AN ANALYSIS OF MONOAMINES IN THE BRAIN OF *Caiman sclerops*

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ABSTRACT

Regional and whole brain concentrations of 5-hydroxy-tryptamine (5-HT), epinepherine (E), norepinepherine (NE) and dopamine (DA) were measured in Caiman sclerops using the methods of Quay (1963) and Cox and Perhach (1973). Whole brain 5-HT concentration averaged 1.59 ± 0.10 ug/g ; regions which were significantly higher than the mean were the medulla, pons and midbrain tegmentum while regions significantly lower than the mean were the olfactory bulbs, cerebellum and cerebral hemispheres. Whole brain E concentration averaged 0.15 ± 0.02 ug/g; the diencephalon was significantly higher than the mean while the cerebral hemispheres were significantly lower than the mean. Whole brain NE concentration averaged 0.29 ± 0.02 ug/g; the diencephalon was significantly higher than the mean while the olfactory bulbs and optic tectum were significantly below the mean. Whole brain DA concentration averaged 0.96 ± 0.06 ug/g; the diencephalon was significantly higher than the mean while the medulla, midbrain tegmentum and cerebellum were significantly below the mean. In whole brain 5-HT constituted 53% of total monoamines and catecholamines 47%. Of the total catecholamines, E constituted 10%, NE 21% and DA 69%.

Treatment with reserpine caused depletion of catecholamines over a period of seven days by which time E and NE were more than 99% depleted. DA reached its lowest level (85% depletion) on the third day. Depletion of 5-HT by reserpine

was less systematic with the greatest depletion (51%) on the third day. In general the brain regions most rapidly and thoruoghly depleted by reserpine were those with the highest initial levels of each amine. Brain areas with low control levels of an amine showed increased levels of that amine after reserpine. Overall physical activity levels were not significantly affected by reserpine.

Para-chlorophenylalanine (PCPA) caused a significant depletion of 5-HT which was of slightly greater magnitude than that produced by reserpine, being 59% complete on day fourteen. E and NE levels were slightly elevated on the third day after PCPA, but were close to control values on the seventh day. Significant depletion of DA was observed, being 80% complete on day seven. Midbrain tegmentum levels of all three catecholamines were significantly elevated on days three and seven following PCPA and maximum on day three. Physical activity levels were significantly increased on the third day after PCPA and were significantly decreased on the seventh day.

Levels of 5-HT, E, and NE were not significantly affected by 6-hydroxydopamine (6-OHDA), but DA was depleted by 63% on the eighth day. Activity levels were significantly reduced.

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INTRODUCTION

Serotonin and catecholamines have been found in the nervous systems of representatives of all major animal phyla and the role of these substances as neurotransmitters has been well-established. The problem of the functional significance of serotonin and catecholamine-containing neurons in the brain has stimulated considerable research in recent years.

Nuclei and tracts in which specific monoamines function as neurotransmitters have been mapped out for the mammalian brain and certain of these pathways have been correlated with specific physiological or behavioral functions, particularly with sleep and alerting mechanisms.

Phylogenetic studies of distribution of monoamines within the brain indicate a general pattern common to all vertebrate classes with certain modifications characteristic of each particular class. By observing these similarities and differences it should be possible to formulate an idea of the evolution of serotonin and catecholamine-containing neuronal systems in the brain.

Of the reports in the literature which deal with reptiles, very few give concentrations of all the brain monoamines for a single species; consequently, comparison of amine levels with one another is difficult and this difficulty is further compounded by the fact that each author divides the brain differently in

performing regional analyses so that it is virtually impossible to make any kind of regional comparison. A systematic qualitative and quantitative analysis in which a single species is used for measurement of all the amines and in which the brain regions used are consistently the same should lead to a clearer picture of monoamine distribution within the reptilian brain and also facilitate phylogenetic comparisons between reptiles and other classes.

Previous studies on crocodilians have shown their brain serotonin concentrations to fall midway between those of birds and mammals and those of other orders of reptiles, a fact which could be of considerable evolutionary significance. It was decided, therefore, that it would be valuable to measure levels and distribution of both serotonin and catecholamines in a single crocodilian species for purposes of phylogenetic comparison.

Many behavioral studies on mammals have involved depletion or enhancement of one or more brain monoamines by means of various drugs, followed by observation of the behavioral consequences of the altered amine levels. Very few studies have been reported in the literature in which drugs of this type were given to non-mammalian species. It was decided, therefore, that an analysis of the effects of drugs of this type on monoamine levels in the reptilian would be very informative, particularly if carried out in conjunction with behavioral studies.

and could ultimately lead to a better understanding of the function and evolution of monoaminergic neuronal systems in the brain.

REVIEW OF THE LITERATURE

The catecholamines epinepherine (E), norepinepherine (NE) and dopamine (DA) as well as the indoleamine serotonin (5-hydroxytryptamine, 5-HT) are of unique importance due to the fact that they are the only neurotransmitters whose localization in particular brain tracts has been established and whose relationship to certain behaviors has been demonstrated.

The physiological effects of adrenalin (epinepherine) were first described by Oliver and Schafer (1895) using extracts of the adrenal gland, and the active principle of these extracts, epinepherine, was first isolated in 1901 by Takemine.

The concept of chemical transmission at synapses was first proposed in 1905 by Elliot, and it was suggested that the substance being released by sympathetic nerve endings might be epinepherine based on the similarity of effects produced by epinepherine to those produced by sympathetic nerve stimulation.

Norepinepherine was synthesized as early as 1904 by Stoltz, who also showed that it was less toxic and equally potent as a pressor substance when compared with E. In 1910, Barger and Dale noted that the actions of NE more closely resembled those of sympathetic nerves than did the actions of E.

Another line of evidence which led to the final recognition of NE as a transmitter substance was the discovery of the enzyme L-DOPA decarboxylase in mammalian tissue by Holtz *et al.* (1938). This enzyme was found to convert dihydroxyphenylalanine

(DOPA) to dopamine, which differed from NE only in the presence of a hydroxyl group. In 1939 it was proposed by Blaschko that NE might be an intermediate formed during the synthesis of E from DA.

In 1946, von Euler et al. demonstrated NE to be the principal catecholamine present in peripheral tissues and adrenergic nerves and it has since been shown to act as a transmitter in the CNS as well.

Since the middle of the nineteenth century it has been known that a substance is present in the blood serum which is capable of causing powerful constriction of smooth muscle. In 1948, this substance was isolated by investigators at the Cleveland Clinic and given the name serotonin. In 1949 Rapport determined that the active principle of this compound was 5-hydroxytryptamine, an indoleamine derived from the amino acid tryptophan.

The finding that 5-HT existed in considerable quantities in the brain led to studies which established its role as a neurotransmitter. Gaddum (1953) showed that the molecular structure of lysergic acid diethlyamide (LSD) is similar to that of 5-HT and that LSD inhibits the action of 5-HT but not that of oxytocin on the rat uterus, possibly indicating that these two substances interact with each other in the brain to produce hallucinatory effects.

Welsh (1954) demonstrated that blocking the action of

acetylcholine on the heart of a gastropod did not affect sensitivity to 5-HT, and he also showed that the response of the heart to acetylcholine was not affected by LSD or ergot alkaloids which block the action of 5-HT, suggesting different receptors for serotonin and acetylcholine; he also suggested that 5-HT might be a neurotransmitter.

Studies by Brodie et al. (1955) on the sedative reserpine produced evidence that this drug acted through liberation of 5-HT in the brain and further supported the suggestion that 5-HT acts as a neurotransmitter and plays an important role in brain activity.

Bioassay was one of the earliest methods employed for the detection of biogenic amines in tissue, but the development of the spectrophotofluorometer and of sensitive fluorescent techniques for assay of tissue monoamines has been a major factor in the rapid advancement of knowledge in this field. In 1955 Bowman and his colleagues devised a method for the spectrophotofluorometric assay of a number of organic compounds, and later that year, members of the same group (Udenfriend et al. 1955) further developed the technique so that it was possible to distinguish between 5-hydroxyindoles and other compounds such as tryptamine and tryptophan which are also found in brain tissue. Members of this group then went on to develop a specific method for determination of 5-HT in brain (Bogdanski et al. 1956) which involved extraction into an organic phase at pH 10

followed by extraction back into an aqueous phase which was then made 3N with HCl for fluorometric examination. Quay (1963) modified the method of Bogdanski by adding four steps of diethyl ether extraction, the last two being at pH 10. This removes interfering indole compounds such as N-acetyl serotonin, 5-hydroxytryptophan, etc. This procedure was used to measure 5-HT concentrations in the present study.

In 1938 it was first discovered by Shaw that catecholamines are strongly adsorbed on aluminum oxide at an alkaline pH but are not adsorbed at an acid pH. This finding was subsequently developed as a method for the isolation of catecholamines from body fluids or tissue extracts. Current methods are based on modifications of the technique developed by Weil-Malherbe and Bone (1952).

The fluorescence of E in alkaline solutions was first reported by Loewi in 1918, but it was not until chemical studies had elucidated the mechanism of the oxidation and rearrangement of catecholamines in alkaline solutions to form fluorescent trihydroxyindole derivatives that the first reliable assay method was described (Lund, 1949).

Since then many modifications of the original procedure have been described, and the more recent methods, such as those developed by Ansell and Beeson (1968) and Cox and Perhach (1973) allow for the differential assay of E, NE and DA in a single sample.

These procedures depend on the differential fluorescence of the monoamines at different activating and exciting wavelengths in a spectrophotofluorometer as well as differing time courses for development and decline of the fluorophore once oxidation has been initiated (Chang, 1964). The assays for catecholamines and serotonin may be combined since only catecholamines and their metabolites are adsorbed onto alumina, while the aqueous phase containing serotonin may be treated by the method of Quay.

Another recently developed technique which has been of great importance for the localization and study of monoamines is the histochemical fluorescence method described by Falck (1962) which permits direct microscopic visualization of monoamines in the tissue. The application of this technique to the CNS has revealed monoaminergic neuronal pathways and cell bodies which were previously unrecognized, and has made possible an extensive mapping of these pathways in the brain. One of the main disadvantages of this method is that it is very difficult to distinguish between the reaction products of E, NE and DA since they all form fluorophores with identical fluorescent properties.

Application of all these various methods has made it possible to study the distribution of biogenic amines throughout the nervous system as well as in other tissues.

Serotonin and related indoleamines are present in many

plants and it is probable that the enzymes required for 5-HT synthesis and metabolism in animals are derived from plant-like ancestors.

Certain protozoa and coelenterates have been shown to contain 5-HT, and in the flatworms, annelids and molluscs high levels of 5-HT have been demonstrated in their nervous systems as well as numerous yellow-fluorescing neurons. (Welsh, 1968).

Serotonin has been found in the central nervous systems of all classes of vertebrates from fish to mammals (Welsh, 1968) as well as in non-neural tissue. In mammals, in fact, the highest 5-HT concentrations are found in the pineal gland and in the enterochromaffin cells of the digestive tract; it has been estimated that in the human the gut contains about 90% of the body's 5-HT with another 8-10% adsorbed on the platelets, while only about 1-2% of the total is present in the central nervous system. (Cooper et al. 1974).

There appear to be no peripheral serotoninergic axons present in vertebrates, as there are in many invertebrates, all 5-HT in neural tissue being confined within the central nervous system.

The 5-HT content of whole brain appears to be higher in amphibians and reptiles than in fishes, birds or mammals.

Brodie et al. (1964) reported a whole brain 5-HT concentration of 9.4 µg/g of tissue in the toad Bufo americanus, and this value is the highest obtained for any vertebrate brain thus

far examined. In general, 5-HT concentrations reported for amphibians are higher than those found in fishes, birds or mammals.

With the exception of the amphibians, the 5-HT content of reptilian brains is higher than that found in any other class of vertebrates. Quay and Wilhoft (1964) measured whole brain 5-HT in three different orders of reptiles and found it to vary from a low of 0.25 μg/g in the alligator to 3.39 μg/g in one species of lizard.

The few studies done on birds have shown their brain 5-HT concentrations to be intermediate between those of reptiles and mammals, Brodie et al. (1964) reporting levels of 0.7 μg/g in the pigeon and 1.0 μg/g in the chicken.

Most values of 5-HT in the whole brain of mammals such as the mouse, rat, guinea pig, rabbit, marmot and monkey range between 0.5 and 0.9 μg/g. These levels are much lower than those generally found in reptiles and amphibians and, on the average, are slightly less than those found in birds. (Welsh, 1968).

5-HT levels in the vertebrate nervous system vary greatly in different parts, but there seems to be a relatively uniform pattern of distribution in all classes. Excepting the pineal gland, the midbrain and hypothalamus contain the highest levels in most species studied, while the cerebellum contains less 5-HT than other parts (Welsh, 1968).

In addition to regional biochemical analysis of 5-HT content,

several techniques have been used in an attempt to localize 5-HT to specific nerve tracts within the brain. The classical neuroanatomical approach has been to produce lesions and follow the distribution of the degenerating axons. Harvey et al. (1963) placed lesions in the median forebrain bundle and observed a dramatic decrease in forebrain 5-HT over the next few weeks, suggesting the presence of a 5-HT containing pathway passing through the lateral hypothalamus.

At about the same time, Falck et al. (1962) perfected the technique of histochemical fluorescence and demonstrated that most 5-HT containing cell bodies were restricted to the raphe nuclei, a narrow group of nuclei lying in the mid-portion of the lower pons and upper medulla, whose fibers followed the path predicted by lesioning studies. This general localization would seem to hold true for all vertebrate species.

Catecholamines, like serotonin, have been demonstrated in a wide variety of living organisms, including several species of plants and fruits (Udenfriend et al. 1959), and one or more of the catecholamines have been found in representatives of all the major animal phyla from protozoa through vertebrates. In the great majority of invertebrates that have been examined, DA is the dominant catecholamine. NE, when present, is at levels much lower than those of DA. In most invertebrates, E appears to be absent. Histochemical fluorescence studies have shown that the catecholamines are present almost exclusively in the nervous

system, and catecholamines have been found in all invertebrate nervous systems from the primitive coelenterates through the highly specialized arthropods (Welsh, 1972).

E and NE have been found in many different organs of the fish including nervous tissue, and DA has also been found in the fish brain. Concentrations reported in the literature exhibit considerable variation (Welsh, 1972).

In amphibians, E is the dominant catecholamine in most organs studied, but in amphibian adrenal glands the concentrations of E and NE are usually similar. In the whole brain, E concentrations reported for the various species range from 0.52 μg/g in an unspecified species of frog (Anton and Sayre, 1962) to 2.4 μg/g in Bufo marinus (Bogdanski et al. 1963).

Whole brain NE concentrations reported for amphibians average about 0.25 μg/g (Anton and Sayre, 1962, Juorio, 1973). Juorio (1973) also reports a DA concentration of approximately 0.4 μg/g in the brain of Rana temporaria, the highest regional concentration being found in the hypothalamus.

In reptiles, DA and NE were present in higher concentrations than E in all organs examined except the adrenal gland, where E either exceeded or was approximately equal to NE.

All three catecholamines have been found in the reptilian brain, E ranging from about 0.1 to 0.3 μg/g (Anton and Sayre 1962). Juorio (1969) reported regional DA concentrations in the brain of a tortoise, these ranging from 0.24 μg/g in the brain-

stem to 0.8 μg/g in the cerebral hemispheres.

In tissues of birds there generally seems to be a higher relative concentration of E than in the corresponding mammalian tissue, but less than in reptilian tissue. The adrenal gland of birds contains discrete cell groups responsible for either E or NE production as does the mammalian adrenal.

Catecholamine concentrations representative of those reported for avian brain would be those of Anton and Sayre (1964) for the pigeon. E concentration was 0.02 μg/g, NE was 0.25 μg/g and DA was 0.29 μg/g.

Naturally, catecholamine distribution has been studied much more extensively in mammals than in any other class of vertebrates. Excluding chromaffin tissue, the tissues in which catecholamines are most concentrated are those having a high degree of sympathetic innervation such as the vas deferens, heart, spleen, etc. (Cooper et al. 1974), and, predictably, NE is the catecholamine found in these regions.

In mammalian brain, the E. concentration is so low as to be negligible. Whole brain NE concentrations given by Iversen (1967) range from 0.16 μg/g in the dog to 0.49 μg/g in the rat, while DA ranges from 0.19 μg/g in the dog to 0.60 μg/g in the rat.

Within most vertebrate nervous systems studied both NE and DA show a characteristic pattern of regional distribution. The highest concentration of NE is almost invariably found in the region of the hypothalamus and other areas of central sympathetic

representation (Cooper et al. 1974).

DA distribution differs markedly from that of NE, suggesting that DA is present not only as a precursor of NE. In fact, DA represents more than 50% of the total catecholamine content of the nervous system of most mammals. The highest levels of DA are found in the neostriatum, nucleus accumbens and tuberculum olfactorium (Cooper et al. 1974).

Except for the single primitive species, Sphenodon punctatum, living reptiles are grouped into three orders: the turtles (Chelonia), the lizards and snakes (Squamata) and the crocodilians (Crocodilia). Several studies have been done in which monoamine concentrations were measured in the brains of selected species and comparisons have been made between the different orders of reptiles as well as between reptiles and other vertebrate classes.

In 1963, Bogdanski et al. conducted studies leading to a phylogenetic comparison of tissue amine levels in various vertebrate classes, including reptiles. The species of reptile used was the lizard Sceloporus cyanogens, and the whole brain of this animal was reported to contain 3.1 μg/g of 5-HT and 1.6 μg/g of NE. Neither E nor DA was measured.

In 1964 Quay and Wilhoft conducted an extensive study of the 5-HT content of reptilian brains and brain regions. Values were given for two species of turtle, four species of lizard, two snakes and the American alligator.

The turtle brains were found to contain 2.10 μg/g of 5-HT

for Terrapene carolina and 2.95 μg/g for Gopherus berlandieri. A regional analysis of brain and pineal of four species of turtle showed relatively high 5-HT concentrations in the tectum, tegmentum, pons and medulla, while concentrations were low in the olfactory bulbs and cerebellum.

Lizard whole brain concentrations ranged from 1.70 μg/g in Sauromalus varius to 3.53 μg/g in Xantusia vigilis. When the brains of six species were divided, the highest 5-HT concentration was generally found in the tegmentum followed by the diencephalon, pons and medulla, with the lowest values again being found in the olfactory bulbs and cerebellum.

Snake whole brains were found to contain 2.44 μg/g in Natrix sipedon and 2.73 μg/g in Thamnophis ordinatus. When regional analysis was carried out using brains of three different species, the diencephalon generally yielded the highest 5-HT concentration, followed by the cerebrum and tectum. Very low 5-HT concentrations were found in the cerebellum and olfactory bulbs.

The 5-HT concentration in the single alligator brain analyzed (Alligator mississippiensis) was reported to be 0.25 μg/g. Regional analysis showed the highest concentrations to be in the tegmentum (1.28 μg/g), followed by the diencephalon (1.22 μg/g) and medulla (0.70 μg/g). The lowest regional concentrations were in the cerebellum (0.07 μg/g) and the olfactory bulbs (0.09 μg/g).

There was considerable variation from species to species within each order both in overall brain concentration and in distribution.

