LOCALIZATION OF MONOAMINE FLUORESCENCE IN THE BRAIN OF THE CAIMAN SCLEROPS

by

Nils O. Lellky Bengtson, B.A., B.S.

THESIS

Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science in Biology
at

THE UNIVERSITY OF HOUSTON
August, 1972

ACKNOWLEDGMENTS

A great deal of help was offered in this study and acknowledgment is made of the extensive instruction and guidance by Dr. Sara E. Huggins, who was instrumental in the successful completion of this project. Portions of the freeze-drying apparatus and the vacuum oven assembly were made with assistance from Mr. John H. Vascu. Special thanks are given to Dr. Georges Ungar for his contribution of dry ice, to Dr. Truman J. Odiorne for his contribution of liquid nitrogen, to Dr. N. Catherine Cominsky for her contribution of facilities for histological staining, and of her technical assistance in this connection, and to Dr. John H. Perry at Baylor University College of Medicine and Dr. Donald C. Kroeger at the University of Texas Dental Branch for their making available fluorescent microscope and camera assemblies. Finally, many thanks are given to Committee members, Dr. N. Catherine Cominsky and Dr. Aubrey P. Kimball for their patience and assistance. This study was supported in part by grant number MH 16894.

LOCALIZATION OF MONOAMINE FLUORESCENCE IN THE BRAIN OF THE CAIMAN SCLEROPS

by

Nils O. Lellky Bengtson, B.A., B.S.

AN ABSTRACT

Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science in Biology
at

THE UNIVERSITY OF HOUSTON
August, 1972

ABSTRACT

This investigation used the Falck-Hillarp histochemical fluorescence technique to locate and map monoamine compounds in the brain of the spectacled caiman, Caiman sclerops. This method which has been used to differentiate between catecholamines and serotonin on the basis of color in a number of animal species was found to work well on the crocodilian brain. The fluorescent cells have been observed to be connected with particular structures including substantia nigra to neostriatum, medulla to olfactory bulb and cortex, medulla to spinal cord, and specific hypothalamic connections. Fluorescent cell groups have been located and assigned numbers in the rat and include A1-4 and B1-3 in the medulla oblongata, A5-7 and B4-6 in the pons, A8-10 and B7-9 in the mesencephalon, and A11-13 in the diencephalon. Fluorescent cell groups were found in the caiman and sufficiently resembled the groups found in the rat to assign these same identifying numbers to groups of cells in the caiman brain. Additional strong fluorescence was observed in the pyriform cortex, hippocampus and in a region of the posterior diencephalic ependyma. A fluorescent cell group observed in the medulla and not previously reported in the rat was given the number "Ax." Dull fluorescence was observed in the basal ganglia and general cortex.

TABLE OF CONTENTS

I. REVIEW OF THE LITERATURE

Cholinergic and monoaminergic neurons constitute only five to ten percent of the total brain neurons but supply much of the information about brain activity and function. The transmitter substances associated with these neurons are the most widely accepted ones, namely acetylcholine, norepinephrine (NA), dopamine (DA), and serotonin (5-HT), and, with the exception of acetylcholine, they possess the common characteristic of forming fluorescent compounds in the presence of formaldehyde. In this connection, two techniques developed during the last decade and used extensively have been very valuable in identifying monoaminergic neurons *in situ* in the brain. One is the autoradiographic localization of labeled NA following its injection into the cerebrospinal fluid, developed by Glowinski and Baldessarini (Anton-Tay, 1971), the other is the histochemical fluorescence technique initiated by Hillarp and developed by Falck and Hillarp (Falck, *et al.*, 1962) which is of immediate interest to this study.

From a chemical viewpoint, the substances which are central to the histochemical fluorescence research are two catecholamines, NA and DA, representing one of a group of similar compounds having a sympathomimetic action, and consisting of a catechol (1, 2-dihydroxybenzene) and an amine as the aliphatic portion (Falck, *et al.*, 1962). In the presence of formaldehyde, NA and DA become intensely fluorescent due to the closure of the side chain by a methylene bridge and subsequent dehydrogenation resulting in the formation of intensely fluorescent and characteristically yellow-green isoquinolines (Eränkö, 1967). Epinephrine, also a catecholamine, plays a minor role in the histochemical fluorescence method due to its negligible fluorescence. Serotonin, on the other hand, is an indoleamine which reacts with formaldehyde to form a beta-carboline compound which exhibits a stronger yellow fluorescence that requires a higher energy reaction; is formed in lesser quantities and is more ultra-violet (UV) light sensitive than the isoquinoline

compounds (Fuxe and Jonsson, 1967). Primary amines with hydroxyl groups in the three and four position thus give intense fluorescence with formaldehyde. Secondary amines like epinephrine react to a much lesser extent and therefore have very weak to negligible fluorescence; no condensation reaction takes place with tertiary amines and amides (Falck, et al., 1962). One characteristic of the NA, DA and 5-HT fluorescence is its notoriously rapid fading, which can be significantly reduced by thorough drying of the tissue in vacuo over phosphorous pentoxide, or by heating for at least one hour at 150° (Falck, et al., 1962). Falck further explains that in this way the isoquinolines and beta-carbolines undergo an acid hydrolysis linkage with protein layers of the tissue resulting in an orientation more receptive to fluorescence. The condensation reaction with primary catecholamines to form fluorescent compounds is well established in organic chemistry and is known as the Pictet-Spengler reaction, a general condensation reaction in which primary and secondary beta-arylethylamines with a carbonyl compound yield a tetrahydroisoquinoline derivitive (Corrodi and Jonsson, 1967). Corrodi and Jonsson also point out that this reaction with NA and DA is quantitative, lending added potential value to the histochemical fluorescence technique. To insist that catecholamine isoquinolines yield highly fluorescent compounds is less than correct since these compounds do not fluoresce in the visible range. Instead, Corrodi and Jonsson describe a "protein promoted" dehydrogenation reaction (catalyzed only by glycine and arginine and their dipeptides), yielding corresponding tautomeric quinoidal forms of the isoquinolines which in the dry protein layer display strong fluorescence in the visible range. Since the distinction between the catecholamines and serotonin can usually be made on the basis of color differences and high UV light sensitivity of the latter, the major problem encountered during fluorescence examination is the distinction between NA and DA. An improvement over the subjective color differentiation between yellow-green NA and gold-green DA is the developed spectrofluorimetric assay results which have been published by

several investigators including Baumgarten (1967). Since maximal excitation of NA is 416 nanometers, the distinction can be made between NA and DA which has its maximum excitation shifted to the left, 412 to 414 nanometers.

The basic histochemical technique with its laboratory procedure has been described in detail by Falck and Hillarp (1962), Anden, et al., (1966a and b), Eränkö (1967) and Corrodi et al., (1966). Hillarp (Corrodi, 1967) is generally credited with the development of catecholamine fluorescence techniques in dry protein layers exposed to formaldehyde, (which followed his attempt to employ the chromaffin method, Falck and Hillarp, 1959). Hillarp, in association with Falck applied this principle to air dried tissue and subsequently to freeze-dried tissue. The procedure involves freezing the tissue in liquid propane, drying in vacuo at -35 degrees Celsius and eventual exposure of the tissue to paraformaldehyde at least for one hour in a closed vessel. Since 1962 various variations and modifications of the standard Falck-Hillarp method have been described, (Snyder, Axelrod and Zweig, 1965; Bjorklund, 1969a; Maickel, 1968; Corrodi and Jonsson, 1967; and, Fuxe, et al., 1970), including the one for this study devised by Masuoka (1969, personal communication). It is important to realize that the histochemical fluorescence technique is relatively sensitive and susceptible to error. The freezing must be rapid, the tissue must be dried at a temperature lower than -30 degrees, the temperature of exposure and water content of the paraformaldehyde must be properly adjusted for each tissue, and avoidance of light and atmospheric humidity must be observed; all these precautions facilitate the achievement of optimal fluorescence with the least diffusion. Further refinements of the technique involving methods of obtaining optimal fluorescence have been reported by Corrodi and Jonsson (1967). For optimal fluorescence reaction with serotonin the use of more severe reaction conditions is necessary, e.g. increased time of exposure to and higher humidity of formaldehyde gas is necessary (Corrodi and Jonsson, 1967 and Fuxe and Jonsson, 1967). The UV light

which activates the fluorescence properties does so by photodecomposition, and it is the great susceptibility of serotonin to photodecomposition that helps to distinguish it from the catecholamines which are more stable to UV light. Mounting tissue in water medium causes weak fluorescence to result by quenching and therefore moisture of any kind should be avoided although Corrodi and Jonsson insist that recovery of fluorescence can be accomplished through dehydration.

With an appreciation of the basis of histochemical fluorescence and a mastery of accompanying techniques, purposeful investigation can be made with the result of elucidation of individual NA, DA and 5-HT central pathways and their organization in the brain. Monoamine cell bodies are located in various places along the brain stem and higher brain centers and their axons are arranged in tracts. These tracts extend from these cell bodies ascending, descending, or both and innervating structures from the telencephalon to the cord. The functional significance of these tracts generally depends upon the type of neuron to neuron contact, i.e. axosomatic, axoaxonic, axodendritic, as well as the particular monoamine that is serving as the transmitter substance (Fuxe, Hokfelt and Ungerstedt, 1970; Andén, et al., 1966a; Dahlström and Fuxe, 1965; Fuxe, 1965; and, Fuxe, et al., 1970). In the rat brain, which serves as the basis for comparison with the caiman brain in this study, there are several pathways widely accepted and associated with each monoamine. On a very general level then, there is a descending bulbospinal tract associated with NA and/or 5-HT (Dahlström and Fuxe, 1965; Fuxe, 1965; and, Dahlström and Fuxe, 1964c); extensive NA and 5-HT groups ascending along the medial forebrain bundle to the limbic system, hypothalamus and preoptic areas (Andén, et al., 1965a and Dahlström and Fuxe, 1964c); a large DA nigro-neostriatal system leading to the caudate and putamen by way of the internal capsule (Fuxe, Hokfelt and Ungerstedt, 1970 and Andén, et al., 1966a, 1965b and 1964); and, hypothalamic catecholamine connections. The hypothalamic connections include a large DA axon group from the arcuate nucleus and periventricular nucleus to the

median eminence, a large norepinephrine axon group originating beyond the mediobasal hypothalamus and leading to the median eminence, and minor tracts that are both small and difficult to identify by transmitter substance (Osborn, 1969; Marley and Stephenson, 1969; Odake, 1967; Barry, 1967; Fuxe and Hokfelt, 1969; and, Fuxe, Hokfelt and Ungerstedt, 1970). An elaboration of these paths is supplied by reports of Dahlström and Fuxe (1964), Fuxe, Hokfelt and Ungerstedt, (1970) and Ungerstedt (1971). These reports supply a basis for classifying cell groups of origin of monoaminergic pathways. Norepinephrine cell groups are designated by the numbers, A1 through A7, dopamine cell groups by numbers A8 through A13, and serotonin groups by the numbers B1 through B9. These are described for the rat but the classification has also been applied to other animals such as the pigeon and rabbit (Fuxe and Ljunggren, 1965, and Dahlström, 1967, respectively).

