

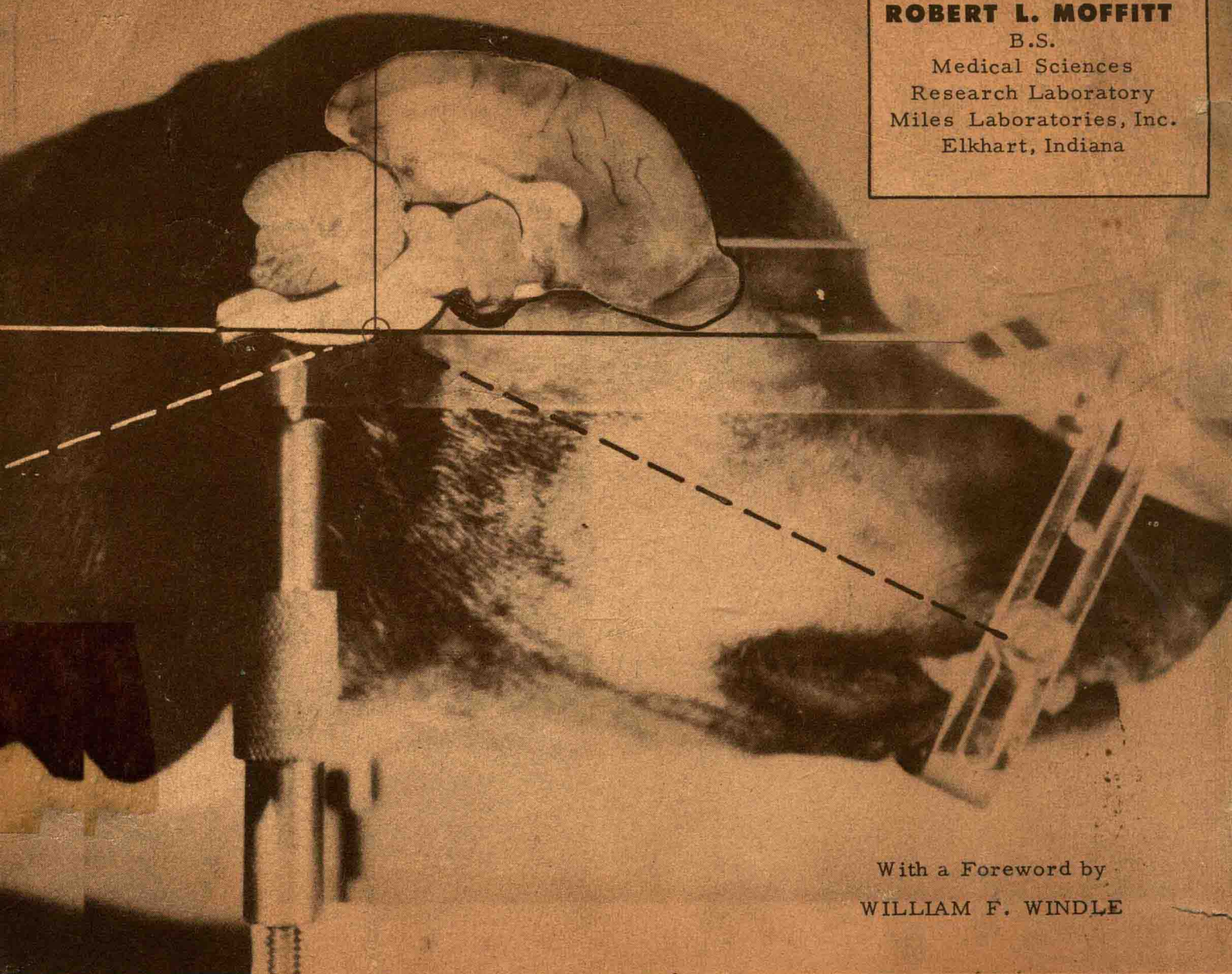
LIM • LIU • MOFFITT

A STEREOTAXIC ATLAS of the DOG'S BRAIN

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With a Foreword by
WILLIAM F. WINDLE

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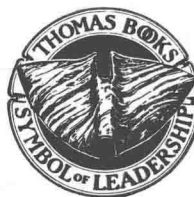
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Foreword

THE EXPERIMENTAL approach to studies of structure and function of the central nervous system is essentially a development of the Twentieth Century. Many investigators have contributed to this movement. A number of new techniques devised by them have added impetus. One of the most significant was the invention of the stereotaxic instrument by R. H. Clarke, which made possible the accurate placement of electrodes in deep structures of the brain. This, as much as anything, established experimental neurology as a primary field of investigation.