In 1974 Rujirekagulwat and Huggins measured whole brain and regional concentrations of 5-HT in the Siamese crocodile, Crocodylus siamensis. Whole brain concentrations were determined to be 0.731 μg/g for large animals (3-4 years old) and 1.481 μg/g for small animals (1 month old). The highest regional concentrations were found in the cerebrum (1.023 μg/g) followed by the diencephalon (0.954 μg/g) and the midbrain tegmentum (0.834 μg/g). The regions with the lowest concentrations were the olfactory bulbs (0.351 μg/g) and the cerebellum (0.429 μg/g). No sex differences were observed, but some seasonal variation was noted.

Doshi et al. (1974) measured whole brain 5-HT concentration in the lizard Anolis carolinensis and found it to vary between 1.83 μg/g and 5.41 μg/g depending on the time of day. No significant sexual differences were noted, but a definite circadian rhythm was indicated.

Anton and Sayre (1964) measured catecholamines in the whole brain of an unspecified species of turtle, finding E to be 0.12 μg/g, NE 0.44 μg/g and DA 0.57 μg/g.

In 1973 Juorio published the results of a study of the distribution of catecholamines in the brain of some lower vertebrates, including two species of tortoise. No whole brain

concentrations were given. In Testudo graeca the highest regional concentrations of both E (4.11 μg/g) and NE (2.66 μg/g) were found in the hypothalamus, followed by the optic lobes (1.16 μg/g) for E and the optic lobes (1.90 μg/g) and medulla (1.91 μg/g) for NE. The highest concentration of DA was found in the nucleus basalis of the cerebral hemispheres (2.98 μg/g) followed by the hypothalamus (1.33 μg/g). The cerebral hemispehers, excluding the nucleus basalis, contained the lowest levels of both E (0.17 μg/g) and NE (0.66 μg/g) while the lowest DA level was present in the medulla (0.38 μg/g).

Many investigations have been undertaken in an attempt to correlate brain catecholamine and/or 5-HT levels with various behavioral states or environmental conditions. In studying these relationships several different strategies may be employed.

First, it is possible to look for naturally occurring changes in amine levels paralleling behavioral states. This method is particularly applicable to the study of circadian and ultradian variations in the animal's sleep-waking cycle, seasonal behavioral changes such as mating or hibernation, and environmental factors such as changes in temperature.

A closely related method involves deliberate alteration of some behavioral or environmental parameter, such as modifying the sleep-waking cycle or subjecting the animal to stress and observing the effect of these manipulations upon the activity of monoamine-containing neurons.

The particular monoamine being tested may also be administered to the animal by various routes and any behavioral effects noted.

Finally, it is possible to alter the neurons themselves by either increasing or decreasing their amine content and observing the resultant effect on behavior. This can be accomplished by lesioning in which neurons are destroyed, or by means of various pharmacological agents which alter the amine content of specific types of neurons.

Circadian variations in both 5-HT and catecholamine levels have been reported in neural and non-neural tissue. Scheving et al. (1968) found a daily fluctuation in 5-HT content in the rat brain, it being lowest during the early part of the evening when activity was highest and maximum during sleep.

In 1972, Scheving et al. also found that blood serum 5-HT levels fluctuate in a manner exactly opposite to that of brain, being highest during activity and lowest during sleep. He included a study of several different environmental conditions which showed that exposure to ether and immobilization had a damping effect of the 5-HT rhythm but that exposure to a novel situation had no effect.

Changes in the catecholamine concentration in the blood and tissues of bony fishes have been observed (Holzbauer and Sharman, 1972). Boehlke et al. (1967) reported diurnal variation in the concentration of NE in the plasma of the channel catfish.

Scheving et al. (1968) reported circadian variations in the concentrations of rat brain catecholamines in which the concentration of a particular amine might vary by 15-20% around the 24 hour mean value. Reis et al. (1968) observed that these fluctuations of NE were localized to certain parts of the brain, but the variation in these parts was up to threefold. Changes in the cerebral catecholamine concentrations may also occur during hibernation (Holzbauer and Sharman, 1972).

These findings suggest possible roles for 5-HT and catecholamines in the mechanisms responsible for either sleep or maintenance of wakefulness, since diurnal or seasonal variations in their levels can be correlated with varying activity levels.

Studies in which monoamine levels were measured during particular behavioral states include those of Aprison et al. (1962) who showed that decreased pecking activity in pigeons produced by an increase in 5-HT in the body was a CNS rather that a peripheral effect. The 5-HT concentration in the telencephalon and diencephalon was directly related to changes in behavior, with the concentration of 5-HT maximum at the time of depressed behavior and returning to normal at the same time as behavior returned to normal. It was suggested that 5-HT might exert its effect on the limbic system and associated structures.

Bliss et al. (1972) subjected mice and rats to various stressful situations and found no change in 5-HT levels but did find increases in 5-hydroxyindoleacetic acid (5-HIAA), a

metabolite of 5-HT, indicating a higher turnover rate for 5-HT.

Sinha (1973) measured 5-HT concentrations in various regions of the cat brain during wakefulness and different stages of sleep. The 5-HT concentration of the cerebral cortex was found to decrease from the awake value after 5 minutes of NREM sleep, as did that of the midbrain, cerebellum and medulla. The 5-HT levels in all areas approximated waking levels after 5 nimutes of REM sleep. The hypothalamus and pons exhibited the opposite pattern, 5-HT concentration increasing during NREM sleep and falling back to waking levels during REM sleep.

In a study of possible ecological factors affecting the 5-HT content of a reptilian brain, Wilhoft and Quay (1965) determined the dffect of temperature on the brain concentration of 5-HT and related amines in the lizard Sceloporus occidentalis. Maintaining animals at 35°C resulted in an increase as compared with animals maintained at 16°C. Thus, the temperature at which poikilothermic animals are maintained can influence the 5-HT content of the CNS.

Quay (1967) has also looked for circadian rhythms in cerebral and brainstem 5-HT concentrations in the turtle, Pseudemys scripta elegans. Due to large individual variations, no clear-cut rhythm was seen. However, at the onset of darkness, an apparent increase in brainstem 5-HT was accompanied by a decrease in cerebral 5-HT.

Quay (1970) evaluated possible factors affecting 5-HT

content in the reptilian brain under natural field conditions. Significant changes in brainstem and cerebral 5-HT appeared to be related mainly to time of day and temperature, and to have different patterns of change on cold and warm days; it was postulated that these changes could constitute or contribute to a central temperature-compensating mechanism in reptiles.

Bliss (1973) studied the relationship between various stresses and behaviors and the 5-HT and DA systems in the brain. It was found that stress due to foot shock did not alter absolute levels of 5-HT, but an activation of 5-HT metabolism was indicated by increased 5-HIAA levels which persisted for some time after the noxious disturbance was removed. It was found that REM sleep deprivation also increased 5-HIAA. DA turnover was ascertained by measuring homovanillic acid (HVA) levels in the brain, and it was found that higher levels of this DA metabolite were present in actively running animals than in quiescent ones. Stressful experiences were also observed to result in an increased turnover of DA even in the absence of significant motor activity. The inference drawn from these data was that in addition to activation of DA neurons in the basal ganglia by muscular movements, DA neurons may play a role in the cerebral circuit for emotionality.

5-HT, along with NE, has been postulated to play a role in temperature regulation in mammalian species (Feldberg and Myers, 1963, 1964). When micro-injected into the preoptic area of the

hypothalamus 5-HT evokes hyperthermia and also causes a release of acetylcholine along the caudal heat production pathway in the diencephalon and mesencephalon. Peripheral cooling of the animal evokes a 2 to 24-fold increase in the release of 5-HT from these areas(Myers, 1973).

It has also been demonstrated that stimulation of the raphe nuclei in the rat causes an elevation of body temperature (Cooper et al. 1974).

In most species, intraventricular injection of NE leads to a decrease in body temperature, and it has been postulated that the NE neurons exert an inhibitory effect on the 5-HT pathways responsible for heat production (Marley and Stephenson, 1972).

The technique of injecting various monoamines so that they become distributed throughout the ventricular system or a portion thereof, or, alternatively, microinfusion into selected brain areas has been used rather extensively to study the central effects of catecholamines (Marley and Stephenson, 1972).

Intracisternal injection of E and NE has been shown to produce sleep followed by deep anesthesia (Leimdorfer, 1950; Kobayashi, 1965). Similar effects have been observed following injection of catecholamines intraventricularly (Marley and Stephenson, 1972).

Myers (1964a) defined a possible locus in cats for the soporific action of E injected intraventricularly, using micro-

injection of the amine into different parts of the brain. When injected into the CSF, anterior, posterior, lateral or medial hypothalamic areas, E in small doses produced a drowsy state, and in large doses produced a deep sleep in which the cats failed to respond to painful stimuli. E injected into other brain areas failed to produce this phenomenon.

Grossman (1968) reported that NE stereotaxically positioned in the midbrain reticular formation of rats significantly reduced locomotor activity and reaction to sensory stimuli.

In baby chicks, E and NE infused into the diencephalon or hypothalamus produced a deep sleep; DA was found to have similar effects provided the chick had been pretreated with a monoamine oxidase (MAO) inhibitor (Marley and Stephenson, 1972).

Catecholamines injected intravenously are generally reported to elicit behavioral arousal, and some investigators (Grunden, 1969; Kulkarni, 1967) have reported a period of brief excitement which preceded the central depressant effect of catecholamines injected intraventricularly or into the brain itself.

Benkert (1969) found that microinjection of NE into the hypothalamus brought about an augmentation of locomotor activity in rats.

Cordeau (1963) injected E through a cannula into the brainstem of cats and found behavioral and electrocortical arousal lasting from 10-20 minutes.

In cat <u>encephale isolé</u> preparations catecholamines evoke

electrocortical arousal. It is uncertain whether the alerting produced by catecholamines is due to direct action on the ascending reticular formation or is secondary to peripheral effects of the amines, such as increased blood pressure (Marley and Stephenson, 1972).

Many of these reports are contradictory; for example, E infused into the hypothalamic region has been found by some investigators to cause behavioral excitation and by others to cause sedation. The only general conclusion which may be drawn from this body of evidence is that catecholamines exert an excitatory effect when injected intravenously and variable effects when injected intraventricularly, intracisternally, or into specific brain regions.

The technique of selectively destroying discrete groups of central neurons by brain lesions has provided a valuable tool both for tracing monoamine pathways and for ascertaining their function through observation of the behavioral effects which are produced when a particular pathway is destroyed.

The association of a monoamine (5-HT) with a certain fiber tract was first demonstrated through lesioning of the median forebrain bundle, a tract which interconnects the hypothalamus, basal telencephalon and midbrain. Destruction of this tract leads to a reduction in brain 5-HT and NE on the side containing the lesion (Heller, 1972).

It has been demonstrated by histochemical fluorescence

studies that the perikarya of 5-HT containing neurons are located in the raphe nuclei. Jouvet (1968, 1969a, 1973) has performed stereotaxic lesioning of the raphe system in cats. Following 80-90% destruction of the raphe system, a state of long-lasting behavioral and EEG arousal was observed during the first 3-4 days. In the weeks which followed, the percentage of time spent in slow-wave sleep (SWS) was very small (10-15%) and paradoxical sleep (PS) was never observed. Partial lesions of the raphe system resulted in a less severe state of insomnia, there being a significant correlation between the extent of the lesion and the intensity of the resulting insomnia, as well as the selective decrease of cerebral 5-HT.

Pujol et al. (1972) reported that following subtotal destruction of the raphe system both synthesis of 5-HT from its precursor 5-hydroxytryptophan (5-HTP) and catabolism of 5-HT to 5-HIAA decreased very rapidly. Injection of 5-HTP did not alter the state of permanent behavioral arousal even though 5-HTP can be decarboxylated to form 5-HT in other neurons by means of the "non-specific" enzyme 5-HTP-DOPA decarboxylase. This would suggest that in order for sleep to occur, 5-HT must be released from 5-HT neurons, and it is not enough merely to have it present in the brain.

The evidence for intervention of catecholaminergic mechanisms in the control of tonic arousal in the cat has been summarized by Jouvet (1973). An increase in the level of cerebral

catecholamines has been shown to induce long-lasting arousal in the cat, whereas inhibition of catecholamine synthesis suppressed the EEG and behavioral arousal which normally follows injection of DL-amphetamine.

NE rather than DA seems to be responsible for cortical arousal. Destruction of the substantia nigra, which results in a more than 90% drop in tele-diencephalic DA levels, does not interfere with cortical EEG arousal although it does affect behavioral waking (Jones et al. 1968).

The same group also found that destruction of the dorsal NE pathway in the mesencephalon significantly reduced the tele-diencephalic NE, increased cortical synchronization, and decreased cortical arousal. Inhibition of catecholamine synthesis after destruction of the raphe nuclei of cats resulted in a reversal of the insomniac state which lasted about 24 hours, after which there was a rapid return to behavioral and EEG insomnia. The suggestion was made that under normal conditions 5-HT 5-HT neurons might exert tonic inhibitory action on some catecholamine neurons at the onset of sleep.

In 1970, Petitjean and Jouvet found that partial destruction of the locus coeruleus or the ascending dorsal NE pathway produced marked hypersomnia with up to 300% increases in both SWS and PS for 5 to 10 days, accompanied by decreased forebrain NE as well as increased turnover of 5-HT. It was postulated that there might also be an inhibitory catecholaminergic pathway

terminating on the raphe system.

Most of the data obtained in the cat favor the hypothesis that the sleep-waking cycle is regulated by two antagonistic systems of neurons: the 5-HT system for inducing sleep and the catecholamine neurons for waking and PS.

One means by which the role of DA in the CNS has been elucidated is through observation of patients with Parkinson's disease, a "ready-made lesion" of the basal ganglia in which DA levels are far below normal. This disease is characterized by extrapyramidal symptomatology such as rigidity, akinesia, tremor, etc.; it can generally be improved by administration of large amounts of DOPA, the immediate precursor of DA (Cooper et al. 1974).

Hubert and van Rossum (1969) described depressed patients who, at autopsy, demonstrated neuropathological changes of Prakinsonism even though they had failed to manifest any symptoms of the disease with the possible exception of the depression itself, which is a common accompaniment of Parkinsonism.

Sandler (1972) suggested that many non-parkinsonian subjects who respond to L-DOPA, such as retarded depressive patients, have a relative DA deficiency; and, in fact, CSF in these patients has been found to contain very low levels of HVA, a metabolite of DA.

Lesions placed in the tegmental area of monkeys and cats

resulted in substantial loss of DA from the substantia nigra and led to parkinsonian symptoms such as hypokinesia, hypotonicity and postural tremor.

One of the first drugs found empirically to be effective as a central tranquilizing agent was the Rauwolfia alkaloid reserpine, originally used mainly as an anti-hypertensive agent.

Release of 5-HT by reserpine was first reported by Pletscher et al. (1955); reserpine-induced depletion of NE in the hypothalamus of the cat was first reported by Holzbauer and Vogt (1956). Since then, reserpine has proved to be a valuable tool in the analysis of the action of monoamines in the CNS. Reserpine produces a characteristic sedative effect and its probable mechanism of action is prevention of incorporation of monoamines into storage vesicles (Glowinski and Axelrod, 1965; Glowinski et al., 1966). The connection between sedation and depletion of brain amines was not immediately obvious because the time course for depletion was not the same as that for sedation, depletion lasting for weeks while the behavioral effects were over within 48-72 hours.

Haggendal and Lindqvist (1964) demonstrated the existence of a very small functional catecholamine storage pool, restoration of which parallels behavioral recovery.

Concentrations of NE, DA, and 5-HT are lowered about the same extent and for the same time period by reserpine (Shore,

1962; Carlsson, 1966). In order to discover whether sedation was due to loss of catecholamines or 5-HT, experiments were performed in which large amounts of precursor amino acids (5-HTP or DOPA) were injected. The sedative effects of reserpine were reversed 15-30 minutes after injection of DOPA in mice (Carlsson et al. 1957), monkeys (Evrett and Toman, 1959), cats (McGeer et al., 1963) but not consistently in rats (Carlsson, 1966). This time interval suggests that reversal was due to synthesis of either NE or DA. DA was suggested to be the most likely since in reserpinized cats behavioral recovery was associated with a return to normal of DA concentration but only a partial recovery of NE. The level of 5-HT was unaffected (McGeer et al., 1963).

Brodie et al. (1956), on the other hand, suggested a causal relationship between sedation and depletion of 5-HT since sedation did not occur after administration of α-methyl-p-tyrosine, which has a lesser effect on 5-HT concentration than reserpine.

The question of the mechanism of action of reserpine has still not been settled satisfactorily. Since the discovery of reserpine, however, various other drugs having more specific actions have been found and these have been used to deplete 5-HT or catecholamines specifically.

Almost all the studies which have been done using reserpine were performed on mammals. Juorio (1973), however, injected

tortoises (Testudo graeca) with 0.5 mg/kg of reserpine. E, NE and DA were markedly depleted both in the hypothalamus and in the cerebral hemispheres, the depletion of DA proceeding much faster than that of either E or NE. DA depletion was also much more complete, approaching 100% by the end of the first day and remaining low for at least 8 days thereafter. NE and E levels were lowest on the eighth day, and were about 25% of the control level. These results were interpreted as evidence that a similar catecholamine storage mechanism exists in both mammals and reptiles. 5-HT levels were not measured.

Brodie and Bogdanski (1964) administered 25 mg/kg of reserpine to frogs (Rana pipiens). At a temperature of 37°C, E was lowered by about 40%, while 5-HT was lowered by about 70%, suggesting a selective effect on 5-HT stores in the frog brain. The time course for depletion was not given, but it was mentioned that reserpine depleted brain amines much more rapidly at a temperature of 37°C than at room temperature. In the frogs, even extremely large doses of reserpine (100 mg.kg) failed to produce sedation. Only after pretreatment with a MAO inhibitor did reserpine sedate the animals.

The 5-HT depleting properties of p-chlorophenylalanine (PCPA) were first described by Koe and Weissman (1966) who also described its mechanism of action. PCPA was found to exert its effects through blockade of the enzyme tryptophan hydroxylase, and consequent blockade of 5-HT synthesis.

Delorme et al. (1966) found that in cats and rats a single injection of 400 mg/kg of PCPA led to an almost total suppression of sleep, the effects becoming evident about 24 hours after injection. Recovery began after about 80 hours, and proceeded gradually with sleep returning to normal on about the 8th-10th day. The insomnia produced by PCPA is very similar to that produced by lesion of the raphe nuclei.

In waking animals, increased irritability, alertness and aggressiveness have been observed after injection of PCPA as well as increased sexual and social activity and sensitivity to pain (Weissman, 1973).

Experiments dealing with the effects of PCPA apparently have been limited to mammalian species, mainly rats, cats, rabbits and monkeys. No reports have been found in the literature of experiments in which PCPA was administered to reptiles.

In 1967 Tranzer and Thoenen discovered that an isomer of NE, 6-hydroxydopamine (6-OHDA) produced a destruction of the terminal endings of peripheral sympathetic neurons. The effect was relatively selective and has been termed a "chemical sympathectomy". When injected into the brain, DA neurons are destroyed along with NE neurons (Bloom et al., 1969; Ungerstedt, 1968). Utresky and Iversen (1969) found that a dose of 5 µg injected intraventricularly was capable of reducing whole brain NE by 30% within two days while 25-50 µg decreased NE by 50% without altering DA concentrations. With higher doses, both

NE and DA decreased simultaneously with the decline in DA being steeper than the drop in NE, until at a total dose of about 500 μg both were reduced by about 80% (Kostrzewa and Jacobowitz, 1974). It appears that 6-OHDA affects NE neurons throughout the CNS with prepontine fibers being somewhat more susceptible to damage or depletion than lower brainstem neurons (Kostrzewa and Jacobowitz, 1974). In the CNS, the damaged catecholamine-containing neurons do not appear to regenerate to any substantial degree. Bloom (1971) found no structural recovery of NE neurons for as long as 8 to 10 months following a single injection of 6-OHDA.

