Zhou, Feng Chiao Simon

INDUCED HOMOTYPIC SPROUTING OF SEROTONERGIC RAPHE-HIPPOCAMPAL SYSTEM AND ITS REGULATION BY CORTICOSTERONE

*City University of New York* PH.D. 1983

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark _✓_.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print _____
3. Photographs with dark background ✓
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

# INDUCED HOMOTYPIC SPROUTING OF SEROTONERGIC RAPHE-HIPPOCAMPAL SYSTEM AND ITS REGULATION BY CORTICOSTERONE

BY FENG C. ZHOU

A dissertation submitted to the Graduate Faculty in Biomedical Science in partial fulfillment of the requirements for the Doctor of Philosophy, The City University of New York.

1983

This manuscript has been read and accepted for the Graduate Faculty in BioMedical Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

8/5/83
Date

Chairman of Examining Committee

8/23/83
Date

Executive Officer

Edward W. Gresik, Ph.D.

Bruce S. McEven, Ph.D.

Pedro Pasik, M.D.

Amy W.H. Yu, Ph.D.

Supervisory Committee

The City University of New York

## ABSTRACT

INDUCED HOMOTYPIC SPROUTING OF SEROTONERGIC
RAPHE-HIPPOCAMPAL SYSTEM
AND ITS REGULATION BY CORTICOSTERONE

by

Feng C. Zhou

Advisor: Dr. Efrain C. Azmitia

Most studies of neuronal plasticity have concentrated on regeneration or heterotypic sprouting. Homotypic srouting has the advantage of restoring both morphological and functional connections with relative ease. A model of homotypic sprouting using the rat raphe-hippocampal (RH) system has demonstrated structure, biochemical, and functional restoration of 5,7-dihydroxytryptamine (5,7-DHT) lesions in this system.

In the present work the 5-HT neurons which project to the dorsal hippocampus (DHipp) via the cingulem bundle (CB) and fimbria fornix (FF) are found to consist of two homologous groups in the median raphe nucleus (MRN). The termination of which have been studied. These fibers terminate in a coherent pattern, and densely innervate the fasciola cineria. These data provide an anatomical contribution to the

RH model of homotypic sprouting.

Three days after 5,7-DHT lesions in the CB, a decrease was observed in the number of 5-HT neurons innervating DHipp (by HRP tracing) and in the fiber density of their projections in the DHipp (by immunocytochemistry). This decrement is not completely reversed until 21-42 days post-lesion. The restitution is a result of proliferation of undamaged 5-HT fibers from the FF. This ia an example of homotypic collateral sprouting.

This homotypic compensatory growth in the DHipp is suppressed by adrenalectomy (ADX). The number of RH neurons remained low 21 days post-CB-lesion in these ADX animals (64% lower than sham). However, the supression is reversed by subcutaneuous implantation of corticosterone pellets.

5,7-DHT lesion in the CB and FF removed most of the MRN innervation to the DHipp. No regeneration from the MRN occurred as late as 42 days post-lesion. However, collateral sprouting from DRN neurons, which normally innervate the ventral hippocampus, is observed innervating the DHipp.

THIS DISSERTATION IS DEDICATED TO

MY PARENTS

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Efrain Azmitia for his support, guidance, and discussions through the course of this research. In addition, my thanks are extended to Dr. Pedro Pasik and Dr. Amy Yu for their encouragement and for making valuable suggestions concerning this project.

My gratitude is extended to the faculty and graduate students of the Department of Anatomy, as well as Mr. Patrick Gannon and Mr. Scott Finley for their friendship and assistance.

I would also like to thank my wife, Paula, for her help, understanding and tremendous love.

TABLE OF CONTENTS

## List Of Tables And Figures

# INTRODUCTION

New growth after damage in the central nervous system (CNS) is gradually accepted as a common phenomenon, but morphological and functional recovery from the damage is far more unusual. Most attempts at inducing regeneration, or the regrowth of lesioned ends of neurons back to their original targets, fail because of glial scar formation (review see Bjorklund and Stenevi, 1979). Fibers sprouting from intact fibers frequently expand within the denervated area. "Heterotypic sprouting", in which sprouting fibers carry different transmitters, results in a chemical inbalance within the terminal field and produces abnormal behaviors (McCouch et al., 1958). The aberrant sprouts have been shown in certain cases to prevent the restoration of the normal balance when the proper chemical fibers regenerate back toward the denervated target (Wiklund and Mollgard, 1979; Bernstein and Bernstein, 1971). "Homotypic sprouting", on the other hand, refers to sprouting fibers that carry the same transmitter, resulting in morphological and functional restoration of the lost connections (Azmitia et al, 1978).

A number of research studies have successfully demonstrated functional recovery by sprouting (Goldberger and Murray, 1974; Lund et al., 1973; Loesche and Steward, 1977; Lynch et al., 1973; Scheff and Cotman, 1977; Steward et al., 1973). Interestingly, all of these cases involve ipsilateral lesions of bilateral projections. Partial functional recovery results from homotypic sprouting of projection fibers from the contralateral site. These studies of sprouting using bilateral innervation models do not allow unequivocal statements to be

made about the nature of the "sprouting signal" since most of the cases involve destruction of multiple chemical systems. Whether sprouting is triggered by the removal of the same fiber type (homotypic) or a different fiber type (heterotypic) cannot be answered.

Present studies provide direct evidence that homotypic sprouting can be triggered by a specific lesion of serotonergic (5-HT) fibers, and the functional abnormality of this neurotoxic lesion is reversed by the homotypic sprouting (Azmitia et al., 1978) from a homologous neuronal group in the raphe-hippocampal system. Furthermore, this homotypic sprouting appears to be regulated by the corticosterone in the circulating blood.

## Plasticity During Development

During the development of the nervous system, neuronal plasticity is a normal procedure. A single neuron can establish multiple connections and eventually make the proper contact with its target cells. It was generally believed that the neuronal plasticity gradually diminished during maturation of the nervous system. However, more recently studies have shown that it is not lost but "dormant". It reacts to many stimuli and accommodate itself to varying circumstances.

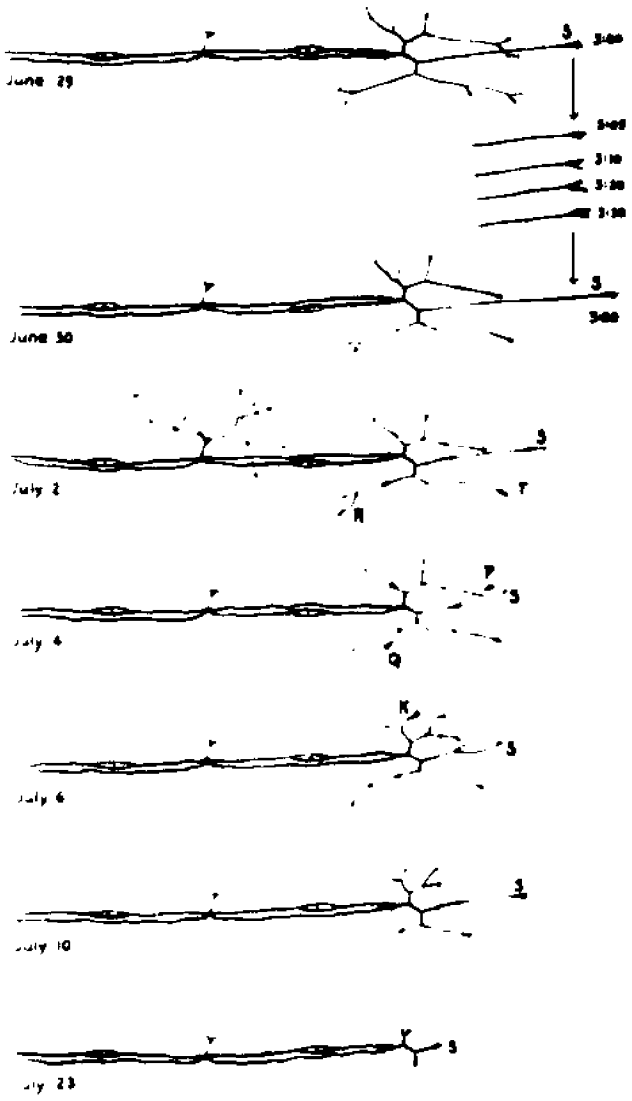
During the developmental stage, nerve cells can sprout axonal branches and absorb old ones in order to change their shape, and make contact with their proper targets. The living sprouts were first seen and described in tissue culture by Harrison (1908, 1910). He observed that the growth cone in the sprout tip was advancing as an amoeba.