Although considerable interest was aroused by the 1908 report of Sir Victor Horsley and R. H. Clarke, in *Brain*, the original instrument was of limited value because its electrodes were glass-coated and friable, and because, in the absence of brain atlases, coordinates for placing lesions accurately in the brains of experimental animals were difficult to determine. Before full advantage could be taken of the new technique, World War I intervened.

Soon after establishment of the Neurological Institute at Northwestern University, S. W. Ranson saw the value of Clarke's stereotaxic instrument and revived its use, reconstructing it from the illustrations in *Brain*. He and W. R. Ingram wrote a preliminary account of how to establish reference points in the cat's brain for their instrument, this appearing in the *Proceedings of the Society of Experimental Biology and Medicine* in 1931. Ranson described the use of the stereotaxic instrument more fully in a later article in *Psychiatrische en Neurologische Bladen* (1934). The method soon became popular and a long series of excellent studies on the diencephalon and other parts of the nervous system followed.

Two important improvements explain the successful revival of this technique. More suitable electrodes were devised and brains were carefully mapped in terms of the stereotaxic coordinates. Ranson's electrodes, coated with baked enamel, could be prepared easily and were more durable than those of Horsley and Clarke. Ranson and his students prepared a topographical atlas of the diencephalic structures of the cat's brain before employing the stereotaxic instrument. This atlas was published in 1932 by Ingram, Hannett and Ranson in the *Journal of Comparative Neurology*.

A number of refinements have been made in stereotaxic instruments during the last twenty-eight years, and several companies now manufacture

them. Furthermore, several atlases have been published. The technique, used originally on the monkey, was soon extended to the cat and other species of experimental animals and is currently being employed to insert electrodes into the human brain. For some strange reason nearly everyone has avoided the dog, physiologists' favorite experimental subject. There is no valid reason why experiments should not be carried out in the dog's brain, and there are many good reasons why they should. It is only necessary to take the proper preliminary steps. These, of course, involve adaptation of the instrument to the animal's head, determination of planes of reference, and most importantly, preparation of an adequate atlas of sections corresponding to the coordinates of the instrument to be used.

It would seem that scientists have avoided the dog in experimental neurology partly because of variability in size and shape of the head, but largely because spade work for investigations had been carried out in other species, in which the anatomy of the brain is better known. Drs. Lim, Liu, and Moffitt were not content to follow this course of least resistance. They have demonstrated that variability in heads need not be an obstacle. Their courage in applying the stereotaxic technique of Horsley and Clarke to the dog is laudable. Many important questions cannot be answered until lesions and stimuli accurately placed in deep structures of the brains of several species are compared. We know that there are differences between the cat and monkey, and between the rat and cat. The possibility of now investigating the dog is most attractive.

WILLIAM F. WINDLE

Bethesda, Maryland

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A Stereotaxic Atlas of the
DOG'S BRAIN

Introduction

WHEN Horsley and Clarke (1908) first described the latter's stereotaxic apparatus, they stated that, "We have not attempted to enlarge it so as to include dogs because in almost every respect the cat's brain is superior to the dog's for elementary neurological purposes; the nerve tracts are better marked, the size of the encephalon is more convenient for serial sections, and, most important of all, cat's heads are of much more uniform size and shape than those of dogs; in fact, the endless variations in the size and shape of dog's heads make them unsuitable for research involving accurate cranio-encephalic topography."

Being situated in locations where dogs were more readily available than cats, we have been compelled over the years to use dogs for investigations involving the central nervous system, an experience which has led to the collection of the material presented in this atlas. By selecting short haired hound-like dogs of limited weight (10 ± 2 kg) and without unduly short or long noses, it has been possible to use them in the Lab-Tronics cat stereotaxic head holder with only minor modifications. The addition of a second pair of earpin clamps 10 cm behind the original pair of earpin clamps on the horizontal bars of the head holder, and of a snout clamp, renders the Lab-Tronics instrument available for the dog without interfering with its use with the cat or other animals (see Figure 1).

