
| HIP | hippocampus |
| HL | nucleus habenula lateralis |
| HM | nucleus habenula medialis |
| IP | nucleus interpeduncularis |
| IV | nucleus interventralis |
| LD | nucleus lateralis dorsalis |
| LGD | nucleus corpus geniculatum lateralis dorsalis |
| LGV | nucleus corpus geniculatum lateralis ventralis |
| LM | lemniscus medialis |
| LME | lamina medullaris externa |
| LP | nucleus lateralis posterior |
| MD | nucleus medialis dorsalis |
| MG | nucleus corpus geniculatum medialis |
| ML | corpus mamillaris lateralis |
| MM | corpus mamillaris medialis |
| MT | fasciculus mamillothalamicus |
| MV | nucleus medialis ventralis |
| NCL | nucleus centralis lateralis |
| NCM | nucleus centralis medialis |
| ND | nucleus Darkschevitch |
| NI | nucleus interstitialis |
| NP | nucleus premamillaris |
| NCP | nucleus commissura posterior |
| NR | nucleus ruber |
| NTO | nucleus tractus opticus |
| OC | nucleus N. oculomotorius |
| OCH | chiasma opticum |
| OCN | N. oculomotorius |
| OT | tractus opticus |
| P | commissura posterior |
| PC | nucleus paracentralis |
| PF | nucleus parafascicularis |
| PM | pedunculus corpus mamillaris |
| PO | area preoptica |
| PTA | nucleus pretectalis anterior |
| PTM | nucleus pretectalis medialis |
| PTP | nucleus pretectalis posterior |
| PUL | pulvinar |
| PUT | putamen |
| PV | nucleus paraventricularis hypothalami |
| PVT | nucleus paraventricularis thalami |
| PYR | cortex pyriformis |
| RE | nucleus reuniens |
| RF | formatio reticularis |
| RH | nucleus rhomboideus |
| RS | tractus rubrospinalis |
| RT | nucleus reticularis thalami |
| SC | colliculus superior |
| SCH | nucleus suprachiasmaticus |
| SG | nucleus suprageniculatus |
| SM | stria medullaris thalami |
| SN | substantia nigra |
| SO | nucleus supraopticus |
| ST | stria terminalis |
| STH | nucleus subthalamicus |
| T | nucleus parataenialis |
| TOL | tractus olfactorius lateralis |
| TS | tractus spinothalamicus |
| TT | tractus tegmentalis centralis |
| VA | nucleus ventralis anterior |
| VL | nucleus ventralis lateralis |
| VM | nucleus ventralis medialis |
| VMH | nucleus hypothalamicus ventralis medialis |
| VPL | nucleus ventralis posterior lateralis |
| VPM | nucleus ventralis posterior medialis |
| ZI | zona incerta |



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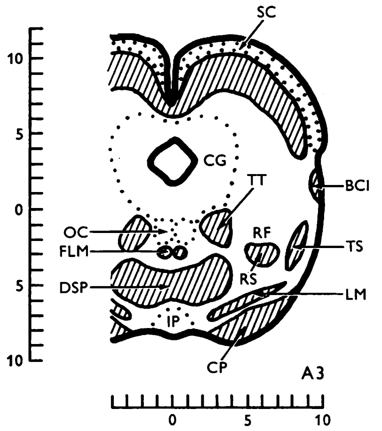

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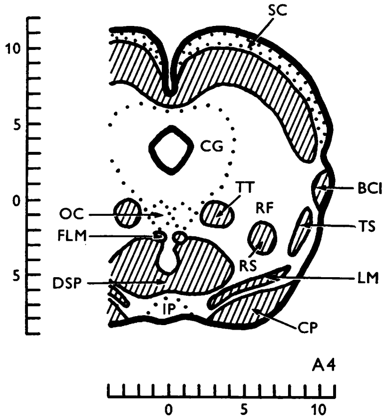

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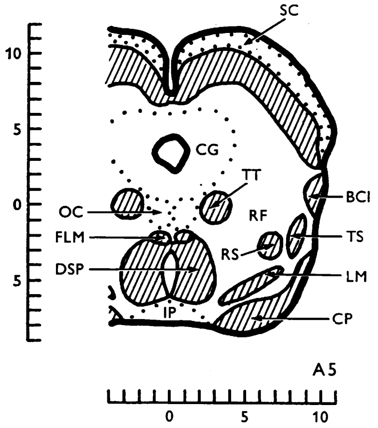