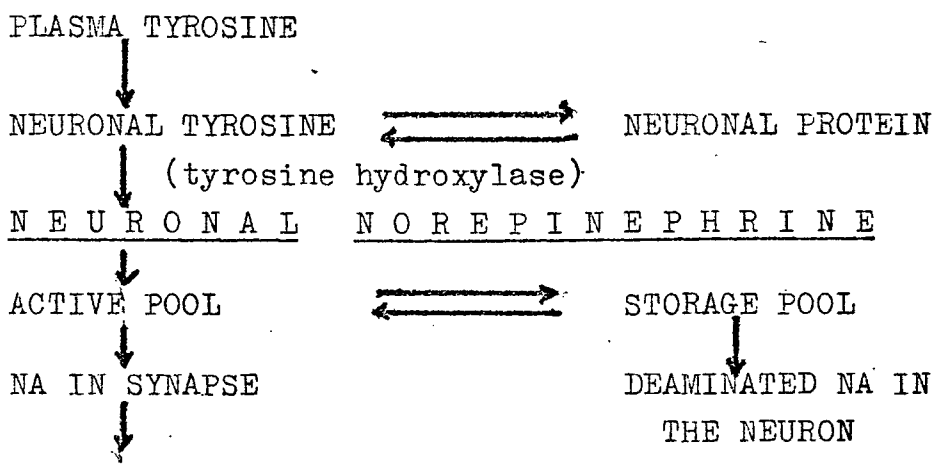
Group A1 consists of small to medium sized cells forming a connected system from the level of the motor decussation to the anterior third of the inferior olivary complex. Group A2 is a small group of small to medium sized cells where the nucleus tractus solitarii, nucleus motorius dorsaliz nervi vagi and nucleus commisuralis are situated. Group A3 has only a few small weakly fluorescent cells found in the nucleus olivarius accessorius dorsalis. Group A4 is found at the level of the nucleus nervi facialis and consists of medium sized cells. Group A5 is a small group of medium sized cells found at the level of the tractus rubro-spinalis especially at the level of the posterior and middle third of the nucleus olivaris superior, medial to outgoing fibers of the nucleus facialis. Group A6 belongs to the locus coeruleus. Group A7 is located around the posterior third of the griseum pontis and consists of medium to large sized cells. Just posterior to the red nucleus is group A8, a large group of medium sized cells within the reticular formation. Within the substantia, mainly within the zona compacta and zona reticulata is the large group of medium sized cells belonging to group A9. The ventrolateral part of this

group forms an indistinct border with the ventromedial part of group A8. A10 represents the largest group in the mesencephalon, located in the area dorsal to the nucleus interpedunculus and running caudally within the posterior part of the nucleus lanearis, as well as cranially within the area corresponding to the nucleus parabrachialis pigmentosus in the cat. Group A11 is a small group located close to the third ventricle medial, dorsal and ventral to the fasciculus retrofluxus within the substantia grisea periventricularis. Group A12 and A13 correspond to the arcuate nucleus and the area surrounding the mammilothalamic tract respectively. Thus, for the dopamine and norepinephrine cell groups, A1 through A4 are in the medulla oblongata, A5 through A7 are in the pons, A8 through A10 are in the mesencephalon and A11 through A13 are in the diencephalon. The serotonin cell groups include B1 within the nucleus raphe pallidus which makes an indistinct border with the cells of group B3. Group B2 is a small group of medium sized cells in the area of the nucleus raphe obscurus. Group B4 consists of a few small to medium sized cells located under the fourth ventricle dorsal to the vestibular nuclei and the nucleus nervi abducens. Within the nucleus raphe pontis are the cells of group B5 found at the level of the nucleus motorius nervi trigemini. Group B6 extends from the fourth ventricle at the anterior end. Within the substantia grisea centralis, mainly in the nucleus dorsalis raphe, is group B7, and extending from the posterior end of the posterior collicle to the caudal third of the interpeduncularis are two large groups, B8 and B9. All the serotonin groups are confined to the posterior brain stem with B1 through B4 in the medulla oblongata, B3, B5 and B6 in the pons and groups B7 through B9 in the mesencephalon (Dahlström and Fuxe, 1964 and Ungerstedt, 1971).

The serotonin cells are generally confined to the raphe nuclei. Furthermore, Dahlström et al. (1962) point out that monoamine cell bodies have low concentrations of catecholamines and serotonin whereas their terminals contain extremely high concentrations of these substances. Generally, the distribution of dopamine is different from that of

norepinephrine in that the highest concentrations of the former will be found in the corpus striatum with only small amounts in other brain areas (Bertler and Rosengren, 1967). In addition, brain areas rich in catecholamines are also rich in DOPA decarboxylase activity, a fact which is easily understood with examination of the catecholamine pathway in which DOPA is converted to dopamine, and dopamine to norepinephrine (Bertler and Rosengren, 1967). Bertler and Rosengren elicit an appreciation for the catecholamine systems by further pointing out that areas of high norepinephrine concentrations are also areas of low dopamine concentration, and the reverse is true. Norepinephrine has concentrations in various parts of the brain but is found all over the brain; dopamine is limited to a few nuclei. Evidence of DOPA decarboxylase activity indicates the formation of catecholamines and in this situation dopamine represents an intermediate of norepinephrine but with a function of its own.

Precursors of the monoamines are amino acids, tyrosine for the catecholamines and tryptophan for serotonin. Anton-Tay and Wurtman discussed monoamine metabolism of the brain in their 1971 paper. They observed that tyrosine is taken from the circulation and its circulating levels show daily fluctuations. The enzyme tyrosine hydroxylase had been found to be rate limiting and regulated by end product inhibition.



Conversion of norepinephrine to epinephrine has been observed in the olfactory bulb, but evidence has not been presented to

indicate either the capacity of the brain for epinephrine formation or a role in neurotransmission. Serotonin is metabolized from tryptophan and its role in monoaminergic metabolism is exemplified by that of norepinephrine above.

The catecholamine and serotonin nerve cells have the same ultrastructure consisting of a granular reticulum and perinuclearly-situated Golgi apparatus (Fuxe, et al.,1966). Amine vesicles in the monoamine cells are formed in association with the Golgi apparatus and close to the nucleus. Of particular interest on the microstructural level is the sympathetic innervation of the pineal body which in the rat is the only innervation to the structure. Serotonin content of the body displays 24 hour cyclic rhythms with norepinephrine (Neff, 1969). Neff has found that the pineal body in the rat contains two serotonin storage areas, specialized cells called pinealocytes, and sympathetic nerve compartments. Further elaboration of monoaminergic structure and function has been observed by Owman (1967). Monoaminergic mechanisms are found in neurons of the vascular wall and endothelium of the brain vessels, both of which form barriers to the movement of certain amines and amino acids across the vessel walls. Enzymes acting on certain monoamines as well as on their precursors can be found in the walls of capillaries of the brain. Eränkö and Härkönen (1964) have discovered that

> some sympathetic ganglion cells may be both adrenergic and cholinergic at the same time. This is of special interest in view of pharmacological observations apparently indicating that stimulation of a cholinergic sympathetic fiber causes a liberation of acetylcholine which in turn may exert its effect by liberation of norepinephrine from the same fiber or an adjacent one.

The use of drugs to alter monoamine activity has contributed to the elucidation of brain function similarly to the way that research of the monoamine pathways have contributed to the clarification of brain organization. Thus Quay (1968) has noted variation in serotonin levels in different parts of the brain and concluded that 5-HT is a probable mediator of the sleep mechanism. Although he

cautions against the effects of handling, environmental and behavioral influences and circadian rhythm, his evidence indicates serotonin to be the major component of regulation of states of sleep and of regulation of the hypothalamic hypophyseal system as well. Papers by Maanen and Smelik (1967) and McKenne, et al. (1966) support this position as does the research of Baldy-Moulinier, et al. (1969) who employed EEG information along with norepinephrine and alpha-methyl-para-tyrosine injections to induce sleep changes. Thus Baldy-Moulinier, and Jouvet (1968) have found that drugs decreasing brain serotonin usually decrease sleep and vice versa. Jouvet selectively destroyed serotonin-containing nerve cells of the raphe system and found a permanent state of wakefulness resulted. Reis and Wurtman (1968) have tried to correlate pineal norepinephrine cyclic rhythm with light and sleep but lack the experimental evidence to support this point conclusively. In other research areas, the use of drugs to alter the monoaminergic activity in the brain has led to some clarification of function. The use of moncamine precursors and monoamine oxidase inhibitors intensifies the monoamines and their fluorescent compounds thus facilitating their examination. Weitzman, Baldy-Moulinier, Jonsson, et al. (1969), Walker (1970), Bartonicek (1967) and Carlsson, Falck and Hillarp (1962) have investigated the use of chemicals to change monoamine fluorescence. Nialamide, nialamide with L-DOPA, and 6-hydroxytryptamine, increase monoamine levels while reserpine, meta tyrosine, L-DOPA with reserpine, and nialamide decrease monoamine levels. The effects of melatonin (Hishikawa, 1969), parachlorophenylalanine (Koella, Feldstein and Czicman, 1968, and Watt, et al., 1969), morphine (Gunne, Jonsson and Fuxe, 1969), lysergic acid diethylamide (Foote, 1969) and ethanol (Corrodi, Fuxe and Hokfelt, 1966) have been reported.

II. MATERIALS AND METHODS

ANIMALS

The animals used in the experimental procedures described below were Caiman sclerops. They were young animals with an overall length of ten to fifteen inches and were obtained from pet centers in the city.

APPARATUS

Freeze-drying Equipment

Among the various techniques developed on the basis of paraformaldehyde-induced fluorescence of brain catecholamines and serotonin, the one of choice for this study was a modification of the standard Falck-Hillarp freeze-dry histochemical technique described in their 1962 paper. In order to achieve a relatively simple, low cost and rapid method of preparing brain tissue for eventual fluorescence examination, a freeze-drying apparatus was built as shown in figures 1 and 2. A Duo-Seal vacuum pump (Sargent-Welch Scientific Company, Skokie, Illinois) provided the necessary vacuum of five to fifteen micra of mercury as measured on a "Televac" (Frederich Company, Bathyres, Pennsylvania). The rest of the apparatus consisted of a refrigeration unit, the cooling coil of which fitted into a Fluo-war flask (Vir-tis Company, Gardiner, N.Y.) filled with methanol; the temperature of the methanol could be maintained at -30 to -35 degrees Celsius. A stainless steel rod, milled at the top to form a depressed tissue chamber and fitted with a broad flat lucite collar, could be lowered into the methanol. Vacuum was applied to the tissue by placing an appropriately blown glass "bell" over the tissue compartment, and connecting the former with the vacuum pump via vacuum tubing through a glass U-trap and through a Virtis Macrotrap. The U-trap fitted into a Dewar flask filled with liquid nitrogen and the Virtis trap was filled with methanol and dry ice. The Televac was inserted immediately in advance of the head nozzle of the vacuum pump enabling accurate reading of vacuum levels. In the course of several freeze-drying "runs"

the apparatus was modified somewhat using improvements worked out by Masuoka (personal communication). To increase the efficiency of the vacuum pump, the vacuum tubing was shortened; the conduction cylinder was replaced with a deep, narrow, stainless steel vessel into the bottom of which a milled stainless steel tissue tray could be lowered; and, the connection of the tissue vessel with the vacuum line was improved by the use of a single unit lead-in valve (Atmo-Vac, Refrigeration for Science, Inc., Oceanside, L.I., N.Y.) having a broad base and fitting the flat rim of the tissue vessel and equipped with a cut-off valve from the vacuum line. In the revised assembly only the Vertis trap was used. In both cases a light weight framework held a drying agent, phosphorus pentoxide, in mesh bottomed trays above the tissue; in the first "set up" only one tray was used, but in the second there was a stack of three. Connections throughout the vacuum line were secured with the use of adjustable tubing clamps and silicon gel. Permanent seals, e.g. the seal on the Virtis trap were made with silicon rubber. Tissues were quick frozen initially by dropping into a basket submerged in a small cylinder containing a liquefied mixture of 90% propane and 10% propylene. The gas mixture was liquefied by conducting it through a coil of copper tubing in a Dewar flask of liquid nitrogen and allowing it to flow into the cylinder which was submerged in the same Dewar flask.

Microtechnique Equipment

In addition to the apparatus already mentioned, the modified Falck-Hillarp method used for this study required the construction of a dry box enabling the handling of dry tissue in a low humidity environment (about 20%), a vacuum oven for low vacuum levels, and a fumehood.

Fluorescence Equipment

Examination of tissue was made in a darkened room using a Leitz fluorescent microscope with a high intensity mercury vapor light source. This included a filter system of UV, UG 5, and BG 12 filters. In order to achieve

maximum resolution a low fluorescence immersion oil was used. An overhead photographic system incorporating a Leica camera made it possible to photograph significant fluorescent areas.

METHODS

Methods of Freeze-drying

The procedure followed in freeze-drying was a modification of the Falck-Hillarp method as used by Masuoka. Equilibration of the paraformaldehyde to be used in the procedure required at least ten days to two weeks. Sulfuric acid and water were mixed in proportions such as to give 30%, 50% and 90% humidity in each of three dissicator jars. The sulfuric acid-water mixture was placed in the base of the jar in a layer of glass beads. A perforated porcelain plate was placed in the shoulder of each jar so that vapor could pass freely throughout the jar. Open petri dishes containing paraformaldehyde were placed on this plate.

Twenty four hours prior to killing, some animals were treated with Nialamide (50mg/kg), Nialamide and L-DOPA (50mg/kg of each) and alpha-methyl-metatyrosine (AMMT, 100 mg/kg). Some animals were also left untreated. Since the goal of this experiment was the localization of fluorescent substances and not the quantification of these materials, no strict schedule of dosage or controls was followed. The quantities given were the same for all treated animals except the first, regardless of the variations in weight.