In order to produce a more selective depletion and/or destruction of only one division of the central catecholamine neuronal systems, various techniques have been used. Administration of a series of low doses has been found to selectively deplete the CNS stores of NE, while administration of certain drugs such as the tricyclic antidepressant desipramine before 6-OHDA injection brings about a selective depletion of DA.

After intraventricular or intracisternal administration of 6-OHDA an effect similar to reserpine sedation was produced (Kostrzewa and Jacobowitz, 1974) in rats. Ungerstedt (1968, 1973) found that a unilateral 6-OHDA-induced lesion of the nigrostriatal pathway in rats induced a dose-dependent rotational behavior toward the lesioned side. Bilateral lesioning of the same pathway produced a state of akinesia, adipsia and aphagia.

These drug-induced effects are similar to the effects produced by electrothermal lesioning of the rat striatum, and have been compared with parkinsonian degeneration of DA neurons.

Shaywitz et al. (1975) used 6-OHDA to deplete brain DA in neonatal rats and found them to be significantly more active than their littermate controls during the period between 2 and 3 weeks of age. As the rat pups approached maturity the hyperactivity diminished and they correlated this finding with the clinical syndrome of minimal brain dysfunction (MBD) in children where hyperactivity is pronounced until 10 or 12 years of age and then diminishes. These children have been found to have significantly reduced HVA levels in the CSF, indicating reduced brain DA concentration. It has therefore been suggested that DA may act as a modulator of excitatory NE activity so that reduction of brain DA removes the constraints and allows activity to occur unchecked.

Most studies using 6-OHDA have also been performed on mammalian species, the only non-mammalian species found in the literature being a frog (Hopkins, 1971) in which 100 mg/kg of 6-OHDA injected into the dorsal lymph sac caused a 45% depletion of NE and a 55% depletion of E after four days. No significant catecholamine depletion occurred in the forebrain. DA levels were not measured.

RESEARCH OBJECTIVES

The object of the present research was to determine regional brain concentrations of the indoleamine 5-HT and the catecholamines E, NE, and DA in a reptile, the crocodilian Caiman sclerops. Very few reports in the literature give concentrations of all four monoamines for a single species of reptile; therefore, comparison of amine levels relative to one another is difficult. This difficulty is further enhanced by the fact that each author divides the brain differently, making regional comparison impossible. A systematic qualitative and quantitative analysis in which a single species is used for measurement of all the amines and in which the brain regions used are consistently the same should lead to a clearer picture of monoamine distribution within the reptilian brain and also facilitate phylogenetic comparisons between reptiles and other classes.

Almost all the studies which have been carried out using drugs which modify brain monoamines have used mammalian species. The only reports found in the literature in which drugs of this type were administered to reptiles involved injection of reserpine in a tortoise (Juorio, 1973) followed by measurement of catecholamine levels in the hypothalamus and cerebral hemispheres. Flanigan (1973) injected caimans with reserpine and observed changes in the EEG but did not measure monoamine levels.

No reports have been found in which either PCPA or 6-OHDA were given to reptiles. Therefore it was decided that these three drugs would be administered to caimans in order to ascertain their effects in a reptile. First, the effect of each drug on regional and whole brain monoamine levels would be determined using the same brain regions as the controls, and a comparison would be made between the time course and pattern of depletion in the reptile with that in mammals.

It was also decided that gross behavioral effects should be monitored in the caiman after drug administration. The parameter chosen was overall activity levels since drug-induced changes in monoamine levels in the mammalian brain can almost always be correlated with changes in locomotor activity.

MATERIALS AND METHODS

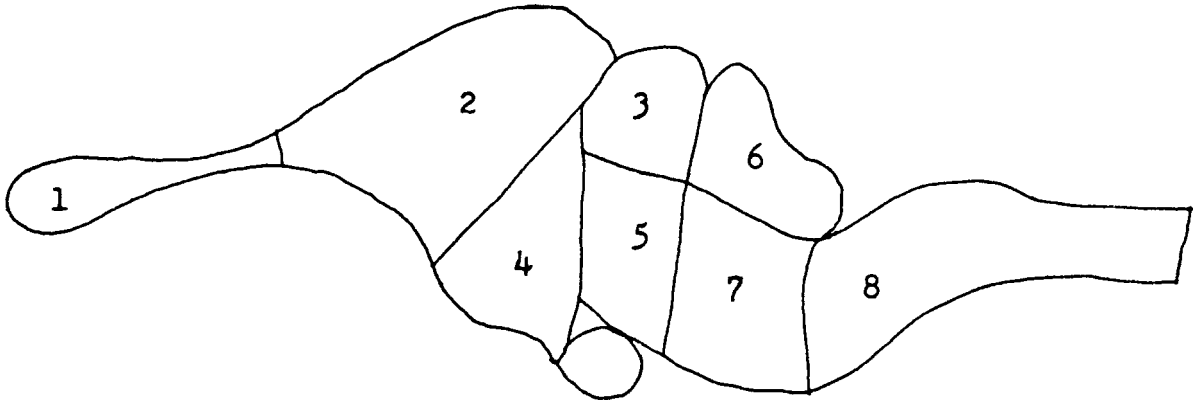
The animals used in this study were young South American caimans, Caiman sclerops, about one to three months of age. They were obtained from commercial sources and a colony was maintained in a large aquarium where they were fed regularly with chicken cut into small pieces. All were males weighing an average of 36.5 g with a range of 27.9g to 60.0g. Length ranged from 21.5 cm to 26.5 cm with an average of 23.6 cm.

In order to rule out any circadian variations in amine levels, all assays were begun at about 12 noon when the animal to be used was killed by decapitation. The brain was removed as quickly as possible and placed on a sheet of aluminum foil in a pan of ice for dissection. The pia mater was removed and the brain was divided into the following regions (Figure 1):

1. Olfactory bulbs and stalks
2. Cerebral hemispheres
3. Optic tectum
4. Diencephalon
5. Midbrain tegmentum
6. Cerebellum
7. Pons
8. Medulla.

Each portion was placed on a pre-weighed sheet of aluminum foil and these were then weighed together on a Mettler balance.

DIVISION OF THE CAIMAN BRAIN INTO REGIONS



1. Olfactory Bulb and Stalk
2. Cerebral Hemispheres
3. Optic Tectum
4. Diencephalon
5. Midbrain Tegmentum
6. Cerebellum
7. Pons
8. Medulla

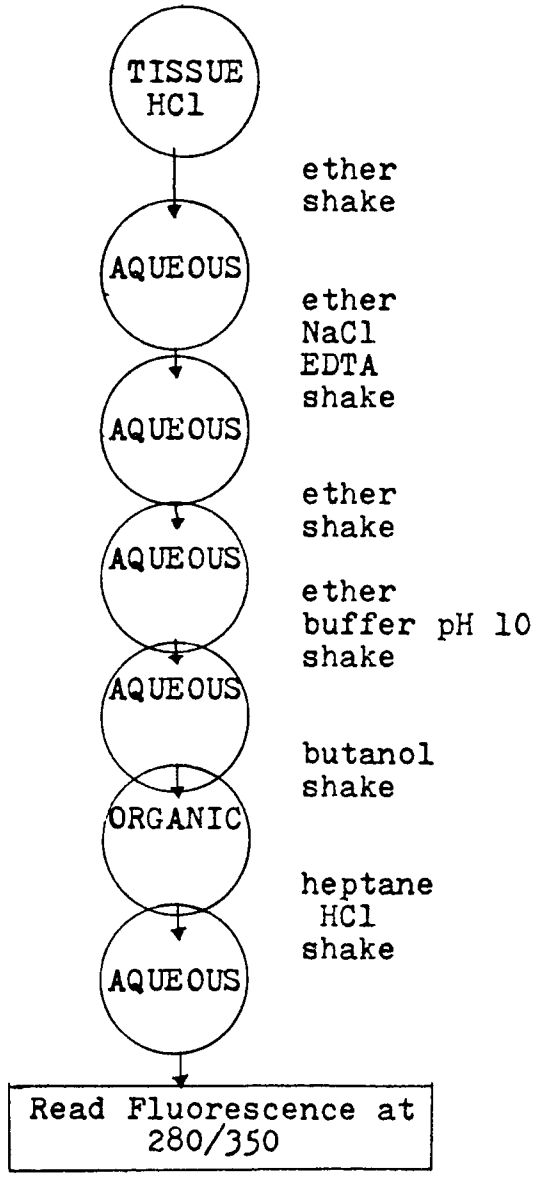
FIGURE 1

The method used for measurement of 5-HT was based upon Quay's (1963) modification of a procedure originally outlined by Bogdanski (1956). The extraction procedure is outlined in Figure 2.

A concentrated standard solution containing 100 µg/ml of 5-HT was prepared from 5-HT-creatinine sulfate dissolved in 0.1 N HCl, and was kept refrigerated when not in use. Solutions which had been stored for more than a week were discarded and new solution was prepared. This standard solution was further diluted for use in preparing internal standards.

Each tissue sample was homogenized in 5 ml of 0.1 N HCl except for the largest one, the cerebral hemispheres. These were homogenized in 12 ml of 0.1 N HCl, and the resulting homogenate was divided into three samples of 4 ml each. The volume of two of these samples was made up to 5 ml by addition of 0.1 N HCl and one of these served as a sample for analysis while another served as a tissue blank. To the third portion, 1 ml of standard solution containing 0.5 ug of 5-HT was added to make an internal standard.

3 ml of ether was added to each of the homogenates and they were shaken for 2 minutes in glass-stoppered centrifuge tubes using a mechanical shaker. The ether phase was discarded and 1 ml of EDTA solution (Quay, 1963), 500 mg NaCl and another 3 ml ether were added. After shaking for 2 minutes, the samples were centrifuged and the ether phase again discarded.



OUTLINE OF PROCEDURE FOR EXTRACTION OF 5-HT FROM TISSUE

FIGURE 2

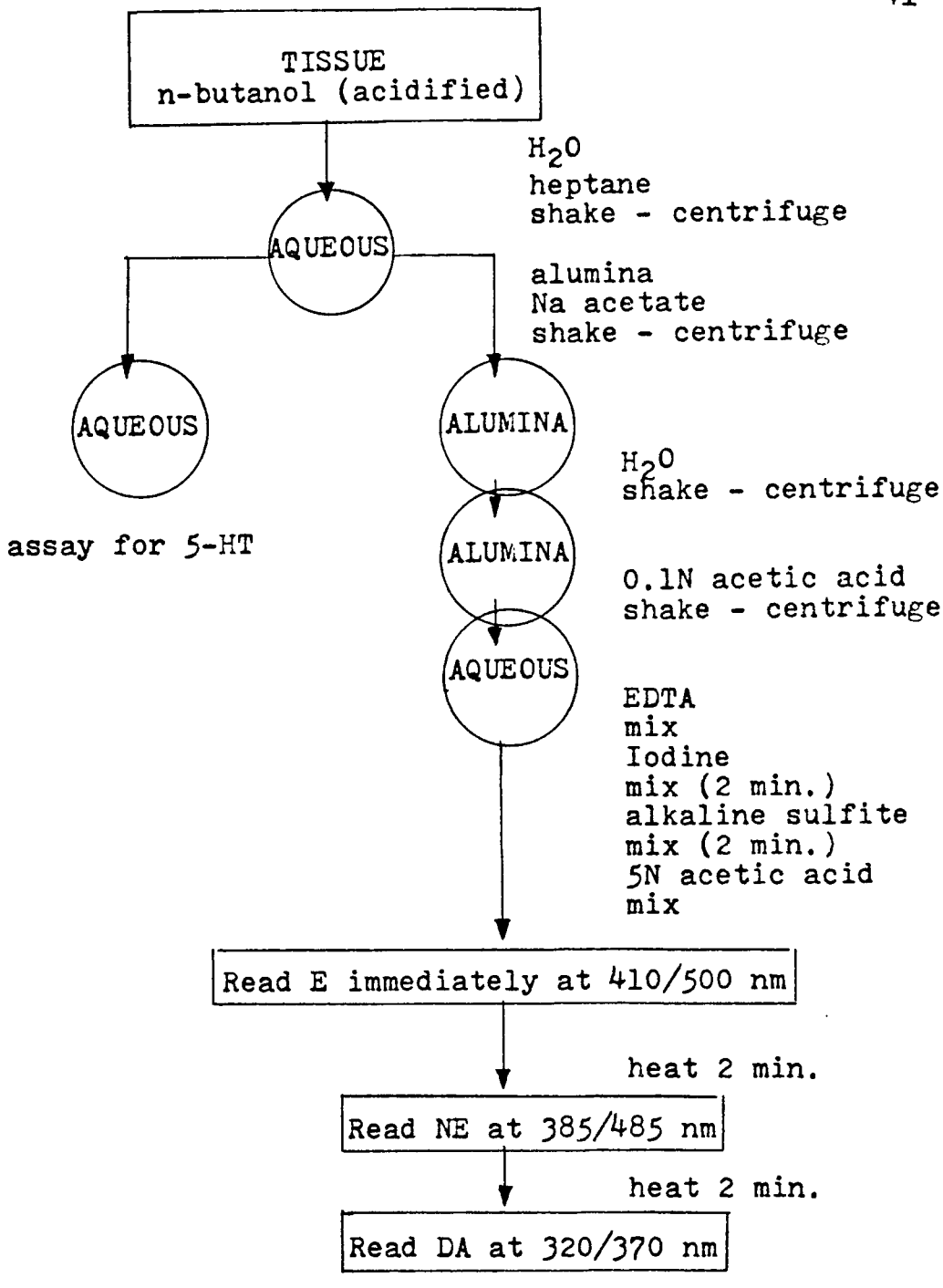
Another 3 ml ether was added, the tubes were shaken for 2 minutes and the ether phase discarded. 3 ml of ether were added once more, together with 1 ml pH 10 buffer (Quay, 1963). The tubes were shaken and the ether phase discarded. 3 ml of N-butanol were than added, the tubes were shaken for 5 minutes and the aqueous phase discarded. The organic phase was added to a centrifuge tube containing 8 ml heptane and 3 ml 0.1 N HCl, and this was shaken for 5 minutes. The organic phase was discarded and the aqueous phase was saved for measurement of fluorescence.

This was accomplished using an Aminco-Bowman spectrophotofluorometer with an excitation wavelength of 280 nm and an emmission wavelength of 350 nm.

The method used for catecholamine assay was described by Cox and Perhach (1973) and allows for the measurement of E, NE and DA in a single sample. The entire procedure is outlined in figure 3.

A concentrated standard solution containing 100 μg/ml of each of the catecholamines was prepared from L-epinepherine bitartrate or L-epinepherine, norepinepherine HCl and dopamine HCl dissolved in 0.1 N acetic acid and kept refrigerated when not in use. This was further diluted for use in preparing internal standards for each assay. Solutions which had been stored for more than a week were discarded.

Each tissue was homogenized in 3 ml of acidified butanol



OUTLINE OF PROCEDURE FOR EXTRACTION OF CATECHOLAMINES FROM TISSUE

FIGURE 3

except for the largest one, the cerebral hemispheres. These were homogenized in 9 ml of acidified n-butanol and divided into three portions, one of which served as the tissue sample for assay, another as a tissue blank and the third as an internal standard containing 0.03 ug of each amine.

The homogenates were added to glass-stoppered centrifuge tubes containing 3 ml distilled water and 5 ml heptane. The tubes were then shaken for 5 minutes using a mechanical shaker and the organic phase was discarded. When 1.ml sodium acetate solution (2 M) and 200 mg alumina were added to the aqueous phase, the tubes were again shaken for ten minutes, centrifuged, and the aqueous phase set aside for assay of 5-HT. Then 2 ml distilled water were added to the alumina, the tubes were shaken for 5 minutes, centrifuged, and the aqueous phase aspirated. Then 4 ml of 0.1 N acetic acid were added to the alumina and the tubes were shaken for ten minutes and centrifuged. Three samples of 1 ml each were taken from the aqueous phase of each tube and the catecholamines were oxidized to improve fluorescence characteristics. Oxidation was brought about by adjusting the pH of the samples to about 6.5 by addition of 0.2 ml EDTA reagent (Chang, 1964), then adding 0.1 ml of 0.1 M iodine to oxidize the monoamines. After exactly two minutes the reaction was stopped by adding 0.2 ml alkaline sulfite solution (Chang, 1964) and exactly 2 minutes later the pH of the solution was adjusted to about 5.4 by addition of 0.2 ml of 5 N acetic acid.

E was assayed by reading fluorescence immediately (activation at 410 nm and emmission at 500 nm).

NE was assayed by reading fluorescence after heating in boiling water for exactly 2 minutes (activation at 385 nm, emmission at 485 nm.)

DA was assayed by reading fluorescence after heating in boiling water for 5 minutes (activation at 320 nm, emmission at 370 nm).

Since fluorescence increased linearly as amine concentration was increased within the concentration range to be measured, it was possible to calculate both 5-HT and catecholamine concentrations by comparison with the internal standards. The amount of standard (S) added to a portion of homogenate from the largest tissue sample is represented by (B - A) where A is "fluorescence units" obtained from the final extract of homogenate alone and B is the "fluorescence units" obtained from the final extract of the portion to which the standard had been added.

Thus, the amine content of the tissue was:

$$\frac{A - \text{Blank}}{B - A} \times S \qquad (1)$$

Tissue amine concentration ($\mu g/g$) was obtained by dividing (1) by the weight of the tissue in g.

All animals to be used in experimental procedures were given injections of drugs at about 12 noon. The drugs were

suspended in either 0.9% saline or in sterile Steroid Suspending Vehicle (SSV)(NaCl 9 mg, Na-carboxymethylcellulose 5 mg, polysorbate 80 0.004 ml, benzyl alcohol 0.009 ml in 1 ml H_2O).

Reserpine was diluted in SSV to a concentration of 1 mg/ml, and the dose injected was 5 mg/kg body weight given intraperitoneally. The control animals used in activity studies received an equal volume of SSV. Animals receiving reserpine were killed at 24 hours, 48 hours, 72 hours, 7 days and 14 days. Activity was recorded on the third day following injection.

PCPA was diluted in SSV to a concentration of 10 mg/ml and the dosage given was 300 mg/kg body weight injected intraperitoneally. The control animals used in activity studies received an equivalent volume of SSV. Animals receiving PCPA were killed at 3 days, 7 days and 14 days. Activity was recorded on the third and seventh days following injection.

The caimans to be used in the 6-OHDA study were pretreated with desmethylimipramine (20 mg/kg, dissolved in 0.9% saline, injected intraperitoneally) followed one hour later by intracisternal administration of 25 ul of a solution containing 100 ug of 6-OHDA per 25 ul of vehicle (0.9% saline + 0.4 mg ascorbic acid per ml). Controls used in activity studies received an equivalent volume of 0.9% saline intraperitoneally followed one hour later by intracisternal administration of vehicle solution.

Activity was measured using an apparatus described by Huggins et al (1973). This consisted of a styrofoam box in

which a wooden platform was mounted on blocks of polyurethane foam so that any movement by the animal would result in movement of the platform also. A geophone, of the type commonly used in geophysical exploration, was anchored to the platform with florist's clay and served as a transducer for movements made by the caimans. It was connected through a high-gain amplifier to a physiograph. Large sponges soaked with water were placed on the platform in order to keep the caimans moist. Recordings were taken for at least 12 consecutive hours from two animals simultaneously, the experimental animal usually being placed in one box and the control in the other.