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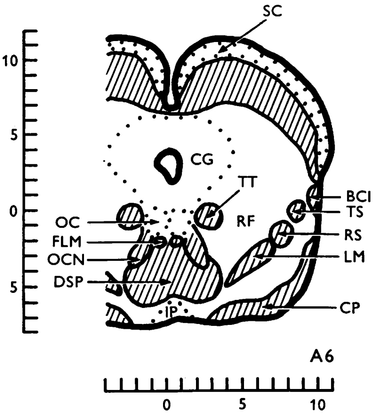

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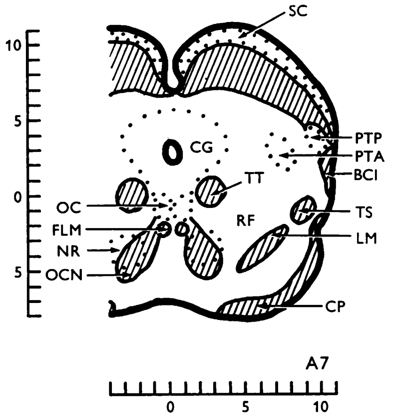
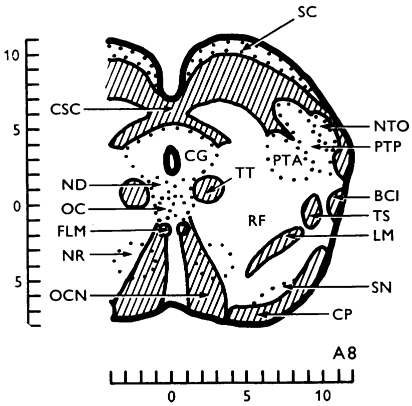


Fig. 7

Figs. 2–30. Tracings from projections of transverse sections of goat brain at 1 mm intervals from 2 to 30 mm rostral to the vertical interaural plane. Scales are in mm.



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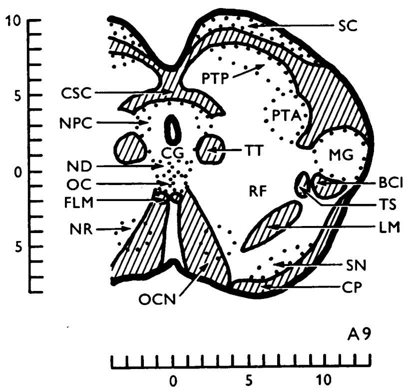

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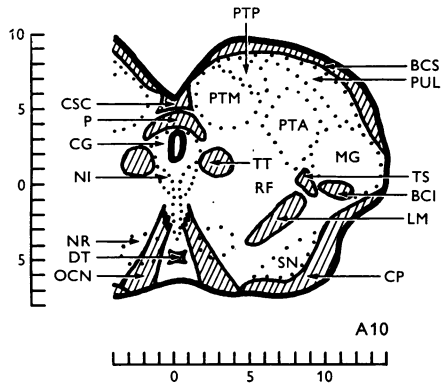
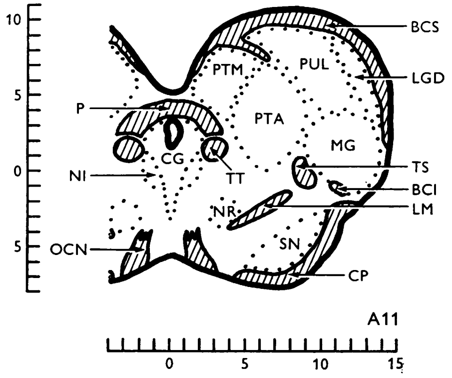


Fig. 10



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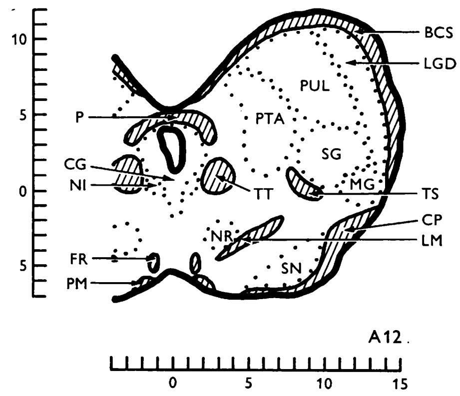

