## A STEREOTAXIC ATLAS OF THE DIENCEPHALON

OF THE SOUTHDOWN SHEEP

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1971

Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1976

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Thesis Approved:


947637

Appreciation is expressed to Dr. David P. Jennings for serving as principle adviser to my Master's program and providing the facilities and support required to perform this study. Further appreciation is extended to Dr. John H. Venable and Dr. Calvin C. Beames, Jr., for their guidance and helpful suggestions.

My friends associated with this department have provided a stimulating and enjoyable atmosphere in which to work. Rather than enumerate, I thank them all. Their moral support and technical assistance have been invaluable.

Further thanks are extended to Mrs. Dixie Jennings, the typist, for her valuable suggestions, and to Mrs. Christine Jennings for her understanding and helpfulness.

My parents have provided help and encouragement when needed. For this and much more, I am very grateful.

During the course of this study, financial aid and support were provided by USPHA GRS RR05567, NIH NS11978, and Department of Physiological Sciences.

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## CHAPTER I

## INTRODUCTION

In 1908 Horsley and Clark introduced a systematic method called stereotaxis for locating structures in the brain. Their method defined a three-dimensional system of coordinates with respect to the brain using external features of the skull as reference points. The development of a useful stereotaxic map has been a consistent and necessary step to the accurate placement of probes into the living brain, such as cannuli for chemical perfusion and electrodes for recording, stimulating, and lesioning.

Sheep are used widely for neuroendocrine research (Traczyk and Przekop, 1963; Radford, 1967; Richard, 1970; Johnson, Zehr, and Moore, 1970; and Haskins, Jennings, and Rogers, 1975). They offer several advantages. Their sleep-waking behavior has been well described and is easily monitored (Ruckenbush, 1972). They are docile and less excitable to experimental manipulation than are monkeys, cats, or dogs, and thus, are easier to restrain. They are large enough to support microelectrode assemblies for recording neuronal activity, and they have a large blood volume, allowing repeated sampling.

Southdown ewes were selected as the breed of choice for microelectrode studies because of their reported skull uniformity, small size, and availability. Their small size made them less expensive to keep than larger breeds and allowed adaptation of an available cat and dog
stereotaxic head-holder to their skull conformation. The Southdown's blocky body and short neck made it relatively easy to restrain during chronic microelectrode studies.

Prior to this study, no stereotaxic atlas has been available for Southdown sheep. Although research on ruminants has created several stereotaxic atlases [goat (Tindal, Knaggs, and Turvey, 1968); water buffalo (Singh, Singh, Soni, and Manchanda, 1972); Préalpes-Du-Sud sheep (Richard, 1967); and Merino sheep (Welento, Szteyn, and Milart, 1964)], skull variation from one species or breed to another is too great to allow their use on the Southdown. The atlas presented herein was constructed for this purpose. It features plates of each section, both unstained and thionin stained, for quick and easy location of nuclear structures in unstained tissue.

## CHAPTER II

## MATERIALS AND METHODS

## Definition of the Stereotaxic <br> Coordinate System

The stereotaxic coordinate system in this atlas is based on the definition of three orthogonal planes: the horizontal, tranverse, and midsagittal reference planes. These three planes intersect at the zero point, a point midway between the tips of the stereotaxic instrument ear bars. The horizontal reference plane lies parallel to the instrument platform. It and the transverse reference plane intersect along the interaural axis (an imaginary line passing through the center of both external auditory meatus). The midsagittal reference plane bisects the interaural axis thus dividing the brain near the midline. The location of any point in this three dimensional system is defined by frontal, lateral, and vertical coordinates (rectilinear measurements of distance from a point to the three reference planes described).

In this atlas, lateral coordinate axes are lines with a vertical coordinate of zero, lying in the horizontal reference plane and orthogonal to the midsagittal plane; vertical coordinate axes are lines with a lateral coordinate of zero, lying in the midsagittal plane, and orthogonal to the horizontal plane; and the single frontal coordinate axis is a line with a lateral and vertical coordinate of zero lying in the horizontal plane and orthogonal to the transverse plane. Lateral, vertical,
and frontal coordinates are distance measurements in millimeters made parallel to these axes from the proper reference plane.

Orientation of the Head

In 1908 Horsley and Clark, using the rhesus monkey, oriented the horizontal reference plane parallel to the interaural-infraorbital plane (a plane passing through the interaural axis and the center of the lower margin of the boney orbit of the eye). This head orientation also has been used for the dog (Lim, Liu, and Moffitt, 1960), the cat (Snider and Niemer, 1961; Bleir, 1961), and many other animals. Because of angular variation between the longitudinal axis of the brainstem and the interaural-infraorbital plane, this orientation could result in the brainstem being other than horizontal in different animals (Oswaldo-Cruz and Rocha-Miranda, 1968).

For the purpose of this atlas the decision was made to tilt the head at an angle making the longitudinal axis of the brainstem parallel with the frontal coordinate axis. In order to determine the brainstem's orientation within the skull, a sheep head was perfused with formalin and refrigerated for one week. Following formalin fixation, the head was cut in the midsagittal plane with a band saw. This demonstrated that the interaural-infraorbital plane intersected the longitudinal axis of the brainstem at approximately a $20^{\circ}$ angle. A difference in height of 24 millimeters between the infraorbital hooks and the ear bars approximately aligned the frontal coordinate axis with the brainstem axis (Figure 1).


Figure 1. Alignment of the Brainstem With the Frontal
Coordinate Axis. e.b.-ear bar; f.c.a.frontal coordinate axis; i.h.-infraorbital hook; v.c.a.-vertical coordinate axis.

## Modification of Head-Holder

Once a desired orientation of the head was determined, modifications of the Baltimore dog and cat head-holder were designed to achieve this orientation and to accommodate the larger sheep head. The distance between ear bar supports was widened from 150 to 216 millimeters and elevated to a position 24 millimeters above the infraorbital hooks (Figures 2 A and 2 B ). The increased height of the ear bar tips above the infraorbital hooks was calculated to position the interaural-infraorbital axis at approximately a $20^{\circ}$ angle to the frontal coordinate axis (Figure 1). The ear bar supports were designed with an extension at the top of the vertical support bar to allow a lateral radiographic view of the sella tursica.