Activity was quantitated by measurement of amplitude of movement and time spent in movement. The scale used was : "high-level activity" with pen excursions in excess of 2 cm; "medium-level activity" with pen excursions between 1 and 2 cm; "low-level activity" with pen movements greater than 1 mm and less than 1 cm and continuing for several pen movements; and "minimal activity" with multiple excursions less than 1 mm or a single excursion less than 1 cm.

RESULTS

Table 1 shows average 5-HT, E, NE and DA concentrations for each region of the brain as well as for the whole brain.

Whole brain 5-HT concentration averaged 1.59 ± 0.10 μg/g. Regional 5-HT concentrations significantly higher than the mean were found in the medulla (3.04 ± 0.19 μg/g; $p < 0.005$), the pons (2.71 ± 0.22 μg/g; $p < 0.005$), and the midbrain tegmentum (2.60 ± 0.24 μg/g; $p < 0.05$). Regional concentrations significantly lower than the mean were found in the olfactory bulbs (0.28 ± 0.09 μg/g; $p < 0.005$), the cerebellum (0.70 ± 0.06 μg/g; $p < 0.005$) and the cerebral hemispheres (1.03 ± 0.10 μg/g; $p < 0.005$).

Whole brain E averaged 0.15 ± 0.02 μg/g. A regional concentration significantly higher than the mean was found in the diencephalon (0.28 ± 0.03 μg/g; $p < 0.05$) while a regional concentration significantly lower than the mean was found in the cerebral hemispheres (0.07 ± 0.01 μg/g; $p < 0.05$).

Whole brain NE concentration averaged o.29 ± 0.02 μg/g. A regional concentration significantly higher than the mean was found in the diencephalon (0.42 ± 0.02 μg/g; $p < 0.01$) while regional concentrations significantly lower than the mean were found in the olfactory bulbs (0.12 ± 0.02 μg/g; $p < 0.005$) and the optic tectum (0.20 ± 0.01 μg/g; $p < 0.01$).

TABLE 1: REGIONAL MONOAMINE CONCENTRATIONS IN THE BRAIN OF Caiman sclerops.

Brain Region	Amine concentration (μg/g wet weight of tissue ± S.E.M.)			
	5-HT (N=5)	E (N=5)	NE (N=5)	DA (N=5)
Olfactory Bulbs and Stalks	0.28 ± 0.09	0.19 ± 0.05	0.12 ± 0.02	0.56 ± 0.16
Cerebral Hemispheres	1.03 ± 0.10	0.07 ± 0.01	0.29 ± 0.02	0.98 ± 0.06
Optic Tectum	1.73 ± 0.32	0.10 ± 0.02	0.20 ± 0.01	0.77 ± 0.05
Diencephalon	1.40 ± 0.14	0.28 ± 0.03	0.42 ± 0.02	3.16 ± 0.23
Midbrain Tegmentum	2.60 ± 0.24	0.21 ± 0.33	0.30 ± 0.03	0.41 ± 0.07
Cerebellum	0.70 ± 0.06	0.13 ± 0.04	0.34 ± 0.03	0.45 ± 0.07
Pons	2.71 ± 0.22	0.18 ± 0.03	0.32 ± 0.04	1.50 ± 0.22
Medulla	3.04 ± 0.19	0.22 ± 0.03	0.25 ± 0.01	0.11 ± 0.03
Whole Brain	1.59 ± 0.10	0.15 ± 0.02	0.29 ± 0.02	0.96 ± 0.06

N = Number of animals

Whole brain DA concentration averaged 0.96 ± 0.06 μg/g. A regional concentration significantly higher than the mean was found in the diencephalon (3.16 ± 0.23 μg/g; $p < 0.005$) while regional concentrations significantly lower than the mean were found in the medulla (0.11 ± 0.03 μg/g; $p < 0.005$), the cerebellum (0.45 ± 0.07 μg/g; $p < 0.005$), and the midbrain tegmentum (0.41 ± 0.07 μg/g; $p < 0.005$).

In whole brain, the ratio of total catecholamines to 5-HT was about 1:1, 5-HT constituting 53% and catecholamines the remainder (Table 2). Regions which contained predominantly 5-HT were the medulla (84%), the midbrain tegmentum (74%) and the optic tectum (62%).

Regions which contained predominantly catecholamines were the olfactory bulbs, with 76% of total monoamines being catecholamine, and the diencephalon where catecholamines made up 73% of the total monoamines measured.

In whole brain, E made up 5% of total monoamines measured, the highest percentage being found in the olfactory bulbs (17%) and the lowest percentages in the cerebral hemispheres (3%), optic tectum (4%) and pons (4%).

In whole brain, NE made up 10% of total monoamines measured, being highest in the cerebellum (21%) and lowest in the pons (7%).

TABLE 2: INDIVIDUAL MONOAMINE CONCENTRATIONS
EXPRESSED AS PERCENT OF TOTAL MONOAMINES IN CAIMAN BRAIN

Brain Region	5-HT	E	NE	DA	Total Concentration ($\mu g/g \pm$ S.E.M.; N=5)
Olfactory Bulbs and Stalks	24%	17%	11%	49%	1.15 ± 0.12
Cerebral Hemispheres	43%	3%	12%	41%	2.37 ± 0.17
Optic Tectum	62%	4%	7%	28%	2.79 ± 0.39
Diencephalon	27%	5%	8%	60%	5.26 ± 0.54
Midbrain Tegmentum	74%	6%	9%	12%	3.52 ± 0.21
Cerebellum	43%	8%	21%	28%	1.62 ± 0.12
Pons	58%	4%	7%	32%	4.71 ± 0.33
Medulla	84%	6%	8%	3%	3.62 ± 0.13
Whole Brain	53%	5%	10%	32%	3.00 ± 0.15

N = Number of animals

DA constituted 32% of total whole brain monoamines measured, being highest in the diencephalon (60%). DA constituted the lowest percentage of total regional monoamines in the medulla (3%) and the midbrain tegmentum (12%).

Of the catecholamines (Table 3), E made up 10% in whole brain, the highest regional percentages being found in the medulla (38%), midbrain tegmentum (23%) and olfactory bulbs (21%). The lowest regional percentages were found in the cerebral hemispheres (5%), diencephalon (7%) and pons (9%).

NE accounted for 21% of total whole brain catecholamines, the highest percentages being found in the medulla (43%), cerebellum (37%) and midbrain tegmentum (32%). The lowest percentages were found in the diencephalon (11%) and pons (16%).

DA made up 69% of total catecholamines in whole brain, the highest regional percentage being found in the diencephalon (82%), optic tectum (72%), pons (75%) and the cerebral hemispheres (73%). The region containing the lowest percentage of DA was the medulla (19%).

The ratio of 5-HT to E and NE alone was 3.6:1 for whole brain.

When reserpine was administered to the caimans, catecholamines

TABLE 3: INDIVIDUAL CATECHOLAMINE CONCENTRATIONS EXPRESSED AS PERCENT OF TOTAL CATECHOLAMINES IN CAIMAN BRAIN

Brain Region	E	NE	DA	Total Concentration ($\mu g/g \pm$ S.E.M.; N=5)
Olfactory Bulbs and Stalks	22%	14%	64%	0.87 ± 0.16
Cerebral Hemispheres	5%	22%	73%	1.35 ± 0.08
Optic Tectum	9%	19%	72%	1.06 ± 0.07
Diencephalon	7%	11%	82%	3.85 ± 0.51
Midbrain Tegmentum	23%	33%	44%	0.92 ± 0.10
Cerebellum	14%	37%	49%	0.92 ± 0.10
Pons	9%	16%	75%	2.00 ± 0.23
Medulla	38%	43%	19%	0.58 ± 0.05
Whole Brain	10%	21%	69%	1.40 ± 0.05

N = Number of animals

were progressively depleted (Figure 4) over a period of 7 days. By the fourteenth day concentrations of all catecholamines were somewhat higher than on the third and seventh days.

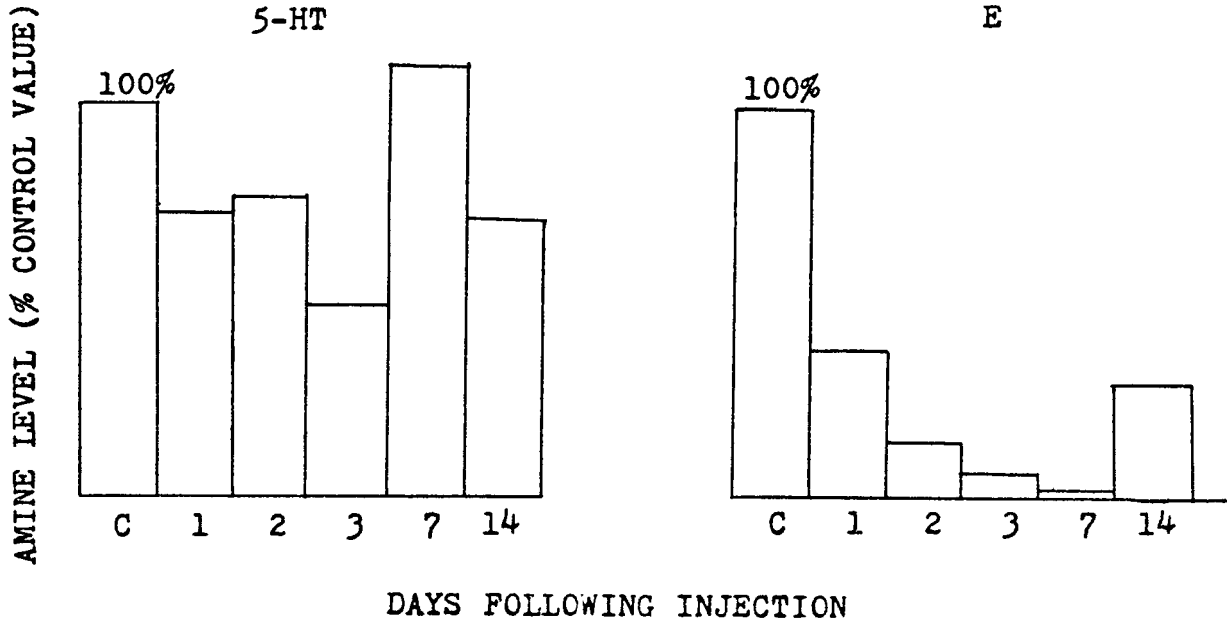
The pattern of depletion was much the same for the three catecholamines, concentrations being about half control levels on the first day following administration of the drug, roughly one-fourth control levels on the second day and around one-tenth of control levels on the third day. On the seventh day, E and NE had further decreased to less than 1% of control levels, but DA had increased somewhat, reaching about one-third of the control level. By the fourteenth day the concentrations of all the catecholamines had increased over the values found on the their and seventh days, being at least 20-30% of control levels.

The most rapid depletion of E (Table 4, Figure 5) appeared to be from the olfactory bulbs and cerebellum. Depletion of NE (Table 5, Figure 6) also appeared to be most rapid from the olfactory bulbs, cerebellum and cerebral hemispheres. Depletion of DA (Table 6, Figure 7) appeared to be most rapid from the olfactory bulbs and cerebellum also.

It is interesting to note that while in control animals DA concentrations in the medulla were well below those found in other regions of the brain, administration of reserpine (Table 6) caused DA concentrations in the medulla to rise

EFFECT OF RESERPINE ON WHOLE BRAIN MONOAMINE CONCENTRATIONS IN

Caiman sclerops



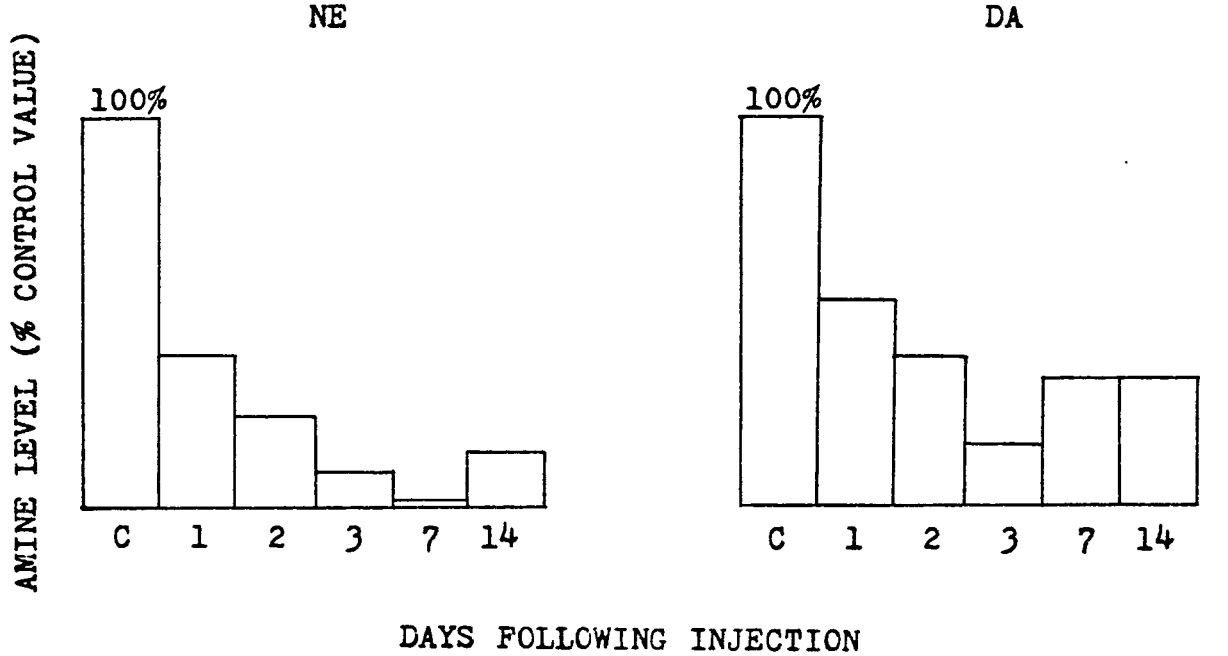


FIGURE 4

TABLE 4: EFFECT OF RESERPINE ON REGIONAL AND WHOLE BRAIN EPINEPHERINE CONCENTRATIONS IN THE CAIMAN

Brain Region	Number of days following injection									
	Control (N=5)	1 (N=2)		2 (N=2)		3 (N=2)		7 (N=2)		14 (N=2)	
		C	P	C	P	C	P	C	P	C	P
Olfactory Bulbs and Stalks	0.19 ± 0.05	0.00	-100%	0.00	-100%	0.00	-100%	0.00	-100%	0.06	-67%
Cerebral Hemispheres	0.07 ± 0.01	0.03	-64%	0.01	-92%*	0.00	-100%*	0.00	-100%*	0.02	-72%*
Optic Tectum	0.10 ± 0.02	0.03	-68%	0.00	-100%*	0.00	-100%*	0.00	-100%*	0.03	-68%
Diencephalon	0.28 ± 0.03	0.08	-72%*	0.04	-87%**	0.02	-93%**	0.00	-100%***	0.06	-80%*
Midbrain Tegmentum	0.21 ± 0.33	0.07	-66%	0.06	-69%	0.06	-72%	0.00	-100%*	0.05	-71%
Cerebellum	0.13 ± 0.04	0.01	-89%	0.01	-92%	0.00	-100%*	0.00	-100%*	0.05	-65%
Pons	0.18 ± 0.03	0.07	-63%	0.04	-78%*	0.00	-100%*	0.00	-100%*	0.06	-65%
Medulla	0.22 ± 0.03	0.10	-53%	0.01	-96%*	0.00	-100%*	0.00	-100%*	0.05	-76%*
Whole Brain	0.15 ± 0.02	0.05	-65%*	0.02	-88%*	0.01	-95%*	0.00	-100%**	0.04	-74%*

C = Concentration in μg/g wet weight of tissue
P = Percentage change from control values
N = Number of animals

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

EFFECT OF RESERPINE ON REGIONAL EPINEPHERINE CONCENTRATIONS IN CAIMAN BRAIN

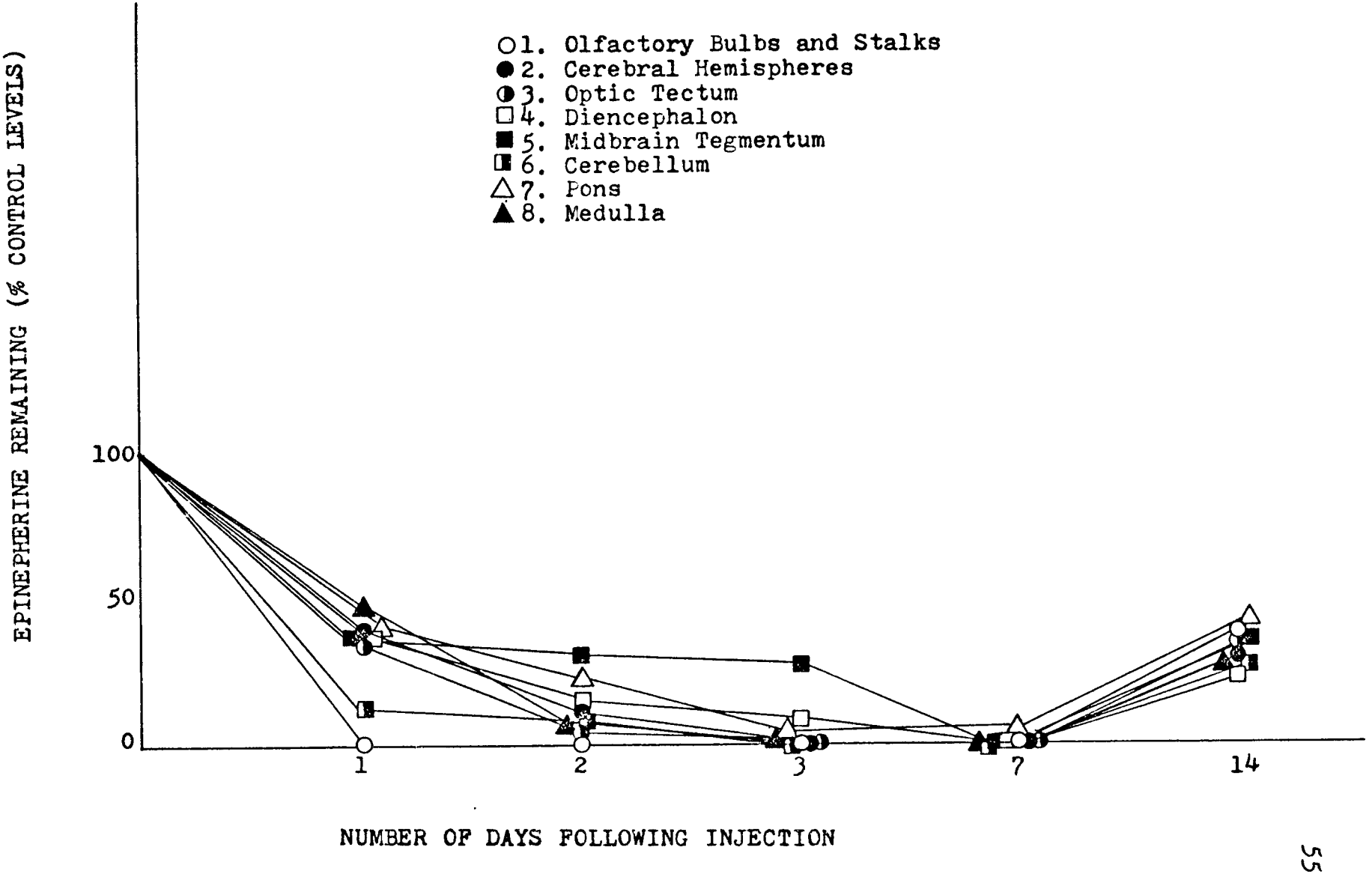


FIGURE 5

TABLE 5: EFFECT OF RESERPINE ON REGIONAL AND WHOLE BRAIN NOREPINEPHERINE CONCENTRATIONS IN THE CAIMAN

Brain Region	Number of days following injection									
	Control (N=5)	1 (N=2)		2 (N=2)		3 (N=2)		7 (N=2)		14 (N=2)	
		C	P	C	P	C	P	C	P	C	P
Olfactory Bulbs and Stalks	0.12 ± 0.02	0.04	-69%	0.00	-100%*	0.00	-100%*	0.00	-100%*	0.00	-100%*
Cerebral Hemispheres	0.29 ± 0.02	0.11	-64%*	0.04	-85%**	0.01	-96%***	0.00	-100%***	0.04	-85%**
Optic Tectum	0.20 ± 0.01	0.14	-31%*	0.06	-69%***	0.02	-89%***	0.03	-82%***	0.05	-77%***
Diencephalon	0.42 ± 0.02	0.12	-71%***	0.11	-74%***	0.05	-88%***	0.00	-100%***	0.01	-97%***
Midbrain Tegmentum	0.30 ± 0.03	0.13	-58%*	0.07	-78%**	0.04	-87%***	0.00	-100%***	0.00	-100%***
Cerebellum	0.34 ± 0.03	0.09	-73%*	0.04	-89%**	0.00	-100%***	0.00	-100%***	0.00	-100%***
Pons	0.32 ± 0.04	0.11	-66%*	0.13	-59%	0.05	-84%*	0.00	-100%*	0.06	-80%*
Medulla	0.25 ± 0.01	0.14	-44%***	0.05	-80%***	0.01	-94%***	0.00	-100%***	0.03	-89%***
Whole Brain	0.29 ± 0.02	0.11	-61%	0.06	-80%	0.02	-92%	0.00	-99%	0.03	-88%