We have found the brain measurements to vary with animal size, and with errors arising from imperfect alignment of parts (electrodes, marking rods) of the stereotaxic head holder itself, or of the saw or knife to the brain during sawing or microsectioning, or from shrinking following removal of the brain from the cranial case, or from measurements of irregular surfaces. Some of these errors have been reduced by photographing gross brain sections *in situ* within the cranium with a millimeter scale alongside, and making measurements from the photographs.

The means and standard deviations of various dimensions of the dog's brain measured in this way are given in Table 1. These were taken from four series of brains, viz.

	Number of Dogs			Mean Weight \pm SD kg
	Mongrels	Beagles	Total	
1. Midline sagittal section of the brain <i>in situ</i> within cranium.	14	3	17	10.1 \pm 1.8
2. Coronal sections of the brain <i>in situ</i> within cranium cut at 5 mm intervals caudal and rostral to the interaural plane.	5	2	7	9.7 \pm 2.3
3. Midline sagittal section of the brain measured after removal from the cranium.	7	0	7	11.6 \pm 2.1
4. Brainstem and subcortex with cerebellar and cerebral cortices removed.	11	0	11	10.5 \pm 1.7
	37	5	42	10.4 \pm 1.9

In sagittal sections, measurements in the longitudinal axis are made from the interaural plane or line and are recorded with the prefix (C) or (R) to indicate whether the structure measured lies caudal or rostral to it. The interaural line represents the longitudinal zero and is labelled CRO. In addition, measurements in the vertical (V) axis are made from the Horsley-Clarke interaural-infraorbital horizontal plane or line (vertical zero). In coronal sections, transverse (T) measurements are made from the midline (transverse zero) and vertical measurements as indicated above. Horizontal sections provide longitudinal (C or R) and transverse (T) measurements, while the brainstem preparations permit measurements of structures not evident in the others. The method of describing the location of structures in the brain in terms of the three co-ordinates C or R, V and T is essentially that recommended by Horsley and Clarke (1908) except that the horizontal zero plane is used in preference to the +10 "basal" plane.

Table 1 gives some idea of the variability of these measurements and indicates that most structures in the dog's brainstem and subcortex may be reached within a millimeter or so of their locations. Table 2 and Figure 7-2 compare the mean longitudinal and vertical measurements taken from gross sagittal sections of 3 beagle (B) and 14 mongrel (M) brains cut *in situ* within the cranium, showing that very small differences are encountered. The majority of dogs used in this atlas has been the subject of experimental stimulation of, or drug injection into, the brainstem or subcortex, or the IIIrd or IVth ventricles.

Thus the microsections C15 to R34 (Figures 23 to 68) are taken from a dog in which the vasomotor component of the myelencephalic sympathetic center was mapped out by stimulation, and gamma quantities of a hypotensive agent in lambda volumes injected into the pressor area (i.e., the area giving the maximum pressor response of 250-300 mm Hg). Because the tracts left by the concentric electrodes and their distension by the drug injections through the hollow inner electrode, marred sections C10 to C6

Figures 28 to 32), these were replaced by equivalent sections from another dog in which this area was not damaged. Examination of section C5 (Figure 33) will reveal an electrode tract ending in a small hemorrhagic area at T1.5-2 to the left of the midline, and V4.5-6.5 above the horizontal zero; this was the most rostral point explored and was beyond the maximum pressor point. Within the pressor area, the bare tips of the insulated concentric electrodes rested at V1.5-2 above the horizontal zero, within the nucleus reticularis gigantocellularis, at about T2 from the midline.

The atlas has been assembled as a guide for the localization of major structures in the brain in physiological or pharmacological experiments in the dog. It does not deal with cytoarchitectonics or the fiber systems of the brain. The material presented covers (a) the stereotaxic instruments and methods for the preparation of gross sections of the brain *in situ* within the cranium, Figures 1-3; (b) gross preparations and sagittal, horizontal and coronal gross sections, with reconstructions of neural structures which form functional systems in the brain, Figures 4-22; and (c) microsections at mm intervals from the obex to the genu of the corpus callosum, with alternate sections stained for fibers and cells.

