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THE HYPOTHALAMUS OF THE ADULT GUINEA PIG IN STEREOTAXIC COORDINATES

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ABSTRACT

A stereotaxis atlas of the hypothalamus of the adult guinea pig was prepared using males of a Hartley strain. The coordinates of the atlas were utilized in a study of hypothalamico-pituitary function in which experimental lesions were accurately placed in a variety of hypothalamic sites. Distances between various sutural landmarks on the skull were recorded, and a correlation was found between these and body weight.

The atlas is presented in view of the paucity of detailed anatomical descriptions of the hypothalamus of the guinea pig and the increasing utilization of this common laboratory animal in stimulation and lesion experiments relating to pituitary function. The only available atlas of the adult guinea pig brain is not suitable for use with a small animal stereotoxic instrument with the standard incissor bar and nose clamp adaptor.

INTRODUCTION

Two stereotaxic atlases of the guinea pig brain have appeared in recent years. In 1964, Luparello, Stein and Park presented an atlas of the hypothalamus of the immature guinea pig that is not suitable for use with the adult animal. More recently, Tindal (1965) published an atlas of the forebrain of the adult guinea pig based upon large (700-830 g), female animals of an unspecified strain. The standard head position in Tindal's atlas is obtained through the utilization of eye bars and a plate clamp found on standard cat-monkey stereotaxis instruments. This method of head placement was found unsatisfactory in our hands.

Before a study of hypothalamico-adenohypophyseal function could be made in the guinea pig, it was necessary to construct an atlas of the adult, guinea-pig hypothalamus with suitable stereotaxic coordinates for placement of experimental lesions. The atlas presented here was utilized successfully in these studies (Benson, '66a, '66b) and was found to be an aid in the placement of small, electrolytic lesions in a variety of hypothalamic sites. The atlas was prepared using animals of a standard, albino strain of guinea pigs, and the head is held by means of an incissor bar and ear plugs similar to those found on most small-animal stereotaxic instruments.

This atlas is presented because of increasing interest in the utilization of the guinea pig in neuroanatomical and neuroendocrinological studies, and the paucity of detailed anatomical descriptions of the adult guinea pig brain. The anatomy of the mammalian hypothalamus has been studied extensively. A few of those contributing more thorough descriptions are Gurdjian (1927) and Krieg (1932) in the rat, Rioch (1929) in the dog and cat, Papez (1932) in the armadillo, Jessup and Shanklin (1940) in the Lebanese coney, Lod (1931) and Bodian (1939, 1940) in the Virginia opossum, Ingram (1940) the hypothalamus of primates, and Diepen (1941) has described the hypothalamus of the sheep. However, no adequate description of the hypothalamus of the guinea pig is available. Frey (1937) only mnetioned the hypothalamic region of the guinea pig, giving illustrations, but no exact description of the relations of its nuclei. Dankmeijer and Nauta (1945) described only the magnocellular nuclei (filifomr and supraoptic) in the guinea pig.

Material and Methods

1. Animals

Eighty-six adult, male, albino guinea pigs of a Hartley strain were used. Bilateral, electrolytic lesions were placed in the hypothalamus of sixty-eight of these. Animals were selected over a wide range of body weight, between 500 and 900 grams. It was subsequently found that animals between 550 and 750 grams were better suited, and the atlas may be used most effectively in animals with a body weight of 650± grams.

All animals were housed in individual cages, maintained in a temperature, humidity, and light controlled room, and fed standard guinea pig lab chow and water *ad libitum*.

2. Fixation and staining of brains

The brains were perfused in the following manner: Each animal was anesthesized with sodium pentobarbital (40 mg/k, i.p.) and the thorax was opened by bilateral, parasaggital incisions through the ribs and intercostal muscles with cranial reflection of the sternum. A clamp was placed on the thoracic aorta and inferior vena cava, the right atrium punctured, and the bloow allowed to pour freely into the thoracic cavity.