Speidel's observations (1933) on living nerves growing into the transparent tails of the tadpoles were as follows (see figure 1):

> "Tadpole no. 602. June 29th, typical ameboid motion is exhibited by one of the terminal myelin-emergent branches (S). June 30th, although quite active, this cone progressed during the 24 hour period only 18 u. July 2nd, sprouts R and T become active. S retracts somewhat. July 4th, P and Q become active. S retracts further. ... July 10th, an active cone again appears at S." (p. 19)

The significance of these observations is that it gives us a vivid continuous picture of nerve growth. Many studies of nerve growth including today's sophisticated ones, are a montage of fragmented states, lacking continous observations of a single studied subject. In development, nerve growth appears not to be a programmed expansion, but a trial and error, or a "withdrawing" and growing process. The growth and retraction reaches a balance in the adult. For example, what he did not describe but had drawn in detail (figure 1) was a collateral sprout emerging from the node of Ranvier during the retracting of sprout S. However, this sprout retracted, when sprout S regained its growth.

Figure 1: Development of a living nerve in the tail of a tadpole. The advancing and retraction of the nerve terminal during the six days of normal growth. The detail is described in page 3.



In the chicken, four million optic fibers are growing toward the lateral geniculate nuclei and tectum on embryonic day 10 and 11. By the time they reach their target on day 18, only 2.4 million fiber remained (Rager, 1978). Degeneration occurred in parallel with growth during day 9 to day 15.

Special attention is drawn to this aspect because in the adult brain sprouting can be induced by damage or degeneration of neighboring nervous elements. The recovery of damaged connection might be similar to the situation in the stages of development.

A previous literature described the growth associated with degeneration in more than 30 species during their development (Ernst 1926; Kallius, 1931; Glucksmann, 1951; Hamburger et al., 1949; Cowan and Wenger, 1967; 1968; Clarke et al., 1976; Piatt, 1946; Prestige, 1967 a,b; Landmesser and Pilar, 1974 a,b; Hughes and LaVelle, 1975; Landmesser, 1976; Chu-Wang and Oppenheim, 1978 a,b; Knyihar et al., 1978; and Rager, 1980; for good review see Ernst, 1926 and Rager, 1981). These reports agreed on two points: (1) Redundancy: More axonal sprouts and more cells are produced than necessary for constructing the adult neuronal system. A consequence of this overproduction is that the sizes of projecting and receiving systems do not match initially. The matching is subsequently brought about by degeneration. (2) Equilibrium: The development as well as the maintenence of the nervous system of vertebrates results more from a balance of forces than from a rigid genetic formulation.

However, at the end of the development, neuronal connection

stabilized and degeneration subsided. Elongation and sprouting is stopped. Ramon y Cajal concluded in 1928: "Once the development was ended the founts of growth and regeneration of the axons and dendrites dried up irrevocably. In adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated." (p. 750)

What has been changed in the brain during the maturation process remained a mystery. Will the matured nervous system be capable to grow again in the adult CNS ?

## Plasticity In The Adult

The adult nervous system does grow, mostly as a result of stimulation. In the early nighteenth centery, Cajal described sprouting buds emerging from damaged axons in the adult brain: "After axotomy, the juveniled axon regained its fluidity. New growth cones reformed. Damaged axons buded and elongated.". "However," he further observed "these processes retracted and axons withered long before re-establishment of original contacts." (1928).

The regeneration of end fibers to reinnervate their lost connections is well documented and reviewed in early developmental stages in the peripheral nervous system (PNS) (Cajal, 1928; Guth, 1956; and Weddell et al., 1941), in spinal cord (Druckman, 1955; Clemente, 1964; Windle and Chamber, 1950; and Puchala and Windle, 1977) and in recent years in monoaminergic systems (Bjorklund and Stenevi, 1979; Bjorklund et al., 1981).

Abortive attempts of regeneration in mammalian CNS were widely encountered. Axotomized neurons regenerate but within two to three weeks the new sprouts are retracted or reabsorbed due to (1) glial scars forming an impenetrable barrier (Windle et al., 1952; Clemente, 1964; and Kao et al., 1977), or (2) post traumatic autoimmunization (Feringa et al., 1973; 1975; and Berry and Riches, 1974).

During the 1950's, major efforts were made to improve regeneration of a traumatic lesion in the spinal cord by reducing the formation of the glial scar (Clemente 1954; Windle and Chambers, 1950; Sugar and Gerard, 1940) using bacterial pyrogens and steroids. These studies met with limited success.

In the meantime, major achievements were made in the discovery that axonal sprouting of "intact neurons" could be induced by the damage of neighboring neural elements in the CNS. The pioneer study was reported by Liu and Chambers (1958), who demonstrated collateral sprouting from intact axons after partial denervation of the descending pathway using the Nauta stain for degenerating axons. A similar phenomenon was observed after partial denervation of sympathetic ganglion by Murray and Thompson (1957). A decade later, Raisman (1969) reported that damage of medial forebrain bundle (MFB) induced collateral sprouting of the septo-hippocampal fibers. Many examples of sprouting axons were demonstrated in the CNS since then: in the spinal cord after lesion of dorsal root (Goldberger and Murray, 1974), in the thalamus after partial denervation of spinal afferents (Wall and Egger, 1971), in the lateral geniculate body after lesioning of its cortical afferents (Goodman and Horel, 1967; Ralston and Chow, 1972; Stenevi et

al., 1972; and Wong-Riley, 1972), in the olfactory tubercle after lesioning of the olfactory bulb (Gilad and Reis, 1979, Reis and Ross, 1973; and Moore et al., 1974), in the cerebellum after lesioning of its peduncle (Pickel et al., 1974), in the superior colliculus after lesioning of retinal afferents (Lund et al., 1971; 1973), in the hippocampus after lesioning of the entorhinal cortex (Cotman et al., 1973; 1977; Lynch et al., 1972; 1976; Steward et al., 1974; 1976; 1978a,b; Storm-Mathisen, 1974; Zimmer et al., 1973; 1975; West et al., 1975; Lee, et al., 1977), after lesioning of the septal afferent (Raisman 1973), and after partial denervation of raphe afferents to the hippocampus (Azmitia et al., 1978).

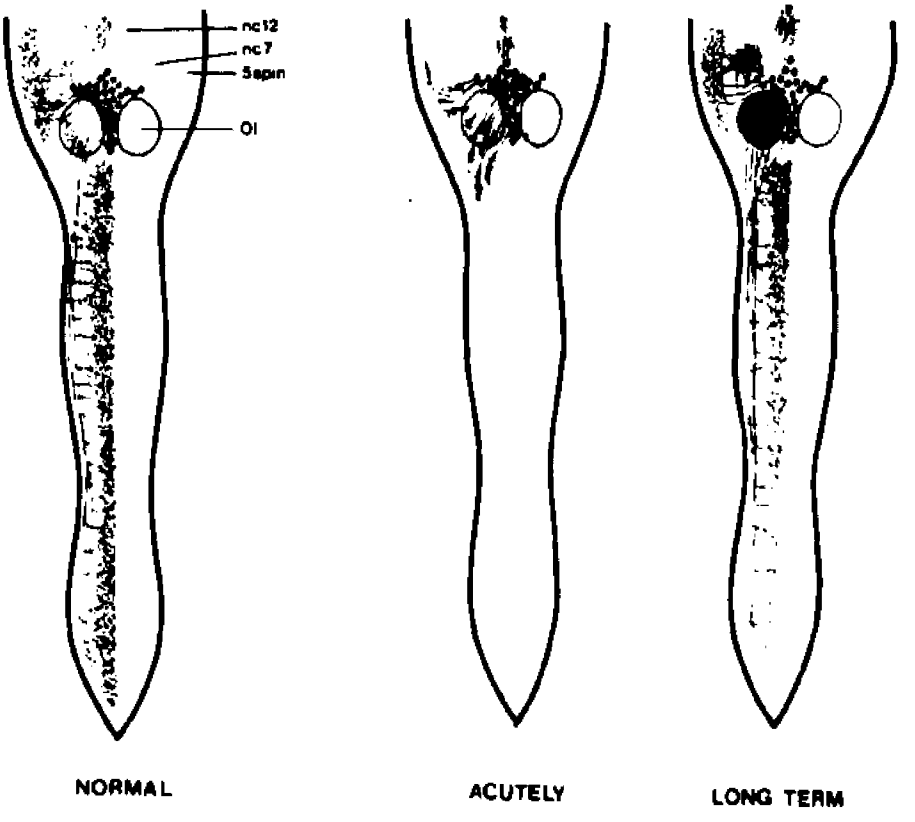
In 1970, chemical neurotoxic lesions were developed achieving a specific and more localized lesion in the CNS. Furthermore, by injection of neurotoxin into the ventricle, the glial scar was greatly reduced (Nygren and Olson, 1977). 6-hydroxydopamine (6-OHDA) was first discovered by Tranzer and Thoenen (1967) for producing selective lesioning of adrenergic neurons. The rationale for this target directed neurotoxic action is that 6-OHDA is generally a cytotoxic agent selectively accumulated by catecholamine (CA) neurons. Therefore, the degenerative action of the compound was limited to the structures that accumulate it. The chemical lesion was extended to kainic acid for selectively lesioning glutaminergic neurons (Cotman et al., 1979), and 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT) for lesioning serotonergic (5-HT) neurons (Baumgarten et al., 1972), and folic acid for lesioning gamma-aminobutyric acid (GABA) neurons (McGeer et al., 1983).