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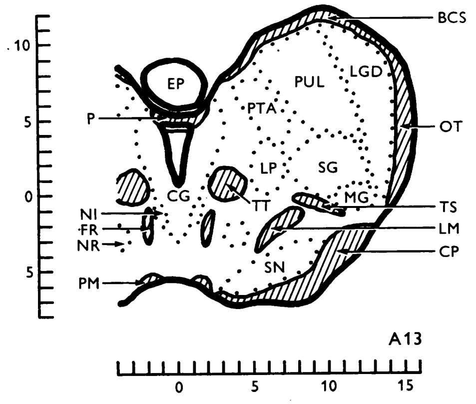

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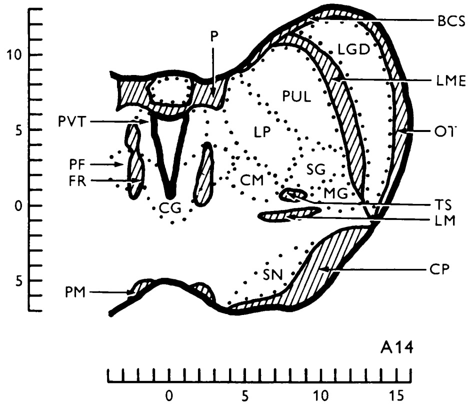

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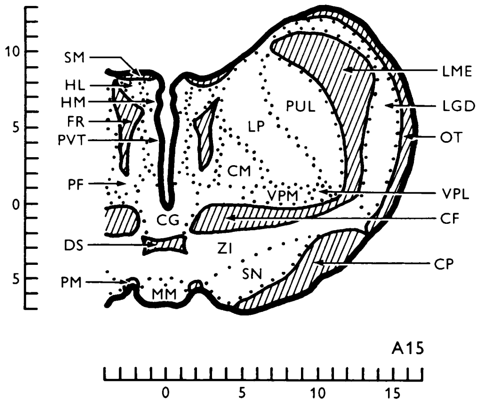

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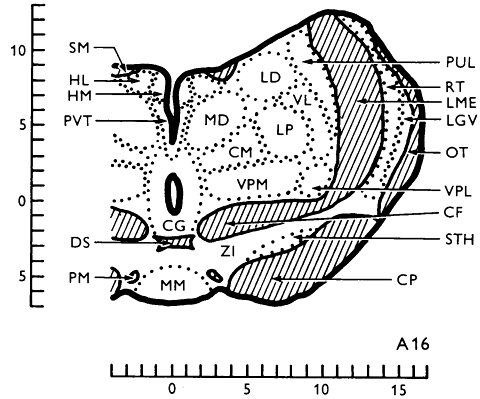

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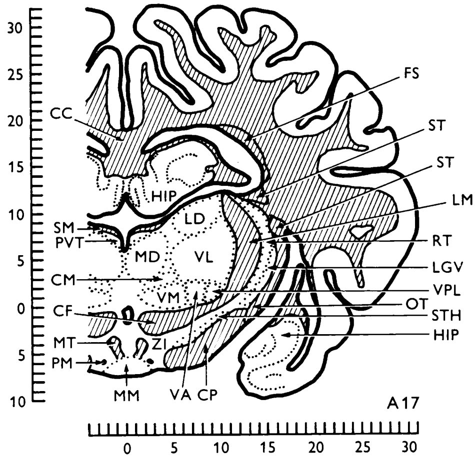

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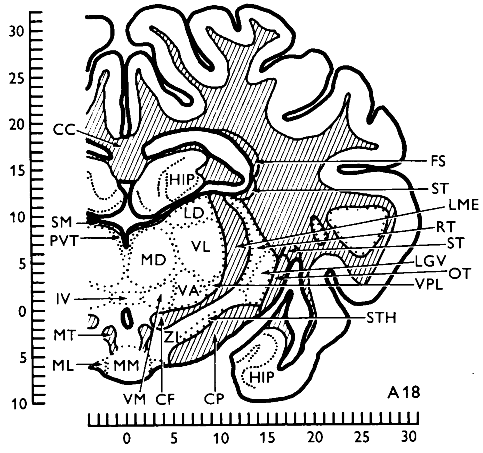