Preparatory to sectioning of the brain *in situ*, the head is perfused through the carotids or through the aorta (after clamping the origin, the descending portion and the subclavians) with about two liters of saline followed by two liters of 10% neutral formalin at about 120 mm Hg pressure. Following decapitation, the head is skinned, and the lower jaw, zygomatic arch and all fascia and soft tissues removed. It is then mounted in a precisely made soft-wood box * with wooden earpins, and vertical and horizontal wooden rods for transfixing the snout. The wooden earpins and rods are positioned with the aid of a stereotaxic vise with steel plates extending from the jaws carrying adjustable bushings for holding the earpins and extra headpins, or for drilling transverse holes in the face or snout (Figures 3-1, 2). After the head is mounted, the box is ruled in the desired planes to provide guides for sawing. The box is then clamped on the moving platform (M) of a Wells "Quikut" band saw (Figure 3-3), with one wall placed against the support plate (P) and the first line to be sawed through aligned against the saw blade. The screw activating the support plate is provided with a large wheel (W) calibrated so that the plate can move the box (containing the head) toward the saw blade at millimeter intervals. The gross sections are carefully wiped and washed under running water to remove tissue debris, placed in 10% formalin and photographed with a millimeter scale on the same plane as soon as possible.

Although freezing permits of greater ease in sawing the head, it is not essential and should be avoided when the brain slices are to be removed for the preparation of microsections. The brain slices, however, may be

* Box is 4.5" wide, 5.5" deep, 9" long, with 0.5" thick walls, fastened with wooden pins. The box is made to fit the 5" stereotaxic vise.

TABLE 1
MEASUREMENTS OF THE DOG BRAIN IN THE (1) LONGITUDINAL, (2) VERTICAL AND (3) TRANSVERSE AXES EMPLOYING THE FOLLOWING ZEROS:
INTERAURAL CORONAL PLANE IN (1), INFRAORBITAL-INTERAURAL HORIZONTAL PLANE IN (2) AND MIDLINE SAGITTAL PLANE IN (3).

Structure or specific site	Longitudinal (interaural zero)	Vertical (horizontal zero)					Transverse (midline zero)	
		Ventral surface of brain	Dorsal surface of brain stem	Dorsal surface of cerebellar cortex	Corpus callosum	Dorsal surface of cerebral cortex	Brain stem or subcortex	Cerebral cortex
Medulla oblongata or myelencephalon C 20 C 15		V 0.9 ± 0.4 ¹		V 26.1 ± 2.7 ¹			T 5.4 ± 0.7 ⁴ T 5.9 ± 1.0 ² T 7.2 ± 0.4 ⁴	
Obex Opening of spinal cord C 10	C 15-14 C 12.2 ± 1.9 ³ C 14.4 ± 1.0 ¹	V 0.4 ± 0.5 ¹	V 7.4 ± 1.3 ¹	V 28.2 ± 1.6 ¹			T 7.4 ± 0.6 ² T 8.0 ± 0.4 ⁴	
Fastigium, middle of C 5	C 7.0 ± 1.6 ¹	V 0.4 ± 0.4 ¹	V 13.0 ± 1.1 ¹	V 28.8 ± 1.4 ¹		V 39 ± 1.7 ¹	T 8.3 ± 1.1 ² T 8.8 ± 0.5 ⁴	T 19.7 ± 3.7 ²
Rostral medulla	C 3							
Interaural zero	0	0		V 27.6 ± 2.4 ¹		V 41 ± 2.4 ¹	T 10.6 ± 0.4 ¹ T 7.2 ± 0.5 ⁴	T 24.4 ± 1.5 ¹
Pons (ventral) Midbrain or mesencephalon (dorsal) Caudal end of inf. colliculi Midline intercollicular groove R 5	C 3-R 10 R 4-R 12 R 3.8 ± 1.0 ⁴ R 4.5 ± 0.4 ¹							
Caudal end of sup. colliculi Rostral border of pons	R 9.2 ± 0.9 ⁴ R 8.7 ± 0.6 ³ R 9.8 ± 0.7 ¹	V 0.3 ± 0.5 ¹	V 18.4 ± 1.2 ¹			V 42 ± 2.1 ¹	T 8.4 ± 1.2 ² T 8.9 ± 0.5 ⁴	T 25.9 ± 1.3 ²

TABLE 1 (Continued)