Using electrical, mechanical and especially chemical lesions, with the aid of histochemical fluorescent methods and transmitter synthesizing enzyme assays, studies of plasticity of monoaminergic system were rapidly developed in the 70's. Several chemical systems have been shown to regenerate and sprout after damage to their axons or their neighboring axons. These include the adrenergic system (NA) (Bjorklund et al., 1971; 1975; 1979; Moore et al., 1971; Katzman et al., 1971; 1977; Stenevi et al., 1972; Nygren et al., 1976; 1977; Pickel et al., 1973; Nadi et al., 1981; McRae-Degueurce et al., 1982), cholinergic system (Cotman et al., 1973; Lynch et al., 1972; Steward and Messenheimer, 1978; Storm-Mathisen, 1974; Bjorklund and stenevi, 1977; 1979; Nadler et al., 1977a), dopaminergic (DA) system (Kazman et al., 1971; Reis and Ross, 1973; Reis et al., 1978; Gilad and Reis, 1979; Tassin et al., 1979; Nadi et al., 1981), gamma-aminobutyric acid (GABA) system (Nadler et al., 1977b; Goldowitz et al., 1982) and in serotonergic system (Bjorklund et al., 1971; 1976; Nobin et al., 1973; Nygren et al., 1974; Wuttke et al., 1977, Wiklund and Bjorklund, 1978; and Azmitia et al., 1978).

An extensive study of plasticity in a monoaminergic system was performed in the 5-HT bulbospinal system after neurotoxin (5,6-DHT or 5,7-DHT) lesions. The regenerating fibers appeared from the surviving proximal axon stumps by 4-5 days after the lesion was induced. The sprouts had the appearance of delicate, smooth, or fine-varicose fibers observed histochemically to have a high fluorescence intensity (Bjorklund et al., 1973, 1979; Nobin et al., 1973). During the subsequent weeks the sprouts grew rapidly to cover progressively wider

areas of the lower medulla and upper spinal cord. By 3-6 months normal or even supranormal levels were established in the entire lower brainstem, and cranial part of the spinal cord. The regenerating 5-HT fibers were able to extend along the spinal cord all the way from the upper cervical segments down to the lumbar spinal cord, a distance at least 5 cm in the rat (figure 2). The biochemical data had shown that the regeneration was paralleled by a recovery of the 5-HT levels and $^{3}$H-5-HT uptake in the spinal cord (Wiklund et al., 1978; 1980). Monoaminergic neurons seem to retain a higher degree of morphological plasticity into adulthood. Most of them being nonmyelinated or weakly myelinated possess significant regenerative capacities, whereas abortive or feeble regeneration may be characteristic only for the long myelinated axons (Emson et al., 1977). Thus, monoaminergic neuron systems may serve as useful models for studying the neuronal growth, regeneration, sprouting and their regulation.

Figure 2: Schematic representation of the regeneration of the bulbospinal serotonin system after 5,6-DHT. Left: projections of the medullary serotonergic cell groups to the gray matter of the spinal cord, inferior olive (OI), spinal trigeminal nucleus (5 spin), facial nucleus (nc 7) and hypoglossal nucleus (nc 12). Middle: extent of axonal degeneration and distribution of the surviving axon stumps acutely after treatment. Right: regenerated axonal paths and terminal systems after long-term survival. The principal neuroanatomical features have been reformed, but there is an abnormal proximo-distal distribution of terminals. In the inferior olive, the regenerated plexa have invaded parts of the nuclear complex, which normally are devoid of serotonergic terminals. (from Wiklund and Bjorklund, 1980).



In the turn of the 70's to 80's, attention was gradually drawn in comparing the chemical characters of sprouting fibers with those of the denervated fibers.

Implantation of chemically appropriate neurons into a chemical deficient brain region has been the newly developed model for studying this process. Azmitia et al (1981) demonstrated that following a 5,7-DHT lesion in 5-HT afferent fibers from the median raphe nucleus, the dorsal hippocampus was denervated of immunocytochemically stained 5-HT fibers. However, four weeks after transplantation of fetal raphe into the raphe afferent of the denervated dorsal hippocampus or adjacent lateral ventricle, the dorsal hippocampus was reinnervated with 5-HT stained fibers derived from the implanted neurons.

Similar studies were performed in nigrostriatal DA system, following 6-OHDA lesion of DA fibers in the unilateral nigrostriatal route. The striatum was deprived of DA fibers and abnormal unilateral rotation subsequently resulted. These deficits were partially compensated by an implant of fetal substantia nigra into denervated striatum neighborhood. Reinnervation of DA fibers in striatum, demonstrated by fluorescence histochemical staining, paralleled the partial reversal of asymmetrical turning in a T-maze (Perlow, 1979; Freed et al., 1980; Dunnett, et al., 1981 a,b; Bjorklund, et al., 1981).

Genetic deficit of vesopressin, an anti-diuretic hormone in hypothalamo-hypophyseal system of Bratteboro rats, were also reported reversed by implant of fetal anterior hypothalamic neurons into the

median eminence (ME) of Baratteboro rats. The growth of the fibers of the implanted neurons into to the ME was demonstrated by immunocytochemical staining. Daily water consumption was dramatically reduced (Gash et al., 1980). Congenital hypogonadism of male mice was similarly partially reversed after implantation of fetal preoptic nucleus (Krieger, et al., 1982).

## Plasticity After Lesion

Although many stimuli are effective to induce plasticity, essentially all studies on adult plasticity have made use of lesions to investigate this phenomenon and its mechanism. On the other hand, the major goal of studying plasticity is to provide insight into its nature and recovery after brain damage. Many type of plasticities were observed. For the convenience of nomenclature, a connection that does not exist in normal animal will be termed "ectopic" in distinction to "normal" connections.

Regeneration, or a reinnervation of disconnected targets from the damaged fiber (figure 3), has previously been described. In many cases, after axotomy of the fibers, the appropriate directions for axotomized axons are prevented by glial scar formation. These fibers do not reach or approach their lost targets but wander in the local region or form ectopic connections. Thus, "regenrative sprouting" was named to distinguish itself from "regeneration" in that the growing

axons do not reestablish connection with their normal targets (Moore, 1974) (see figure 3).

Bernstein and Bernsteins' (1971) electron microscopic study of regrowth in the transected spinal cord provided evidence that such regenerative sprouting produce abundant synaptic contacts in the gray matter proximal to the lesion. The possible functional significance of this synaptic reorganization is obscure. Regenerative sprouting by optic axons of goldfish was examined distal to the site of a crush. Normally, optic nerves of goldfish contain approximately $2x10^5$ myelinated axons. The number of axonal processses increased fourfold during the first 3 month post-lesion and then returned toward pre-lesioned number 3 months later (Murray, 1982). The excess number of axonal processes provide excess synaptic contacts and persisted for several months. The regenerative sprouting fibers with ectopic contacts are diminished overtime, yet the regeneration fibers with appropriate contacts are retained. This is somewhat similar to what we have described in the developmental stage (see p. 5). The return of normal visual function is temporally associated with the reduction of excess number of processes and synaptic (ectopic) contacts.