A few milliliters of physiological saline were introduced into the left ventricle through a small hypodermic needle and rubber tube fitted to a reservoir stationed on a shelf approximately a meter above the animals. This was followed by approximately 50 milliliters of a buffered, 10% (v/v) formalin solution, introduced slowly from a second reservoir on the sanne shelf. The perfusion period lasted approximately 30 minutes, the rate of fiow being adjusted with a screw-type clamp

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fitted onto the rubber tubing. After perfusion the brains were removed and stored in neutral, 10% formalin.

After washing in water, the brains were dehydrated by bathing in a graded series of ethyl alcohol solutions (50-80-95%, v/v) to a final day in absolute alcohol with several changes. Next the brains were allowed to remain for twenty-four hours in a 50-50 (v/v) mixture of absolute alcohol and diethyl ether, and subsequently run through dilute cellodin (cellulose nitrate, 2-3%, wt/v) and a thick (10%) cellodin solution. The brains, with 14% celloidin about them, were mounted on a fiber block, hardened in an atmosphere of chloroform, and stored in 80% alcohol.

All celloidin embedded brains were sectioned with the sliding microtome at 100 micra in frontal plane corresponding to that of the atlas. Serial sections were numbered, and every fifth section was stained with a 1% (wt/v) aqueous solution of to'uidin blue and permanently mounted on glass microscope slides.

An additional four brains, having been perfused and fixed in neutral formalin, were thoroughly washed and placed in Bouin's fixative. After removal of the picric acid by repetitious washing in 70% alcohol, these were dehydrated, embedded in paraffin, and after serial sectioning at 20 micra were stained by a silver method for paraffin sections.

3. Electrolytic coagulation lesions

Electrolytic coagulation lesions were placed in the hypothalamus with the aid of a Kopf stereotaxic instrument equipped with small-animal ear plugs and an adjustable incissor bar and nose clamp. Previous studies indicated that direct current in the range of 3 ma passed in a circuit between a monopolar electrode acutely placed in the brain and a silver indifferent lead in the rectum produced a lesion approximately 1.0 to 1.5 mm in diameter when allowed to flow for a period of 20 seconds. The size of the lesion was graded down by reducing the duration of current flow while keeping the amperage constant. The coagulating electrode was constructed by the repeated dipping of a small, straight, steel, suturing needle in an insulating varnish. The insulation was removed from approximately 1.0 mm of the pointed tip.

Preparation of the Atlas

1. Standard head position

By definition the midsagittal plane passes through bregma, the midpoint on the interaural line, and the junction of the frontal and nasal bones at the midline. A horizontal plane was established that was perpendicular to the midsagittal plane and passed through bregma and the frontonasal suture in the midline. In the standard head position any frontal plans was perpendicular to these two planes.

An adult, male guinea pig was chosen that weighed 650 grams. After anesthesia the animal's ears were slit to expose each external auditory meatus, the ear bars were inserted, and the head of the animal was centered by symmetrical adjustment of the ear bars. The head was brought to the standard position by dorsoventral adjustment of the movable incissor bar and nose clamp until bregma and the frontonasal suture were in a horizontal plane that was perpendicular to the vertical line passing through the interaural point (refer to Figure 1.)

With the head in the standard position two stiff wires, 0.7 mm in diameter, were placed on the right and left side at the anterio-posterior zero (OAP) or interaural frontal plane, 5.0 mm on either side of the midline (±5L). The positions were marked on the skull and openings were made in the valvarium with a small dental burr and electric drill. The wires were lowered to a point 10.0 mm above the interaural horizontal plane. A single wire was placed 10.0 mm anterior to the interaural frontal plane (10A). This wire was brought into a position 2.0 mm off the midline on the right (+2L) and lowered vertically to a point 13.0 mm (+8V) above the interaural horizontal plane. Additionally, a wire was placed at the midline (OL) 6.0 mm posterior to the interaural frontal plane (6P) and was taken vertically to a point 2.0 mm (—3V) above the horizontal interaural plane.