The increased distance between ear bar supports necessitated extensions for the ear bars as shown in Figure 3. The extensions are in the form of sleeves that fit snugly over the original tips and add an extra 22 millimeters to each ear bar. The ear bar sleeves maintain a diameter of six millimeters and have blunt tips to prevent perforation of the tympanic membrane。

In addition to the ear bars, the other point of fixation is furnished by the infraorbital hooks; maxillary lifters force the infraorbital arches up into this point of fixation. As illustrated in Figure 2D, both the infraorbital hooks and the maxillary lifters were originally on the same bar extending from one side of the moveable platform to the other. The maxillary lifters were left unchanged; however, because of the large snout of the animal, bilateral supports for the infraorbital hooks were necessary (Figure 2C). The modified head-holder is shown in Figure 4.


Figure 2. Modifications of the Head-Holder. A. Original ear bar support; B. Modified ear bar support; C. Modified infraorbital hook support; D. Original support for infraorbital hooks and maxillary lifters.


Figure 3. Modification of the Ear Bars. The sleeve (top illustration) fits snugly over the end of the original ear bar.


Figure 4. Placement of Head in the Head-Holder. e.b.-ear bar; i.h.-infraorbital hook; 1.e.-1esion electrode; m.d.micro drive; m.1.-maxillary lifter; m.pf.-moveable platform; s.pf.-stationary platform.

## Positioning the Sheep Head in Head-Holder

Before positioning each animal in the head-holder, stereotaxic zero coordinates were determined. The moveable platform was fastened to the stationary platform runners and the ear bars were centered. The electrode was fastened to the electrode microdrive and its tip was centered between the ear bars so that frontal, lateral, and vertical "zero coordinates" could be determined. After determining coordinate readings for the electrode tip at zero position, the electrode was moved to one side to allow the sheep head to be placed in the head-holder. General anesthesia was administered to the animal throughout the time it was positioned in the head-holder.

In the experiences of this laboratory, correct placement of both ear bars was the first and most critical step for reasonable stereotaxic accuracy. When sliding the ear bar into an ear canal one must test the tendency of its tip to slip into other cartilagenous depressions; however, care should be taken to avoid damaging the cartilage (Richard, 1967). In agreement with other investigations on the goat, we have found that if damage is sustained, it can be quite difficult to place the ear bars properly (Tindal, et al., 1968). Once the first ear bar is correctly seated, care must be taken to insure it remains seated while fastening it to the ear bar support. The alternate ear bar is then handled in a similar way. Once both ear bars are seated and fastened in the ear bar holders, the head can be centered by sliding the ear bars in the supports. A lapse in pressure at this point has been a prelude to difficult replacement.

With the head centered, a few checks for accurate ear bar placement were routinely made. Movement of the head was attempted with restrained
vigor. The skull was examined to see how level and straight it appeared. The calibration reading on the ear bars was compared with previous readings, our readings being $32 \pm 3$ millimeters. If the previous checks agreed, the infraorbital hooks were positioned at the lowest point of the infraorbital arch. The head was forced up against the hooks by the maxillary lifters and given its final check. The correct angle between the upper edge of the head-holder and the rostral edge of the nose was approximately $125^{\circ}$. Examining the tilt of the head and the centering of the nose from the front was helpful when an error in placement appeared likely as checked by other methods, but in itself, it could occasionally be misleading.

## Lesioning Procedure

A craniotomy was extended with bone rongeurs sufficiently to make a series of paired bilateral lesions at orthogonally determined points five to ten millimeters apart. The lesion points extended from rostral of the diencephalon caudalward to the mamillary bodies. Rostral to the area being studied a set of four lesions was placed for use as reference points when positioning the formalin-fixed diencephalon in the cryostat. Lesions were made by passing a direct current through a glass insulated stainless steel electrode, thus depositing $\mathrm{Fe}^{++}$ions in the tissue at the uninsulated electrode tip. During subsequent perfusion, the $\mathrm{Fe}^{++}$ions were reacted with sodium ferrocyanide producing Prussian blue spots approximately one millimeter in diameter or less.

## Perfusion

After lesioning, both carotid arteries were cannulated for perfusion, and the jugular veins or right atrium were lanced to permit adequate drainage of the vascular system. The animal was perfused first with 4000 milliliters of normal saline to clear the vascular system and prevent clotting. This was followed by 2000 milliliters of isotonic saline containing ten per cent formalin and two per cent sodium ferrocyanide. Once perfused, the head was removed and refrigerated. At the end of a week, the partially fixed brain was removed from the skull, and a block of tissue containing the diencephalon was placed in a jar of formalin for ten days.

## Sectioning

Following fixation, the brainstem was serially sectioned on an International Equipment Co. Model CTD cryostat at minus six to minus eight degrees Centigrade. The tissue block was mounted with its ventral surface towards the cutting edge of the blade and was tilted until the rostral set of four lesions was being cut in the same section. This positioning of the block insured that sections were cut perpendicular to the frontal coordinate axis. One hundred micron thick sections were cut and mounted directly on slides coated with a solution of 0.5 per cent gelatin and 0.05 per cent chrome alum. Every fifth section was used for the atlas.

Photography of Unstained Sections

While the tissue section was still wet, it was mounted in a photographic enlarger in a manner similar to using a film negative in an
enlarger (Guzmán, Alcarez, and Fernández, 1958). The image was recorded on number four contrast photographic paper. Due to the opaqueness of the myelin relative to the transparency of grey matter, the resulting reversal print gave good definition of myelinated areas. Prints did not have to be made immediately since sections could be kept moist for several minutes by placing them in a covered staining dish containing a small amount of formalin. Besides allowing more time to work with the sections, this procedure facilitated further binding to the gelatin coated slide prior to thionin staining. Air bubbles were a problem that was never completely eliminated, but could be reduced considerably by floating portions of the sections free of the slides and brushing away debris.