Collateral sprouting is broadly defined to refer to any "ectopic" innervation from an undamaged neuron or fiber into a denervated zone or new territory. Many areas of the brain were reported to preserve such potentiality (see previous reference, p. 8 and figure 3).

A neuron, separated from its principal targets by a traumatic lesion, reacted as though it were in developmental stage, thus

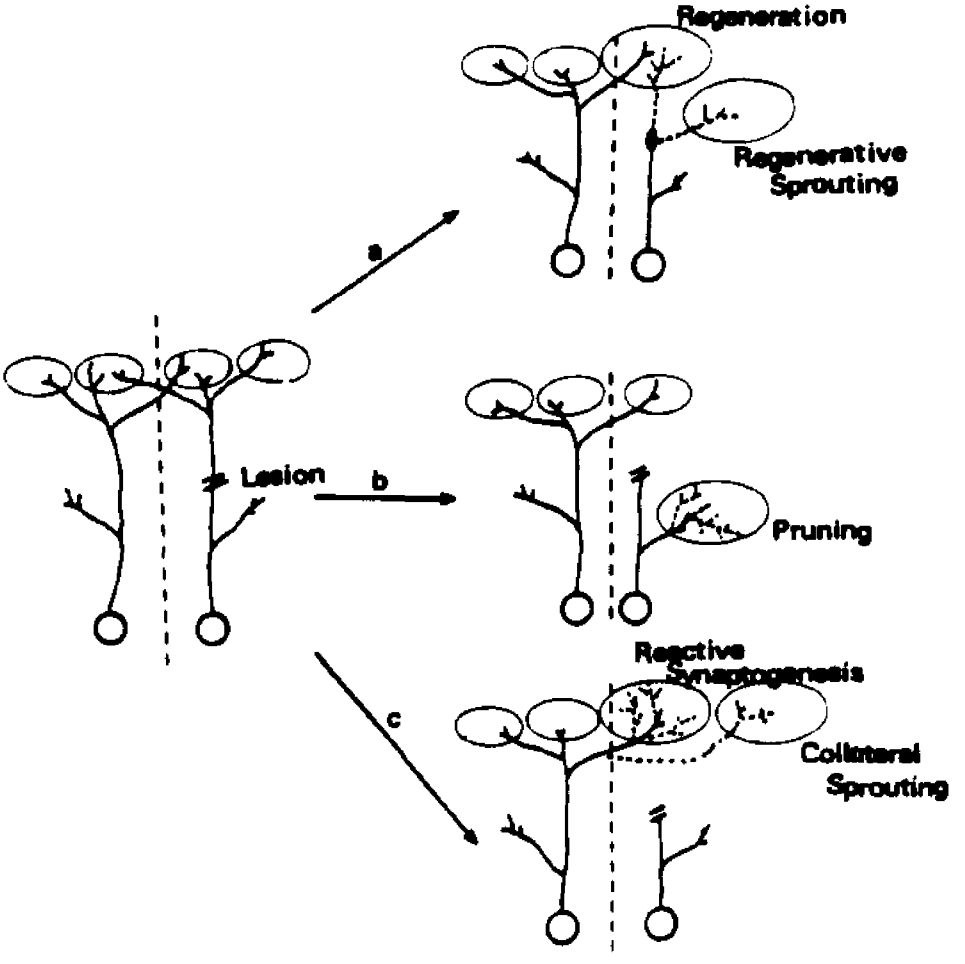
producing a "shunt" sprouting in the proximal region in compensation for the loss of its distal arborizations. Schneider (1973) refered to such a shunted sprouting as a "pruning effect" (figure 3).

Lesions preventing the growth of one branch of the neuron resulted in a compensatory overgrowth from other branches (Devor and Schneider 1975). This phenomenon suggests that a lesion of one part of the axonal tree may trigger sprouting from the intact terminals of the same neuron. Besides Schneider's obervation on the visual system, this phenomenon was observed mainly in locus ceruleus (LC) neurons which have many collateral projections to different brain regions. The LC fibers sprout in the hippocampus (Pickel, 1974); in the neocortex (Robinson et al., 1977); in the different areas of cerebellum (Robinson et al., 1977) and in the olfactory tubercle (Reis and Ross 1973; Gilad and Reis, 1979) after having "pruned" their collaterals in the cerebellum.

A group of partial denervated neurons can be reinnervated by the survived afferent which are normally located within the denervated zone. This process is called "reactive synatogenesis" (Cotman 1976) (see figure 3). Since reinnervation fibers of this type are located in the adjacent vicinity of the denervated zone. Several models have been reported of this type. In the medial septum, removal of cholinergic hippocampal afferent causes an expansion of the aminergic afferent fibers which ascend along the MFB. On the other hand, transection of MFB to the septum caused sprouting of cholinergic fibers from the hippocampus in the medial septum (Raisman, 1969; Raisman and Field, 1973). In another model, unilateral lesion of the entorhinal cortex

denervated 60% of the synapses on the granule cells of the dentate gyrus. The lost input is replaced by fibers from the septum (Lynch et al., 1972; Cotman et al., 1973; Stom-Methisen, 1974; Steward and Vinsant, 1978) and from the ipsilateral and contralateral CA4 input (Zimmer et al., 1973; West et al., 1975; Lee et al., 1977). In all these cases, the reinnervating fibers contained different chemical transmitters from those of denervated fibers, and thus, functional significance is limited. However, reactive synaptogenesis originating from fibers of homologous neurons can produce partial functional restoration in the lesioned adult brain (see next section).

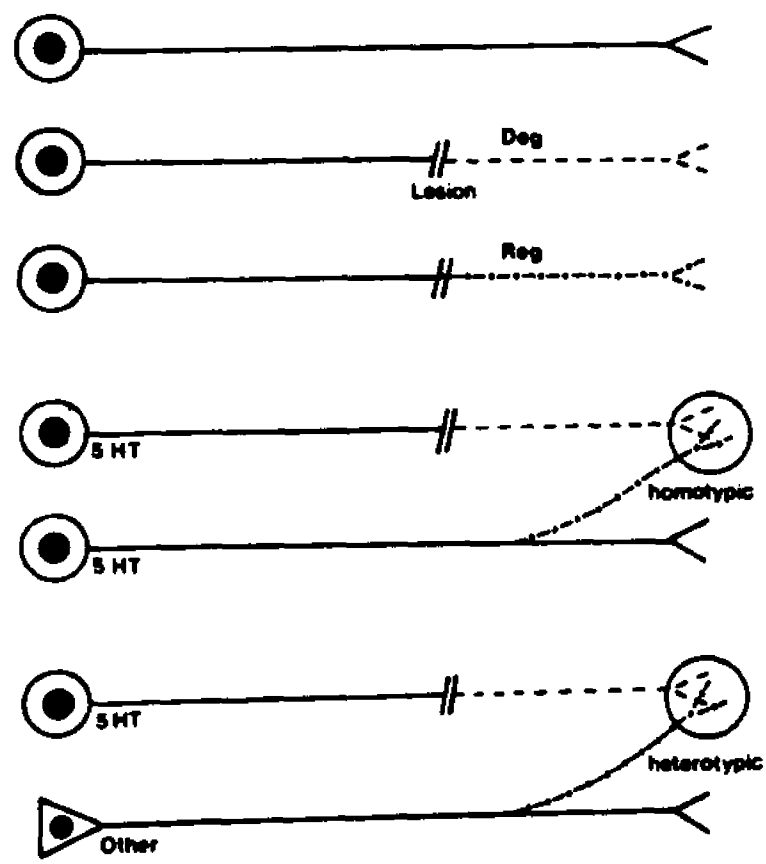
Figure 3: Schematic representation of different type of regrowth in response to a lesion in one side of the bilateral projecting nerves. The dash lines represent midline. The doted lines represent growing fibers after lesion. Oval circles represent the area of innervation. (a) Regeneration is the regrowth of the damaged fibers back to denervated area, while regenerative sprouting fibers grow toward ectopic area. (b) The growth of the collateral fibers after damage to axon of a same neuron is called pruning. (c) The regrowth of undamaged fibers in response to damage of neighboring fibers is generally called collateral sprouting, while the compensatory regrowth of undamaged fibers which are located within denervated zone is defined as reactive synaptogenesis.



Homotypic And Heterotypic Sprouting

Homotypic sprouting is a compensatory growth of undamaged fibers which carry the same transmitter as the damaged fibers, while heterotypic sprouting is the growth of a different chemical fiber type in response to damage of neighboring fibers (figure 4).