With these wires in place the animal was opened along the ventral abdominal wall and thorax and perfused with saline and neutral, 10% formalin as described above. After two hours fixation time the wires were elevated, and the head was allowed to sit overnight in a fresh solution of 10%, neutral formalin. On the following day the head was again placed in the stereotaxic instrument, and a small straight steel rod (0.75 mm in diameter) was inserted from behind in a horizontal plane 5.0 mm (OV) above the interaural horizontal plane at the midline. With the rod in place the bone was removed and the brain placed in formalin for a 48 hour period. The indwelling rod was used for orientation of the brain for sectioning in a frontal plane and its trajectory for establishing a zero horizontal plane.

The wire tracts were well preserved in this brain, and the anterior-posterior distances between them coincided with the numbered and counted, 100 micra sections, indicating that essentially little shrinkage of the tissue occurred. Every fifth sections, after staining and mounting, was placed in a photographic enlarger, and a negative print was made, ten times the actual size by direct measurement. Millimeter coordinates were drawn on these prints in the vertical and lateral planes. The anterior-posterior coordinates were established by the wire tracts at the interaural frontal plane and marked in millimeters in front (A) or behind (P) that point. For the working atlas a series of india ink tracings were made at 0.5 mm intervals through the hypothalamic area beginning at a level where the posterior mammillary nucleus and cerebral peduncles are adjacent and extending to a level in the preoptic area where the optic chiasma and the anterior commissure are in frontal plane.

A system of coordinates was established about the interaural point. The vertical zero (OV) passes through the optic chiasma and is 5.0 mm above the horizontal plane passing through the interaural point. The anterio-posterior zero (OAP) is at the frontal plane passing through the interaural line. The lateral zero (OL) is at the midsagittal plane (see Figure 1.).

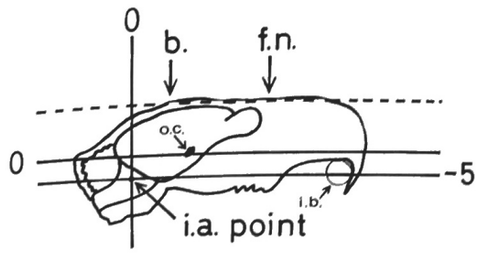

Fig. 1. Standard head position. The standard head position is derived by vertical adjustment of the incisor bar (i.b.) until bregma (b.) and the frontonasal suture (f.n.) are in a horizontal plane as indicated by the broken line. This horizontal plane is perpendicular to the anterior-posterior zero which passes through the interaural point (i.a. point). The vertical zero passes through the optic chiasma (o.c.) and is 5.0 mm above the horizontal plane passing through the interaural point.

2. Measurement of sutural landmarks in the skull.

Measurements of sutural landmarks were recorded on forty guinea pigs of various weights. With the head in the standard position the distances to the three confluences of bony sutures, i.e. lambda, bregma, and the frontonasal suture, were measured from the interaural frontal plane. Measurements are presented in Table 1. The distance from the interaural plane to the bregma and to the frontonasal suture was seen to increase in direct relationship with the increment in total body weight, while the distance to lambda decreased. When the mean body weights were plotted against the mean distances shown in Table 1, a straight line was obtained indicating a constant rate of growth and correlation between body weight and intersutural distances. The vertical distance to the horizontal zero plane was observed to be related to body weight in a similar fashion.

These data suggest that the skull of the guinea pig continues to enlarge after the animal reaches 500 g. The distance from lambda to the frontonasal suture (which might be taken as an index of overall brain length) was observed to be significantly different ($p < 0.01$) between animals weighing between 600-700 g and animals weighing between 805-815. This indicates that more precise and reproductable localization of neutral structures may be made with the coordinates of this atlas when close attention is paid to body weight. This was borne out in studies in which the atlas was employed.