R 10		V 4.1 ± 1.7 ¹	V 17.8 ± 1.1 ¹	V 27.5 ± 1.2 ¹	V 43 ± 1.8 ¹	T 7.5 ± 0.8 ² T 6.5 ± 0.3 ⁴	T 25.3 ± 2.0 ²
Midline midbrain-thalamic junction	R 11.8 ± 0.8 ¹ R 13-R 25						
Thalamus	R 14.3 ± 1.4 ³ R 14.8 ± 0.9 ¹						
Caudal end of mammillary bodies							
R 15		V 4.2 ± 2.1 ¹		V 27.3 ± 1.5 ¹	V 44 ± 1.7 ¹		
Rostral end of sup. colliculi	R 16.4 ± 1.9 ⁴ R 15-R 25						
Hypothalamus		V 4.8 ± 1.2 ¹		V 25.3 ± 1.7 ¹	V 43 ± 1.6 ¹		T 21.7 ± 1.2 ²
R 20	R 24.2 ± 1.3 ³ R 24.9 ± 0.8 ¹		V 12.2 ± 1.1 ¹				
Anterior commissure, middle of			V 5.3 ± 1.2 ¹				
Optic chiasma, middle of		V 4.1 ± 1.1 ¹ V 5.1 ± 1.3 ¹		V 23.6 ± 1.6 ¹ V 22.7 ± 1.5 ¹	V 42 ± 1.4 ¹ V 40 ± 1.8 ¹		T 21.0 ± 1.2 ² T 20.9 ± 1.3 ²
R 25							
R 30							
Ventral surface: genu of corpus callosum			V 15.6 ± 1.7 ¹				
Rostral end of genu of corpus callosum	R 33.2 ± 2.2 ³ R 34.1 ± 1.5 ¹	V 4.7 ± 0.9 ¹			V 38 ± 1.7 ¹		
R 35							
Rostral end of caudate nucleus	R 38.4 ± 5.0 ⁴	V 3.2 ± 1.0 ¹ V 1.3 ± 0.7 ¹ V 1.4 ± 1.5 ¹ V 3.1 ± 3.6 ¹			V 37 ± 2.0 ¹ V 34 ± 2.2 ¹ V 28 ± 3.5 ¹ V 19 ± 9.0 ¹		
R 40							
R 45							
R 50							
R 55							
Frontal pole	R 53.0 ± 2.0 ³ R 55.1 ± 1.6 ¹						

All measurements are in mm, giving mean ± SD distances from the respective zero. C, caudal; R, rostral to interaural plane; V, vertical or dorsal to infraorbital-interaural horizontal plane; T, transverse or lateral to the midline sagittal plane.

Measurements have been made from four types of brain preparations listed on page 4.

TABLE 2
A COMPARISON OF MEAN LONGITUDINAL AND VERTICAL MEASUREMENTS TAKEN FROM GROSS
SAGITTAL SECTIONS OF THREE BEAGLE AND FOURTEEN MONGREL DOG BRAINS *in Situ*

Structure or specific site	Longitudi- nal (interaural zero)		Vertical (horizontal zero)									
			Ventral surface of brain		Dorsal surface brain stem		Dorsal surface of cerebellar cortex		Corpus callosum		Dorsal surface of cerebral cortex	
	B	M	B	M	B	M	B	M	B	M	B	M
C 15			0.8	0.9			26.5	26.1				
Opening of spinal cord	14.2	14.4			6.6	7.6						
C 10			-0.9	-0.3			28.3	28.2				
Fastigium, middle of	6.7	7.1			12.5	13.1						
C 5			-0.7	-0.3			29.2	28.8			40.0	39.0
Interaural zero	0	0	0	0			25.5	28.0			41.0	42.0
Midline intercollic- ular groove	4.2	4.6			19.2	18.2						
R 5			0.8	0.2							43.0	43.0
Rostral border of pons	9.1	10.0										
R 10			5.8	3.7					27.7	27.4	44.0	43.0
Midline midbrain- thalamic junction	11.8	11.8			18.3	17.7						
Caudal end of mammillary bodies	14.2	15.0										
R 15			3.8	4.3					27.1	27.4	43.0	43.0
R 20			2.3	2.9					25.2	25.3	42.0	40.0
Midpoint of anterior commissure	24.8	25.0			12.6	12.2						
Midpoint of optic chiasma					5.8	5.2						
R 25			4.6	4.0					24.1	23.5	42.0	42.0
R 30			5.8	4.9					23.6	22.6	40.0	40.0
Ventral surface of corpus callosum			15.7	15.6								
Rostral end of genu corpus callosum	34.5	34.0										
R 35			5.0	4.6							38.0	38.0
R 40			3.7	3.0							37.0	37.0
R 45			1.0	1.3							34.0	33.0
R 50			1.3	1.4							25.0	27.0
R 55			1.0	3.6							19.0	18.0
Frontal pole	54.7	55.2										