C = Concentration in μg/g wet weight of tissue
P = Percentage change from control values
N = Number of animals

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

EFFECT OF RESERPINE ON REGIONAL NOREPINEPHERINE CONCENTRATIONS IN CAIMAN BRAIN

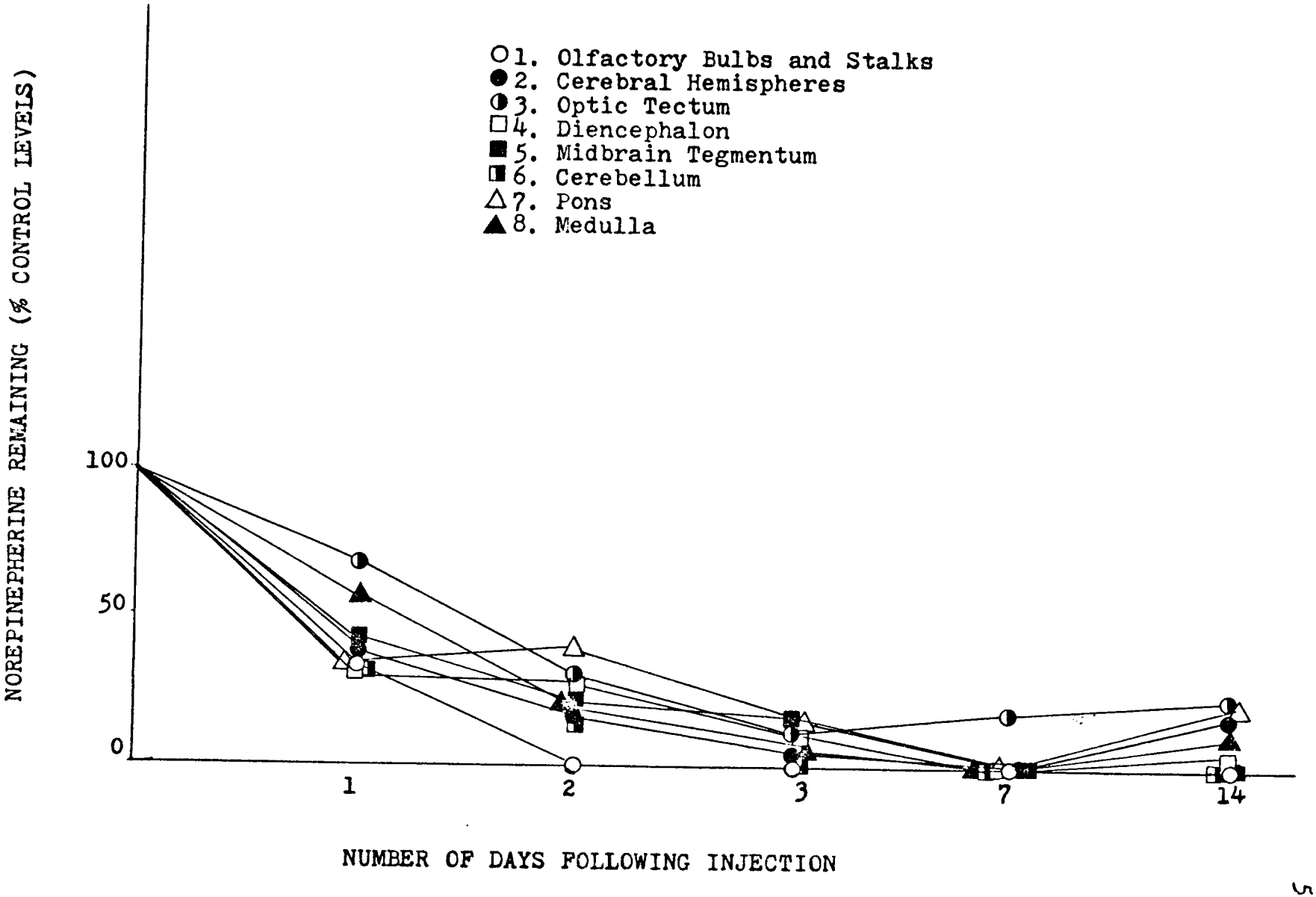


FIGURE 6

TABLE 6: EFFECT OF RESERPINE ON REGIONAL AND WHOLE BRAIN DOPAMINE CONCENTRATION IN THE CAIMAN

Brain Region	Control (N=5)	Number of days following injection									
		1 (N=2)		2 (N=2)		3 (N=2)		7 (N=2)		14 (N=2)	
		C	P	C	P	C	P	C	P	C	P
Olfactory Bulbs and Stalks	0.56 ± 0.16	0.01	-98%*	0.00	-100%*	0.01	-98%*	0.00	-100%*	0.00	-100%*
Cerebral Hemispheres	0.98 ± 0.06	0.58	-41%*	0.42	-56%**	0.00	-100%***	0.12	-88%***	0.27	-72%***
Optic Tectum	0.77 ± 0.05	0.35	-54%*	0.32	-58%*	0.03	-96%***	0.47	-39%*	0.35	-54%*
Diencephalon	3.16 ± 0.23	0.75	-76%***	0.38	-88%***	0.61	-81%***	0.73	-77%***	0.28	-91%***
Midbrain Tegmentum	0.41 ± 0.07	0.30	-27%	0.44	+ 8%	0.10	-75%	0.34	-16%	0.45	+10%
Cerebellum	0.45 ± 0.07	0.36	-20%	0.07	-84%	0.04	-91%*	0.00	-100%*	0.47	+4%
Pons	1.50 ± 0.22	0.33	-78%*	0.57	-62%	0.38	-75%*	0.61	-59%	0.37	-75%*
Medulla	0.11 ± 0.03	0.47	+334%***	0.29	+171%*	0.32	+193%*	0.00	-100%	0.39	+259%**
Whole Brain	0.96 ± 0.06	0.50	-49%*	0.35	-63%**	0.14	-85%***	0.30	-68%**	0.31	-68%**

C = Concentration in μg/g wet weight of tissue
P = Percentage change from control values
N = Number of animals

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

EFFECT OF RESERPINE ON REGIONAL DOPAMINE CONCENTRATIONS IN CAIMAN BRAIN

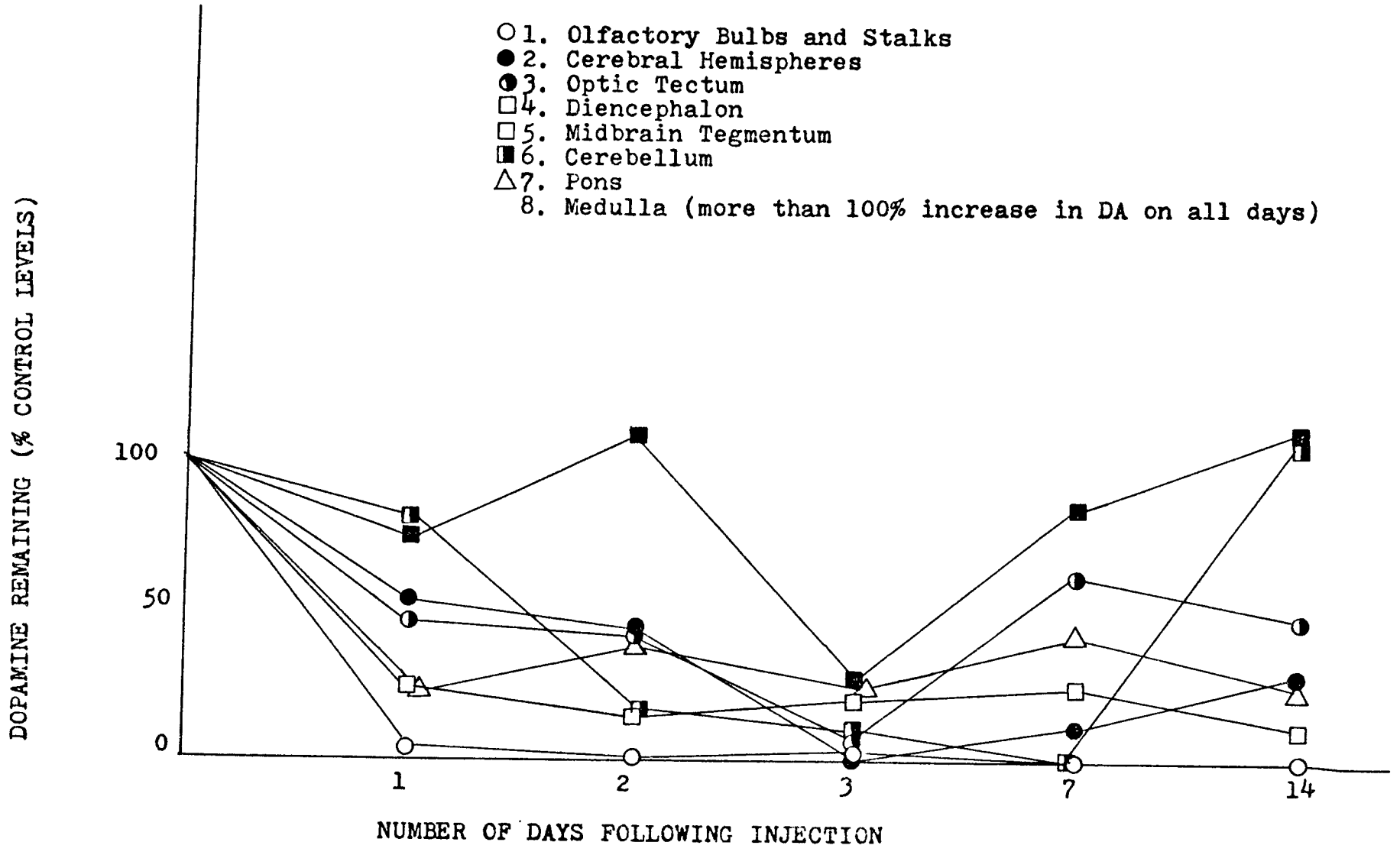


FIGURE 7

significantly above control levels and remain significantly elevated for at least 14 days following treatment.

Reserpine-induced depletion of 5-HT (Table 7, Figure 8) was not nearly as complete as was depletion of catecholamines, and the time course it followed was somewhat erratic. The greatest depletion was found on the third day following administration of the drug, the concentration present at this time being 49% of the control level. On the seventh day, whole brain 5-HT concentration was 12% above the control value and on the fourteenth day was 70% of control level.

When PCPA was administered to the caimans, 5-HT was significantly depleted (Table 8, Figures 9 and 10), though levels never dropped much below half of control values. On the third day following PCPA administration, whole brain 5-HT concentration had fallen to 54% of the control value and at fourteen days was only 41% of the control level. The most rapid and uniform depletion of 5-HT appeared to be from the optic tectum, midbrain tegmentum, medulla and pons. Depletion in other areas was not as complete and followed a more erratic course. In fact, the olfactory bulbs and cerebellum, which in control animals normally contain the lowest 5-HT concentrations, exhibited elevated 5-HT levels in animals treated with PCPA.

The effect of PCPA on catecholamines (Tables 9 and 10, Figure 9) appeared very erratic, with decreased levels in some brain

TABLE 7: EFFECT OF RESERPINE ON REGIONAL AND WHOLE BRAIN 5-HT CONCENTRATIONS IN THE CAIMAN

Brain Region	Control (N=5)	Number of days following injection									
		1 (N=2)		2 (N=2)		3 (N=2)		7 (N=2)		14 (N=2)	
		C	P	C	P	C	P	C	P	C	P
Olfactory Bulbs and Stalks	0.28 ± 0.09	0.13	-54%	0.36	+28%	0.09	-69%	0.58	+108%	0.14	-51%
Cerebral Hemispheres	1.03 ± 0.10	0.58	-43%	0.99	-4%	0.53	-49%*	1.53	+48%*	0.97	-6%
Optic Tectum	1.73 ± 0.32	1.06	-38%	1.19	-31%	0.88	-49%	2.77	+60%	1.47	-15%
Diencephalon	1.40 ± 0.14	1.37	-3%	1.45	+4%	0.41	-71%*	1.44	+3%	1.48	+5%
Midbrain Tegmentum	2.60 ± 0.24	1.46	-44%	1.27	-51%*	0.62	-76%*	1.17	-55%*	1.15	-56%*
Cerebellum	0.70 ± 0.06	1.27	+82%*	0.57	-18%	0.76	+8%	2.40	+243%***	1.22	+74%*
Pons	2.71 ± 0.22	1.34	-51%*	1.82	-33%	1.50	-45%*	2.19	-19%	1.57	-42%
Medulla	3.04 ± 0.19	2.30	-24%	1.18	-61%**	1.71	-44%*	2.87	-6%	2.78	-9%
Whole Brain	1.59 ± 0.10	1.14	-29%	1.12	-23%	0.78	-51%**	1.78	+12%	1.12	-30%

C = Concentration in μg/g wet weight of tissue
P = Percentage change from control values
N = Number of animals