3. The atlas

A stereotaxic atlas of the adult guinea pig hypothalamus is presented in Figures 2 through 10. These are photographs of original india ink tracings of frontal sections chosen at 0.5 mm intervals from the mammillary nuclei to the level of the optic chiasma. The figure in the upper right hand corner followed by the letter "A" indicates the distance in millimeters anterior or rostral to the interaural, frontal plane. The figures in vertical series on the left and below represent millimeters. The interaural point, not shown, is at —5.0 (±5V).

TABLE 1 Measurements of Sutural Landmarks in the Guinea Pig Skull

Weight In Grams	Number of Animals	Mean Weight	Distance From Interaural Plane To: (in mm±S.E.) Bregma	Fronto-nasal	Lambda	Vertical Distance from Bregma to Horizontal Zero (OV) (in mm±S.E.)
501-550	6	522	7.9±0.2	28.9±0.5	10.4±0.5	9.3±0.9
551-600	8	581	7.9±0.5	29.4±1.2	10.7±0.5	9.2±1.0
601-650	7	621	8.0±0.6	29.0±2.5	10.8±1.0	9.0±0.3
651-700	10	677	8.6±1.3	30.2±1.4	10.0±0.8	9.6±0.7
701-750	1	720	8.9—	31.3—	9.8—	10.0—
801-850	4	830	9.2±0.9	31.0±0.6	10.2±0.7	10.3±0.5
851-915	4	904	10.1±0.7	32.1±0.4	9.6±0.8	10.1±0.6

		Distance from Lambda to Frontonasal Suture (in mm) Mean	S.E.	
601-700	17	40.0	±0.33	$p < 0.01$
805-915	8	41.4	±0.39	

ABBREVIATIONS USED ON FIGURES 2-10

AA	Area amygdaliodea anterior
ABL	Nucleus amygdaloideus basalis pars lateralis
ACO	Nucleus amygdaloideus corticalis
AD	Nucleus anterodorsalis thalami
AHA	Area anterior hypothalami
AL	Nucleus anmygaloideus lateralis
AM	Nucleus anteromedialis thalami
AME	Eminentia mediana pars anterior
ARH	Nucleus arcuatus hypothalami
AV	Nucleus anteroventralis thalami
BCA	Nucleus proprius commissurae anterior
CA	Commissura anterior
CC	Corpus callosum
CE	Capsula externa
CH	Commissura hippocampi (Commissura fornicis)
CI	Capsula interna
CL	Nucleus subthalamicus
CLA	Claustrum
CPU	Nucleus caudatus / Putamen
DM	Nucleus dorsomedialis hypothalami
FI	Fimbria hippocampi
FIL	Nucleus filiformis hypothalami (Nucleus paraventricularis hypothalami)
FLD	Fasciculus longitudinalis dorsalis
FR	Fissura rhinalis
FX	Fornix
GE	Nucleus gelatinosus thalami
GD	Gyrus dentatus
GL	Corpus geniculatum laterale
GP	Globus pallidus
HL	Nucleus habenularis lateralis
HM	Nucleus habenularis medialis
HP	Hippocampus (Cornu ammonis)
INF	Infundibulum
LM	Nucleus mammillaris lateralis
LHA	Area lateralis hypothalami
LS	Nucleus lateralis septi
LT	Nucleus lateralis thalami
MD	Nucleus mediodorsalis thalami
MFB	Fasciculus medialis telencephali (Medial forebrain bundle)
ML	Lemniscus medialis
MM	Nucleus mammillaris medialis
MP	Pedunculus mammillaris
MS	Nucleus medialis septi
MT	Tractus mammillothalamicus
OC	Chiasma opticum
OT	Tractus opticus
PC	Pedunculus cerebri
PF	Nucleus parafascicularis thalami
PH	Nucleus posterior hypothalami
PIR	Cortex piriformis
PM	Nucleus mammillaris posterior
PMD	Nucleus premammillaris dorsalis
PME	Eminentia mediana pars posterior
PMV	Nucleus premammillaris ventralis
POA	Area preoptica
PT	Nucleus parataenialis thalami
PV	Nucleus paraventricularis thalami
PVG	Substantia grisea periventricularis
RE	Nucleus reuniens thalami
RH	Nucleus rhomboideus thalami
RT	Nucleus reticularis thalami
SC	Nucleus suprachiasmaticus
SM	Stria medullaris thalami
SN	Substantia nigra
SO	Nucleus supraopticus hypothalami
ST	Stria terminalis
SUM	Area supramammillaris
TH	Thalamus
TI	Tractus infundibularis
TOL	Tractus olfactorius lateralis
TT	Tractus mammillotegmentalis
V	Ventriculus cerebri
VA	Nucleus ventralis thalami pars anterior
VD	Nucleus ventralis thalami pars dorsomedialis
VE	Nucleus ventralis thalami
VM	Nucleus ventromedialis hypothalami
ZI	Zona incerta