## Thionin Staining

The tissue sections are allowed to dry prior to thionin staining. Thionin is an acidophilic stain which probably binds the phosphate groups of nucleic acids thus defining nuclear areas. The pH of the stain is approximately 3.2 which gives a negative charge to the primary phosphate groups of nucleic acids having a pK of about 2 (Pearse, 1961). The organic acids with a higher pK than 3.2 will not take up the stain, thus increasing the specificity of the dye. The staining procedure is as follows.

Concentrate Stain: 0.1 Gm . Thionin 400 ml . Water

8 drops Acetic Acid
Stain Bath: $\quad 80 \mathrm{ml}$. of Stain Concentrate 240 ml . of Water

5 drops of Acetic Acid

1. Dioxane Bath - 15 minutes
2. Distilled Water - 3 fast dips
3. Thionin Stain - 20 minutes
4. Dioxane and Absolute Alcohol (1:1) - 2 minutes
5. Dioxane and Absolute Alcohol (1:1) - 5 minutes
6. Dioxane and Absolute Alcohol (1:1) - Until desired decoloration
7. Dioxane Bath - 15 seconds agitated
8. Dioxane Bath - 15 seconds agitated
9. Xylene - 2 minutes or until milky film removed
10. Xylene - 5 minutes
11. Xylene - 5 minutes

Note: the relatively large thickness of the tissue sections necessitated a long penetration time for the stain; this time could be shortened by removal of the lipid with butanol and chloroform prior to staining.

## Photography of Stained Sections

The stained sections were photographed with Leitz Aristophot macrodia equipment and a bellows camera that were well-aligned to eliminate light scatter in the photographic system. A condenser with an 80 millimeter focal length and a Summar 80 millimeter 1:4.5 Ernst Leitz Wetzlar lens were used in this system which utilized transmitted illumination of the slide. Between the light source and the stage, Kodak Wratten gelatin filters 90 and 73 were placed to improve the contrast of stained and unstained areas. The Kodak Wratten 90 filter transmits 1ight between 550 and 640 nanometers with peak transmittance at 580 nanometers, and the Kodak Wratten 73 filter transmits light between 560 and 610 nanometers
with peak transmittance at 570 nanometers. Both filters transmit light at wave lengths that are absorbed by thionin which absorbs the most light between 500 and 625 nanometers with peak absorbance at 596 nanometers (Gurr, 1971). Thus by use of filters, the degree of light absorbance by the stained areas over the unstained areas is increased greatly.

Kodak Contrast Process Pan, a wide spectrum film was used to photograph the slide. The film was selected for its steep "gamma" curve which provided high contrast, and not for its spectral sensitivity which extends below 400 and above 640 nanometers. The film was developed with Kodak D-11 developer at a temperature of 68 degrees Fahrenheit for four minutes. Prints of these negatives were made using a number four filter and Dupont Velour Black four contrast paper.

## Determination of Frontal Coordinates

Frontal coordinates for the atlas were determined by the following steps.

1. Percent shrinkage was calculated for each of five brainstems. The lesion frontal coordinates and the number of 100 micron sections between lesions were compared. There should be 40 sections between lesions placed 4 millimeters apart; if there is fewer than 40 sections, then the distance between the lesions has shrunk.
(1- $\left.\frac{\text { number of sections between lesions }}{\text { distance between lesions in } \mathrm{mm} \times 10}\right) \times 100=$ per cent shrinkage
2. Actual thickness of fresh tissue per 100 micron section was calculated.
$100+$ per cent shrinkage $=$ fresh tissue section thickness in microns
3. Frontal positions of the commissura anterior, nucleus supraopticus, and thalamic adhesion were calculated using frontal lesion coordinates and corrected tissue section thickness.
4. Mean frontal coordinates for the previous named structures were calculated from the five brains.
5. A scalar profile of these mean structural coordinates was matched for best fit with a scalar profile of identical structures displayed in the atlas. The resulting match dictated frontal coordinates used in the atlas.

## Determination of Vertical Coordinate Axes

Vertical coordinates were determined by reconstructing the brainstem in acetate overlays. The overlays were traced from the photographs made with the wet tissue sections; the lesions were marked on the overlays as they occurred in the brainstem. Tacks were passed through the stack of overlays so that they passed close to all lesion sites. The overlays were then matched with their respective photograph, and the vertical coordinate axis was determined from the tack holes. To help illustrate the vertical variation expected between sheep, coordinates for the bottom midline of four Southdown sheep skulls were plotted.

Determination of Lateral Coordinate Axes

The lateral coordinate axes were centered about the midine of each section. A scale was determined from bilateral lesions spread 12 millimeters apart.

## Determination of Nomenclature

Nomenclature was based on a communication from Dr. James E. Breazile to fellow members of the Subcommittee on Histologic Nomenclature of the International Committee on Veterinary Anatomical Nomenclature (ICVAN), concerning terminology for nervous tissue and for the central nervous system, dated July 22, 1971. This latter group operates under the authority of the World Veterinary Congress. This nomenclature was followed as closely as possible; however, this was a preliminary list and did not appear to be complete. Structures in the atlas not appearing on the list are as follows: the chiasma opticum, the tractus opticus, the area hypothalamica caudalis, the nucleus pretectalis anterior, and the commissura caudalis. The chiasma opticum, the tractus opticus, and the commissura caudalis appear to have been overlooked, but the area hypothalamica caudalis and the nucleus pretectalis anterior may have been present in the list as the area hypothalamica dorsocaudalis and the nucleus pretectalis medialis, respectively. The term area hypothalamica caudalis was based on hypothalamic divisions by Haymaker, Anderson, and Nauta (1969) which was used as the final authority on hypothalamic organization. The term nucleus pretectalis anterior was based on its agreement with Richard (1970) and Rose (1942). Boundaries of the area hypothalamica caudalis, area hypothalamica dorsalis, area hypothalamica lateralis, area septalis, area preoptica, nucleus hypothalamicus dorsomedialis, and nucleus hypothalamicus rostralis were intended to be conservative, positioning the mass of the structures in known locations and leaving off identification of that structure when its nuclear pattern seemed to disappear. Crosby, Humphrey, and Lauer (1962, p. 311) was
often used to clear up conflicts on nomenclature, especially concerning thalamic divisions and the nucleus mamillaris lateralis.