* p 0.05
** p 0.01
*** p 0.005

EFFECT OF RESERPINE ON REGIONAL 5-HT CONCENTRATIONS IN THE CAIMAN BRAIN

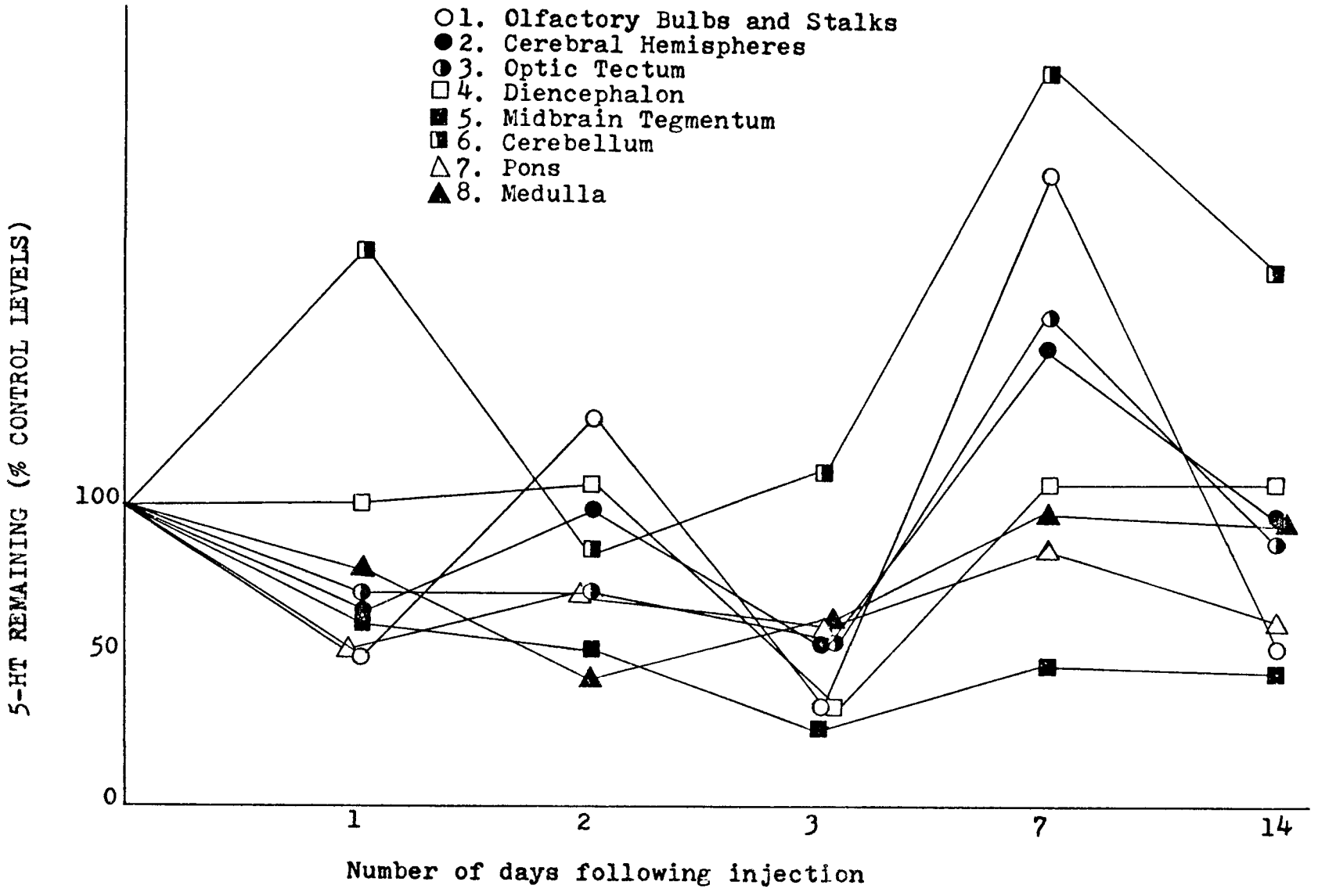


FIGURE 8

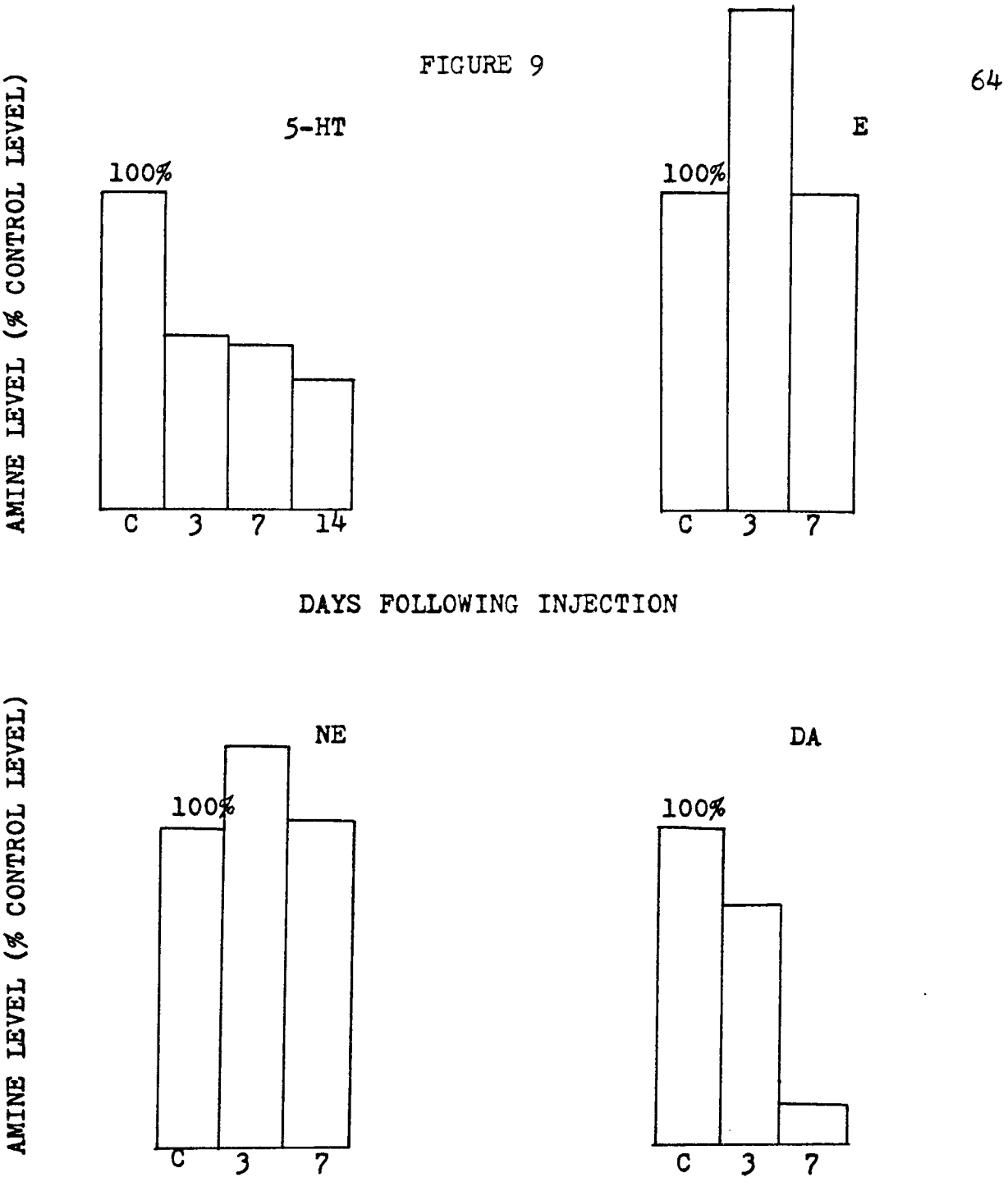
TABLE 8: EFFECT OF PCPA ON REGIONAL AND WHOLE BRAIN 5-HT CONCENTRATIONS IN THE CAIMAN

Brain Region	Number of days following injection							
	Control (N=5)	1 (N=2)		7 (N=2)		14 (N=2)	
		C	P	C	P	C	P
Olfactory Bulbs and Stalks	0.28 ± 0.09	0.49	+77%	0.31	+11%	0.25	-12%
Cerebral Hemispheres	1.03 ± 0.10	0.58	-43%	0.89	-13%	0.77	-25%
Optic Tectum	1.73 ± 0.32	0.66	-62%	0.56	-68%	0.39	-77%
Diencephalon	1.40 ± 0.14	0.78	-48%	0.90	-36%	0.49	-65%*
Midbrain Tegmentum	2.60 ± 0.24	1.15	-56%*	0.71	-73%*	0.62	-76%*
Cerebellum	0.70 ± -.06	0.62	-12%	0.77	+11%	0.27	-61%*
Pons	2.71 ± 0.22	1.78	-34%	0.78	-71%**	0.90	-67%*
Medulla	3.04 ± 0.19	1.68	-45%*	0.86	-72%***	0.75	-75%***
Whole Brain	1.59 ± 0.10	0.86	-46%*	0.81	-49%*	0.65	-59%**

C = Concentration in μg/g wet weight of tissue
P = Percentage change from control values
N = Number of animals

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

FIGURE 9



EFFECT OF PCPA ON WHOLE BRAIN MONOAMINE CONCENTRATIONS IN THE CAIMAN

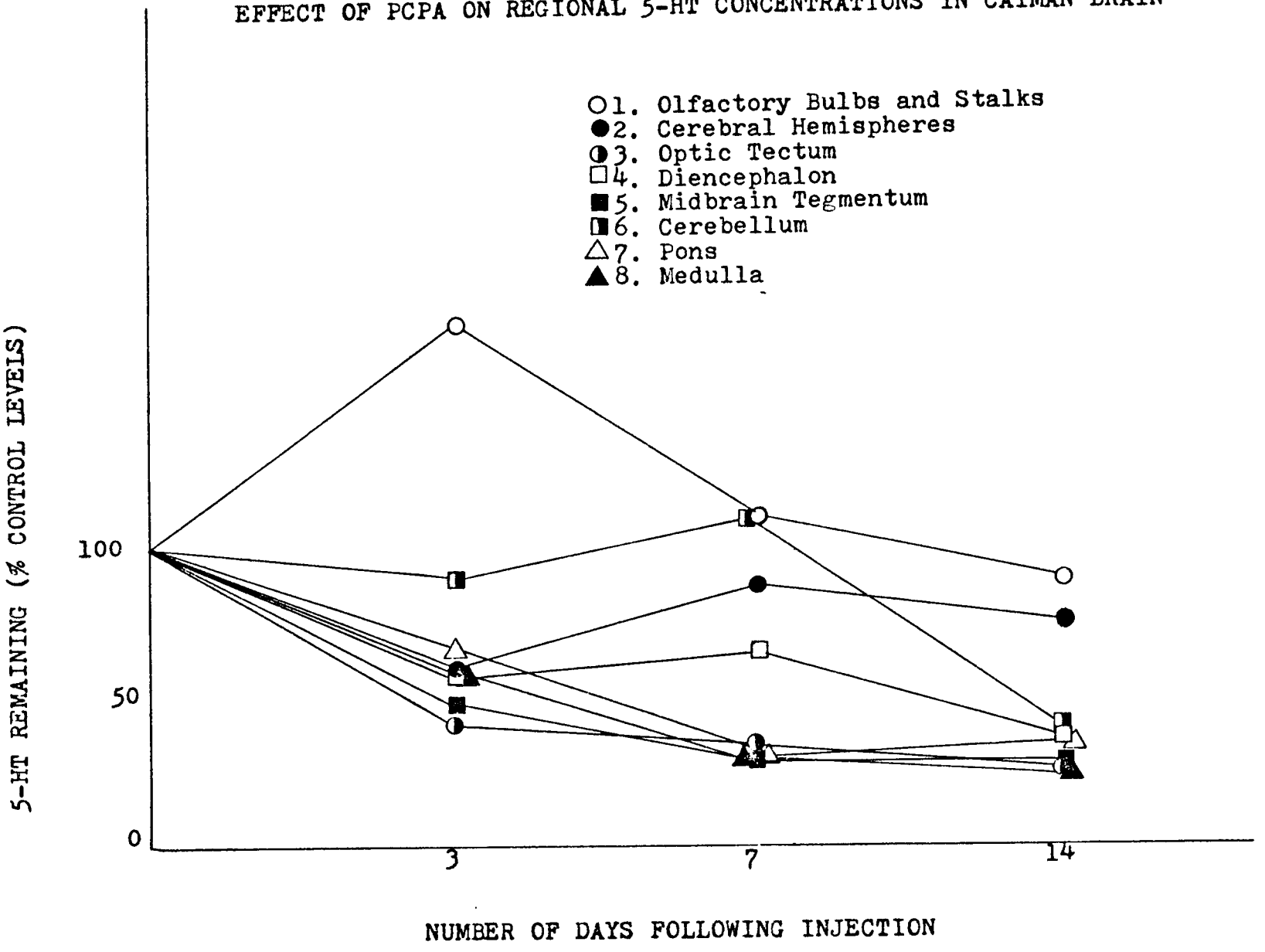

EFFECT OF PCPA ON REGIONAL 5-HT CONCENTRATIONS IN CAIMAN BRAIN
1. Olfactory Bulbs and Stalks
2. Cerebral Hemispheres
3. Optic Tectum
4. Diencephalon
5. Midbrain Tegmentum
6. Cerebellum
7. Pons
8. Medulla
5-HT REMAINING (% CONTROL LEVELS)
100
50
0
3
7
14
NUMBER OF DAYS FOLLOWING INJECTION

FIGURE 10

TABLE 9: EFFECT OF PCPA ON REGIONAL AND WHOLE BRAIN CATECHOLAMINE CONCENTRATIONS IN THE CAIMAN 3 DAYS POST-INJECTION

Brain Region	E Control (N=5)	E (N=1) C	E (N=1) P	NE Control (N=5)	NE (N=1) C	NE (N=1) P	DA Control (N=5)	DA (N=1) C	DA (N=1) P
Olfactory Bulbs and Stalks	0.19 ± 0.05	0.32	+69%	0.12 ± 0.02	0.06	-52%	0.56 ± 0.16	0.00	-100%*
Cerebral Hemispheres	0.07 ± 0.01	0.19	+164%***	0.29 ± 0.02	0.36	+24%	0.98 ± 0.06	0.50	-49%*
Optic Tectum	0.10 ± 0.02	0.14	+40%	0.20 ± 0.01	0.24	+23%	0.77 ± 0.05	0.66	-14%
Diencephalon	0.28 ± 0.03	0.33	+18%	0.42 ± 0.02	0.42	+ 1%	3.16 ± 0.23	1.08	-66%**
Midbrain Tegmentum	0.21 ± 0.33	0.59	+177%***	0.30 ± 0.03	0.79	+163%***	0.41 ± 0.07	0.94	+131%*
Cerebellum	0.13 ± 0.04	0.19	+41%*	0.34 ± 0.03	0.38	+12%	0.45 ± 0.07	0.00	-100%*
Pons	0.18 ± 0.03	0.26	+45%	0.32 ± 0.04	0.46	+42%	1.50 ± 0.22	2.68	+79%*
Medulla	0.22 ± 0.03	0.18	-18%	0.25 ± 0.01	0.33	+34%***	0.11 ± 0.03	0.11	+4%
Whole Brain	0.15 ± 0.02	0.23	+59%*	0.29 ± 0.02	0.37	+26%	0.96 ± 0.06	0.72	-26%

C = Concentration in μg/g wet weight of tissue
P = Percentage change from control values
N = Number of animals

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

TABLE 10: EFFECT OF PCPA ON REGIONAL AND WHOLE BRAIN CATECHOLAMINE CONCENTRATIONS IN THE CAIMAN 7 DAYS POST-INJECTION

Brain Region	E Control (N=5)	E (N=1) C	E (N=1) P	NE Control (N=5)	NE (N=1) C	NE (N=1) P	DA Control (N=5)	DA (N=1) C	DA (N=1) P
Olfactory Bulbs and Stalks	0.19 ± 0.05	0.00	-100%	0.12 ± 0.02	0.00	-100%*	0.56 ± 0.16	0.26	-54%
Cerebral Hemispheres	0.07 ± 0.01	0.09	+23%	0.29 ± 0.02	0.28	- 4%	0.98 ± 0.06	0.00	-100%***
Optic Tectum	0.10 ± 0.02	0.07	-27%	0.20 ± 0.01	0.24	+20%	0.77 ± 0.05	0.82	+7%
Diencephalon	0.28 ± 0.03	0.35	+23%	0.42 ± 0.02	0.45	+9%	3.16 ± 0.23	0.00	-100%***
Midbrain Tegmentum	0.21 ± 0.33	0.52	+145%**	0.30 ± 0.03	0.58	+93%***	0.41 ± 0.07	0.50	+22%
Cerebellum	0.13 ± 0.04	0.00	-100%*	0.34 ± 0.03	0.16	-54%	0.45 ± 0.22	0.00	-100%*
Pons	0.18 ± 0.03	0.15	-18%	0.32 ± 0.04	0.35	+9%	1.50 ± 0.22	0.00	-100%*
Medulla	0.22 ± 0.03	0.09	-2%	0.25 ± 0.01	0.30	+3%	0.11 ± 0.03	0.67	+517%***
Whole Brain	0.15 ± 0.02	0.14	-2%	0.29 ± 0.02	0.30	+3%	0.96 ± 0.06	0.19	-81%***

C = Concentration in μg/g wet weight of tissue
P = Percentage change from control values
N = Number of animals

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

regions and enhanced levels in others. The overall trend was for E and NE levels (Figures 11 and 12) to be initially enhanced and then later reduced. Levels of both E and NE in the midbrain tegmentum were increased substantially, being at least 90% above control values on both the third and seventh days following injection.

In the case of DA (Figure 13), however, significant depletion occurred, concentrations for whole brain being decreased by 25% on the third day and more than 80% on the seventh day. Thus, whole brain DA concentration was more complete in animals given PCPA than was depletion of any other amine, since 5-HT was decreased by just under 60% . Regions which exhibited significantly elevated DA levels despite the overall depletion were the medulla and midbrain tegmentum.

When 6-OHDA was given to caimans, (Table 10, Figures 14 and 15) there was no significant alteration in whole brain levels of 5-HT, E, or NE although E levels did appear slightly elevated. Whole brain DA concentration decreased significantly to 36.7% of the control value, and its concentration was decreased in all brain regions.

Table 12 summarizes the results of the activity studies on caimans which had received the various drugs. The effect of reserpine on activity levels was not statistically significant, but the trend was for activity to be slightly below control values for all levels.