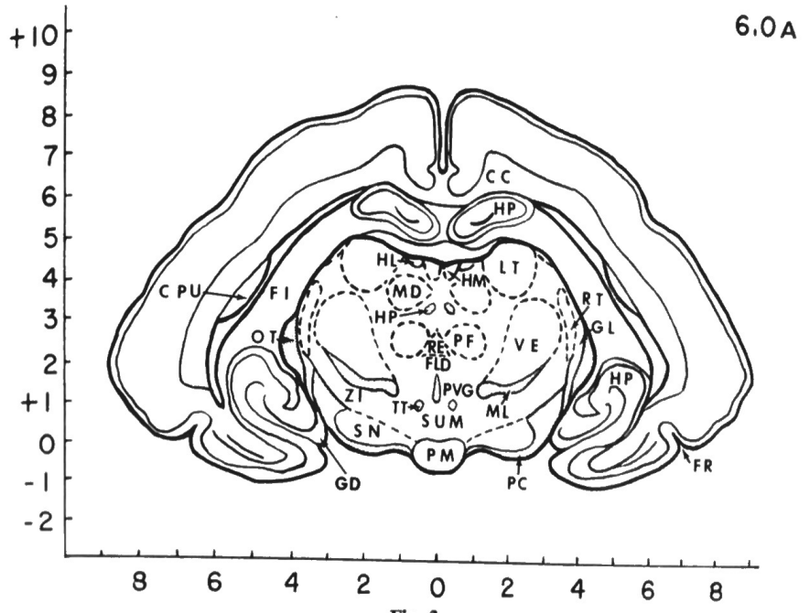

Fig. 2

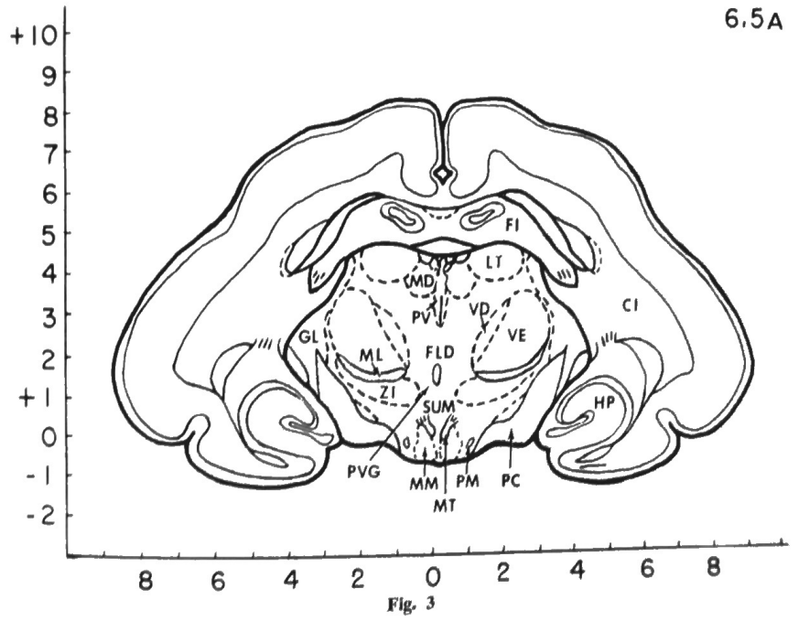

Fig. 3

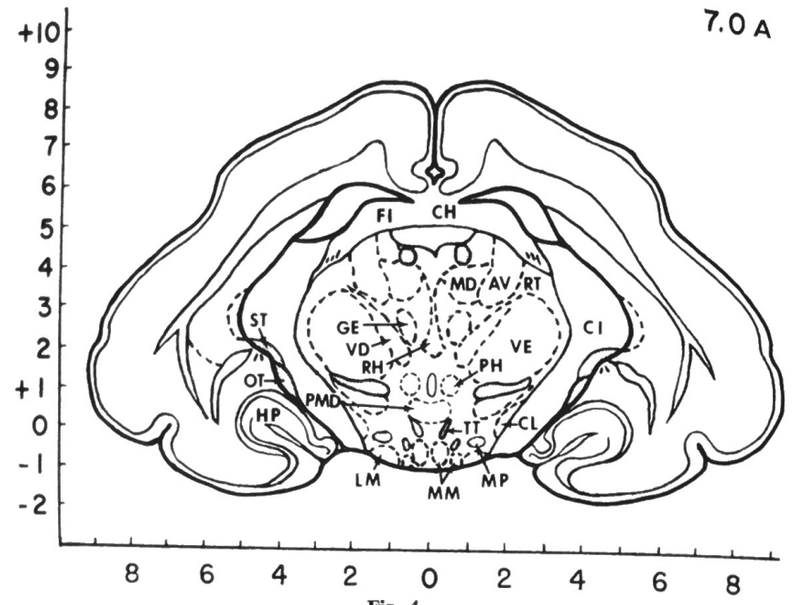