RESULTS

Using data obtained from five Southdown ewes, variation in frontal, lateral, and vertical coordinates was examined. The standard deviation for commissura anterior, nucleus supraopticus, and thalamic adhesion frontal coordinates was held to a maximum of 1.30 millimeters as shown in Table I.

TABLE I
FRONTAL COORDINATE MEANS AND STANDARD DEVIATIONS OF SELECTED STRUCTURES

| Structure | Mean Frontal <br> Coordinates | Standard <br> Deviation |
| :---: | :---: | :---: |
| Commissura Anterior |  |  |
| Anterior Edge | 30.9 mm | 0.57 mm |
| Posterior Edge | 30.1 |  |

${ }^{1}$ Three sheep were used instead of five.

Stereotaxic accuracy in the lateral axis was examined from two different perspectives. First, lateral coordinate variation for the commissura anterior and the nucleus supraopticus was calculated with reference to each sectional midline (Table II). Second, accuracy in the lateral placement of lesions was measured relative to the midline (Table III).

TABLE II
LATERAL COORDINATE MEANS AND STANDARD DEVIATIONS
OF SELECTED STRUCTURES

| Structure | Mean Lateral <br> Coordinates | Standard <br> Deviation |
| :---: | :---: | :---: |
| Commissura Anterior |  |  |
| Lateral Edge | 2.7 mm | 0.35 mm |
| Nucleus Supraopticus |  | 0.48 |
| Medial Edge | 2.8 | 0.91 |

TABLE III
MEAN LATERAL ERROR IN LESION PLACEMENT

| Animal <br> Studied | Average Lateral Error <br> for Lesion Sets | Standard <br> Deviation | Number of <br> Lesion Sets |
| :--- | :---: | :---: | :---: |
| Sheep 3 | 0.61 mm | 0.34 mm | 4 |
| Sheep 8 | 1.50 | 0.35 | 2 |
| Sheep 11 | 0.54 | 0.25 | 6 |

Vertical variation in the midsagittal floor of the cranium is examined in Figure 5. This reflects the result of different tilts for each head, the maximum effect on vertical variation being furthest away from the ear bars, about which the head rotates.

Using the method of transparent overlays described on page 16, positions of lesion sets were projected onto adjacent tissue sections. This method provided examination of the vertical coordinates for the commissura anterior and the nucleus supraopticus (Table IV). With variation in tilt being a factor in the standard deviation, these rostrally located structures should give an indication of the maximum vertical variation to be expected.

The list of structures identified, their abbreviations, and the series of forty-two plates follows on pages 26-71.

TABLE IV
VERTICAL COORDINATE MEANS AND STANDARD DEVIATIONS OF SELECTED STRUCTURES

| Structure | Mean Vertical <br> Coordinates | Standard <br> Deviation |
| :---: | :---: | :---: |
| Commissura Anterior |  |  |
| Dorsal Edge | +7.7 mm | 2.80 mm |
| Ventral Edge | +6.5 | 2.90 |
| Nucleus Supraopticus |  |  |
| Dorsal Edge | +2.3 | 3.38 |
| Ventral Edge | +0.8 | 3.64 |



Figure 5. Vertical Variation in the Midsagittal Floor of the Cranium. Measurements in millimeters were taken from four sheep relative to the zero point (center of interaural axis). Lines were discontinued where partial destruction of the cranium or interfering structures made measurements impossible; the direction in which the cranial floor continued was indicated by arrows. f.c.a.-frontal coordinate axis; s.t.-sella tursica; v.c.a.-vertical coordinate axis

## CHAPTER IV

## DISCUSSION AND CONCLUSION

The realiability of introducing electrodes into the hypothalamus using only a stereotaxic atlas has been questioned by Larsson (1954) and Anderson, Persson, and Ström (1960), who emphasize the necessity of radiological control. Concurrent investigations in this laboratory on Southdown sheep have required radiographic confirmation for critical placement of electrodes. This is understandable since there is variability in skull configuration and consequent variability in the related coordinate systems' alignment with the brain.

However the accuracy of this stereotaxic atlas has proven to be within useful limits. The maximum standard deviations for frontal, lateral, and vertical coordinates are 1.30 millimeters (Table I), 1.50 millimeters (Table III), and 3.64 millimeters (Table IV), respectively.

One reason for the small frontal standard deviation was that in addition to defining frontal and vertical coordinates, the lesions provided a means of checking for shrinkage. For each brain, tissue shrinkage was calculated and used to adjust its frontal coordinates. The atlas' frontal coordinates were then matched with average frontal coordinates for the commissura anterior, the nucleus supraopticus, and the thalamic adhesion.

Lateral variation in nuclear structures appeared to be quite small (Table II). The main source of error was in determining the midline
relative to head orientation in the head-holder. This resulted in a lateral placement error as great as 1.50 millimeters (Table III). Use of the sagittal sinus as a lateral reference would eliminate dependence on skull uniformity, and should improve accuracy.

The zero vertical coordinates for each plate were determined directly from the brain used in the atlas. The method of transparent overlays described on page 16 was used to project lesion positions onto adjacent tissue sections. Vertical coordinates were then determined relative to the lesions. This method was used in determining the mean vertical coordinate and the standard deviation for the commissura anterior and the nucleus supraopticus (Table IV). These standard deviations ranged from 2.80 to 3.64 millimeters, with the atlas' coordinates differing approximately 2.5 millimeters from the mean.