Figure 4: Homotypic sprouting is the sprouting of a fibers which carry the same transmitter as the damaged fiber. Heterotypic sprouting fibers, on the other hand, carry different transmitter as compared to the damaged one. Dash lines represent degenerating or degenerated fibers. Dash and dotted combined lines represent regrowing fibers.



Previous studies on collateral sprouting fall into two general categories:

1. Heterotypic convergence: Sprouting of mixed afferents take place in response to partial denervation of a group of mixed afferents arising from a different brain region. Such sprouting has been demonstrated in the spinal cord after removal of dorsal root afferents (Liu and Chamber, 1958; Goldberger and Murray, 1974), in cerebellum after removal of the inferior and middle peduncles (Pickel et al., 1974) and in the septum after removal of the medial forebrain bundle (Raisman, 1969; Raisman and Field, 1973).

2. Bilateral convergence: Denervation of ipsilateral afferents to a given region results in sprouting of the contralateral afferents from the homologous area of the opposite side of the brain (figure 3). Examples include the unilateral lesion of the entorhinal inputs to the hippocampus (Lynch et al., 1976; Cotman and Nadler, 1978; and Stewards and Loesche, 1977), the optic nerve inputs to the ventral lateral geniculate (Goodman et al., 1973; Lund et al., 1973), the vestibular nerve inputs to the vestibular nucleus (Dieringer and Precht, 1977; 1979a,b; Pretch et al., 1966) and in the red nucleus after deafferentation of cerebellar fibers (Nakamura et al., 1974; Murakami et al., 1976). In these well-known studies, reinnervating fibers are all derived from the homologous neurons in the contralateral side, which normally has minor innervations to the denervated area.

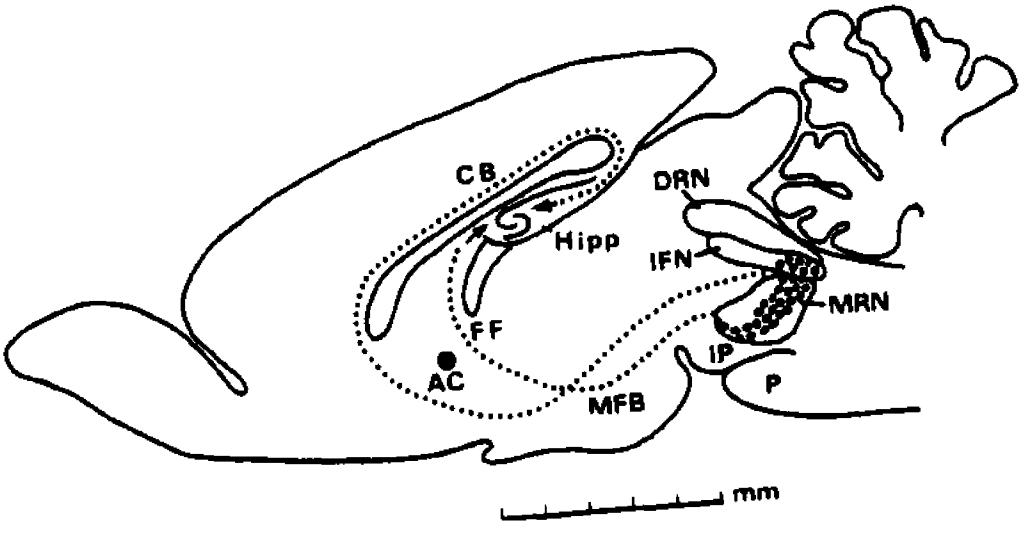
These two categories do not make a clear distinction between heterotypic and homotypic sprouting because the lesion destroys

numerous chemical fiber types.

A model of homotypic collateral sprouting has been established using the serotonergic afferents to the hippocampus of the adult rat (Azmitia et al, 1978). The dorsal hippocampus (DHipp) of the rat normally receives two separate and anatomically distinct serotonergic projections from the midbrain raphe (MR) (Azmitia, 1978; Zhou and Azmitia, 1983); (1) a supracallosal pathway which ascends in the MFB and uses the cingulum bundle (CB) to innervate the hippocampus and (2) an infracallosal projection which ascends in the MFB but joins with the fornix-fimbria (FF) to innervate the dorsal hippocampus (figure 5) (Azmitia and Segal, 1978). This arrangement of fibers, separated by the corpus callosum, allows the destruction of one pathway while leaving the second pathway essentially undamaged. Furthermore, microinjections of the specific serotonergic neurotoxic drug, 5,7-DHT limit the damage to a single fiber type when used with desipramine (DMI, a noradrenergic reuptake blocker) pretreated rats.

This neurotoxin model is used to study the reorganization of midbrain 5-HT cells projecting to the dorsal hippocampus using the CB and FF pathways. Interestingly, these two routes are shared by at least three chemical systems: (1) the noradrenergic cells in the locus coeruleus, (2) the cholinergic cells in the septum, and (3) the serotonergic cells in the midbrain raphe nuclei. The present studies are to confirm the specific action of 5,7-DHT on serotonergic neurons in the MR, and to observe the dynamic changes in the population and topography of 5-HT cells in the MR and density of 5-HT fibers in the DHipp after microinjections of 5,7-DHT into the CB and/or FF.

Figure 5: The dorsal hippocampus of rats normally receives 5-HT innervation from MRN and IFN. The 5-HT neurons in these two nuclei project through medial forbrain bundle (MFB) and then divided into infracallosal pathway, the FF, and supracallosal pathway, the CB, before innervating dorsal hippocampus. AC: anterior commissure, IP: interpeduncular nucleus, P: pons.



Glucocorticoid Effect On Neuronal Regrowth

Hormonal steroids may play a key role in modulating neuronal plasticity. Sex steroids have been implicated in the increased sprouting response of female versus male rats to fimbrial transection (Loy and Milner, 1980). Estrogen, testosterone, triiodothyronine and corticosterone have been shown to regulate neuronal plasticity in the central and peripheral nervous system. Estrogen has been demonstrated to facilitate axonal sprouting and synaptic regeneration in the deafferented hypothalamus of female rats (Matsumoto and Arai, 1979). Triiodothyronine has been observed to stimulate axon regeneration across incised wounds in the telencephalon of rats (Fertig et al., 1971; Heinicke, 1977). Testosterone was observed to promote axonal regeneration (Yu and Srinivasan, 1981; and Yu, 1982). Glucocorticoids have been shown to inhibit the outgrowth of neuronal processes in the cultured sympathetic neurons (Unsicker et al., 1978), and to retard the lesion-induced cholinergic axon sprouting in the rat hippocampal dentate gyrus (Scheff and Cotman 1980, 1982). On the other hand, glucocorticoids have been reported to facilitate growth of ganglion cells in the newborn rats (Costa et al., 1974) as well as to increase the population of small intensely fluorescent cells in sympathetic ganglia (Eranko et al., 1972). Furthermore, glucocorticoids have been shown to play a "permissive" role in normal development of 5-HT neurons in the midbrain (Sze, 1980).

Serotonergic neurons appear to be directly influenced by adrenal steroid hormones. Glucocorticoids have been reported to elevate 5-HT synthesis in the telencephalon of normal (Millard et al., 1972) and in

the brainstem of adrenalectomized rats (Azmitia et al., 1970); to acutely elevate 5-HT levels in the midbrain, hypothalamus, and amygdala of normal rats and reverse the decrease in 5-HT content in the midbrain and hypothalamus of adrenalectomized rats (Telegdy and Vermes, 1975); to depress whole brain 5-HT levels of normal animals (Curzon and Green, 1968); to enhance tryptophan uptake into synaptosomes prepared from whole brain (Neckers and Sze, 1975); to normalize the decrease in 5-HT uptake and release by synaptosomes of hypothalami of adrenalectomized rats (Vermes et al., 1976); and to inhibit monoamine oxidase activity in brains of normal animals (Parvez and Parvez, 1973). The presence of glucocorticoids may be responsible for the increase in activity of the rate-limiting enzyme for 5-HT synthesis, tryptophan hydroxylase, seen in the whole brain following ethanol intoxication (Sze and Neckers, 1974). Moreover, corticosterone appears to be necessary for the normal developmental rise of tryptophan hydroxylase beyond 12 days postnatally in the rat (Sze, 1980). Thus, glucocorticoids appear to play a role in the functional maintenance of serotonergic neurons.