Fig. 4

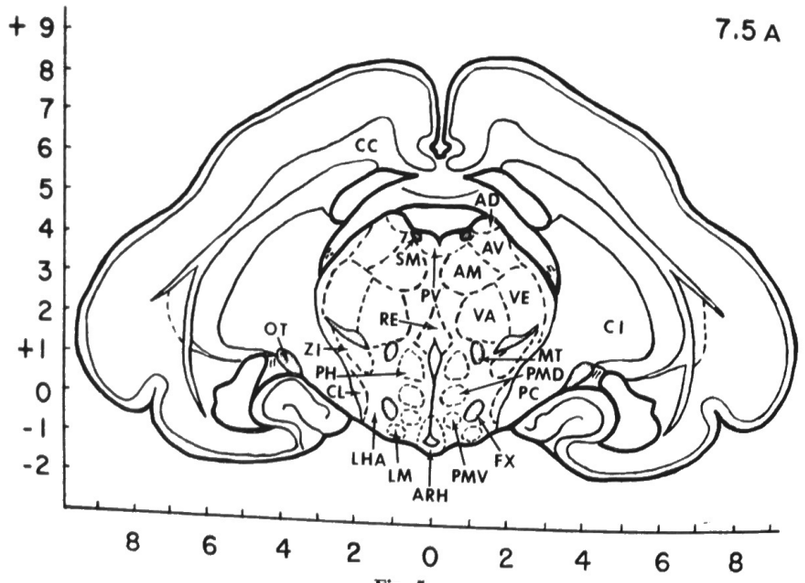

Fig. 5

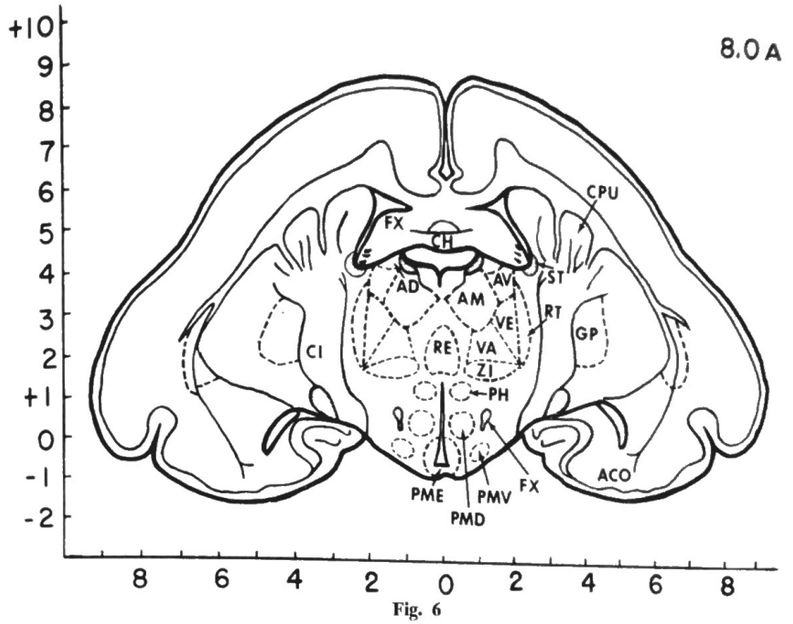

Fig. 6

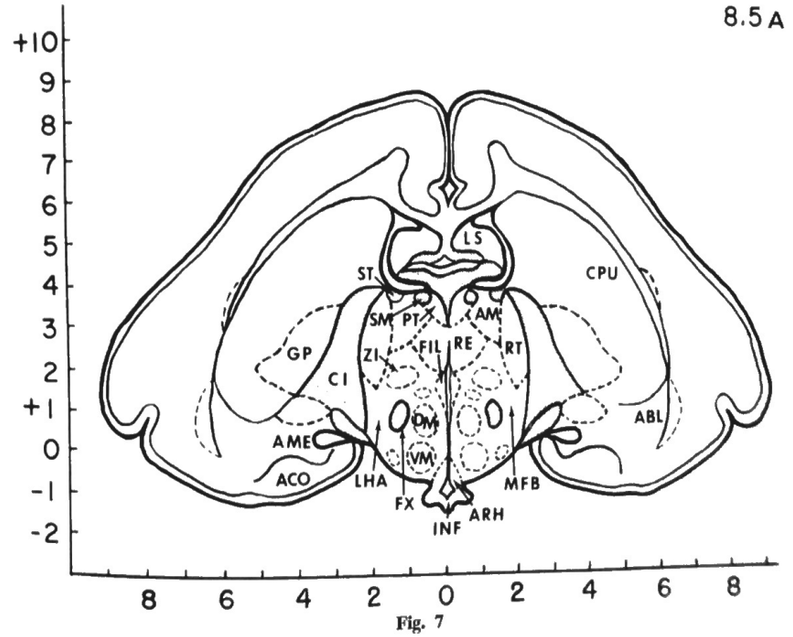