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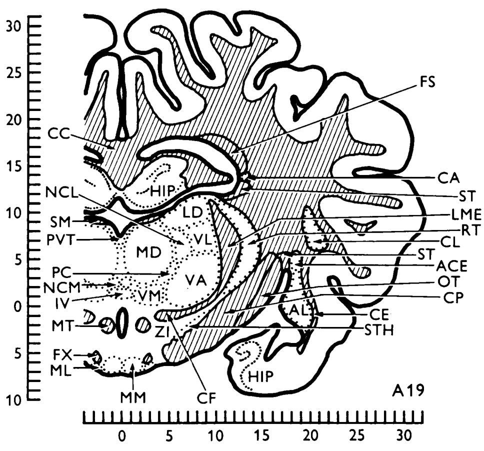

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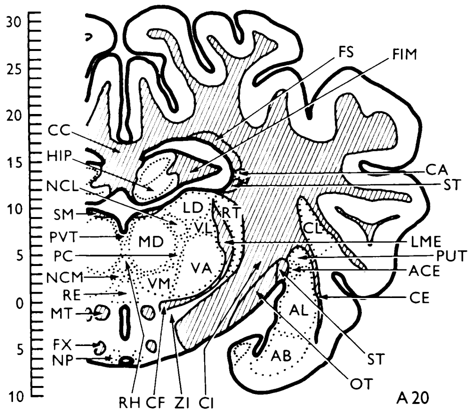

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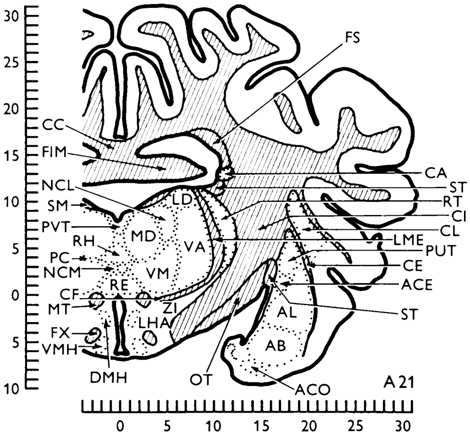

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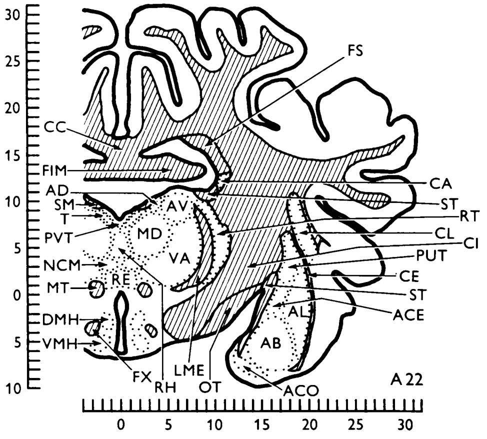

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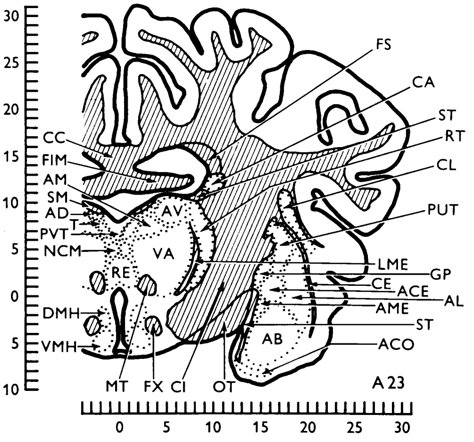