On the day freezing was begun, a Dewar flask was filled with liquid nitrogen. The copper tubing coil and delivery spout and the insert for the propane-propylene mixture were placed in the Dewar flask. The gas mixture liquefied as it passed through the cold tubing and flowed into the insert cylinder (Figure 2).

When the conduction cylinder was used it was pre-cooled in a deep freezer while the methanol was being pre-cooled in the refrigeration unit, and when the deep tissue vessel was used, it, together with the tissue tray, was allowed to pre-cool in the methanol of the cooling unit. The temperature of the methanol was -35 to -30 degrees.

Numbered small pieces of Whatman #50 nonabsorbant filter paper were arranged near a pan filled with ice. The animal was killed using a rat guillotine, and working in the ice, the brain and cord were removed as quickly as possible and attached to the numbered filter paper pieces and dropped in the liquid propane-propylene. In later experiments whole brains were wrapped in Saran Wrap (Dow Chemical) permitting better identification of areas of fluorescence. When all needed tissues were frozen, the basket was lifted and the tissue was placed either in the tissue compartment of the conduction coil or in the tissue tray and lowered into the freezing vessel. The phosphorus pentoxide trays were put in place and covers secured and connected with the vacuum pump. The level of the vacuum was lowered to five to fifteen micra of mercury; this vacuum level was maintained for 72 hours during which time the trap was kept full of methanol and dry ice.

When the conduction rod was used, it was necessary to warm the rod slowly before the vacuum was bled. Otherwise the refrigeration unit was simply turned off. In both cases the vacuum bled slowly through a U-tube filled with silica gel.

Microtechnique Methods

Working quickly in the dry box, the tissue was then removed to a small platform in a screwcapped jar containing paraformaldehyde of desired humidity and placed in an oven in a hood, capped and maintained at 65 to 75 degrees. Several different schedules were tried for the paraformaldehyde exposure, ranging from one or two hours to eighteen hours. During this time, the non-fluorescent paraffin was melted and degassed for 30 minutes at 55 degrees.

Working in the dry box at a humidity of 20%, the tissue was placed in the tissue molds and immediately covered with the degassed paraffin. The molds were then removed, cooled, transferred to the oven and allowed to stand for 15 to 30 minutes under a low (two to four pounds) vacuum. The molds were then placed in the dry box for storage until use.

Fluorescence Methods

The paraffin blocks were mounted on wooden blocks and then sectioned on a standard rotary advance microtome at a thickness of seven micra. To avoid losing fluorescence which is rapidly destroyed by light and diffuses out with atmospheric humidity, only a few sections were made at a time. At each region in the block two sections were made, one for the immediate microfluorescence examination and one for subsequent histological staining and examination with light microscope. Sections for fluorescence examination were placed on glass microslides and heated on a hotplate to smooth out the tissue. Initially the tissue sections taken from the paraffin blocks were badly folded and wrinkled. The wrinkled sections were straightened by increasing the angle of the microtome blade to the paraffin block and by slow heating on the hotplate. Histological staining of the heat-fixed tissue sections presented a problem because tissue often came off in xylene. Failure to achieve proper results with a hexane-based toluidine blue staining method prompted the processing of tissue for histological staining separately from those for fluorescence examination. In this case the tissue to be stained was wet mounted onto the slide in a water bath. Thus the wet-mounted tissue could be stained using a routine hematoxylin and eosin staining technique with excellent results.

A bioscope tracing of each section of tissue was made and used to map fluorescent areas. An exposure time of three and one half minutes gave acceptable photographic results when Kodak High Speed Ektochrome daylight color reversal film (EH 135-36) was used. Staining of tissue for use with the light microscope was with the standard hematoxylin and eosin staining technique. Slides were left in the hematoxylin for three minutes and counterstained in the eosin for one and one half minutes: rinses consisted of varying concentrations of alcohol, acetic acid, ammonium hydroxide and xylene solutions.

Method of Study

Interpretation of results was significantly aided by the use of several references, especially Ariens-Kappers, Huber and Crosby (1936), Huber and Crosby (1926), Crosby (1916), Tuge (1932), Fuxe (1965), Hillarp, et al., (1966), Ungerstedt (1971) and Andén (1966a and b). The older papers provided the anatomical basis of identification; the more recent papers provided useful diagrammatic and definitive basis for NA, DA and 5-HT pathways. Stained reference slides were made available in the laboratory and were also found to be useful in the interpretation of results.

FIGURE 1. Freeze-drying Apparatus. Tissue tray(s) were placed in the stainless steel tissue vessel (A), which was resting in methanol (B) cooled by the refrigeration unit (C). Vacuum was secured by covering the tissue vessel and turning on the vacuum pump (D). Along the vacuum line there was the "Atmo-vac" (E), trap (F) filled with dry ice and methanol, and the "Televac" (G). Temperature of the methanol around the tissue vessel was measured by a thermometer (H).

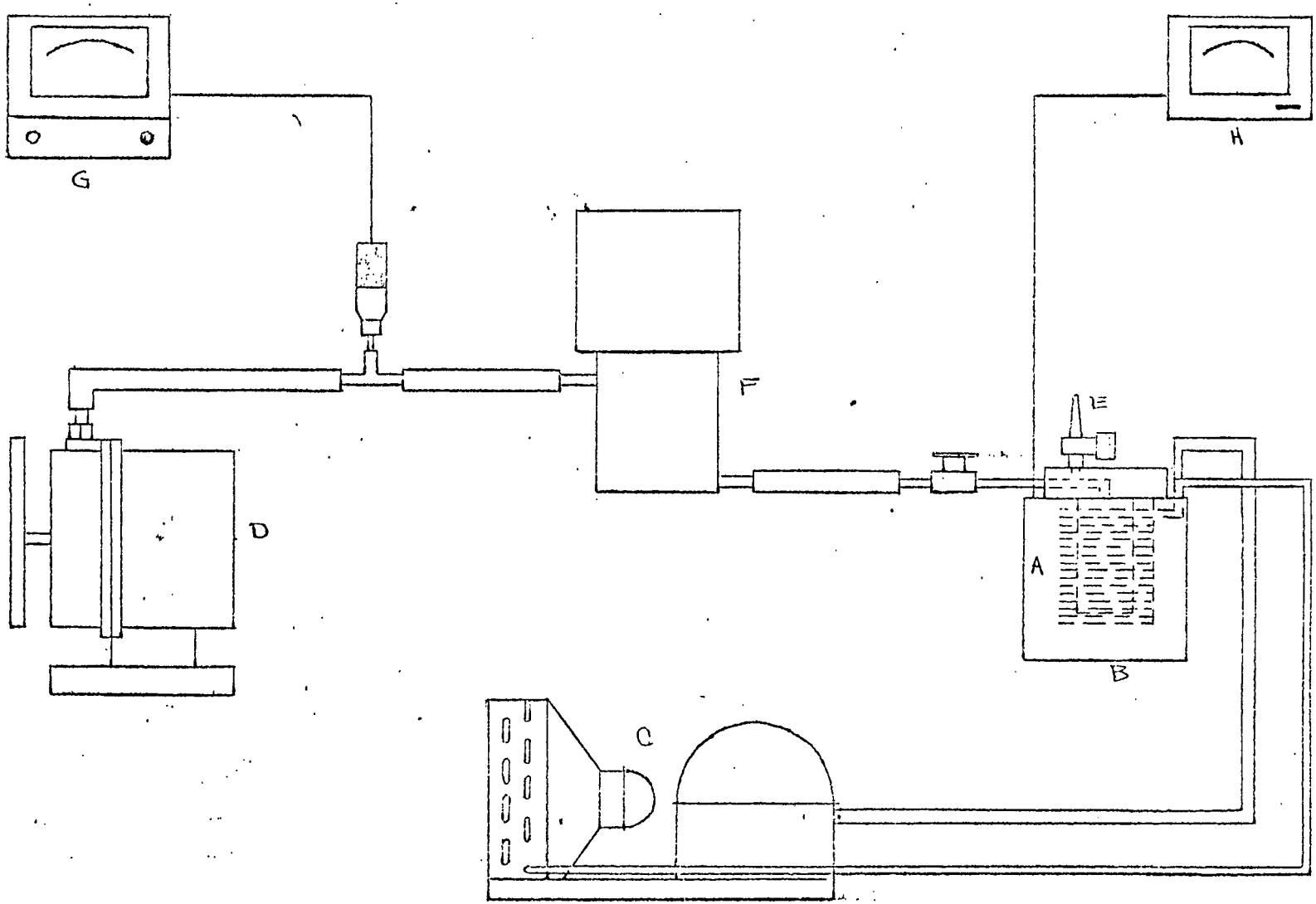
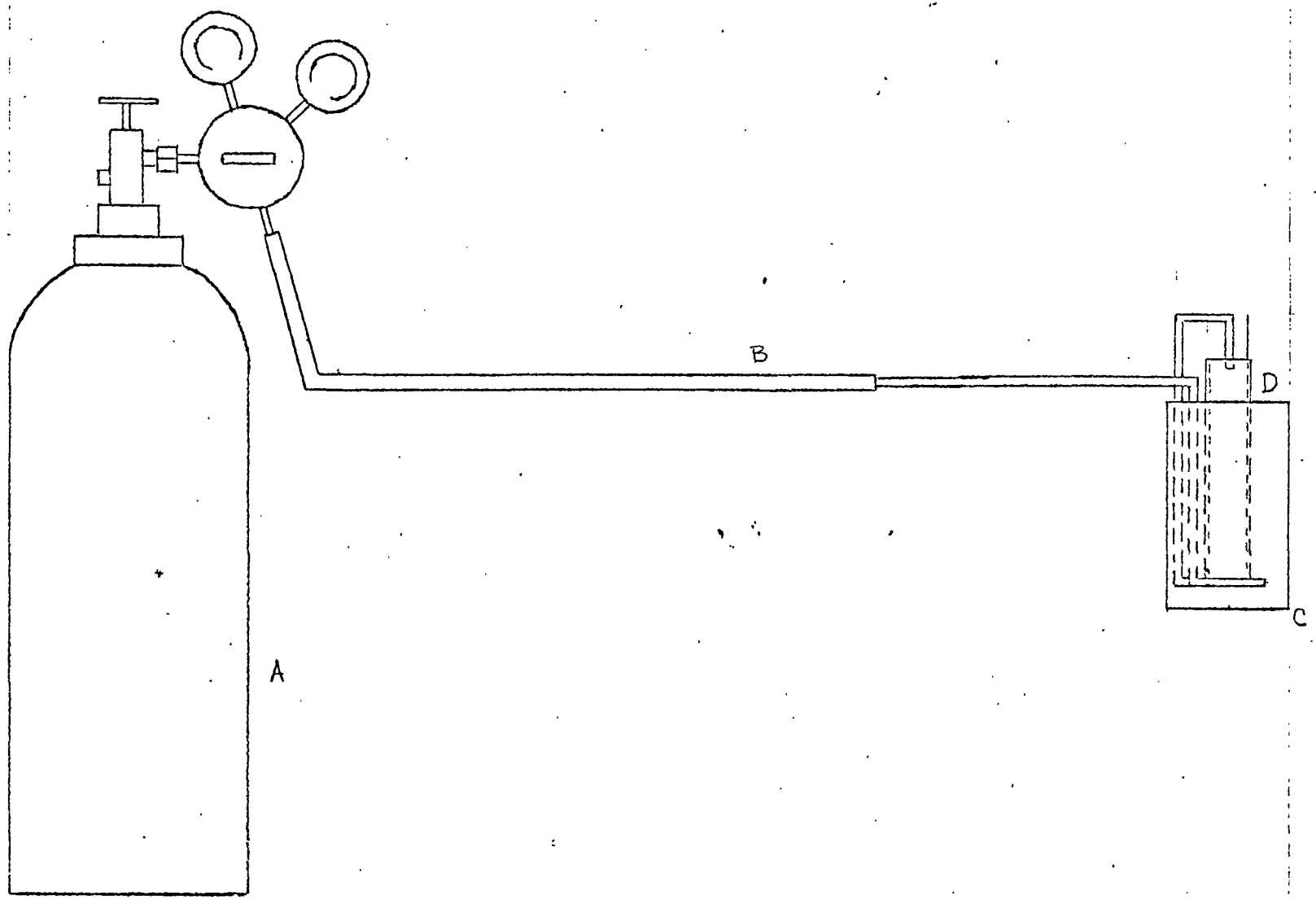

G
H
F
E
D
A
B
C

FIGURE 2. Apparatus for Liquefication of Propane-Propylene Gas. Gas was allowed to flow from the tank (A), through copper tubing (B) that led into the Dewar flask (C) containing liquid nitrogen. As the gas liquefied in the copper tubing, it flowed up and into a collecting cylinder (D), that was placed in the Dewar flask.