Fig. 7

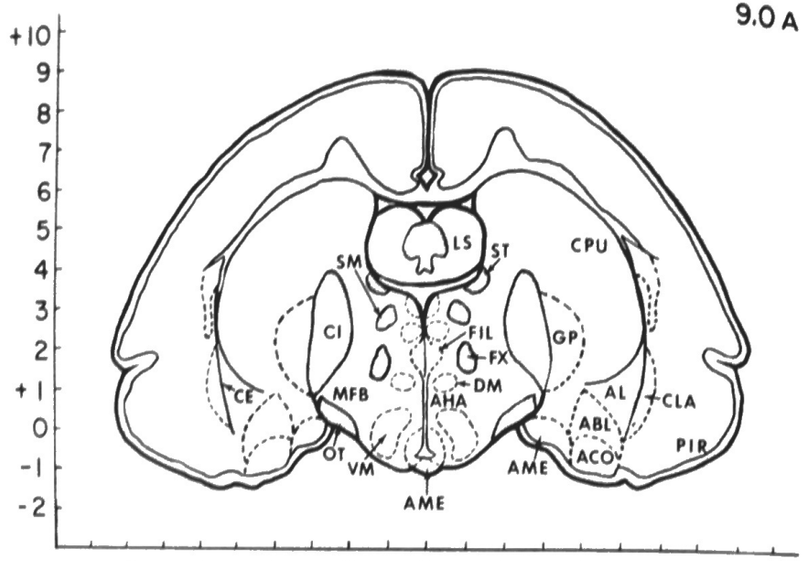

Fig. 8

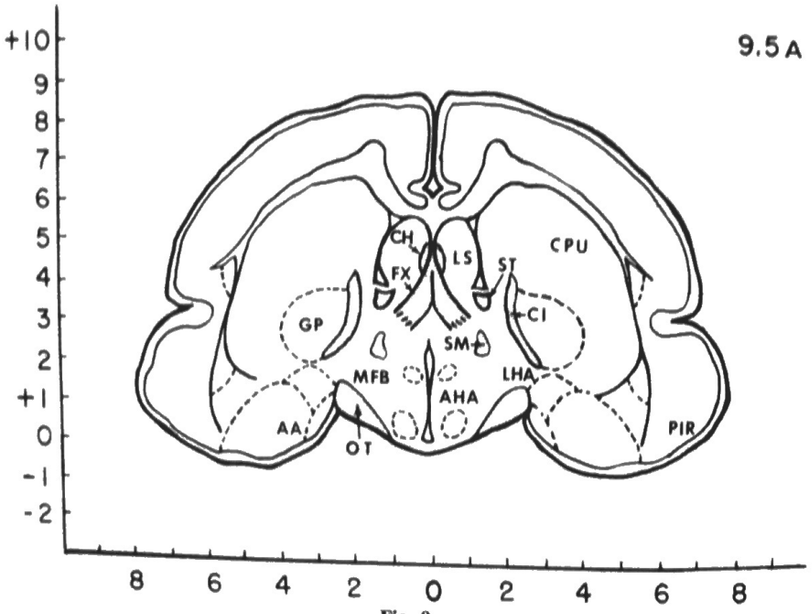

Fig. 9