Calculations for these rostrally located structures give an indication of the maximum vertical variation to be expected. Small differences in head tilt from one sheep to the next contribute to this variation. The maximum effect would be furthest away from the ear bars, about which the head rotates. The result of this rotary variation is illustrated in Figure 5 showing vertical variation in the midsagittal floor of the cranium. This should reflect the vertical variation expected in the closely associated brainstem.

The head-holder alone was the source of some uncertainty. Due to a limited budget and nonavailability of a commercial sheep head-holder, an available Baltimore cat and dog stereotaxic head-holder had to be modified for sheep, the cost of modifications being closely scrutinized. Therefore the ear bar supports were made of aluminum, a soft, easily machined metal. A more rigid and heavier metal would have been better.

A slight bending and twisting of the ear bar supports occurred when the ear bars were pushed in and fastened; the twisting was a natural result of the lateral pressure from the ear bars on the frontal extension of the ear bar supports. This extension structurally weakened the head-holder design and should be incorporated only if necessary for radiographic control in future designs.

Stereotaxic accuracy of the atlas is an area of principle concern; however, this atlas also provides comparative plates of unstained and thionin stained sections which can be used for quick location of structures on other animals than Southdown sheep. It is certainly most applicable to sheep and may be easier to obtain than the other two sheep atlases referenced (Richard, 1969; Welento, et al., 1964), one being a publication in French of the Paris Institut National de la Recherche Agronomique and the other in Volume 124 of Anatomischer Anzliger.

In summary, the resulting stereotaxic atlas identifies and locates major diencephalic structures of the Southdown ewe in reference to a three-dimensional coordinate system. The described head-holder modifications, made to accommodate sheep in a Baltimore cat and dog head-holder, were adequate for accurate stereotaxis. However, small improvements in accuracy might be obtained by using a more rigid material than aluminum in constructing these modifications. The photographic comparison of unstained and thionin stained sections can be a useful tool for locating structures in neurological studies.

## CHAPTER V

## STEREOTAXIC ATLAS

The following structures were identified.

| Plates | Abbreviation | Structure |
| ---: | :--- | :--- |
| 20-26 | Ahc |  |
| 14-21 | Area hypothalamica caudalis |  |
| $9-16$ | Ah1 | Area hypothalamica dorsalis |
| $1-7$ | Ap | Area hypothalamica lateralis |
| $1-7$ | As | Area septalis |
| $1-9$ | Ci | Capsula interna |
| $2-10$ | Co | Chiasma opticum |
| $38-40$ | Cso | Colliculus rostralis stratum opticum |
| $38-42$ | Csz | Colliculus rostralis stratum zonale |
| $1-7$ | Ca | Commissura anterior |
| $31-42$ | Ccd | Commissura caudalis |
| $38-42$ | Ccr | Commissura colliculorum rostralis |
| 30 | Ch | Commissura habenularum |
| $26-29$ | Csm | Commissura supramamillaris |
| $1-24$ | Cc | Corpus callosum |
| $32-42$ | Cs | Corpus subcommissuralis |
| $26-33$ | Fh | Fasciculus habenulointerpeduncularis |


| Plates | Abbreviation | Structure |
| :---: | :---: | :---: |
| 1-27 | Fos | Fasciculus occipitoanterior superior |
|  |  | Fasciculus retroflexus see Fasciculus habenulo- |
|  |  | interpeduncularis |
|  |  | Fasciculus subcallosal see Fasciculus occipitoanterior superior |
| 24-32 | FF | Fields of Forel |
| 5-24 | F | Fornix |
| 31-38 | Pi | Glandula pineale |
| 1-8 | Gp | Globus pallidus |
| 25-42 | H | Hippocampus |
| 12-36 | Lme | Lamina medullaris thalami externa |
| 12-27 | Lmi | Lamina medullaris thalami interna |
| 40-41 | No | Nuclei oculomotorii |
| 11-19 | Nad | Nucleus anterior dorsalis |
| 15-16 | Nam | Nucleus anterior medialis |
| 12-19 | Nav | Nucleus anterior ventralis |
|  |  | Nucleus arcuatus see Nucleus infundibularis |
|  |  | Nucleus of Cajal see Nucleus interstitialis |
| 1-27 | Nc | Nucleus caudatus |
| 18-21 | Ncm | Nucleus centralis medialis |
| 16-27 | Ndt | Nucleus dorsomedialis thalami |
| 27-36 | Ng1 | Nucleus geniculatus lateralis |
| 30-41 | Ngm | Nucleus geniculatus medialis |
| 24-29 | Nh1 | Nucleus habenularis lateralis |
| 23-29 | Nhm | Nucleus habenularis medialis |
| 16-18 | Nhd | Nucleus hypothalamicus dorsomedialis |


| Plates | Abbreviation | Structure |
| :---: | :---: | :---: |
| 8-13 | Nhr | Nucleus hypothalamicus rostralis |
| 13-17 | Nhv | Nucleus hypothalamicus ventromedialis |
| 12-20 | Ni | Nucleus infundibularis |
| 35-38 | Nit | Nucleus interstitialis |
| 23-33 | N1c | Nucleus lateralis caudalis |
| 17-28 | N1d | Nucleus lateralis dorsalis |
| 21-28 | Nm1 | Nucleus mamillaris lateralis |
| 2-29 | Nmm | Nucleus mamillaris medialis |
| 6-13 | Npv | Nucleus paraventricularis |
| 8-29 | Npt | Nucleus paraventricularis thalami |
| 32-38 | Npa | Nucleus pretectalis anterior |
| 29-39 | Npu | Nucleus pulvinaris |
| 10-27 | Nrt | Nucleus reticularis thalami |
| 13-18 | Nru | Nucleus reuniens thalami |
| 14-16 | Nrh | Nucleus rhomboidieus thalami |
| 33-42 | Nr | Nucleus ruber |
| 29-42 | Nsn | Nucleus substantia nigra |
| 23-28 | Nst | Nucleus subthalamicum |
| 5-8 | Nsc | Nucleus suprachiasmaticus |
| 28-36 | Nsg | Nucleus suprageniculatus |
| 22-27 | Nsm | Nucleus supramamillaris |
| 6-10 | Nso | Nucleus supraopticus |
| 24-29 | Nve | Nucleus ventralis caudalis |
| 19-28 | Nv1 | Nucleus ventralis lateralis |
| 11-23 | Nvr | Nucleus ventralis rostralis |
| 18-42 | Pc | Pedunculus cerebri |