A
B
C
D

III. RESULTS

TECHNIQUE

The fluorescence observed, mapped and photographed (Figures 3-13) indicate the successful application of the Falck-Hillarp histochemical fluorescence technique with the modifications of Masuoka to Crocodilian brain tissue. On the basis of clearly separable green and yellow fluorescences, the results confirm the presence of and proper differentiation between the catecholamines and serotonin respectively, and justify proceding with the Crocodilian brain monoamine mapping. Monoamine fluorescence was modified by the pretreatment; the controls displayed overall green to dull yellow-green, Nialamide-treated animals displayed light green to yellow (Figure 3), and the animals treated with Nialamide and L-DOPA displayed yellow to bright gold (Figure 4). In the latter case diffusion was prominent in a few areas and color differentiation was somewhat hampered by uneveness of quality. The success of fluorescence examination and identification however was greatly aided by the MAO inhibitor and catecholamine precursor, especially in the smaller, less noticeable cell groups.

FLUORESCENT CELL BODY GROUPS

Medulla Oblongata

Beginning at the caudal end of the brain and working forward, the first fluorescent groups to appear included a well-defined area of green fluorescent, small to medium sized cells located just below the horizontal axis and on the margin of the medulla (Figure 5). Medial to the first group and also located on the margin was a similarly well-defined group of medium sized green fluorescent cells. This group as well as the first froup extended forward in the medulla for a considerable distance. At the level of the posterior limit of these green cell groups in the medulla there were two bright yellow-green to yellow cell groups found along the midsagittal plane between the fourth ventricle and the basilar artery. One group, the first to appear, was located

in the middle third of the area. It consisted of medium sized yellow fluorescent cells in a double row parallel to and on either side of the raphe. The second yellow cell group appeared at the level of the posterior fourth of the cerebellum and was also found at the midline (Figure 6). It was located below the first yellow group and extended to the basilar artery and spread laterally, following the margin for a short distance. It contained large cells along the midline giving way to medium sized cells as the group spread out. As the fluorescence examination continued further into the medulla, the lateral expansions of this group became wider approaching but remaining quite separate from the green cell groups mentioned. At the level of the appearance of the more ventral of the yellow cell groups, a third and fourth green cell group were observed. One was found midway between the green cell group in the lateral position and the dorsal yellow cell group (Figure 7). This was an extensive group of medium sized cells centrally located in the reticular formation. The fourth green cell group was found just ventral to the lateral borders of the fourth ventricle. This group extends forward for a short distance. At this level of the medulla oblongata the lateral and ventrolateral green cell groups were no longer present.

Pons

Inferior to the middle third of the cerebellum, fluorescence was limited to the more dorsal midline yellow group, the green cell group located near the fourth ventricle, and a fifth green cell group between the locations formerly occupied by the lateral and ventrolateral green cell groups. This fifth group contained small to medium sized cells and by its location suggested a merging and forward continuation of the marginal green cell groups. At the level of the anterior third of the cerebellum the original midline yellow cell group finally disappeared resulting in a short segment in which midline yellow fluorescence was totally lacking. Yellow fluorescence reappeared however, in the form of four sequential groups located beneath the fourth ventricle and

extending into the posterior mesencephalon. At the same time, the original green cell group located in the area around the ventricle gave way to two more green cell groups which shifted to a more lateral position in the pons, retaining their dorsal location even with or just dorsal to the level of the ventricle. The first of these groups contained small to medium sized cells and began at a level corresponding to the middle of the cerebellum, extending forward for a short distance. The second group was located beneath the anterior third of the cerebellum and was also limited in its caudal to rostral extent. This group was intensely fluorescent, the most compact of the dorsal green groups, and contained medium sized cells (Figure 8). Of the four sequential yellow groups, the first three were in the pons and the most posterior group began at the level of the middle cerebellum. The posterior group was small and contained small cells, then came a short segment with no fluorescence and followed by the second group of the sequence. This second group was also small and short in extent and consisted of small and round cells. Another short segment without fluorescence came next and then the third group of the yellow cells appeared. This group was the last onein the pontine midline and contained small round cells. Just before the anterior margin of the pons was reached, a seventh group of green cells and endings was observed (Figure 9). It was located in a position corresponding to an area medial and dorsal to that occupied by the lateral and ventrolateral groups of the medulla respectively and contained medium sized cells in a small faint group.

Mesencephalon

The first fluorescence observed at the level of the posterior mesencephalon was that belonging to the fourth group of yellow cells located in the sequential line beneath the fourth ventricle and then beneath the cerebral aqueduct. Like the three groups that preceded it, this group was relatively small and consisted of medium sized cells (Figure 10). The rest of the mesencephalic fluorescence consisted of large, diffuse green and yellow areas. In the posterior part of the

mesencephalon a large mass of green and yellow fluorescence was observed in the ventrolateral tegmentum. First to appear were some yellow cells of medium size which were quickly joined by green cells and fibers. Once this group of green and yellow cells had appeared, another group consisting of green cells only was seen along the midline; it had a broad base ventrally and tapered dorsally along the midline. This group was compact but extensive, leading forward in the brain well past the anterior limits of the green and yellow cell group. Near the forward termination of this green cell group, a second group of mixed green and yellow medium sized cells was observed, again in the ventrolateral tegmentum. This group however, began first with green cells with the yellow cells immediately following (Figure 11). The group was as diffuse as the first mixed cell group and extended to a level posterior to the hypothalamus.

Diencephalon

Fluorescence in the form of cell groups in the diencephalon was limited to the periventricular or hypothalamic area. Three groups of green cells were seen around the third ventricle at roughly the same level, the posterior half to two-thirds of the hypothalamus. All three were fairly compact. There was one group in the ventral region of the hypothalamus along either side of the midline, one situated in the dorsal area of the hypothalamus and the third one which was located dorsolateral with respect to the second group.

Telencephalon

Fluorescent cells in the telencephalon were confined to the pyriform cortex, hippocampus and basal ganglia. The pyriform cortex contained intense green cells and fibers generally concentrated in the ventrolateral extreme (Figure 12). Cells were of medium size. The hippocampus consisted of intensely fluorescent medium sized cells and fibers especially dorsal in the ventromedial area. The basal ganglia displayed a very extensive and scattered pale dull

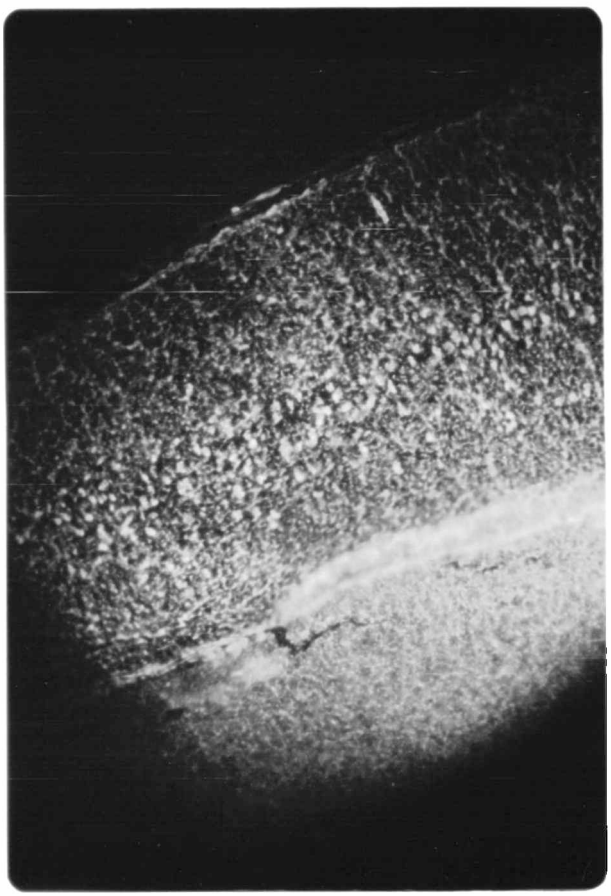
green fluorescence throughout the formation. This fluorescence was observed to begin ventral and lateral to the general cortex but eventually was seen throughout the basal ganglia.

Fluorescent Ependyma

Located posterior to the hypothalamic green cell groups was a fluorescent area in the ependyma of the third ventricle containing both green and yellow fluorescence and concentrated in a limited area but extending for some distance in the third ventricle (Figure 13). In the tissue area adjacent to this fluorescent ependymal area were green and yellow cells and an extensive fiber network.

FIGURE 3. Section of Cerebral Cortex in Nialamide and L-DOPA Pretreated Caiman. Extensive fibers and varicosities fill the cortical band and contrast the underlying basal ganglia. Magnification is 10X.

FIGURE 4. Section of Cerebral Cortex in Nialamide Pretreated Caiman. This section is in the same area and level of the telencephalon as in figure 3. Magnification is 10X.



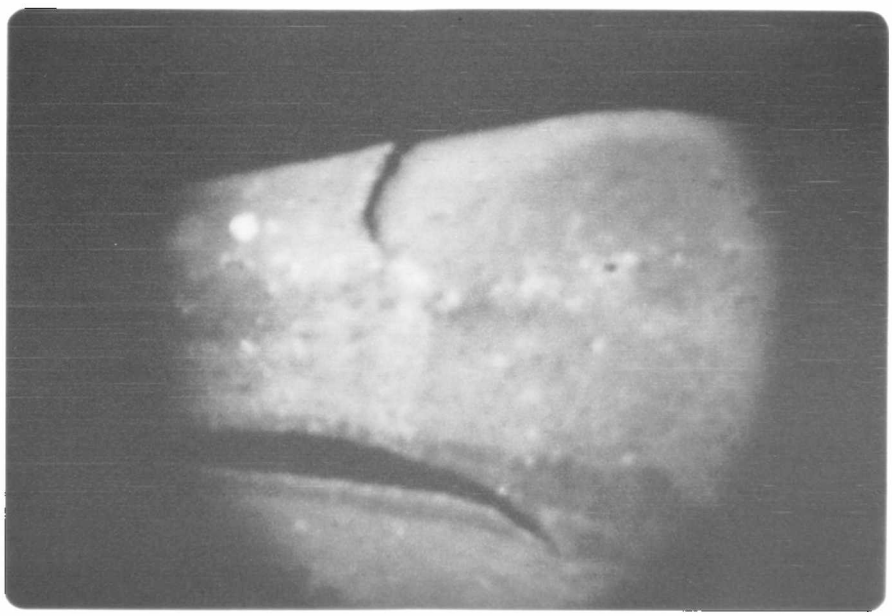


FIGURE 5. Single Prominent Cell and Fluorescent Endings. This section is apparently in the lateral reticular group in the medulla of the caiman and is designated as belonging to group A1 of Dahlström and Fuxe (1964b). Magnification is 40X.

FIGURE 6. Group of Crowded Serotoninergic Cells. This group of cells exemplifies the intense yellow fluorescence observed in the caiman brain. These cells are located along the projection of the ventral medullary yellow cell group located in the inferior reticular nucleus "a" of ~~Ariens~~-Kappers, and group B-3 of Dahlström and Fuxe. Magnification is 10X.