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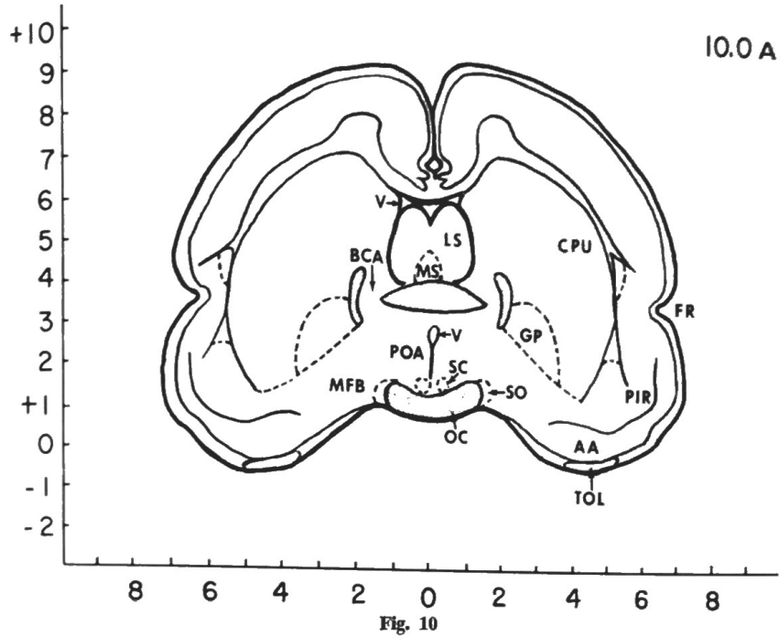

Fig. 10

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