| Plates | Abbreviation | Structure |
| :---: | :---: | :---: |
| 1-11 | P | Putamen |
|  |  | Stratum periventriculare see Substantia grisea centralis |
| 7-29 | Sm | Stria medullaris thalami |
| 5-27 | St | Stria terminalis |
| 27-42 | Sgc | Substantia grisea centralis |
| 14-25 | Tm | Tractus mamillothalamicus |
| 11-22, | To | Tractus opticus |
| 38-41 |  |  |
| 32-42 | Tt | Tractus tegmenti centralis |
| 14-25 | Zi | Zona incerta |



Fasciculus occipitoanterior superior

Nucleus caudatus

Area septalis
Putamen

Globus pallidus Area preoptica

Plate 1. Section at Frontal 33.6. A. Unstained section;
B. Thionin stained section.


Plate 2. Section at Frontal 33.1. A. Unstained section;
B. Thionin stained section.


Plate 3. Section at Frontal 32.5. A. Unstained section; B. Thionin stained section.


Plate 4. Section at Frontal 32.0. A. Unstained section;
B. Thionin stained section.


Plate 5. Section at Frontal 31.5. A. Unstained section;
B. Thionin stained section.


Plate 6. Section at Frontal 31.0. A. Unstained section;
B. Thionin stained section.


Corpus callosum
Capsula interna
Stria terminalis
Stria medullaris thalami Fornix

Commissura anterior

## Chiasma opticum

Fasciculus occipitoanterior superior
Nucleus caudatus
Fornix
Area septalis
Putamen
Globus pallidus
Fornix

Area preoptica
Nucleus paraventricularis Nucleus suprachiasmaticus Nucleus supraopticus

Plate 7. Section at Frontal 30.4. A. Unstained section; B. Thionin stained section.


Corpus callosum Capsula interna Fornix
Stria Terminalis
Stria medullaris thalami

Fornix

Chiasma opticum


Fasciculus occipitoanterior superior

## Nucleus caudatus

## Putamen

Nucleus paraventricularis thalami
Globus pallidus

Nucleus paraventricularis Nucleus hypothelamicus rostralis
Nucleus suprachiasmaticus Nucleus supraopticus

Plate 8. Section at Frontal 29.9. A. Unstained section; B. Thionin stained section.


Plate 9. Section at Frontal 29.3. A. Unstained section;
B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis Stria medullaris thalami

Fornix

Chiasma opticum


## Fasciculus occipitoanterier

 superiorNucleus caudatus
Putamen
Nucleus paraventricularis thalani
Nucleus reticularis thalami

Nucleus paraventricularis Nucleus hypothalamicus rostralis
Area hypothalamica lateralis Nucleus supraopticus

Plate 10. Section at Frontal 28.8. A. Unstained section; B. Thionin stained section.


Plate 11. Section at Frontal 28.3. A. Unstained section; B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis
Stria medullaris thalami Lamina medullaris thalami interna
Lamina medullaris thalami externa

Fornix

Tractus opticus


Fasciculus occipitoanterior superior
Nucleus caudatus
Nucleus anterior dorsalis Nucleus anterior ventralis Nucleus anterior medialis Nucleus paraventricularis thalami
Nucleus ventralis rostralis Nucleus reticularis thalami

Nucleus paraventricularis
Nucleus hypothalamicus rostralis
Area hypothalamica lateralis Nucleus infundibularis

Plate 12. Section at Frontal 27.7. A. Unstained section;
B. Thionin stained section.


101510 mm


Fasciculus occipitoanterior superior
Nucleus caudatus
Nucleus anterior dorsalis Nucleus anterior ventralis Nucleus anterior medialis Nucleus paraventricularis thalami
Nucleus reticularis thalami Nucleus ventralis rostralis Nucleus reuniens thalami

Nucleus paraventricularis Nucleus hypothalamicus rostralis
Area hypothalamica lateralis Nucleus hypothalamicus ventromedialis Nucleus infundibularis

Plate 13. Section at Frontal 27.2. A. Unstained section; B. Thionin stained section.


Plate 14. Section at Frontal 26.7. A. Unstained section;
B. Thionin stained section.


Plate 15. Section at Frontal 26.1. A. Unstained section; B. Thionin stained section.


Plate 16. Section at Frontal 25.6. A. Unstained section; B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis
Stria medullaris thalami Lamina medullaris thalami interna
Lamina medullaris thalami externa

Tractus mamillothalamicus

Formix
Tractus opticus

Fasciculus occipitoanterior superior

## Nucleus caudatus

Nucleus anterior dorsalis
Nucleus lateralis dorsalis Nucleus anterior ventralis Nucleus paraventricularis thalemi
Nucleus dorsomedialis thalami Nucleus ventralis rostralis Nucleus reticularis thalami Nucleus reumiens thalami Area hypothalamica dorsalis Zona incerta
Nucleus hypothalamicus dorsomedialis
Nucleus hypothalamicus ventromedialis
Nucleus infundibularis

Plate 17. Section at Frontal 25.1. A. Unstained section; B. Thionin stained section.