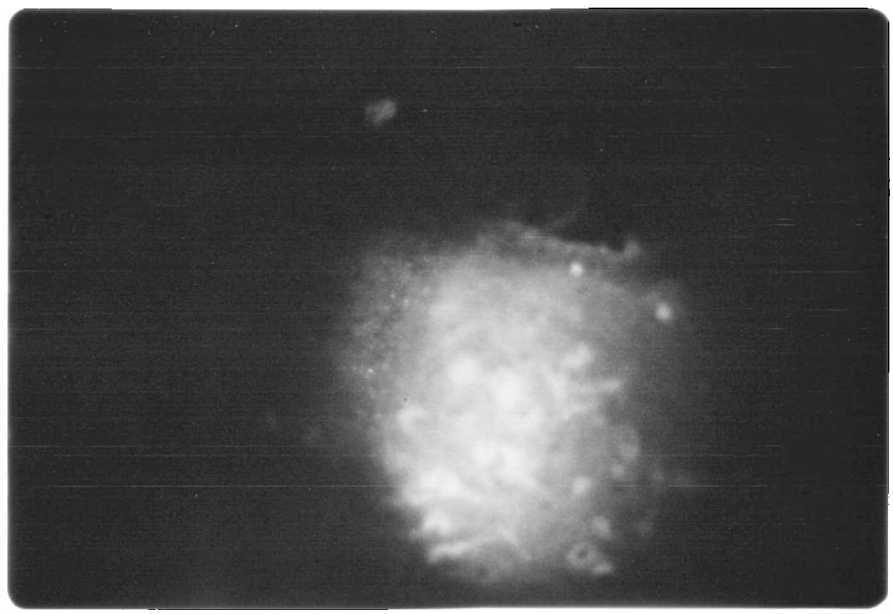
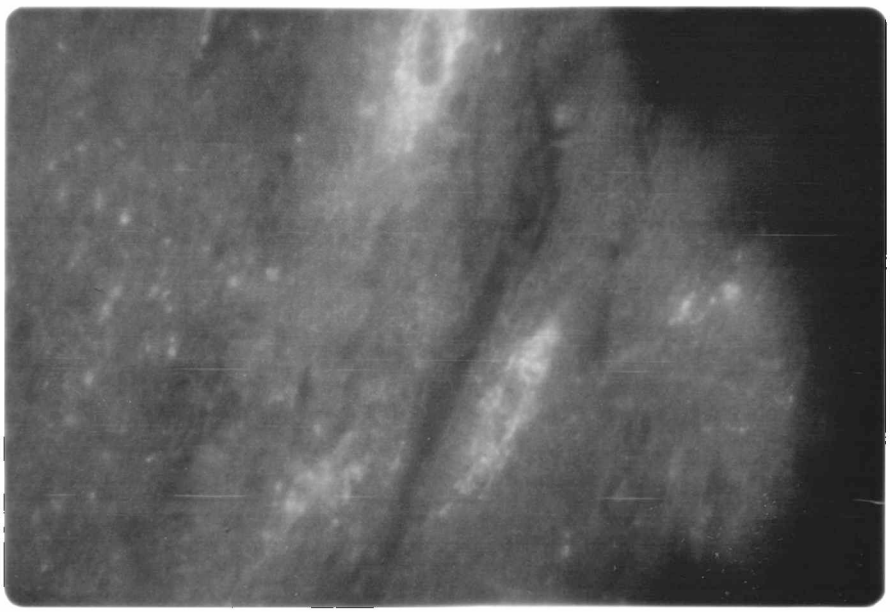


FIGURE 7. Detail of Cells and Fluorescent Endings. These cells are located in the ventral superior reticular nucleus of the medulla and are designated as belonging to group A3 of Dahlström and Fuxe. Close examination reveals the presence of NA granules in the cell bodies. Magnification of this figure is 40X.

FIGURE 8. Detail of NA Cells. These cells are in the pons, apparently in the locus coeruleus or its homolog, and are designated as belonging to group A6 of Dahlström and Fuxe. They are observed to be prolific and are grouped in such a manner as to indicate overall same direction of orientation. Excellent detail of NA granules is observed. Magnification is 40X.



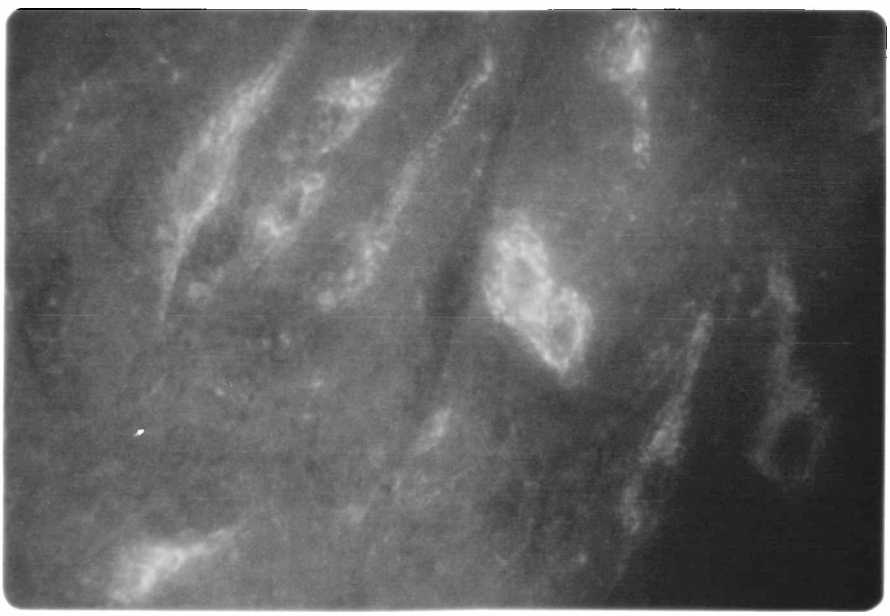
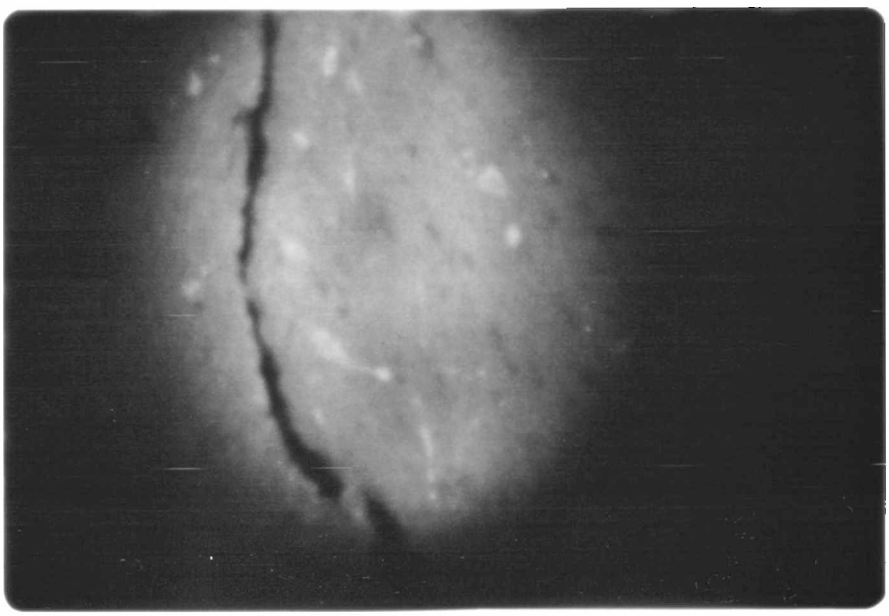


FIGURE 9. Detail of NA Cells. This group of cells is located in the anterior pons within the pontine gray. Although the resolution is not excellent, cells and their extending axons are observed. These cells apparently correspond to group A7 of Dahlström and Fuxe. Magnification is 10X.

FIGURE 10. Serotonin Fluorescence. Paraffin overlying this section obscures cells located within the substantia grisea paraventriculare and apparently belong to the group identified as B7 by Dahlström and Fuxe. Magnification is 10X



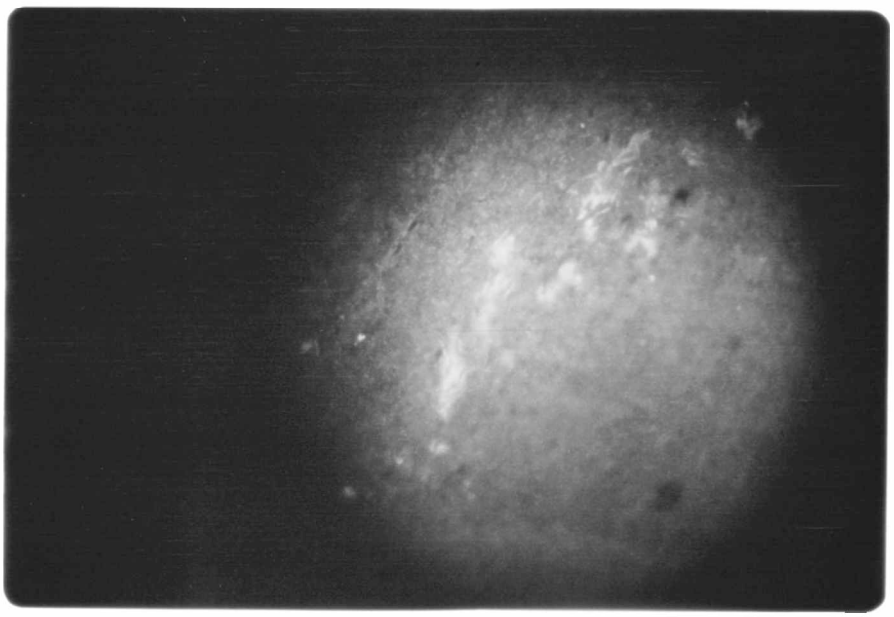
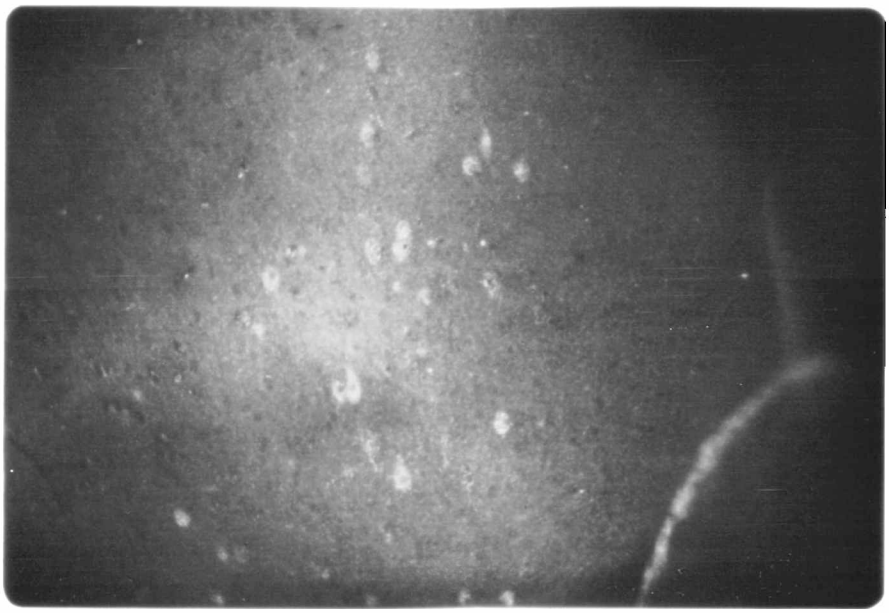


FIGURE 11. Cellular Detail in the Mesencephalon. Serotonin granules are observable in some of these cells found around the entopeduncularis and designated as belonging to group B9 of Dahlström and Fuxe. Magnification is 10X.

FIGURE 12. Section of Pyriform Cortex. Due to the posterior extension of the pyriform, it is observed in a lateral position in sections as far posterior as the anterior mesencephalon. Strong fluorescence is observed throughout this part of the cortex. Magnification is 10X.



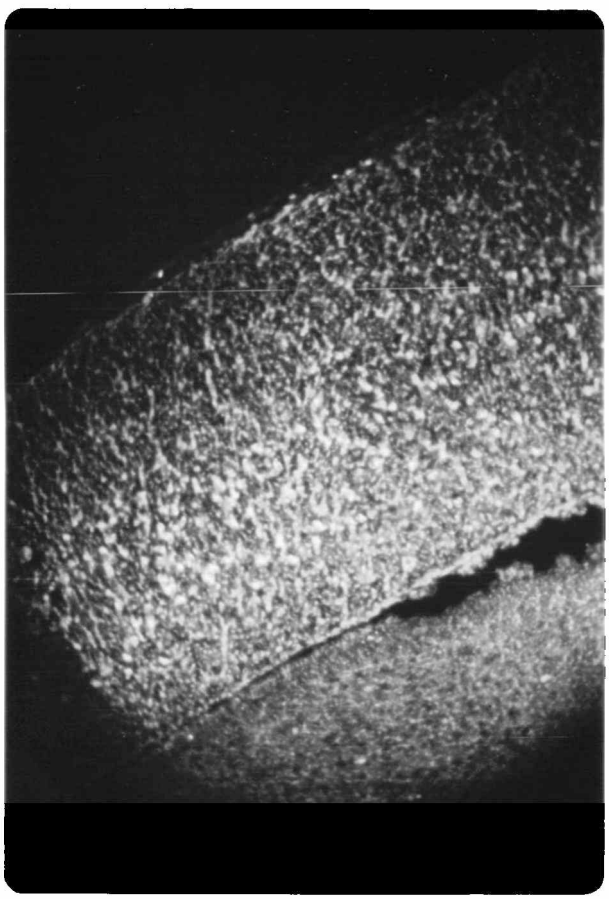
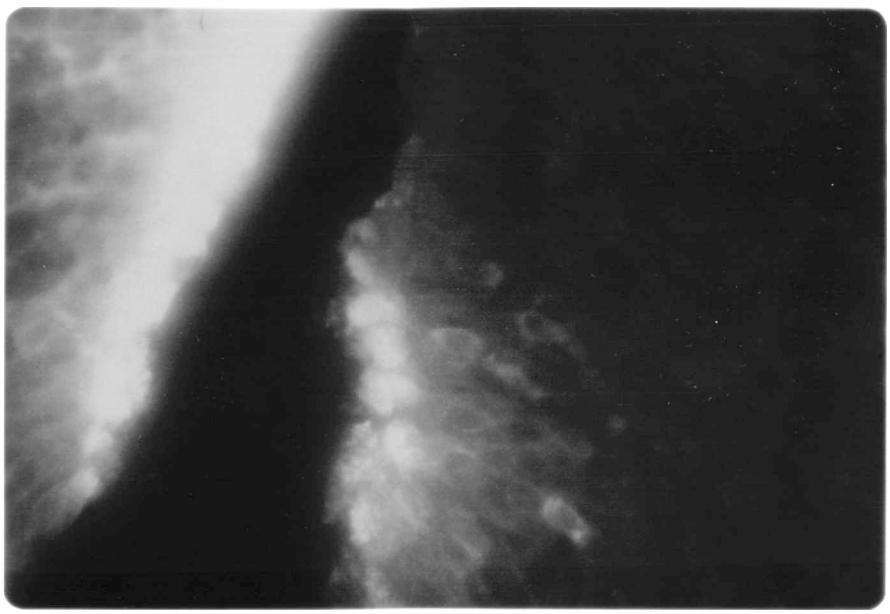