In the brain, high affinity receptors for corticosterone are concentrated mostly in pyramidal cells of the cornu Ammonis and granular cells of the dentate gyrus in the hippocampus (McEwen et al., 1970). Furthermore, corticosteroids modify not only the metabolism (Withrow et al., 1972), but also the electrical activity of hippocampal neurons (Segal 1976; Taylor et al., 1971). Work by Lynch and colleagues (Etgen et al., 1979) suggests that the action of glucocorticoids in the hippocampus may involve alteration of the synthesis and/or degradation of tubulin. A recent study (Cousin et

al., 1982) has shown corticosterone to acutely increase ornithine decarboxylase activity in the hippocampus in a dose-dependent manner. Ornithine decarboxylase is noted to have an important role in RNA and protein synthesis via synthesis of polyamines.

Thus, evidence exists for a specific interaction between serotonin and corticosterone and between the hippocampus and corticosterone. The effect of the corticosterone in the sprouting of serotonergic raphe-hippocampal system is studied.

## Serotonergic System In The CNS

Serotonin (5-hydroxytryptamin, 5-HT) was first detected in gastrointestinal mucosa by Vialli and Erspamer in 1933 as an anti-peristalsis substance and named "enteramine". Later, Rapport (1949) isolated a substance from ox blood, which could cause vasoconstriction, and named it serotonin, a serum factor. Finally, enteramine and serotonin were shown to share the same chemical nature, 5-hydroxytryptamine (Rand and Reid, 1951; Raid and Reid, 1952 and Erspamer and Asero, 1952). A year later, 5-HT was found present biochemically in the dog brain by Twarog and Page in 1953. Bogdansky et al., (1956) further confirmed using fluorimetry, that 5-HT is present in significant quantity in the mesencephalon and diencephalon (especially high in the hypothalamus). Since then, its mechanism of synthesis and storage in the neurons (figure 6 and 7), release from presynaptic terminals, action in the post-synaptic element, and

inactivation by a antagonist were studied. Today 5-HT is widely accepted as a neurotransmitter in the CNS.

Figure 6: Diagram showing the synthesis and degradation of 5-HT. Adapted from Azmitia, 1978.

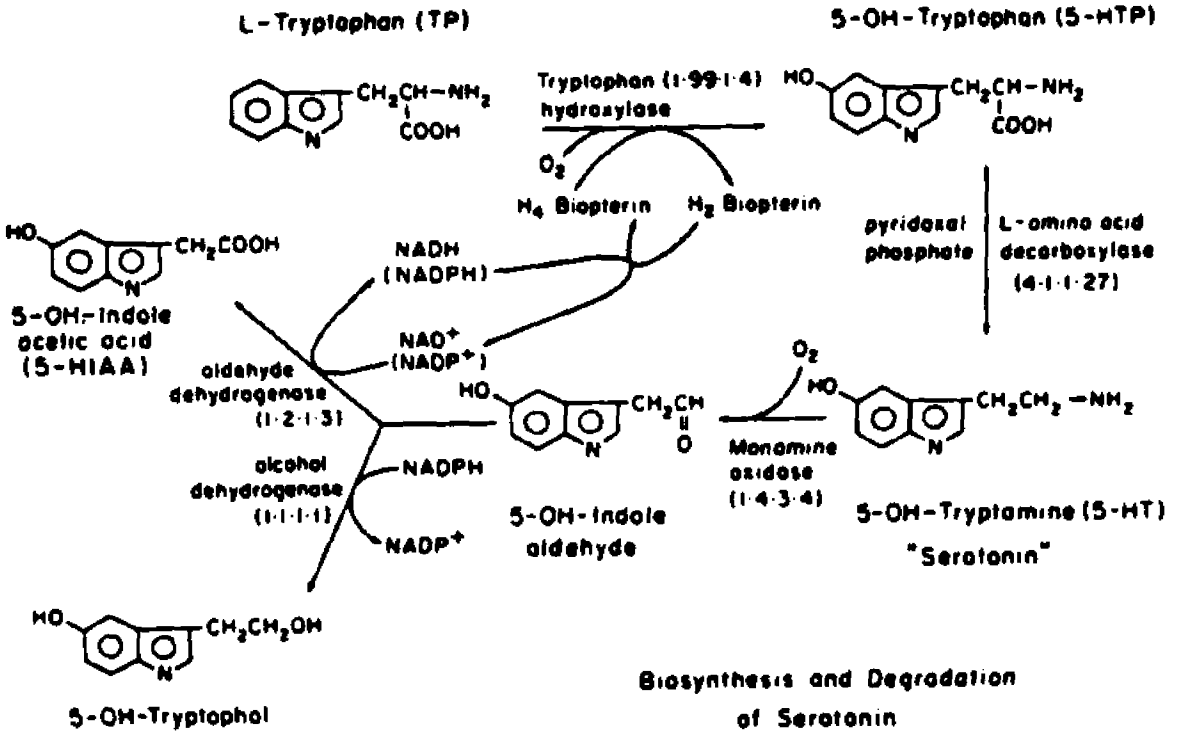
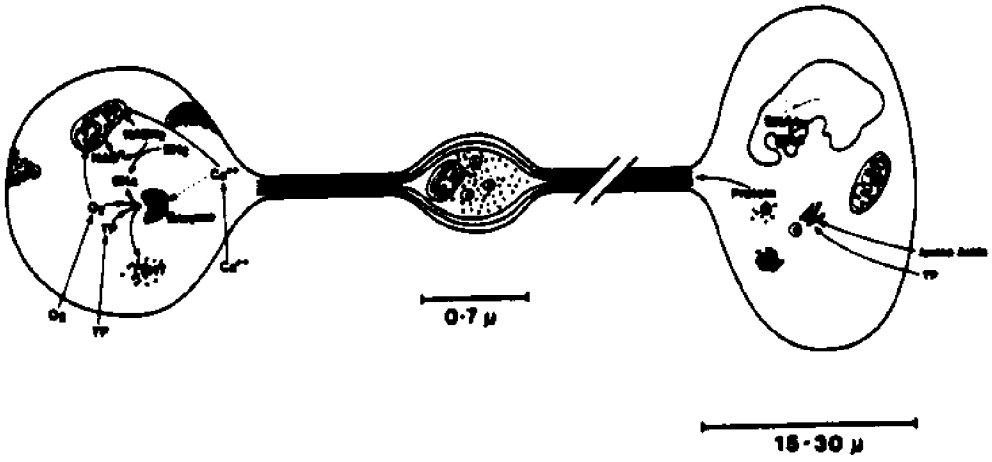


Figure 7: Schematic view of a 5-HT-synthesizing neuron as described in figure 6. Adapted from Azmitia, (1978).



Dahlstrom and Fuxe demonstrated, in 1964 and 1965, the presence of 5-HT neurons in the brain stem, using formaldehyde-induced fluorescence. Nine main nuclear groups were identified to contain 5-HT and designated B1-B9. These nuclei of 5-HT cells were later reconfirmed by immunocytochemical technique (Lidov et al., 1980; and Steinbush, 1981) with similar distribution. An additional group of 5-HT cells (besides B1-B9) in the hypothalamus was identified recently using this method (Frankfurd and Azmitia, 1981).

Descending 5-HT system mainly arises from the nuclei pallidus, obscurus and magnus raphe (B1-B3), and few from the nucleus reticularis and the nucleus reticularis pontis pars ventralis (B5) (Dahlstrom and Fux, 1965; Fuxe, 1965; Ungerstedt, 1971; Martin, 1982; Humbertson 1979; Steinbush, 1981). The 5-HT cells in these nuclei project to the pontine reticular formation, dorsal tegmental nuclei, caudal central gray, neocerebellum, locus cerlureus, and the length of the spinal cord. A few 5-HT cells in nucleus raphe dorsalis (DRN, B7) reach exclusively to the cervical level (Martin, 1982; Bowker, 1981). These 5-HT bulbospinal neurons project to defined areas in lamina I, II, VII-X as well as lamina III-VI of the spinal cord (Crutcher, 1977; Humbertson et al., 1979; Martin et al., 1982).