Corpus callosum

## Fornix

Stria terminalis
Stria medullaris thalami Lamina medullaris thalami interna
Lamina medullaris thalami externa

Tractus mamillothalamicus

Fornix
Pedunculus cerebri
Tractus opticus


Fasciculus occipitoanterior superior
Nucleus caudatus
Nucleus anterior dorsalis Nucleus lateralis dorsalis Nucleus anterior ventralis Nucleus paraventricularis thalami
Nucleus dorsomedialis thalami Nucleus reticularis thalami Nucleus ventralis rostralis Nucleus centralis medialis Nucleus reuniens thalami Area hypothalamica dorsalis Zona incerta Nucleus hypothalamicus dorsomedialis
Nucleus infundibularis

Plate 18. Section at Frontal 24.5. A. Unstained section; B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis
Stria medullaris thalami
Lamina medullaris thalami interna
Lamina medullaris thalami externa

Tractus Mamillothalamicus

Fornix
Tractus opticus
Pedunculus cerebri


Fasciculus occipitoanterior superior Nucleus caudatus Nucleus anterior dorsalis Nucleus lateralis dorsalis Nucleus anterior ventralis Nucleus ventralis lateralis

Nucleus dorsomedialis thalami Nucleus paraventricularis thalami
Nucleus ventralis rostralis
Nucleus centralis medialis Area hypothalamica dorsaiis Zona incerta
Area hypothalamica caudalis Nucleus Infundibularis

Plate 19. Section at Frontal 24.0. A. Unstained section;
B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis
Stria medullaris thalami
Lamina medullaris thalami interna
Lamina medullaris thalami externa

## Tractus Mamillothalamicus

Tractus opticus
Fornix
Pedunculus cerebri


Fasciculus occipitoanterior superior
Nucleus caudatus
Nucleus lateralis dorsalis Nucleus paraventricularis thalami
Nucleus reticularis thalami Nucleus ventralis lateralis Nucleus dorsomedialis thalami

Nucleus ventralis rostralis Nucleus centralis medialis Area hypothalamica dorsalis Zona incerta
Area hypothalamica caudalis
Nucleus mamillaris medialis
Nucleus infundibularis

Plate 20. Section at Frontal 23.5. A. Unstained section; B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis
Stria medullaris thalami
Lamina medullaris thalami
interna
Lamina medullaris thalami
externa

Tractus mamillothalamicus
Tractus opticus
Fornix
Pedunculus cerebri


Fasciculus occipitoanterior superior
Nucleus caudatus
Nucleus lateralis dorsalis Nucleus paraventricularis thalami
Nucleus ventralis lateralis Nucleus dorsomedialis thalami

Nucleus reticularis thalamı
Nucleus ventralis rostralis Nucleus centralis medialis Area hypothalamica dorsalis Zona incerta Area hypothalamica caudalis

Nucleus mamillaris medialis Nucleus mamillaris lateralis

Plate 21. Section at Frontal 22.9. A. Unstained section;
B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis
Stria medullaris thalami

Lamina medullaris thalami interna
Lamina medullaris thalami externa

Tractus mamillothalamicus Tractus opticus

Fornix
Pedunculus cerebri


Fasciculus occipitoanterior superior Nucleus caudatus

Nucleus lateralis dorsalis Nucleus paraventricularis thalami
Nucleus ventralis 1ateralis Nucleus dorsomedialis thalami Nucleus reticularis thalami Nucleus ventralis rostralis

Area hypothalamica caudalis Zona incerta
Nucleus supramamillaris Nucleus mamillaris medialis Nucleus mamillaris 1ateralis

Plate 22. Section at Frontal 22.4. A. Unstained section; B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis
Stria medullaris thalami Lamina medullaris thalami externa

Lamina medullaris thalami interna

Tractus mamillothalamicus
Fornix
Pedunculus cerebri


## Fasciculus occipitoanterior superior

Nucleus caudatus
Nucleus lateralis dorsalis Nucleus habenularis medialis Nucleus lateralis caudalis Nucleus reticularis thalami Nucleus ventralis lateralis Nucleus paraventricularis thalami
Nucleus dorsomedialis thalami
Nucleus ventralis rostralis Area hypothalamica caudalis Zona incerta
Nucleus supramamillaris Nucleus subthalamicum Nucleus mamillaris medialis Nucleus mamillaris lateralis

Plate 23. Section at Frontal 21.9. A. Unstained section; B. Thionin stained section.


Corpus callosum
Fornix
Stria terminalis
Stria medullaris thalami

Lamina medullaris thalami interna
Lamina medullaris thalami externa

## Tractus mamillothalamicus

Pedunculus cerebri


## Fasciculus occipitoanterior

 superior
## Nucleus caudatus

Nucleus lateralis dorsalis
Nucleus habenularis medialis
Nucleus habenularis lateralis
Nucleus lateralis caudalis
Nucleus ventralis lateralis Nucleus dorsomedialis thalami Nucleus paraventricularis thalami
Nucleus ventralis caudalis Area hypothalamica caudalis Fields of Forel

## Zona incerta

Nucleus supramamillaris Nucleus subthalamicum Nucleus mamillaris medialis Nucleus mamillaris lateralis

Plate 24. Section at Frontal 21.3. A. Unstained section; B. Thionin stained section.


Hippocampus
Stria terminalis

Stria medullaris thalami

Lamina medullaris thalami interna Lamina medullaris thalami externa

Tractus mamillothalamicus
Pedunculus cerebri


Fasciculus occipitoanterior superior
Nucleus caudatus
Nucleus lateralis dorsalis Nucleus habenularis medialis Nucleus habenularis lateralis Nucleus reticularis thalami Nucleus lateralis caudalis Nucleus ventralis lateralis Nucleus dorsomedialis thalami Nucleus paraventricularis thalami
Nucleus ventralis caudalis Area hypothalamica caudalis Zona incerta
Fields of Forel
Nucleus supramamillaris
Nuclet:s subthalamicum
Nucleus mamillaris medialis Nucleus mamillaris lateralis

Plate 25. Section at Frontal 20.8. A. Unstained section; B. Thionin stained section.


Plate 26. Section at Frontal 20.3. A. Unstained section; B. Thionin stained section.


Plate 27. Section at Frontal 19.7. A. Unstained section; B. Thionin stained section.