FIGURE 13. Vascular Ependyma. This figure shows the strongly fluorescent section of the diencephalic ependyma located in what is called organum vasculosum in the pigeon (Fuxe and Ljunggren, 1965). Fluorescent material is observed extending from either side of the ependyma and both serotoninergic and catecholaminergic fluorescence is evident. Magnification is 40X.



IV. DISCUSSION

FLUORESCENT CELL GROUPS

Much of the localization of monoamine fluorescence in the brain has been done with the rat (Dahlström and Fuxe, 1964a and b; Arbuthnott, 1966; and, Ungerstedt, 1971). Other fluorescence studies have been made using pigeon (Fuxe and Ljunggren, 1965) and rabbit (Dahlström, 1967), and prompts the assumption that monoamine fluorescence follows a phylogenetic pattern in the vertebrates. On this assumption then, explanation and identification of monoamine fluorescence in the caiman brain is offered in the following review of the fluorescent areas observed using previous accounts of fluorescence in other vertebrate brains as a basis for comparison.

Medulla Oblongata

The first two green fluorescent areas observed in the medulla correspond to reticular nuclei, the group designated as lateral in the results being in the position of the lateral reticular group with the ventrolateral group in an area suggesting the inferior reticular nucleus "b" (Ariens-Kappers, Huber and Crosby, 1936, p. 654). The lateral group corresponds anatomically to the group A1 of Dahlström and Fuxe (1964c). Both the lateral group in the caiman and the A1 group of the rat contain norepinephrine as the prominent transmitter substance, therefore this has been designated A1 (Figures 14 and 16). The fact that the ventrolateral group is absent in any of the fluorescence maps of the rat may indicate the lateral shifting of this group in higher vertebrates, thus joining it to the lateral or A1 group, a medial shifting into the lateral projection of a midline group, or, even the presence of an extra fluorescent area in crocodilians. For this reason the ventrolateral group is here referred to as "Ax," (Figures 14 and 16). A third green cell group is observed in the area of motor nucleus of the vagus nerve. It is in a location corresponding to the A2 nucleus of Dahlström and Fuxe and further, is one of the three cranial nerve nuclei reported to have strong

fluorescence by Dahlström and Fuxe (1964a). Dahlström and Fuxe (1964c) discuss groups B1 through B3 in sufficient detail to assume that these groups correspond to the midline yellow cell groups of the medulla of the caiman. However, there is a major difference in the more ventral groups because the pyramidal tract is absent in the caiman. Whereas the B1 group of the rat is located along the midline and extends laterally along the dorsal border of the pyramidal tract, in the absence of the pyramidal tract these lateral extensions would not be expected to be separated in the caiman and therefore the single lateral extension in the caiman appears to be logical. Whether cells of two groups, B1 and B3, are actually present in the caiman is difficult to prove conclusively but the possibility of a mixed cell group containing B1 and B3 cells is reasonable especially when the mixed cell groups of the mesencephalon are recalled. Both B1 and B3 belong to the nucleus raphe pallidus in the rat which corresponds to the inferior reticular nucleus "a" of Ariens-Kappers (p. 654) in the crocodilians. The more dorsal yellow cell group corresponds to the B2 group which is in the nucleus raphe obscurus in the rat and part of the superior parvocellular nucleus of raphe in the caiman (Ariens-Kappers, p. 655). The fourth cell group appears in the ventral superior reticular nucleus (Ariens-Kappers, p. 655). On the basis of location alone this nucleus would appear to correspond to Dahlström and Fuxe group A3; in the caiman the group contrasts that described in the rat with the former group being quite large and extensive. It seems that once the A3 group appears the midline yellow cell groups assume a more dorsal position and at the same time decrease significantly in size, extending for a short distance in the area of the medial reticular nucleus of the caiman (Ariens-Kappers, p. 755). The fifth green cell group of the medulla oblongata was found in the ventricular area, and compares well with the group identified as group A4 in the rat. Both lie ventral to the cerebellar nuclei suggesting that it is possibly in the area occupied by the nucleus of the brachium conjuntivum (Ariens-Kappers, p. 755).

Pons

The sixth green cell group lies lateral to the nucleus olivaris superioris in a position comparable to group A5 which lies adjacent to the fibers of the rubrospinal tract in the rat. This group is found in the superior olivary nucleus in the caiman also. This group is in direct line with the more posterior groups identified as A1 and Ax and apparently contributes some fibers to the tract extending forward from these fluorescent nuclei. The seventh green group found lateral to the fourth ventricle is apparently located in an area corresponding to the locus coeruleus or group A6 of the rat. This group in the caiman consisted of the same compact and intensely fluorescent cells as described for the area in the rat (Dahlström and Fuxe, 1964b), and found in non-pigmented cells thought to be the forerunners of the locus coeruleus (Ariens-Kappers, p. 654). The sequential positions of the three dorsal pontine yellow cell groups is observed to have a parallel in the rat. In both animals the groups are small and located under the ventricle in the midline. Reference is made to the three groups as B4 through B6. Group B4 is located just dorsal to the vestibular nuclei at the level of the abducens. The caiman differs in the location of this group in that the cells of the group are found ventral to the vestibular nuclei and not in the dorsal position due to the dorsal and inward turning of the nucleus laminaris, a nucleus with cochlear and/or vestibular relationships, having an avian but no mammalian homolog (Ariens-Kappers, pp 473 and 474). In spite of the difference in position however, the B4 group of the rat is thought to correspond to the first yellow cell group in the ventricular area. Group B5 is found in the nucleus raphe pontis in the rat and apparently corresponds to the position of the second yellow cell group of the pontine sequence in the grey matter immediately overlying the MLF in the anterior pons and in a nucleus not found to be previously described. Both B6 and the corresponding group in the caiman lie just under the fourth ventricle in the grey matter of the anterior pons. Lying in an area corresponding to the primitive pontine grey of the pigeon (Fuxe and Ljunggren, 1965), and

also to group A7 in the reticular formation in the posterior one third of the griseum pontis of the rat is the eighth green cell group and the most anterior green group in the pons of the caiman.

Mesencephalon

The initial fluorescence of the mesencephalon is located in the substantia grisea paraventriculare, and since it corresponds well with the group B7 of the rat, located in the homologous substantia grisea centralis, they are probably related fluorescent groups. The first mixed cell group is easily identified as being located in the reticular formation. The yellow group is located in an area corresponding to the nucleus medians raphe in the rat; the green cell group is in a location around the medial lemniscus in a position corresponding to the level just behind the red nucleus in the rat. On this basis, the cell groups are identified as A8 and B8 for the green and yellow cells respectively. Shanklin suggested many years ago that the nucleus entopeduncularis of reptiles was homologous to the substantia nigra (Ariens-Kappers, p. 986). These groups apparently correspond to the groups A9 (the green group in the substantia nigra of the rat) and B9 (the yellow group in the medial lemniscus of the rat). Connecting these two groups in the caiman as is the case for the rat, there is a green cell group extending from the midline in the tegmentum and located dorsal to the nucleus interpeduncularis, the cell group labeled as A10 in the rat. This group seems to be in the accessory nucleus of cranial nerve III, the homolog of the Edinger-Westphal nucleus of mammals (Ariens-Kappers, p. 565).

Diencephalon

The green cell groups similar to those in the hypothalamus or the rat are found in the caiman. There is a marked difference however in the respective structures of the hypothalamus. The arcuatus, containing one of the cell groups in the rat, is absent in the caiman. In both animals however, all three groups are found near the midline and more

or less in the vicinity of the periventricular nucleus. The most dorsal of the three is A11 of the rat, probably homologous to a group found in the medial region of the lateral hypothalamic nucleus of the caiman or in the dorsal periventricular nucleus (Ariens-Kappers, p. 996). The cell group ventral to A11 is A12, located in the nucleus ventralis hypothalamis in the caiman, and in the arcuate nucleus of the rat. A13, which is associated here with the lateral group of the three, is located in the nucleus lateralis hypothalamus in the caiman, and near the dorsomedial nucleus in the rat. Group A11 is found in the habenulopeduncular fasciculus in the caiman, and in the fasciculus retrofluxus and substantia grisea periventricularis in the rat.

Vascular Ependyma

The fluorescent ependymal area, found in the diencephalic ventricle, has been described by Fuxe and Ljunggren (1965) in the pigeon where it is termed organum vasculosum. The extension of green and yellow fluorescence into the immediately surrounding tissue suggests a secretory function although such a role has not been described in the caiman.

Telencephalon

The primitive cortex, which is relatively very much reduced in the rat as a result of the development of the neocortex, is quite extensive in the caiman. It is therefore understood that much of the fluorescence associated with the pyriform cortex and hippocampus in the caiman is relatively absent in the rat cortex. A spectacular array of cells and fibers is found in the pyriform cortex of the caiman and extends from the level of the anterior mesencephalon, where it curves around to the ventrolateral position from the telencephalon. The hippocampus is also strikingly fluorescent with fibers and cells. The general cortex is by contrast quite dull although it contains some fluorescent cells and endings. The basal ganglia display a similarly dull fluorescence which is confined to the cellular material. It is in this connection that there is very little evidence of fluorescent fiber connections in the basal ganglia.

LOCATING MONOAMINE FLUORESCENCE

Norepinephrine is mainly localized in the brain stem and differs greatly from the distribution of dopamine (Bertler and Rosengren, 1959). Stereotaxic lesions and subsequent study of anterograde/retrograde degeneration (Andén, 1966a and b), electrothermic lesions resulting in axonal accumulation of amines (Dahlström and Fuxe, 1964b; Hillarp, Fuxe and Dahlström, 1966), and 6-hydroxydopamine resulting in degeneration of central catecholamine neurons have been used (Fuxe, Hokfelt and Ungerstedt, 1970; Fuxe, 1968) to obtain pathway information which is the next logical step once localization of fluorescent cell groups has been done. It is interesting to note that Fuxe (1968) has been able to locate "unspecific afferents" in the cortex of the rat which represent forward projections of fibers from the non-specific thalamic nuclei. Dahlström and Fuxe (1964a) have found that only the trigeminal, nucleus tractus solitarius, dorsal motor nucleus of the vagus and the Edinger-Westphal nucleus contain much fluorescence. The other cranial nerves contain sparse fluorescent innervation or none at all. From a structural point of view, study of pathway fluorescence can reveal varicosities, i.e. enlargements of the axon (such as is seen in Figure 3) that may represent storage areas (Fuxe and Hokfelt, 1969).