EFFECT OF PUPA ON REGIONAL EPINEPHERINE CONCENTRATIONS IN CAIMAN BRAIN

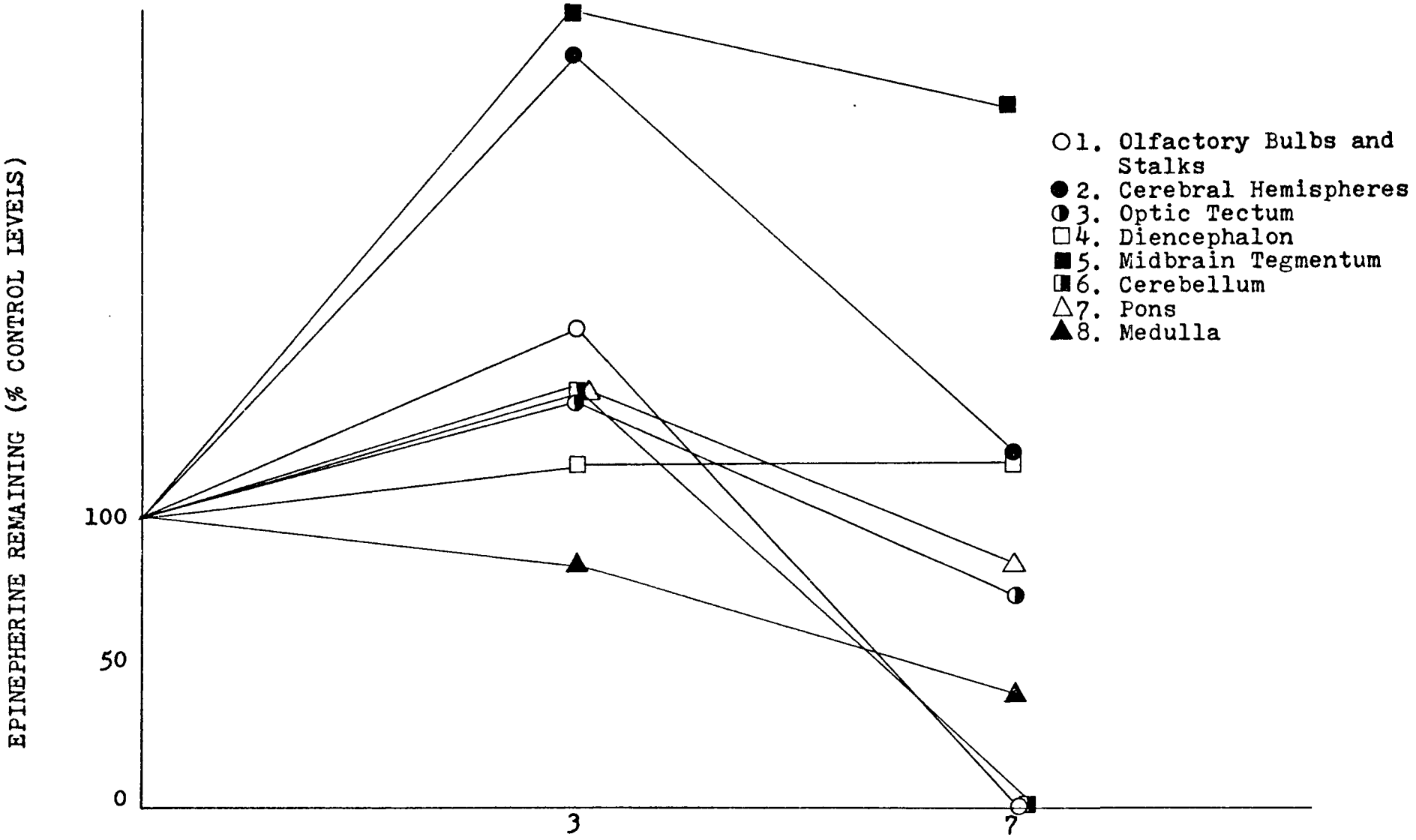


FIGURE 11

EFFECT OF PCPA ON REGIONAL NOREPINEPHERINE CONCENTRATIONS IN CAIMAN BRAIN

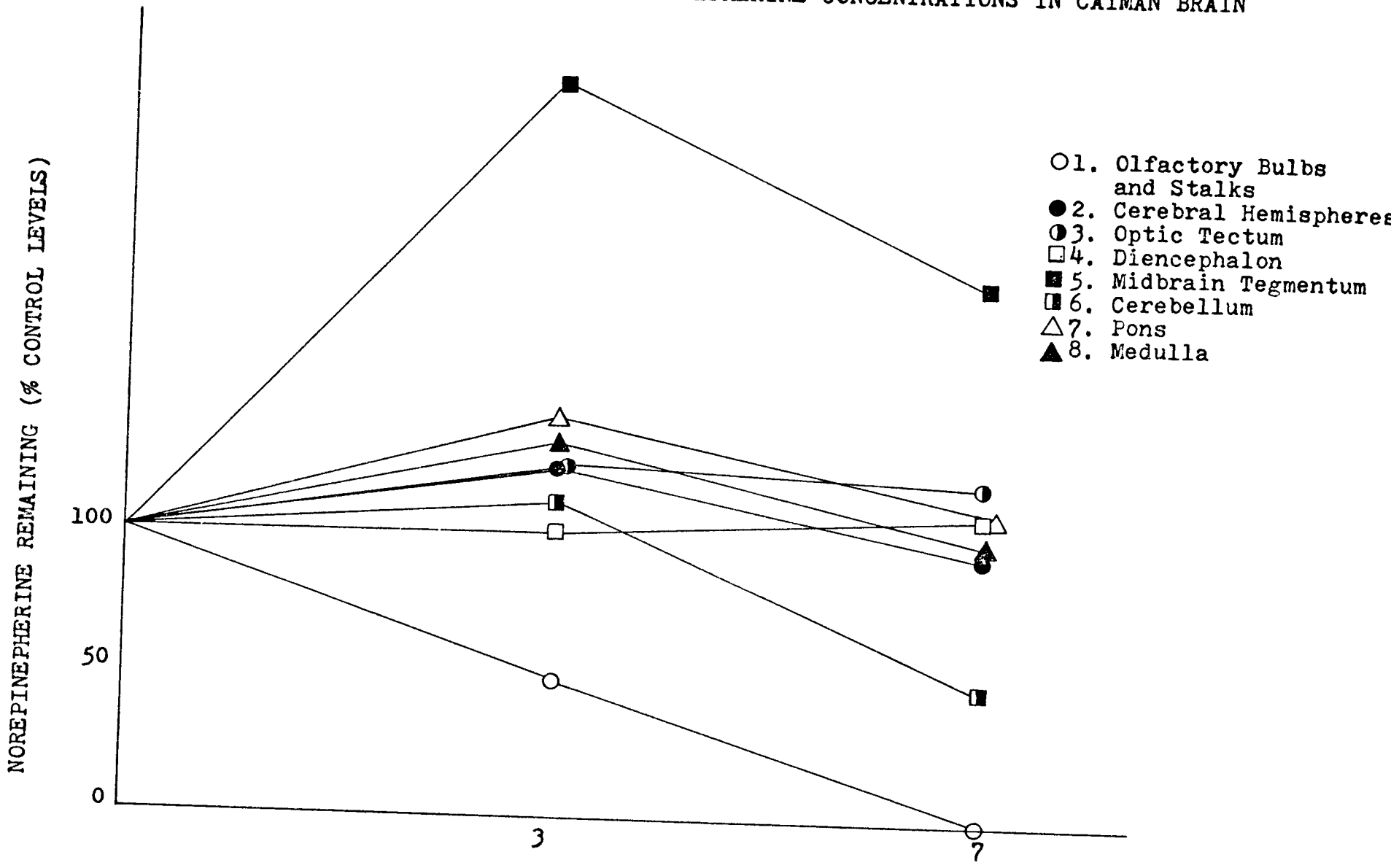


FIGURE 12

EFFECT OF PCPA ON REGIONAL DOPAMINE CONCENTRATIONS IN CAIMAN BRAIN

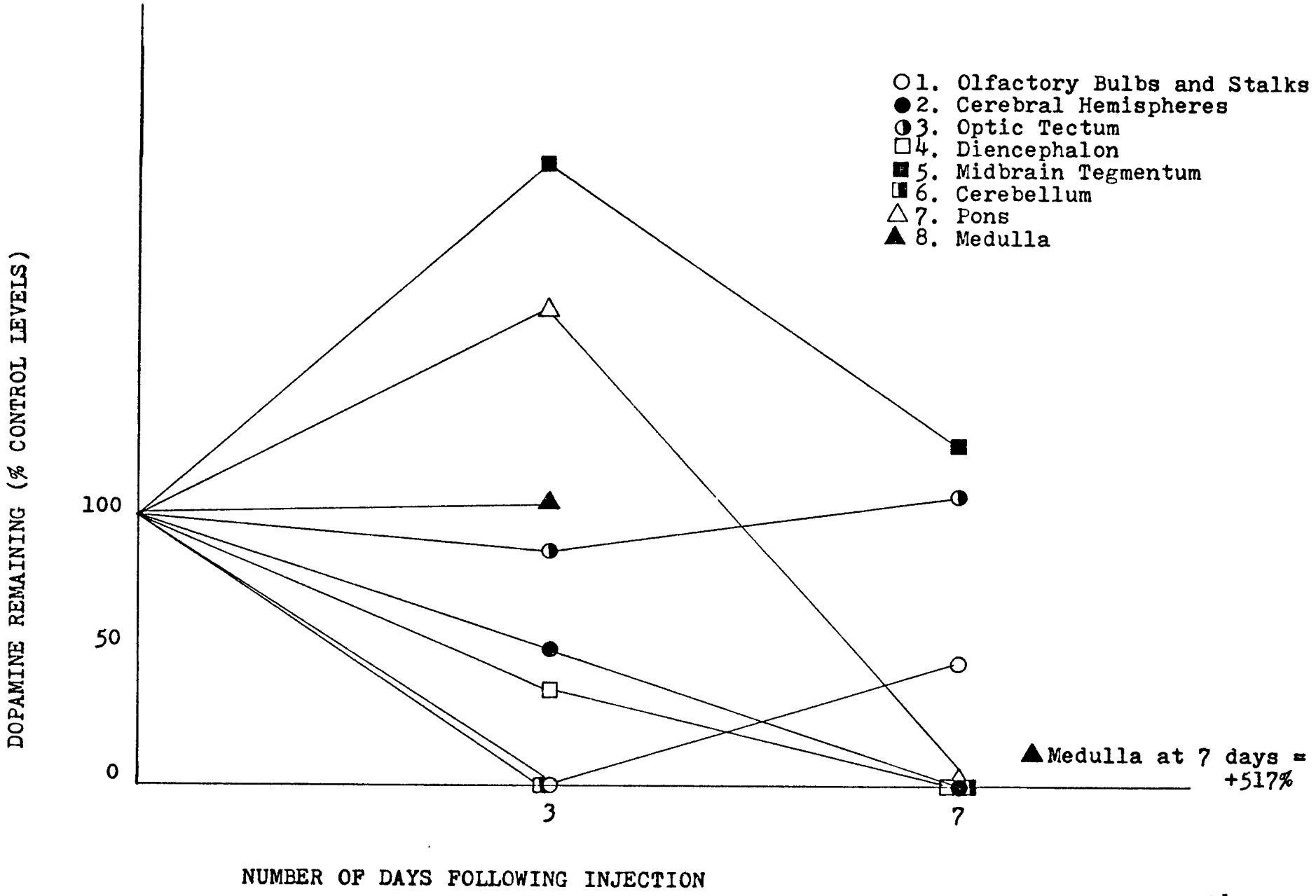


FIGURE 13

TABLE 11: EFFECT OF 6-OHDA ON REGIONAL AND WHOLE BRAIN MONOAMINE CONCENTRATIONS IN THE CAIMAN 8 DAYS POST-INJECTION

Brain Region	5-HT (N=2)		E (N=2)		NE (N=2)		DA (N=2)	
	C	P	C	P	C	P	C	P
Olfactory Bulbs and Stalks	0.38	+37%	0.13	-33%	0.05	-38%	0.00	-100%***
Cerebral Hemispheres	0.98	-4%	0.07	+8%	0.27	-7%	0.36	-63%**
Optic Tectum	1.42	-17%	0.09	-3%	0.18	-9%	0.19	-76%**
Diencephalon	1.43	+2%	0.32	+12%	0.40	-7%	0.27	-91%***
Midbrain Tegmentum	2.51	-4%	0.29	+36%	0.48	+58%*	0.66	-62%
Cerebellum	1.20	+72%*	0.19	+46%	0.26	-24%	0.40	-12%
Pons	3.45	+27%	0.34	+89%	0.46	+45%	0.78	-48%
Medulla	2.92	+4%	0.22	+2%	0.35	+39%***	0.67	-38%
Whole Brain	1.65	+3%	0.19	+31%	0.30	+4%	0.35	-63%**

C = Concentration in μg/g wet weight of tissue
P = Percentage change over control value
N = Number of animals

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

EFFECT OF 6-OHD ON WHOLE BRAIN MONOAMINES IN CAIMAN BRAIN

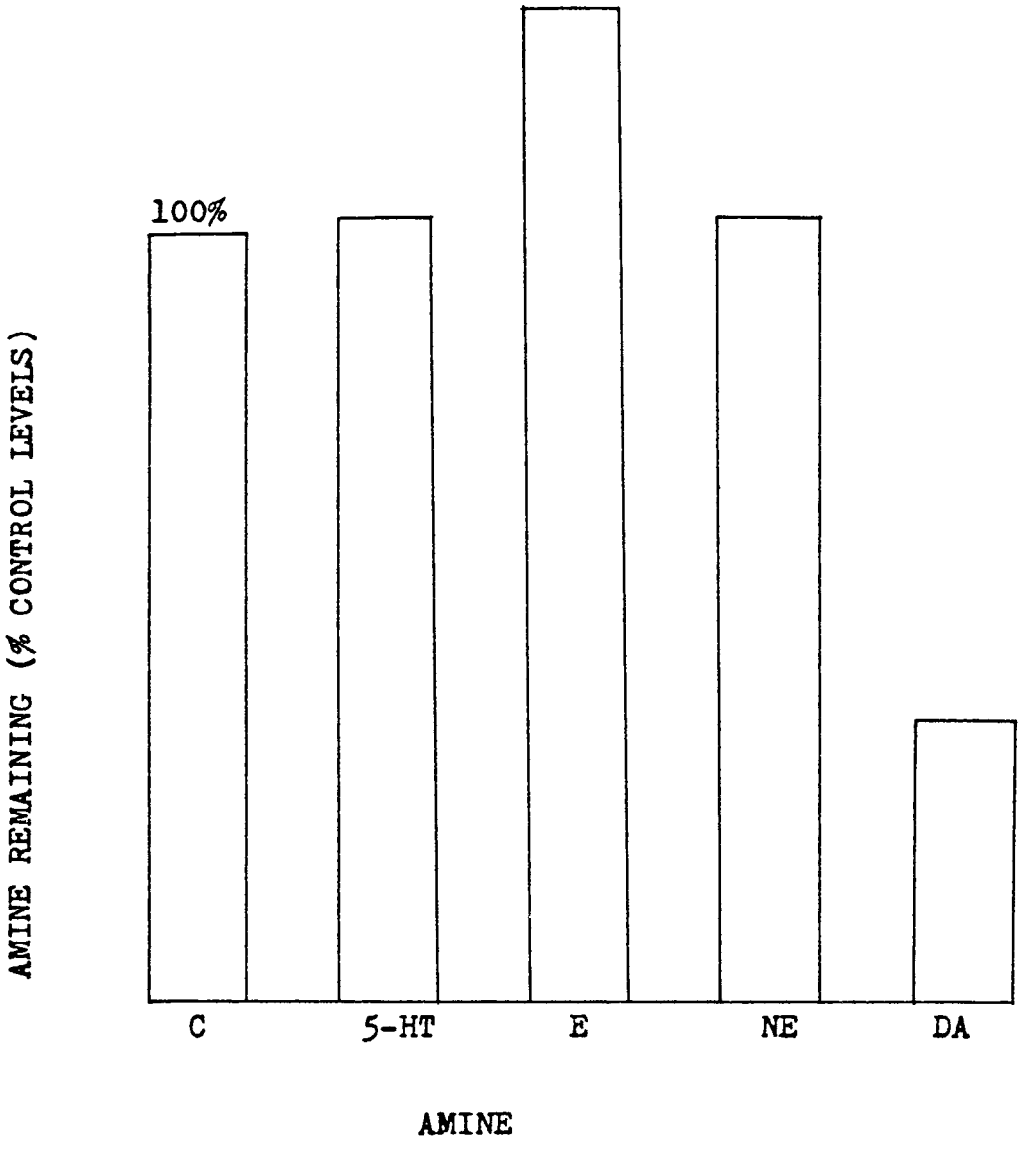


FIGURE 14

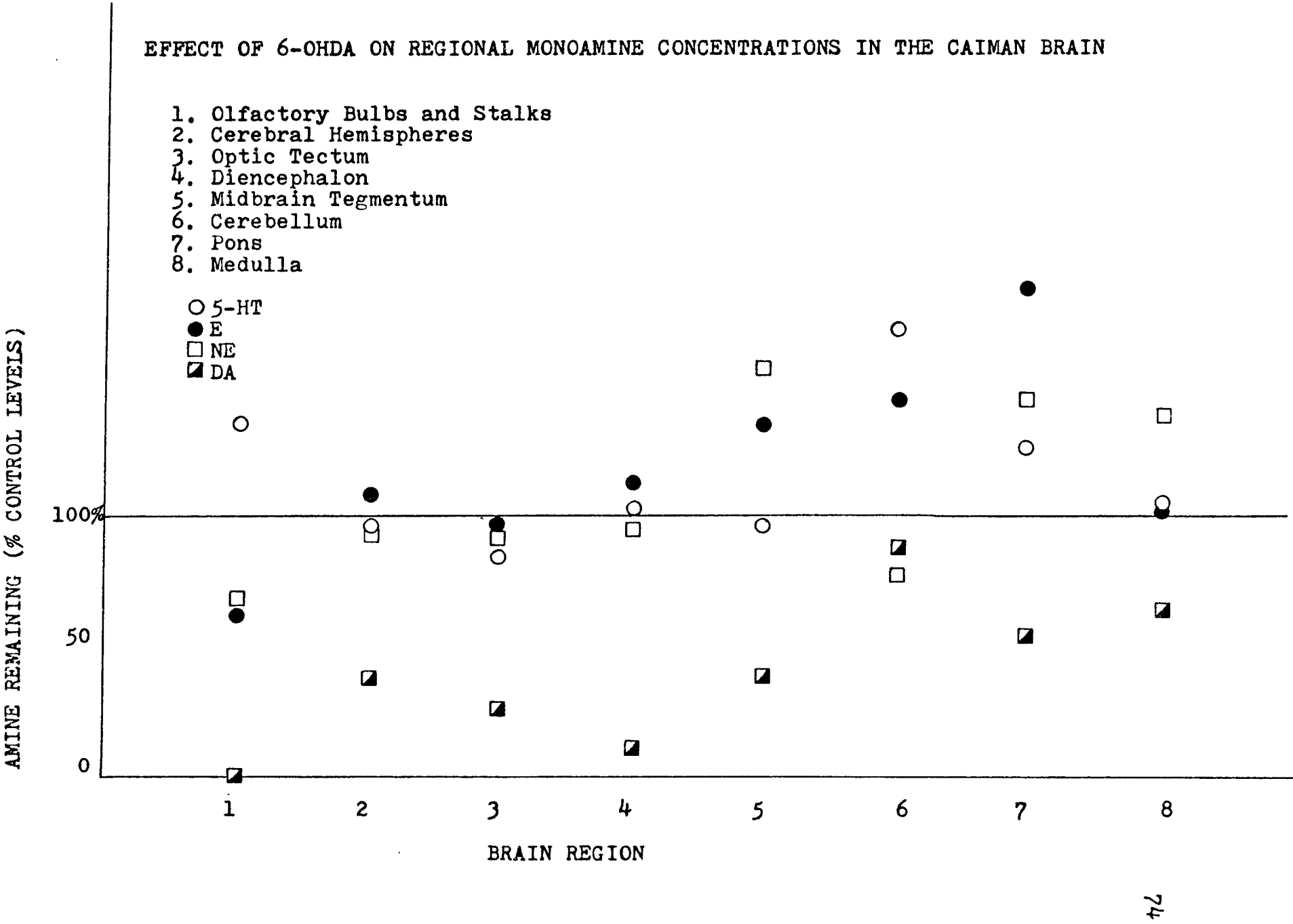

EFFECT OF 6-OHDA ON REGIONAL MONOAMINE CONCENTRATIONS IN THE CAIMAN BRAIN
1. Olfactory Bulbs and Stalks
2. Cerebral Hemispheres
3. Optic Tectum
4. Diencephalon
5. Midbrain Tegmentum
6. Cerebellum
7. Pons
8. Medulla
5-HT
E
NE
DA
AMINE REMAINING (% CONTROL LEVELS)
100%
50
0
1
2
3
4
5
6
7
8
BRAIN REGION

FIGURE 15

TABLE 12: EFFECT OF DRUGS ON ACTIVITY LEVELS IN THE CAIMAN

Drug	Minimal Activity	Low Level Activity	Medium Level Activity	High level Activity
CONTROL (36) N=4	14.97 ± 2.04	19.13 ± 3.80	5.91 ± 1.12	3.64 ± 0.50
RESERPINE 3 DAYS (14) N=1	9.29 ± 1.53*	11.71 ± 2.23	4.57 ± 0.85	2.71 ± 0.52
PCPA 3 DAYS (17) N=1	23.55 ± 2.72*	40.65 ± 6.76**	20.24 ± 3.73***	15.82 ± 3.82***
PCPA 7 DAYS (21) N=1	6.48 ± 1.52***	5.90 ± 2.48***	1.86 ± 0.81***	0.57 ± 0.15***
6-OHDA (31) 8 DAYS N=2	10.55 ± 1.38**	5.29 ± 0.87***	1.13 ± 0.28***	0.77 ± 0.34***

Activity measured as average number of activity bursts per hour ± S.E.M.
Number in parentheses indicates number of one-hour periods
N = Number of animals
* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

When animals were given PCPA, the number of activity bursts at all levels doubled or tripled on the third day, but dropped to less than half of control values on the seventh day.

The effect of 6-OHDA on activity was very similar to that of PCPA on the seventh day, activity at all levels dropping to less than half that of control animals.

DISCUSSION

The whole brain 5-HT concentration found in the caiman (1.59 µg/g) fell within the range reported in the literature for reptiles, being somewhat low compared to most species of turtles and lizards, but rather high compared with concentrations in other crocodilians. Quay and Wilhoft (1964) reported a whole brain 5-HT concentration of 0.25 µg/g in Alligator mississippiensis while Rujirekagulwat and Huggins (1974) found whole brain of Crocodylus siamensis to contain 0.731 µg/g in 3-4 year old animals and 1.481 µg/g in one month old animals. There would appear, therefore, to be considerable age-dependent differences in 5-HT concentrations in crocodilians, levels being initially high and decreasing as the animal matures. The caimans used in our study were comparable in age to the one month old crocodiles used by Rujirekagulwat and Huggins, and the whole brain 5-HT concentrations are remarkably similar for the two species. Quay and Wilhoft do not specify the age of the alligator which they analyzed, but the size indicated usually means a 1-2 year old animal.

These data suggest that the 5-HT concentration of crocodilian brains is midway between that of birds and of most reptiles, being roughly twice that found in birds and half that found in turtles and lizards. These differences could be due to differences in the size of the cerebral hemispheres relative to the

brainstem, since it is generally in the brainstem that 5-HT concentrations are the highest. Therefore as the size of the cerebral hemispheres increases, whole brain 5-HT decreases.

In most mammalian species studied, the midbrain and hypothalamus have been found to contain the highest 5-HT concentrations, while the cerebellum contains very low concentrations. In caimans, the lowest 5-HT concentrations were found in the olfactory bulbs and cerebellum, while all areas of the brainstem were found to contain high concentrations, the highest being in the medulla, followed by the pons, midbrain tegmentum and optic tectum in that order. This pattern would seem to indicate some type of ascending serotoninergic pathway with its cell bodies located in the region of the medulla and possibly the pons and/or the tegmentum, its fibers spreading to the optic tectum, diencephalon and cerebral hemispheres.