Hippocampus

Stria medullaris thalami

Fasciculus habenulointerpeduncularis
Lamina medullaris thalami externa

Fields of Forel

Pedunculus cerebri


Nucleus lateralis dorsalis
Nucleus lateralis caudalis Nucleus habenularis medialis Nucleus habenularis lateralis Nucleus ventralis lateralis Nucleus paraventricularis thalami
Nucleus suprageniculatus Nucleus geniculatus lateralis Nucleus ventralis caudalis Substantia grisea centralis

Commissura supramamillaris
Nucleus subthalamicum Nucleus mamillaris lateralis Nucleus mamillaris medialis

Plate 28. Section at Frontal 19.2. A. Unstained section;
B. Thionin stained section.


Hippocampus

Stria medullaris thalami
Lamina medullaris thalami externa
Fasciculus habenulointerpeduncularis

Fields of Forel

Pedinculus cerebri


Nucleus lateralis caudalis Nucleus habenularis medialis Nucleus habenularis 1ateralis Nucleus pulvinaris Nuclevs suprageniculatus Nuclous paraventricularis thalmi
Nucleus geniculatus lateralis Nucleus ventralis caudalis Substantia grisea centralis

Commissura supramamillaris Nucleus substantia nigra

Nucleus mamillaris medialis

Plate 29. Section at Frontal 18.6. A. Unstained section; B. Thionin stained section.



Plate 31. Section at Frontal 17.6. A. Unstained section;
B. Thionin stained section.


Plate 32. Section at Frontal 17.0. A. Unstained section;
B. Thionin stained section.


Nucleus lateralis caudalis
Nucleus pulvinaris
Nucleus geniculatus lateralis Nucleus pretectalis anterior Corpus subcommissuralis
Substantia grisea centralis
Nucleus suprageniculatus
Tractus tegmenti centralis
Nucleus geniculatus medialis Nucleus ruber
Fasciculus habenulointerpeduncularis
Nucleus substantia nigra

Plate 33. Section at Frontal 16.5. A. Unstained section; B. Thionin stained section.


Hippocampus

Glandula pineale

Commissura caudalis
Lamina medullaris thalami externa

Pedunculus cerebri


Nucleus pulvinaris
Nucleus geniculatus lateralis
Nucleus pretectalis anterior
Corpus subcommissuralis
Substantia grisea centralis
Tractus tegmenti centralis
Nucleus suprageniculatus
Nucleus geniculatus medialis
Nucleus ruber
Nucleus substantia nigra

Plate 34. Section at Frontal 16.0. A. Unstained section;
B. Thionin stained section.


Hippocampus

Glandula pineale

Comnissura caudalis
Lamina medullaris thalami
externa

Pedunculus cerebri


Nucleus pulvinaris
Nucleus geniculatus lateralis Nucleus pretectalis anterior Corpus subcommissuralis
Substantia grisea centralis
Nucleus suprageniculatus
Tractus tegmenti centralis
Nucleus interstitialis
Nucleus geniculatus medialis
Nucleus ruber
Nucleus substantia nigra

Plate 35. Section at Frontal 15.4. A. Unstained section;
B. Thionin stained section.


Plate 36. Section at Frontal 14.9. A. Unstained section; B. Thionin stained section.


Plate 37. Section at Frontal 14.4. A. Unstained section; B. Thionin stained section.


Plate 38. Section at Frontal 13.8. A. Unstained section;
B. Thionin stained section.


Plate 39. Section at Frontal 13.3. A. Unstained section; B. Thionin stained section.


Hippocampus

## Commissura colliculorum

rostralis
Commissura caudalis

## Pedunculus cerebri



Colliculus rostralis stratum zonale Colliculus rostralis stratum opticum

Tractus opticus
Corpus subcommissuralis
Substantia grisea centralis
Nucleus geniculatus medialis Tractus tegmenti centralis

Nucleus ruber
Nucleus substantia nigra

Plate 40. Section at Frontal 12.8. A. Unstained section;
B. Thionin stained section.


Hippocampus

Commissura colliculorum
rostralis
Commissura caudalis

Pedunculus cerebri


Colliculus rostralis stratum zonale

Tractus opticus
Corpus subcommissuralis Substantia grisea centralis

Nucleus geniculatus medialis Tractus tegmenti centralis Nuclei oculomotorii

Nucleus ruber

Nucleus substantia nigra

Plate 41. Section at Frontal 12.2. A. Unstained section;
B. Thionin stained section.


Plate 42. Section at Frontal 11.7. A. Unstained section;
B. Thionin stained section.

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## APPENDIX

FRONTAL COORDINATES OF SELECTED STRUCTURES

| Sheep \# | Frontal Coordinates |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Commissura Anterior |  | Thalamic Adhesion |  | Nucleus Supraopticus |  |
|  | Rostral | Caudal | Rostral | Caudal | Rostral | Caudal |
|  | Edge | Edge | Edge | Edge | Edge | Edge |
| 3 | 30.5 | 29.6 | 27.8 | 20.6 |  |  |
| right side |  |  |  |  | 29.1 | 27.2 |
| left side |  |  |  |  | 29.1 | 27.2 |
| 5 | 30.3 | 29.4 | 27.6 | -- |  |  |
| right side |  |  |  |  | 29.1 | 27.7 |
| left side |  |  |  |  | 29.4 | 28.3 |
| 6 | 31.3 | 30.4 | 27.9 | ---- |  |  |
| right side |  |  |  |  | 30.9 | 29.0 |
| left side |  |  |  |  | 30.5 | 28.4 |
| 8 | 30.9 | 30.1 | 28.1 | 20.6 |  |  |
| right side |  |  |  |  | 30.7 | 28.6 |
| left side |  |  |  |  | 30.5 | 28.3 |
| 11 | 31.7 | 31.1 | 28.2 | 21.5 |  |  |
| right side |  |  |  |  | 32.3 | 28.1 |
| left side |  |  |  |  | 32.7 | 28.1 |
| Mean | 30.90 | 30.10 | 27.92 | 20.90 | 30.43 | 28.09 |

\&<br>VITA<br>John Miles Rogers<br>Candidate for the Degree of<br>Master of Science

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