APPLICATION OF MONOAMINE FLUORESCENCE

Once fluorescence study of a brain has developed to the point that a map with cell groups and fiber tracts can be constructed, application of such information can be made to elucidate the function of the different sets of fibers and cells. Fuxe, Hokfelt and Ungerstedt have done extensive research into the function of monoamine pathways and can be summarized as follows: 1) Degeneration of the nigro-neostriatal DA path causes hypokinesia and rigidity; stimulation causes stereotyped behavior. Experiments with neuroleptic drugs indicate the normal activity of the nigro-neostriatal system is required for normal thought processes. 2) The tubero-infundibular DA path is responsible for the inhibition of the release of LHRF from the median eminence. 3) The

central NA paths involve states of wakefulness and drive. Stimulation of the ascending NA paths results in increased wakefulness and drive, aggression, locomotion and hyperactivity. 4) The ventral ascending path is responsible for ACTH, GH, ADH and oxytocin secretion. 5) The bulbospinal NA path controls somatic and autonomic reflexes. 6) Decreased levels of 5-HT in the central 5-HT paths result in depressed states and wakefulness; increased 5-HT levels result in hallucinations. Another role is in the inhibition of sexual behavior and LH secretion. 7) The descending bulbospinal 5-HT controls somatic and autonomic reflex control especially in the lumbosacral part.

FIGURE 14. Diagram of NA Fluorescent Cell Groups in the Caiman Brain at the Midsagittal Level. Groups are numbered to correspond with the nomenclature of Dahlström and Fuxe and Ungerstedt (1971), and are drawn to show relative posterior to anterior extent. Diagram is modified from Chaisson (1962, p. 49).

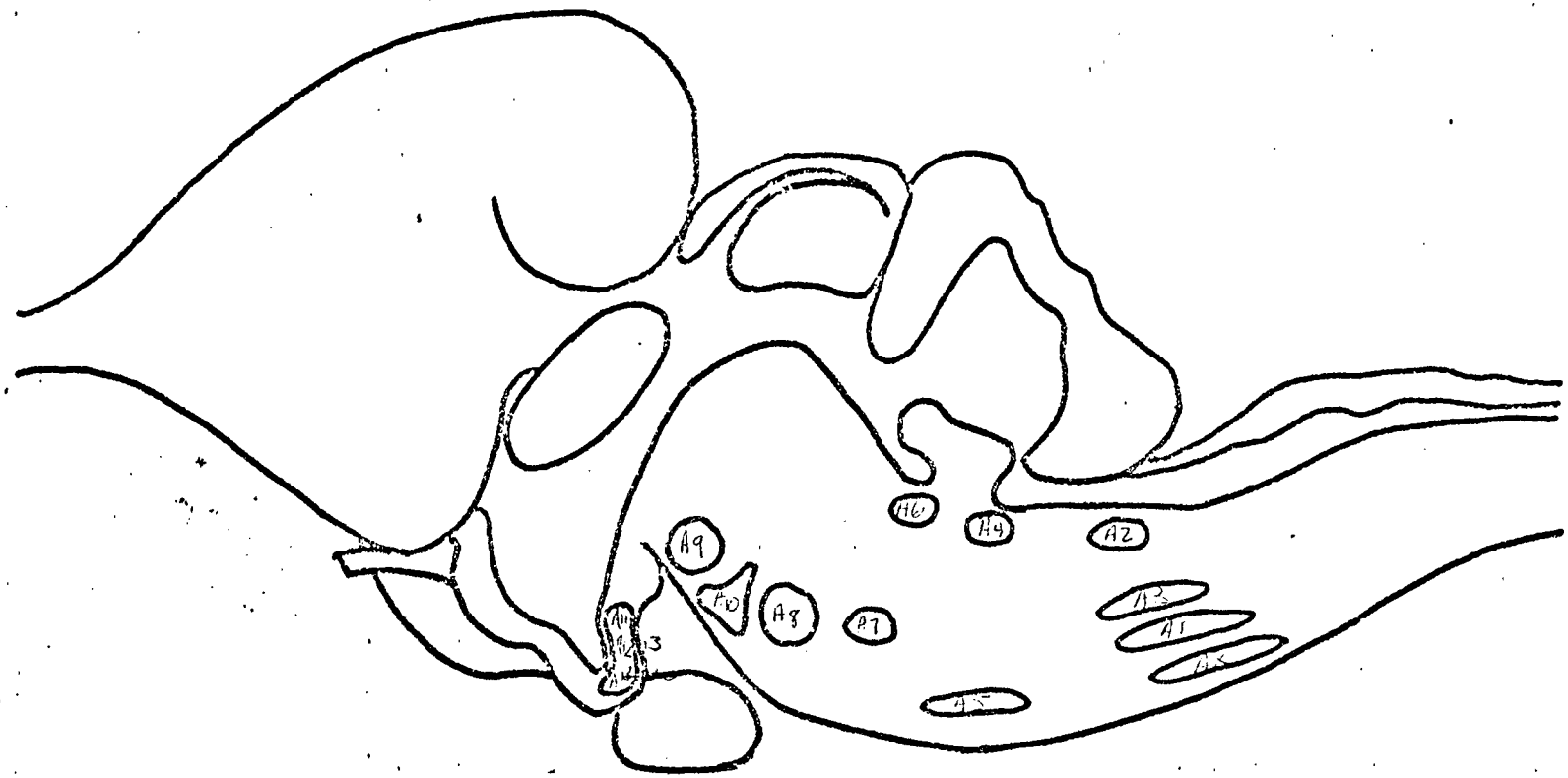

A9
A10
A8
A7
A6
A4
A2
A3
A1
A5

FIGURE 15. Diagram of 5-HT Fluorescent Cell Groups in the Caiman Brain at the Midsagittal Level. Groups are numbered to correspond with the nomenclature of Dahlström and Fuxe and Ungerstedt (1971), and are drawn to show relative posterior to anterior extent. Diagram is modified from Chaisson (1962, p. 49).

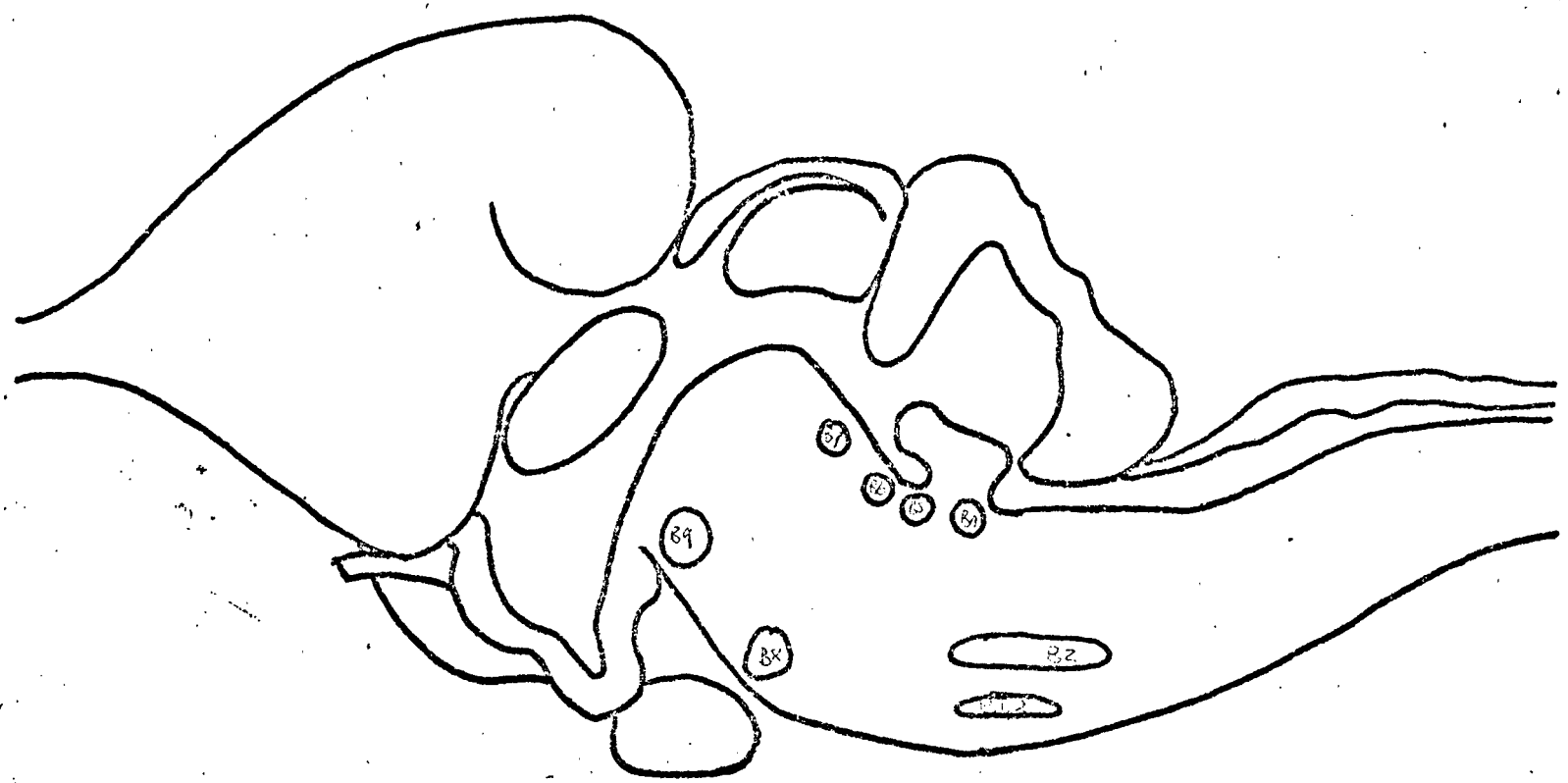
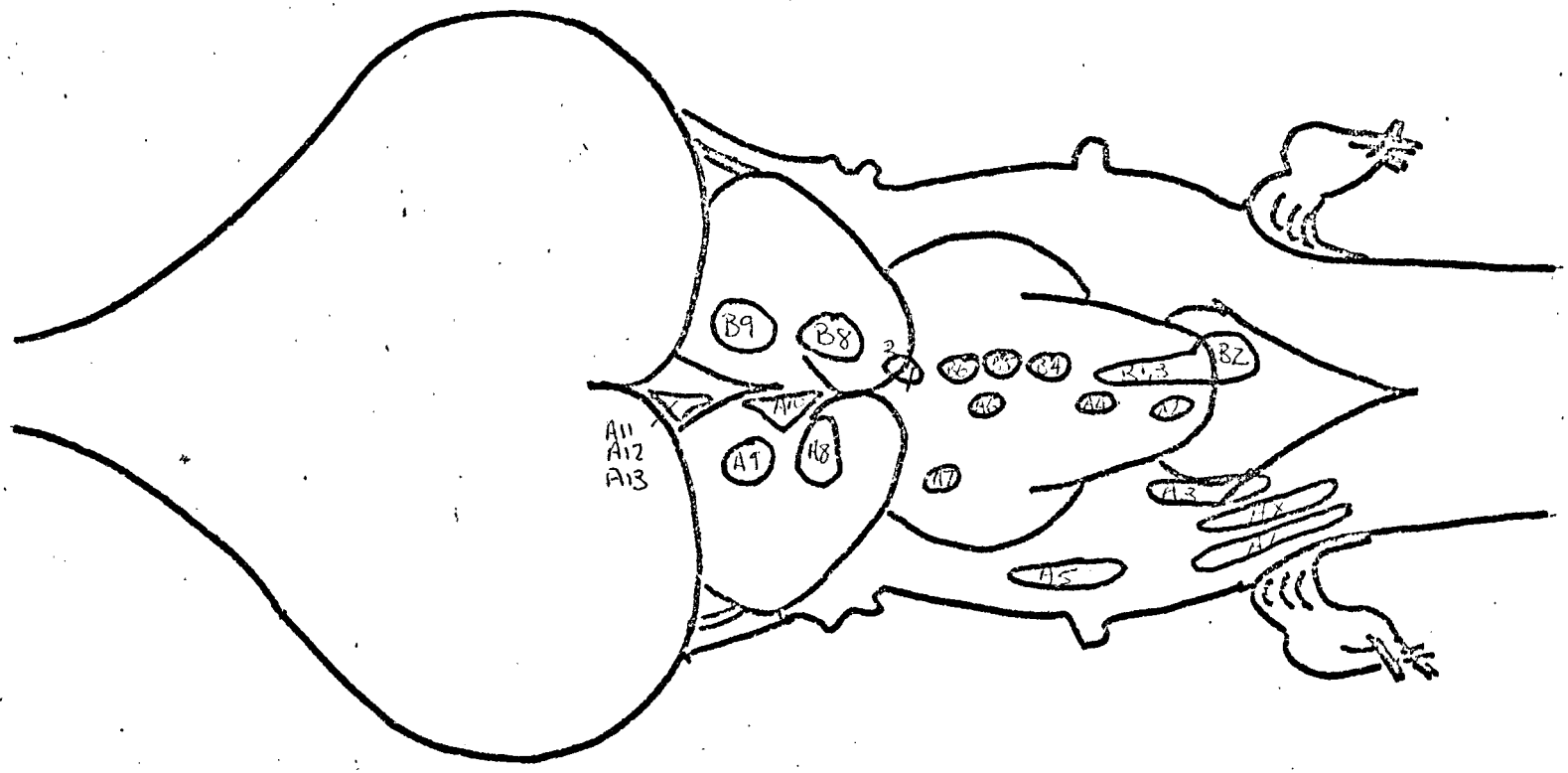

B7
B6
B5
B4
B9
B8
B2

FIGURE 16. Diagram of Catecholamine and 5-HT fluorescence Cell Groups in the Caiman Brain as Viewed from the Dorsal Side. Groups are numbered to correspond with the nomenclature of Dahlström and Fuxe and Ungerstedt (1971), and are drawn to show relative posterior to anterior extent. Diagram is modified from Chaisson (1962, p. 48).