In their analysis of the alligator brain, Quay and Wilhoft found the lowest concentrations of 5-HT in the olfactory bulbs and cerebellum and the highest in the tegmentum and diencephalon. The lowest 5-HT concentrations found by Rujirekagulwat and Huggins in the Siamese crocodile were also in the olfactory bulbs and cerebellum, while the highest levels were found in the cerebrum followed by the diencephalon and tegmentum.

The olfactory bulbs and cerebellum seem to be universally low in 5-HT content, and this holds true for all vertebrates. However, the areas which are high in 5-HT seem to vary

appreciably from one species to another. The brain region which appears to be consistently high in all these studies is the midbrain tegmentum, and it is possible that this area is the site of a major 5-HT containing tract in the caiman as well as in other species.

The two brain regions in which 5-HT constituted more than 70% of the total monoamines present were, in fact, the medulla (84%) and the midbrain tegmentum (74%). Unpublished histochemical fluorescence studies on caiman brain by Bengtson and Huggins (1972) have demonstrated large amounts of yellow fluorescence, presumably 5-HT, in the region of the medulla and pons, probably in the raphe nuclei where cell bodies of 5-HT neurons have been demonstrated (Falck et al., 1962).

5-HT constituted over half of the total monoamine content of caiman whole brain. When compared to the catecholamines E and NE, the ratio of 5-HT to catecholamine was about 3.6:1, and this is in keeping with the ratios reported by Bogdanski et al. (1963) who found a ratio of 1:1 in mammals and a ratio of at least 2:1 in birds, reptiles and amphibia, animals lower on the phylogenetic scale having a higher ratio of 5-HT to E and NE.

It has been suggested that the high levels and interspecific variability of 5-HT levels in poikilothermic animals may be related to special features in the thermoregulation and thermosensitivity in these animals. Quay et al. (1970) found

temperature-related changes in lizard brain 5-HT levels, and they proposed that these changes represented a central temperature compensating mechanism, facilitating response by 5-HT receptor neurons on cool days and inhibiting such neurons on warm days. This system could well be the forerunner of the 5-HT system postulated to be present in the hypothalamus of homeotherms and to function in control of body temperature.

Significant quantities of E were found in the brain of the caiman, where it constituted about 5% of total monoamines and about 10% of catecholamines present.

The whole brain E concentration found (0.15 $\mu g/g$) is very close to that found by Anton and Sayre (1962) who reported a whole brain E concentration of 0.10 $\mu g/g$ in an unspecified species of alligator. This is the only report found in the literature in which brain catecholamines were analyzed in a crocodilian. Brodie and Bogdanski (1964) reported an E concentration of 0.37 $\mu g/g$ in whole brain of the lizard Sceloporus. In amphibians, E has been found to exceed the amount of NE present in whole brain, and concentrations as high as 2.4 $\mu g/g$ have been found in the toad Bufo marinus. Bird and mammalian brains have been found to contain only negligible amounts of E, being slightly higher in birds than in mammals.

The caiman would seem, therefore, to occupy the same position on the phylogenetic scale with respect to its brain E content

as it does with respect to its brain 5-HT content; that is, intermediate between most other reptiles and birds.

In the caiman, the brain region containing the highest E concentration was the diencephalon, followed by the medulla, midbrain tegmentum and olfactory bulbs. The lowest E concentration was in the cerebral hemispheres.

Juorio (1973) reported that in the brain of the tortoise Testudo graeca the highest E concentration was in the hypothalamus followed by the optic lobes, while the lowest was in the cerebral hemispheres. Olfactory bulb concentrations were not reported. Our data for the caiman indicate a similar pattern with high E levels in the region of the diencephalon and low levels in the cerebral hemispheres.

E constituted a higher percentage of total monoamines in the olfactory bulbs (17%) than it did in any other region. The relatively high E concentrations found here considered together with the fact that the enzyme phenylethanolamine-N-methyl transferase, responsible for the formation of E from NE, has been found in mammalian olfactory bulbs points to a possible role for E in the process of olfaction.

The fact that the cerebral hemispheres contained the lowest E levels could in part account for the inverse relationship between an animal's position on the phylogenetic scale and the level of brain E.

Whole brain NE concentration in the caiman (0.29 μg/g) was also very close to that found by Anton and Sayre for their alligator (0.28 μg/g). It was considerably less than the concentration found by Bogdanski et al. in the lizard S. cyanogenys (1.6 μg/g) or that reported by Brodie and Bogdanski in the lizard Sceloporos (1.3 μg/g) and the turtle Terrapene (1.8 μg/g). Whole brain NE concentrations in birds and mammals generally range between 0.1 μg/g and 0.5 μg/g, and the crocodilians would seem to more nearly fit into this range than into the range reported for the other orders of reptiles. In this respect the amine concentration in the caiman brain more closely resembles that of mammals than that of other reptiles.

The highest regional NE concentration in the caiman was found in the diencephalon followed by the cerebellum and pons. The lowest NE concentrations were found in the olfactory bulbs followed by the optic tectum. Juorio (1973) reported that in Testudo graeca the highest regional NE concentrations were found in the hypothalamus followed by the medulla and optic lobes. No NE levels were reported for the cerebellum.

In birds and mammals as well as amphibians (Juorio,1973) the hypothalamic region contains by far the greatest concentrations of NE. Our findings in the caiman indicate that this species is no exception to the general pattern of NE distribution.

Nevertheless, NE levels in the diencephalon of the caiman

were not as markedly high in comparison to other regions as they are in most other species, where NE is the dominant catecholamine for this region. In the caiman, NE constituted only 11% of the total catecholamines present in the diencephalon with E accounting for 7% and DA 82%. In the caiman, NE is the only monoamine which is not present in concentrations higher than in the mammalian brain and the possibility exists that some pathways in which NE normally is the transmitter may use another monoamine, either DA or E, instead.

DA concentration in caiman whole brain averaged 0.96 μg/g. Anton and Sayre (1964) reported a whole brain DA concentration in an unspecified species of turtle to be 0.57 μg/g and in the pigeon to be 0.29 μg/g. DA concentrations in mammalian species generally range from about 0.2 μg/g to 0.6 μg/g for whole brain. Thus, the DA concentrations found in the caiman seem to be rather high compared to birds, mammals and other reptiles.

DA concentration in the caiman was highest in the diencephalon (3.16 μg/g) where it was lamost three times as high as in any other area and constituted 60% of total monoamines. The lowest DA concentrations were found in the medulla and the midbrain tegmentum, where DA constituted 3% and 11% of total monoamines respectively.

Juorio (1973) reported the highest DA concentrations in the brain of the tortoise to be in the nucleus basalis of the

cerebral hemispheres (2.98 μg/g) as it is in birds and mammals. In Rana temporaria, however, the highest DA concentration was found in the hypothalamus (1.24 μg/g) where it equalled the concentration of NE. This pattern also held true in the goldfish where the highest DA concentrations were also found in the hypothalamus, and here DA exceeded NE, being 1.80 μg/g and 1.28 μg/g respectively. The same situation apparently exists in the caiman brain, and in this respect the caiman appears closer to the more primitive vertebrates than to birds and mammals. Juorio (1973) suggested that in the fish hypothalamus, and presumably in the amphibian hypothalamus as well, DA may replace either E or NE as a neurotransmitter.

When reserpine was given to the caimans, the overall effect was a progressive general depletion of monoamines. Whole brain levels of all four amines dropped progressively for the first seven days following administration of reserpine but had begun to rise somewhat on the fourteenth day, indicating that recovery had begun by this time. 5-HT and DA reached their lowest levels on the third day following injection while E and NE were lowest on the seventh day. Depletion of E and NE was virtually complete, being more than 99% on the seventh day. Approximately 85% of DA was depleted on the third day, indicating that either DA is not as completely decreased as the other catecholamines or that the time of maximum depletion occurred sometime between the third and seventh day at a

time when no measurement was made. 5-HT did not appear to be depleted as completely or as systematically as the catecholamines, never falling much below 50% of control values. In fact, levels on the first, third and fourteenth days were at least 70% of control values. These data seem to indicate that reserpine may be at least partially selective for depletion of catecholamines in the caiman brain. This pattern is exactly the opposite of that observed by Brodie et al. (1964) in the frog, where reserpine appeared to deplete 5-HT stores selectively.

In general, the brain regions which were depleted most rapidly and thoroughly were those areas which contained the highest levels of each amine initially. The olfactory bulbs and diencephalon were the regions in which the most significant E depletion occurred, NE depletion was most significant in the diencephalon and medulla, while DA depletion was most significant in the diencephalon and cerebral hemispheres. The only brain regions in which depletion of 5-HT was significant and systematic were the midbrain tegmentum and, to a lesser extent, the medulla. In both of these areas, 5-HT constitutes a very high percentage of total amines.

Another phenomenon observed, particularly in the case of DA and 5-HT, was that brain areas which normally contain very low amounts of an amine actually showed increased levels of that amine following reserpine administration. Areas which

normally contained intermediate concentrations of an amine might show a slight increase or a slight decrease and this apparently fluctuated from day to day. DA levels in the medulla after reserpine treatment represent the most dramatic illustration of this phenomenon. Normally, DA levels here are extremely low, there being less DA here than anywhere else in the brain. After reserpine treatment, however, DA in this region increases by about 200-300% and remains at this elevated level from the first through the fourteenth days after injection. The same phenomenon occurs, but to a lesser extent, in the case of 5-HT in the cerebellum, olfactory bulbs and diencephalon.

The presumed mechanism of action of reserpine is through impairment of the active transport of amines into storage granules (Carlsson et al. 1962) thereby causing them to be released in excessive amounts and subsequently inactivated. If it is indeed true that reserpine exerts a differential effect on 5-HT and catecholamine-containing neurons in the caiman brain, then it is possible that uptake of amines into 5-HT neurons may be at least partially functional in a reserpine-treated animal. It has been shown that monoaminergic neurons are capable of taking up amines other than the one which they synthesize (Iversen, 1967). The metabolic rate of a poikilothermic animal such as the caiman is very low compared to that of a mammal, and it is conceivable that MAO or

other enzymes responsible for the inactivation of catecholamines function at a slow enough rate to allow diffusion of a small part of the DA released to areas which contain 5-HT neurons. These neurons could then take up and store DA, thereby increasing its level in the region. This suggestion is further supported by the fact that DA levels in the midbrain tegmentum are never very significantly reduced by reserpine, and may even increase above control levels; the midbrain tegmentum is also an area which contains a very high percentage of 5-HT in control animals.

Reserpine evidently does cause some 5-HT release from areas where it is highly concentrated and it is possible that some of the 5-HT thus released could diffuse to areas which do not normally contain much 5-HT and there be taken up into 5-HT neurons, catecholamine neurons or some other type of cell.

Overall physical activity levels were not significantly affected by reserpine, although a general trend toward a reduction in activity could be noted. This finding is in agreement with Juorio (1973) who observed no changes in the gross behavior of tortoises given reserpine, and with Brodie and Bogdanski (1964) who observed no sedation in frogs given reserpine. It is known that if reserpine is administered to mammals in small doses over a long period of time it is possible to deplete prectically all the brain amines without causing behavioral symptoms (Cooper et al. 1974). It is possible that

in the caiman, because of its slow metabolic rate, a single large dose of reserpine might act in the same way as a series of small doses administered to a mammal.

When PCPA was administered to the caimans the 5-HT depletion it produced was much more consistent and of slightly greater magnitude than the depletion produced by reserpine. Whole brain 5-HT progressively decreased during the fourteen days following administration of the drug, depletion being greatest on the fourteenth day and nearly 60% complete. PCPA also produced significant changes in whole brain catecholamine levels. On the third day following administration, whole brain E levels had actually increased by nearly 60%. NE had also increased by about 25% while DA had decreased by about 25%. The latter two changes were not highly significant, but were indicative of general trends. By the seventh day following PCPA administration levels of both E and NE were very close to control values whail that of DA was decreased by more than 80%. At this time the whole brain 5-HT was not quite 50% depleted.

As was the case with reserpine, PCPA induced 5-HT depletion was most rapid and complete in the areas which normally contain high levels of the amine, especially in the midbrain tegmentum and medulla, and to a lesser extent in the pons and optic tectum. Regions which normally have low 5-HT levels such as the cerebellum and olfactory bulbs did not vary greatly over control

levels for the first seven days, but by the fourteenth day did show a slight decrease in 5-HT levels.

On the third day following PCPA administration, E and NE levels were elevated in practically every brain region, but particularly in the midbrain tegmentum where they increased by nearly 200% over control values. In the cerebral hemispheres an increase of E only, of about the same magnitude, was observed. By the seventh day, E and NE showed signs of depletion in all areas except the midbrain tegmentum, where they were still roughly 100% above control levels.

On the other hand, DA was significantly depleted in all brain regions except for the midbrain tegmentum, pons and medulla as soon as the third day following PCPA injection. The DA level in the midbrain tegmentum was roughly 100% above control values for this area, while levels in the pons and medulla did not differ significantly from control values. By the seventh day DA was near control value or significantly depleted in all areas except the medulla where it was about 5 times the normal level. However, the amount of DA found in the caiman medulla is very small compared to other areas of the brain, and the absolute increase in DA content was not very great. At this time both E and NE levels were normal or slightly depleted throughout most of the brain while 5-HT was close to 60% depleted and DA was over 80% depleted.

The mechanism of action of the drug PCPA has been shown to

be through inhibition of the enzyme tryptophan hydroxylase, leading to selective 5-HT depletion in the mammal. The initial step in catecholamine synthesis also involves hydroxylation of an amino acid, tyrosine, to form DOPA, which in turn is acted upon by a relatively non-specific enzyme, amino acid decarboxylase, to form DA. In the mammalian system, amino acid decarboxylase acts upon 5-hydroxytryptophan to form 5-HT as well as upon DOPA to form DA. It is possible that in the caiman there is a single enzyme which operates in a similar fashion to hydroxylate both tryptophan and tyrosine and which is inhibited by PCPA. This would explain the widespread depletion of both DA and 5-HT, and the more gradual depletion of E and NE. If this is the case, it is conceivable that in lower vertebrates there exists a single "aromatic amino acid hydroxylase" from which the more specific mammalian forms evolved.

The existence of such an enzyme does not explain the initial rises in E and NE levels, however. Jouvet (1973) has postulated the existence of two antagonistic ascending systems of neurons in the cat; 5-HT neurons for inducing sleep and catecholaminergic neurons for maintaining wakefulness and for indicing paradoxical sleep. If such a system exists in the caiman, it could be that these two systems are mutually inhibitory. Therefore, inactivation of one of these systems would cause increased activity in the other. The transmitter

most likely involved in the catecholaminergic pathway would be either E or NE or both. Since depletion of 5-HT occurs before depletion of either E or NE in the caiman, this would tend to remove some of the inhibitory influence from the catecholaminergic pathway, causing increased synthesis of transmitter as well as increased activity of these neurons. This could include increased synthesis of transmitter as well as increased release, leading to elevated catecholamine levels in the area of this pathway. The catecholamines thus synthesized would probably be formed from pre-existing DA stores; as these are exhausted, they are not replaced completely due to inhibition of the enzyme responsible for DA formation and there is a gradual fall in E and NE levels.

Activity studies on caimans which had received PCPA further support this suggestion. On the third day following PCPA injection, activity levels were found to be extremely high with the animals in a constant state of arousal. This is the same type of behavior observed in mammals which have received PCPA and in which catecholamine levels remain within the normal range. This arousal, then, could be attributed to a removal of 5-HT inhibition on the catecholaminergic pathway responsible for arousal. On the seventh day, however, the activity level of caimans receiving PCPA was reduced significantly below the control value. At this time E and NE levels had fallen to normal or slightly below for the brain as a whole even though

they were still elevated in the midbrain tegmentum. DA, however, which had not been significantly reduced on the third day, was now depleted to less than 20% of normal levels; in fact in the cerebral hemispheres and the diencephalon no DA was detectable at this time.

DA pathways in the cerebrum of mammals have been correlated with motor activity, and after lesioning of these pathways or selective depletion of DA motor disturbances akin to parkinsonism have been observed to result. One manifestation of these disturbances is generally a lack of motor activity. It is possible that even though arousal levels in the PCPA-treated caimans were near normal depletion of DA from pathways controlling motor activity was directly responsible for their lack of movement.

When caimans were pre-treated with desmethylimipramine, which in mammals selectively blocks uptake into NE neurons, and subsequently were injected with 6-OHDA, this resulted in significant DA depletion in all regions of the brain, being especially marked in the region of the diencephalon, cerebral hemispheres, optic tectum and midbrain tegmentum. Overall DA levels had dropped by more than 60% by the end of one week. The levels of E, NE and 5-HT were not significantly affected although E appeared to be somewhat elevated.

Shaywitz (1975) found that in juvenile rats administration of 6-OHDA and selective DA depletion led to hyperactivity.

The caimans used in the study with 6-OHDA were also juveniles, being about one month old, but the effects of DA depletion on activity levels were the opposite of those reported by Shaywitz.

Activity in 6-OHDA treated caimans was significantly lower than activity in the controls, the levels being very similar to those observed in animals treated with PCPA in which DA was depleted. These results may be compared to those obtained after DA depletion or lesions of the striatum in adult mammals and the observed hypoactivity in the caimans can be taken as an indication of possible impairment of motor functioning.

If this is not the case, a further possibility might be that DA is the neurotransmitter involved in the ascending pathway for arousal in the caiman and that its depletion leads to a removal of inhibitory influences on the 5-HT pathway which induces sleep or a similar state.

SUMMARY

1. Regional and whole brain concentrations of 5-HT, E, NE and DA were measured using the methods of Quay (1963) and Cox and Perhach (1973).

2. Average whole brain 5-HT concentration was 1.59 ± 0.10 ug/g. Regions significantly higher than the mean were the medulla, pons and midbrain tegmentum. Regions significantly lower than the mean were the olfactory bulbs, cerebellum and cerebral hemispheres.

3. Average whole brain E concentration was 0.15 ± 0.02 ug/g. The concentration in the diencephalon was significantly higher than the mean while the concentration in the cerebral hemispheres was significantly lower than the mean.

4. Average whole brain NE concentration was 0.29 ± 0.02 ug/g. The concentration in the diencephalon was significantly higher than the mean while the concentrations in the olfactory bulbs and optic tectum were significantly below the mean.

5. Average whole brain DA concentration was 0.96 ± 0.06 ug/g. The concentration in the diencephalon was significantly higher than the mean; regions with concentrations significantly lower than the mean were the medulla, midbrain tegmentum and cerebellum.

6. In whole brain, 5-HT constituted 53% of total monoamines, and catecholamines 47%.

7. Of total whole brain catecholamines, E constituted 10%, NE 21% and DA 69%.

8. Treatment with reserpine caused depletion of all monoamines; E and NE reached their lowest level on the seventh day after injection (more than 99% depletion). DA reached its lowest level on the third day after injection (85% depletion). 5-HT reached its lowest level on the third day after injection also (51% depletion).

9. Physical activity levels were not significantly affected by reserpine.

10. PCPA caused progressive depletion of 5-HT and DA. On the fourteenth day after injection depletion of 5-HT was 59% complete and on the seventh day after injection DA depletion was 81% complete.

11. PCPA resulted in elevated levels of E and NE on the third day after injection with concentrations returning to near normal levels on the seventh day.

12. PCPA resulted in increased physical activity levels on the third day after injection and decreased activity levels on the seventh day after injection.

13. 6-OHDA had no significant effect on levels of E, NE, or 5-HT. DA was depleted by 63%.

14. 5-OHDA resulted in reduced physical activity levels.

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