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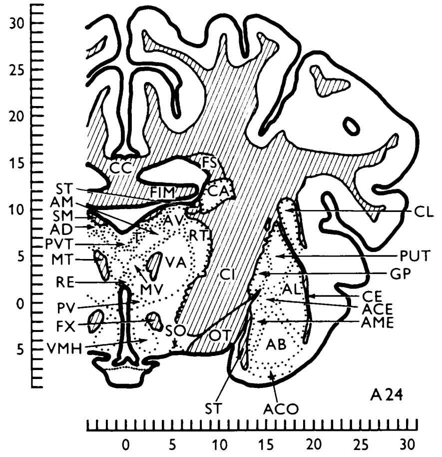

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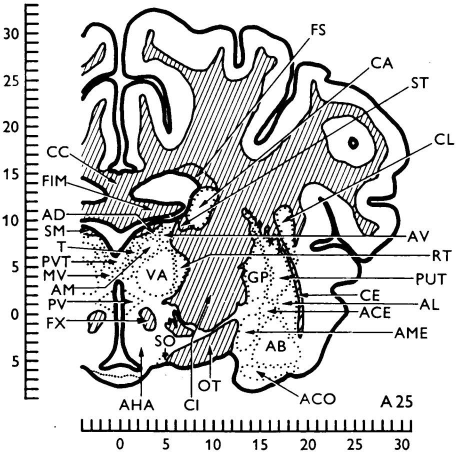

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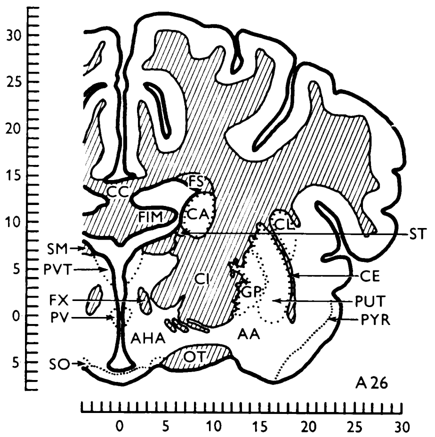

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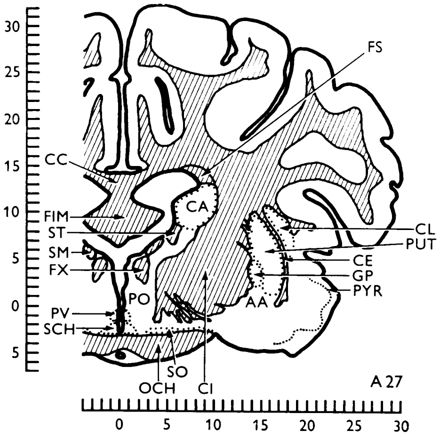
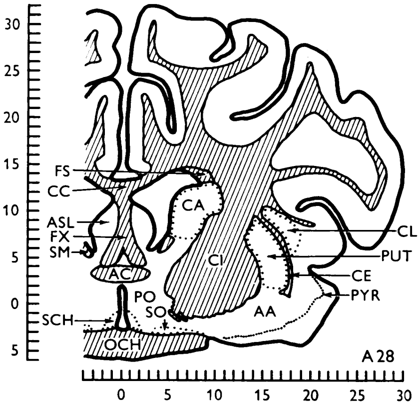


Fig. 27



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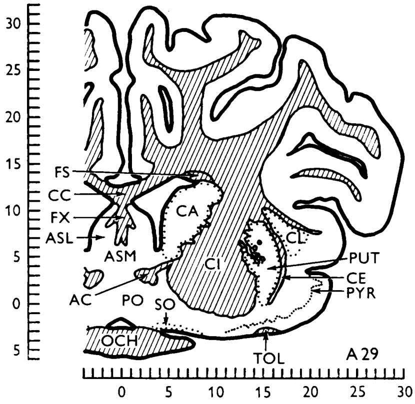

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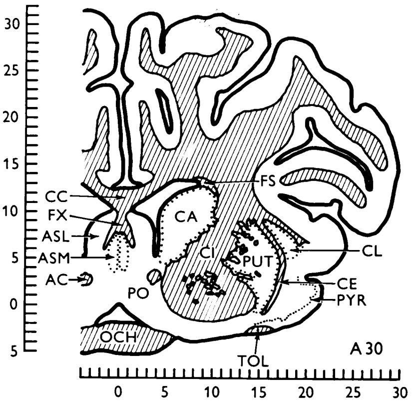

Fig. 30

ably constant, and subsequent unpublished experiments *in vivo* have shown that it is possible to implant electrodes in basal hypothalamic structures with an error of usually not more, and sometimes less, than 1 mm.