B—data from the brains of three pure-bred beagles weighing 10.2 ± 0.6 kg.

M—data from the brains of fourteen mongrel dogs weighing 10.0 ± 2.0 kg.

All measurements are in mm.

safely frozen by dry ice for microtome sectioning without destruction of tissue structure by ice crystals. Serial microscopic sections, 50 microns thick are cut frozen or in celloidin. Two adjacent sections at 0.5 mm intervals are stained, one by the Weil and the other by the Nissl method. A series of 46 micro coronal sections at millimeter intervals have been photographed and half sections of Weil and Nissl preparations mounted together. A line drawing of each such photograph has been prepared and labelled. These are printed at a magnification of $\times 4$.^{*} The photographs and drawings of the gross sections and reconstructions are printed in natural size ($\times 1$). It should be noted that although all scales are ruled in mm

^{*} Note that the upper photographs in Figs. 56-61, 65 and 67 are 2-3% larger than the other microphotographs.

and cm, the numbers on the black rulers refer to cm, while those on the "white" drawn scales refer to mm: all measurements are given in mm.

Two lines are drawn in each section along the two axes from which co-ordinates can be measured. The horizontal line passes through the interaural-infraorbital plane or vertical zero in coronal and sagittal sections. The vertical or second line, which is at right angles to the horizontal, passes through the midline or transverse zero in coronal and horizontal sections, and through the interaural line or longitudinal zero in sagittal sections. The reproductions should be sufficiently accurate to permit the direct measurement of the co-ordinates of any structure from these lines in any photograph or drawing without reference to the scale other than remembering that gross preparations are reproduced in natural size and microsections four times larger.

In labelling, the circles used for indicating location have usually been placed at about the center of the structure, but this has not always been feasible. Where the boundaries of the structure are not well defined, as in the case of intermingled fiber tracts or ill defined nuclei, the labelled location is admittedly an estimate. Abbreviations have been employed only when unavoidable. They are given in the index as such or are indicated by placing the part of the full name abbreviated in parentheses. The index provides a key to the planes in which the structure occurs as well as the figures and pages in which the structures appear.

It was intended to use the 1955 Paris Nomina Anatomica entirely for nomenclature, but lack of suitable terms to cover some of the structures of the dog's brain has compelled us to use other terms.

Since the publication of Horsley and Clarke's (1908) original paper on the stereotaxic instrument and method, in which the dog is mentioned only to be dismissed on the grounds of variability, or more correctly since the time Ranson and his school began to use the stereotaxic method (*circa* 1930), the cat (and monkey) has almost entirely replaced the dog in experimental neurology. Consequently there are few modern references on the dog's brain. Among these, the following have been consulted. Papez' (1929) comparative neurology, which contains good material on the dog, Grunthal (1929) and Rioch (1929-1931) on the dog's hypothalamus and diencephalon respectively, André-Thomas (1940) on the dog's cerebellum, Campbell (1905) and Woolsey (1952) on mapping the dog's cerebral cortex, Kaada (1951) on the dog's limbic system and Jewel and Verney (1957) on the dog's diencephalon and neurohypophysis. Much information has also been obtained from Kappers, Huber and Crosby (1936) comparative neuroanatomy and from atlases and monographs on the cat and human brain, viz., Hess' (1924-1956) hypothalamus and thalamus, Spiegel and Wycis' (1952) stereoencephalotomy, Singer and Yakolev's (1954) sagittal sections of the human brain, Jasper and Ajmone-Marsan's (1955) stereotaxic atlas of the diencephalon of the cat, Brodal's (1956) reticular forma-

tion in the cat, and Leontovich and Mering's (1956) data on the topography of the dog's brain.

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