The majority of 5-HT ascending projections arise from the dorsal (B7) and median raphe (B8). Studies using autoradiographic tracing (Conrad et al., 1974; Bobillier et al., 1976, 1979; Taber et al., 1976; Parent et al., 1981), in combination with lesions (Fuxe and Josson, 1974; Halaris et al.,1976; Moore et al., 1978 and Azmitia and Segal, 1978), using immunocytochemical technique (Lidov et al., 1980;

Steinbusch, 1981; Saavedra et al., 1983) and in combination with marker retrograde tracing (Kohler and Chan-Palay, 1981) have contributed to the knowledge of the ascending 5-HT system in the adult rats. These 5-HT cells have been shown to project via six major tracts to the caudate-putamen, palludum, temporoparietal cortex, thalamus, hypothalamus, subthalamus, suprachiasmatic nuclei, brain stem, substantia nigra, interpeduncular nucleus, mammillary body, amygdala, nucleus accumbens, piriform cortex, cingulate cortex, habenulum, olfactory tubercle, cerebral ventricles, septum and hippocampus.

## 5-HT Raphe-Hippocampus System

The hippocampus is a pivotal structure in the limbic-midbrain system. This brain region integrates at least five chemically specific afferents: histamine system from mecencephalic reticular formation and lateral hypothalamus (Schwartz, 1975), glutamate and aspartate system from the entorhinal cortex (Iversen and Storm-Mathisen, 1976; Nadler et al., 1976; White et al., 1977), acetylcholine system from septum (Schute and Lewis, 1961; Cotman et al., 1973; Mosko et al., 1973), catecholamine system from the locus ceruleous (Blackstad et al., 1967, Swanson and Hartman, 1975; Jones and Moore, 1977; Moore and Halaris, 1975), and serotonin system from the midbrain raphe (Azmitia and Segal, 1978, Zhou and Azmitia, 1983). The chemical balance among these hippocampal afferents enables the animal to compare expected stimuli with actual stimuli (Gray, 1982). Any disruption of these transmitter systems would be expected to affect the normal function of the hippocampus. Intracerebral microinjections of the specific

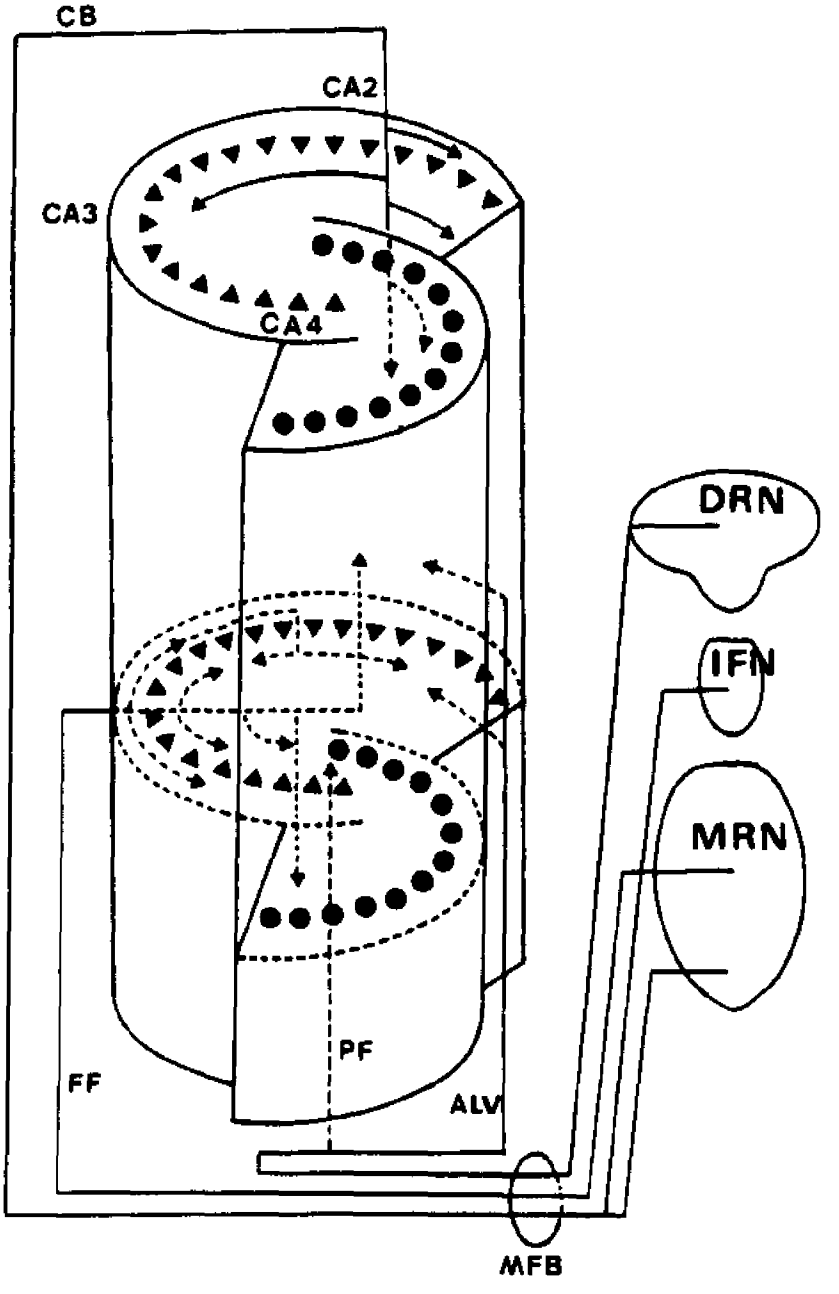
serotonergic neurotoxins to the lesion mibrain raphe 5-HT neurons or removal of the 5-HT afferents travelling within the fornix-fimbria and/or cingulum bundle leads to disruption of many of the known hippocampal functions--nocturnal locomotor activity, spontaneous alternation, theta activity (Jacob, 1975; McNughton et al., 1980; Williams and Azmitia, 1981) and "the behavioral gating" of the entorhinal afferents (Srebro et al., 1982; Winson and Abzug, 1977). Furthermore, the median raphe stimulation results in a potentiation of responses (population spike) in the dentate gyrus produced by the perforant path stimulation (Assaf and Miller 1978). Similar raphe stimulation results in a long latency, long duration inhibition of hippocampal pyramidal cells which can be antagonized by the 5-HT receptor antagonists methysergide and cyproheptadine and blocked by PCPA treatment (Segal 1975, 1976). The granule cells of the dentate gyrus have been shown to be inhibited by either iontophoretic application of 5-HT (Assaf et al 1981) or by stimulation of the median raphe (Assaf and Miller 1978). Finally, spontaneous activity of hippocampal pyramidal cells is inhibited by iontophoresis of 5-HT (Segal 1975). Thus, serotonergic projection from the midbrain raphe is a crucial chemical component in the regulation of normal hippocampal function.

The hippocampus of the rats normally receives 5-HT projections from the midbrain raphe (MR) via three distinct routes (figure 8) (Moore and Halaris, 1975; Azmitia and Segal, 1978). Fibers from the median raphe nucleus (MRN) reach the hippocampus by way of the fornix-fimbria and the cingulum bundle, whereas fibers from the dorsal

raphe nucleus (DRN) enter the hippocampus by way of the entorhinal cortex via the perforant pathway and alveus (Azmitia, 1978).

5-HT fibers (figure 8) travelling in the cingulum bundle are distributed mainly to the dorsal hippocampus, principally to the stratum lacunosom-moleculare and the polymorphic layer (esp. infragranular layer) of the dentate gyrus and sparsely to the stratum radiatum and oriens of CA1 (Moore and Halaris, 1975; Azmitia et al., 1978; Lidov et al., 1981). Projections from the median raphe carried by the fornix-fimbria are distributed to both the dorsal and ventral hippocampus in the regions of the stratum radiatum and oriens of CA2-CA4, with some fibers reaching the infragranular layer of dentate gyrus (Azmitia, 1978; Azmitia and Marovitz, 1980). Dorsal raphe fibers innervate predominantly the molecular region of the dentate gyrus, most heavily in the ventral hippocampus (Azmitia and Segal, 1978).

Figure 8: The hippocampus of the rats normally recieves 5-HT projections from the midbrain raphe nuclei via three distinct routes. Fibers from the MRN reach the hippocampus by way of the CB and the FF, whereas fibers from the dorsal raphe nucleus enter the hippocampus by way of perforant pathway (PF) and alveus (ALV). The 5-HT terminal distribution in the hippocampus is described in the text, page 30. Triangle represents pyramidal cell. Circle represents granular cell of dentate gyrus. MFB: medial forebrain bundle.