B9
B8
A11
A12
A13
A7
A8
B2
A5

V. CONCLUSIONS

The histochemical fluorescence freeze-dry technique was used to examine nonoamine fluorescence in the brain of the Caiman sclerops. The results of this examination in conjunction with neuroanatomical study indicate the following:

1. The histochemical fluorescence technique of Falck and Hillarp is reproduceable in the laboratory and of practical application to the crocodilian brain.
2. The caiman brain contains definite concentrations of fluorescent cells.
3. These fluorescent sites correspond to cell groups identified in the rat, and with the exception of obvious neuroanatomical differences, the fluorescent monoamine cell groups discussed in the rat and identified as A1-13 and B1-9 are found in the caiman with reasonable fidelity with respect to location,cell size and mediator substance.
4. Fluorescent areas not previously reported in the rat are observed in the caiman. One is a group of cells in the inferior reticular nucleus ("a" of Ariens-Kappers, et al., 1936), and given the number, "Ax." The other is an intense area in the ependyma of the third ventricle in a position suggesting the organum vasculosum in the pigeon (Fuxe and Ljunggren, 1965), which may be secretory in function.
5. Definite fluorescence patterns are observed in the telencephalon. The pyriform cortex and hippocampus display intensely fluorescent cells and fibers; the general cortex and basal ganglia display a dull fluorescence.

VI. LITERATURE CITED

Andén, N.-E., et al. Demonstration and mapping out of nigro-neostriatal dopamine neurons. Life Sci. 3:523-530. 1964.

Andén, N.-E., et al. Mapping out of catecholamine and 5-hydroxy-tryptamine neurons innervating the telencephalon. Life Sci. 4:1275-1279. 1965a.

Andén, N.-E., et al. Further evidence for the presence of nigro-neostriatal dopamine neurons in the rat. Am. J. Anat. 116:329-334. 1965b.

Andén, N.-E., et al. Ascending monoamine neurons to the telencephalon and diencephalon. Acta physiol. scand. 67:313-326. 1966a.

Andén, N.-E., et al. Ascending noradrenaline neurons from the pons and the medulla oblongata. Experientia. 22: 44-45. 1966b.

Andén, N.-E., et al. Monoamine pathways to the cerebellum and cerebral cortex. Experientia. 23:838-839. 1967.

Anton-Tay, F. and R. Wurtman. Brain monoamines and endocrine function, in L. Martini and W. Ganong (editors). Frontiers in neuroendocrinology. Oxford University Press: New York. 45-66. 1971.

Arbuthnott, G. A histochemical study of catecholamines in the somatosensory area of rat cerebral cortex. J. physiol. 186:118P-119P. 1966.

Ariens-Kappers, C., G. Huber and E. Crosby. The comparative anatomy of the nervous system of vertebrates including man: 2 volumes. The MacMillan Company: New York. 1936.

Baldy-Moulinier, M., et al. Organization of sleeping and waking in the cat during the 24 hours: pharmacological changes, in the article, Joint meeting of l'association d'EEG et de neurophysiologie clinique de lange francaise. Electroenceph. Clin. Neurophysiol. 27:104-109. 1969.

Barry, J. Mise en evidence par stereotaxie du transport hypothalamique et telencephalique des monoamines elaborees ar niveau du mesencephale. C.R. Soc. Biol. 161:2182. 1967.

Bartonicek, V. Comparison of five clinically used inhibitors of MAO using the fluorescence microscopy method. Med. Pharmacol. exp. 16:142. 1967.

Baumgarten, H., et al. Microspectrofluoremetric differentiation between primary and secondary catecholamines in tissues. Life Sci. 6:2465-2468. 1967.

Bertler, A. and E. Rosengren. Occurrence and distribution of catecholamines in the brain. Acta physiol. scand. 47:350-361. 1959.

Bjorklund, A. An improvement of the histochemical fluorescence method for monoamines. Observations of varying exciteability of fluorophores in different nerve fibres. J. histochem. cytochem. 16:717. 1968.

Carlsson, A., B. Falck and N.-A. Hillarp. Cellular localization of brain monoamines. Acta physiol. scand. 56: Suppl. 196, 1-28. 1962.

Carlsson, A. and M. Lindqvist. In-vivo decarboxylation of a-methyl DOPA and a-methyl metatyrosine. Acta physiol. scand. 54:87-94. 1962.

Chaisson, R. Laboratory anatomy of the alligator. W.C. Brown Co. Dubuque. 48-49. 1962.

Corrodi, H., K. Fuxe and T. Hokfelt. The effect of ethanol on the activity of central catecholamine neurones in rat brain. J. pharm. pharmacol. 18:821-823. 1966.

Corrodi, H. and G. Jonsson. The formaldehyde fluorescence method for the histochemical demonstration of biogenic monoamines. A review on the methodology. J histochem cytochem. 15:65-78. 1967.

Crosby, E. The forebrain of Alligator mississippiensis. J. Comp neurol. 27:325-402. 1916.

Dahlström, A and K. Fuxe. Localization of monoamines in the lower brain stem. Experientia. 20:398-399. 1964a.

Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in cell bodies of brain stem neurons. Acta physiol. scänd. 62:Suppl. 232, 1-55ff. 1964b.

Dahlström, A., et al. Ascending systems of catecholamine neurons from the lower brain stem. Acta physiol scand. 62:485-486. 1962.

Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal nervous systems. Acta physiol. scand. 64:Suppl. 247, 1-36ff. 1965.

Dahlström, A. The interneuronal distribution of noradrenaline and the transport and life span of amine storage vesicles in the sympathetic adrenergic neuron. Naunun Schniedeberg Arch. Pharm. Exp. path. 257:93-99. 1967.

Eränkö, O. and M. Harkonen. Noradrenalin and acetylcholinesterase in sympathetic ganglion cells of the rat. Acta physiol. scand. 62:485-486. 1962.

Eränkö, O. The practical histochemical demonstration of catecholamines by formaldehyde-induced fluorescence method. J. royal micro. soc. 87: part 2, 259-276. 1967.

Falck, B. and N.-A. Hillarp. On the cellular localization of catecholamines in the brain. Acta anat. 38:277-279. 1959.

Falck, B., et al. Fluorescence of catecholamines and related compounds condensed with formaldehyde. J. histochem. cytochem. 10:348-354. 1962.

Foote, W., et al. Comparison of effects of LSD and amphetamine on midbrain raphe units. Nature. 222:5193, 567-569. 1969.

Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. IV. The distribution of monoamine terminals in the central nervous system. Acta physiol. scand. 64:Suppl. 247, 36-85ff. 1965.

Fuxe, K. and L. Ljunggren. Cellular localization of monoamines in the upper brain stem of the pigeon. J. Comp. Neurol. 125:355-381. 1965.

Fuxe, K. and G. Jonsson. A modification of the histochemical fluorescence method for the improved localization of 5-hydroxytryptamine. Histochemie. 11:161-166. 1967.

Fuxe, K. and T. Hokfelt. Catecholamines in the hypothalamus and pituitary gland, in W. Ganong and L. Martini (editors). Frontiers in neuroendocrinology. Oxford University Press: New York. 47-96. 1969.

Fuxe, K., B. Hamberger and T. Hokfelt. Distribution of noradrenaline nerve terminals in cortical areas of the rat. Brain res. 8:125-131. 1968.

Fuxe, K., T. Hokfelt and U. Ungerstedt. Morphological and functional aspects of central monoamine neurons. Int. rev. neurobiol. 13:93-106. 1970.

Fuxe, K., et al. A fluorescence and electron microscope study of central monoamine nerve cells. Anat rec. 155:33-40. 1966.

Fuxe, K., et al. Studies on uptake of intraventricularly administered tritiated noradrenalin and 5-OH-T with combined fluorescence histochemical and autoradiographic technique. Histochemie. 16:186-191. 1968.

Fuxe, K., et al. Fluorescence microscopy in neuroanatomy, in W. Nauta and S. Ebbeson (editors). Contemporary research methods in neuroanatomy. Springer-Verlag: New York. 275-314. 1970.

Gunne, L., G. Jonsson and K. Fuxe. Effects of morphine intoxication on brain catecholamine neurons. Europ. J. pharm. 5:338-342. 1969.

Hillarp, N.-A., K. Fuxe and A. Dahlstrom. Demonstration and mapping of central neurons containing dopamine, noradrenalin, 5-hydroxytryptamine and their reactions to psychopharmica. Pharm. rev. 18:727-741. 1966.

Hishikawa, Y. Natural and melatonin-induced sleep in young chickens--a behavioral and electrographic study. Exp. Brain res. 7:84-94. 1969.

Huber, G. and E. Crosby. On thalamic and tectal nuclei and fiber paths in the brain of the American alligator. J. Comp. Neurol. 40:92-227. 1926.

Jonsson, G., et al. 6-hydroxytryptamine--a new tool in monoamine histochemistry. Brain res. 13:190-195. 1969.

Jouvet, M. Insominia and decrease of cerebral 5-hydroxy-tryptamine after destruction of the raphe system in the cat. Advan pharm. 6B:265-279. 1968.

Koella, W., A. Feldstein and J. Czicman. Effects of para-chlorophenylalanine on the sleep of cats. Electroenceph. clin. neurophysiol. 25:481-490. 1968.

Maanen, J. and P. Smelik. Depletion of monoamines in the hypothalamus and prolactin secretion. Acta physiol. pharmacol. neerl. 14:519-520. 1967.

McKenne, C., et al. Concentrations of 5-HT in rat brain and pineal hypophesectimized and cortisol administration. Neuroendo. 1:251-256. 1966.

Maickel, R., et al. A method for the determination of serotonin and norepinephrine in discrete areas of the brain. Int. J. neuropharmacol. 7:275-288. 1968.

Marley, E. and J. Stephenson. Effects of some catecholamines infused into the hypothalamus of young chickens, in R. Barrett and E. Costa. Procedings of British pharmacological society. Brit. J. pharm. 36:191P-217P. 1969.

Neff, N., et al. Kinetic and fluorescent histochemical analysis of the serotonin compartments in rat pineal gland. Euro. J. pharm. 5:348-356. 1969.

Odake, G. Fluorescence microscopy of catecholamine-containing neurons of hypothalamohypophyseal system. Z. zellforsch. 82:46-48. 1967.

Osborn, L., et al. Experimental studies on catecholamines in the medial eminence and arcuate nucleus of rabbits, in Proceedings of the anatomical society of Great Britain and Ireland. J. Anat. 106:201-204. 1969.

Owman, C. Monoaminergic barrier mechanisms in brain vessels. Bibl. anat. 8:46-50. 1967.

Quay, W. Differences in circadian rhythm in 5-hydroxy-tryptamine according to brain region. Amer. J. psych. 215:6, 1448-1453. 1968.

Reis, D. and R. Wurtman. Diurnal changes in brain noradrenalin. Life sci. 7:91-98. 1968.

Snyder, S., J. Axelrod and M. Zweig. A sensitive and specific fluorescence assay for tissue serotonin. Biochem. pharmacol. 14:831-835. 1965.

Tuge, H. Somatic motor mechanism in the midbrain and medulla oblongata of Chrysemys elegans (Wied). J. Comp. Neurol. 55:185-271. 1932.

Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta physiol. scand. Suppl. 367, 1-44. 1971.

Walker, R., et al. Effect of drugs on excitatory and inhibitory potentials in Helix aspera. Experientia. 27: 281-282. 1970.

Weast, R., S. Selby and C. Hodgman (editors). C.R.C. Handbook of Chemistry and Physics, 46th edition. Chemical Rubber Company: Cleveland.

Wyatt, R., et al. Effects of para-chlorophenylalanine on sleep in man. Electro clin. neurophysiol. 27:529-532. 1969.