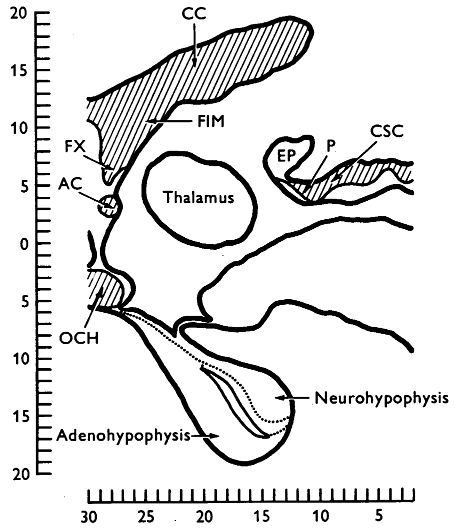

Fig. 31

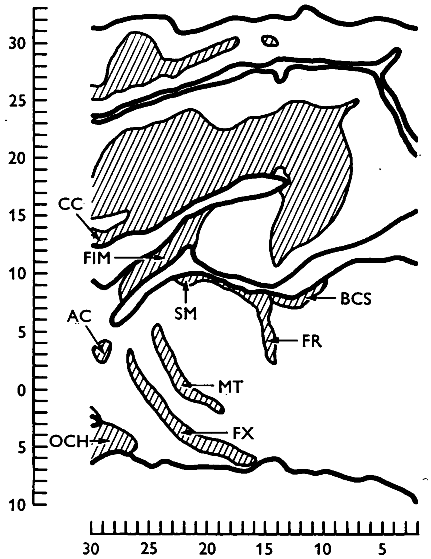

Fig. 32

Fig. 31. Mid-sagittal representation of goat brain, constructed from the transverse planes of the atlas. Scales are in mm.

Fig. 32. Sagittal representation of goat brain at 3 mm lateral to the mid-line, constructed from the transverse planes of the atlas. Scales are in mm.

## DISCUSSION

The small ruminant was used for studies of brain function by Andersson (1951), who used X-rays to localize electrodes in the goat and sheep. Cooper, Daniel & Whitteridge (1953) utilized the stereotaxic-coordinate approach in the young goat, while, more recently, Traczyk & Przekop (1963) described a stereotaxic method for the sheep, based on that of Cooper *et al.* (1953), and Richard (1967), using a similar technique, has published an atlas of stereotaxic coordinates for the sheep brain. X-ray localization of electrodes in the sheep brain has also been used by Clegg & Ganong (1960) and by Radford (1967), while methods involving functional localization have been reported for placement of electrodes in the hypothalamus of the goat (Andersson, Persson & Ström, 1960; Baile, Mahoney & Mayer, 1967), but such methods are only applicable to brain structures where some immediate response, motor or otherwise, can be elicited by electrical stimulation.

In our hands, the stereotaxic-coordinate approach has proved to be satisfactory. However, as pointed out by Richard (1967) for the sheep, extreme care should be taken when inserting the ear bars to avoid damaging the cartilage of the ear. If damage is caused, then it becomes difficult to make a correct insertion of the bars and the head may become displaced by 2–3 mm in relation to the anterior–posterior coordinates. Bearing this reservation in mind, although the absolute errors involved in stereotaxy in the goat may be larger than those encountered in some of the small laboratory animals, when the size of the goat brain and structures within it are taken into account, then, in terms of placing an electrode tip in a given structure, the relative accuracy of the stereotaxic technique in the goat has proved to be as satisfactory as it is for the small laboratory animal.

## SUMMARY

A stereotaxic atlas has been prepared of the forebrain of the adult goat. The coordinates used were the vertical interaural plane (APO) and the horizontal plane (HO) intersecting the interaural point and passing forwards 25 mm above the lower margin of the orbit. The atlas consists of drawings of transverse sections through the brain at 1 mm intervals from Anterior 2 mm to Anterior 30 mm, together with two sagittal reconstructions, one in the mid-line and one 3 mm lateral to the midline.

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