# MATERIAL AND METHODS

## MATERIALS

### 1. Experimental Animals

Female Sprague-Dawley rats (Zivic Miller Labs, Allison Park, PA) weighing approximately 220-250g were used for all studies. Microinjection of 5,7-DHT and horseradish peroxidase, adrenalectomies, and sham operations were performed in the laboratory. Animals were housed in cages (1-3 per cage) unless otherwise stated. The animals were maintained in the central animal facility of the Mount Sinai Medical Center on a 24 hours controlled light cycle (12 hr light/ 12 hr dark). They were fed Purina rat chow, and had free access to tap water (.8% saline for adrenalectomized rats). The animals were maintained in a room separated from the main part of the lab.

### 2. Chemicals and Drugs

Horseradish peroxidase (HRP), 5,7-dihydroxytryptamine (5,7-DHT), Pargyline, 3,3',5,5'-tetramethyl benzidine (TMB), 3,3'-diaminobenzidine (DAB), L-tryptophan, and corticosterone were purchased from the Sigma Chemical Co.(St. Louis, Mo). Other drugs were purchased from the following companies: Desipramine hydrochloride, Merrill Labs (Cincinnati, OH); Chloropent, Fort Dodge Co. (Fort Dodge, Iowa); Neosporin Aerosol, Burroughs Wellcome (Research Triangle Park, NC).

## Methods

### 1. Neurotoxic Lesion

The purpose of the neurotoxic lesion is to produce localized, specific damage of the 5-HT fibers, while leaving other types of fibers and the brain tissue milieu essentially undamaged. Furthermore, this chemical lesion produces less glial scarring which prevents regrowing fibers from approaching their lost targets.

#### A. Neurotoxin

Both 5,6-DHT and 5,7-DHT have been shown to have a relatively selective cytotoxic action on central 5-HT neurons (figure 9)(Baumgarten et. al., 1972; 1973; 1975). Although 5,6-DHT has been shown to have a rather selective degenerative action on 5-HT neurons, with limited effects on catecholamine (CA) neurons, this compound caused a substantial degree of nonspecific damage to non-monoamine nerve cells. 5,7-DHT is a better tool than 5,6-DHT, because it has a higher neurotoxic potency for 5-HT neurons and more limited nonspecific cytotoxic effects on non-monoaminergic neurons. On the other hand, 5,7-DHT is more neurotoxic action to NA neurons than 5,6-DHT (Baumgarten et al., 1973; Bjorklund et al., 1973b; Jacoby et al., 1974) but this limitation can be circumvented by pretreatment with desipramine, a NA neuron uptake blocker (Bjorklund et al., 1975; Gerson and Baldessarini, 1975). Thus, 5,7-DHT can be used to produce rather selective denervation of 5-HT neurons.

Figure 9: The structure of 5,6- and 5,7-dihydroxytryptamine (5,6- and 5,7-DHT) as compared to 5-hydroxytryptamine (5-HT). Adapted from Baumgarten et al., (1977).

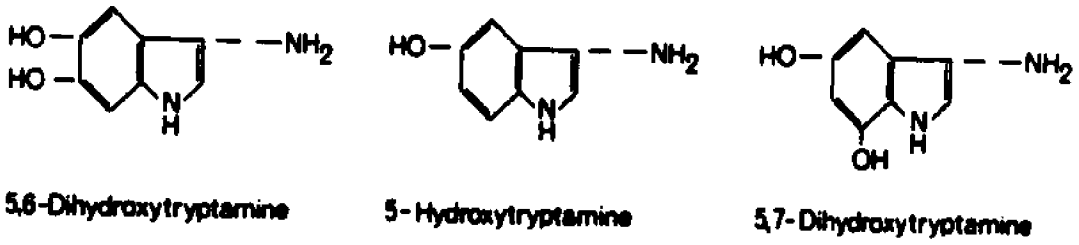
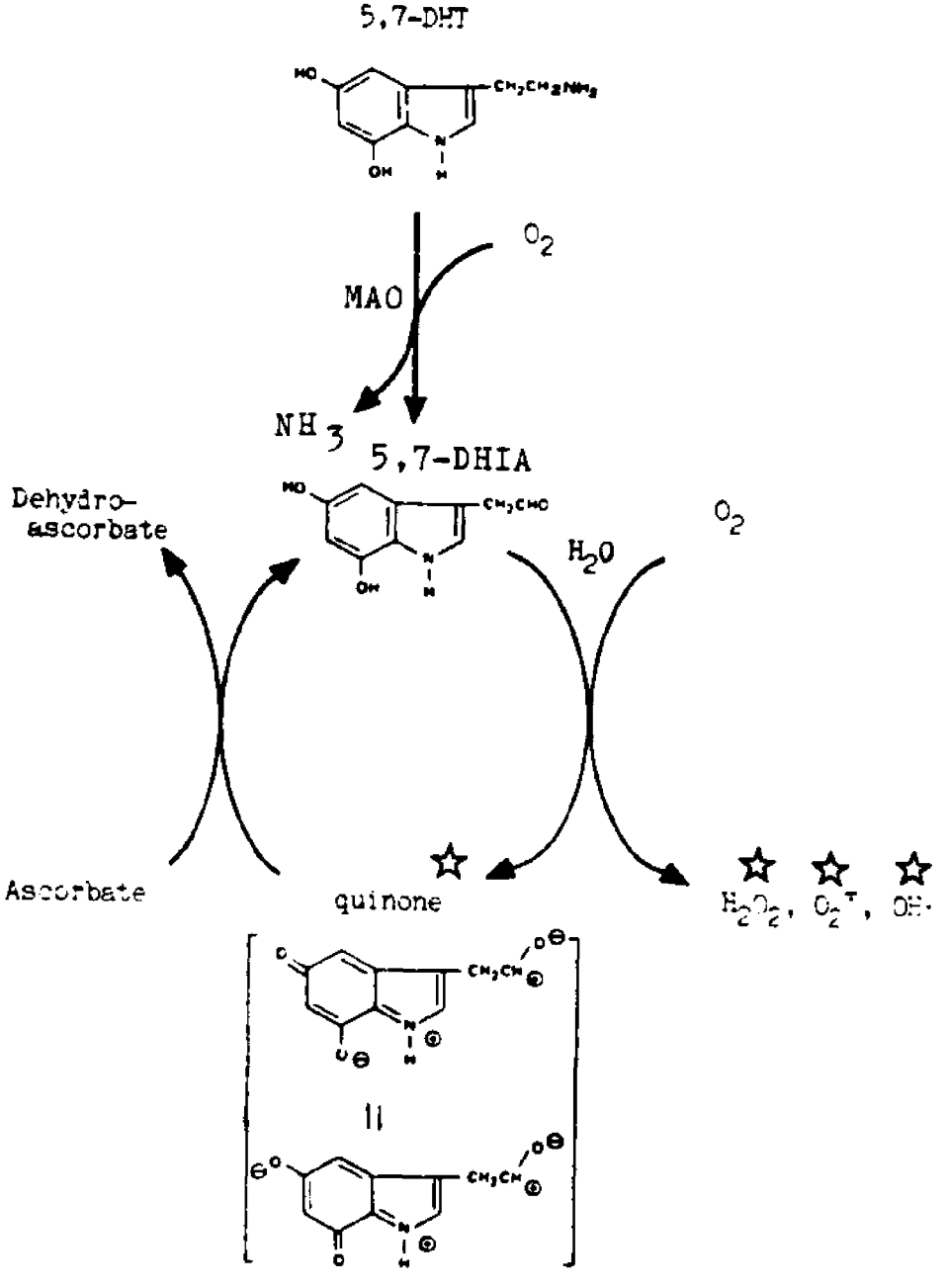


Figure 10: The scheme of hypothetic reaction for the oxidation of 5,7-DHT with formation of 5,7-DHT quinones, $H_2O_2$, superoxide ($O_2^-$), and hydroxy (OH·) radicals. The potentially cytotoxic compounds are denoted with stars. MAO: monoamine oxidase, 5,7-DHIA: 5,7-dihydoxyindole-acetaldehyde.



The selectivity of the cytotoxic effect of 5,7-DHT is derived from the specificity of the active uptake mechanism for the compound at the plasma membrane of the nerve terminal. The cytotoxic action occurs when a critical intraneuronal concentration of the amine is achieved, through the action of this transport mechanism. The cytotoxic effect of 5,7-DHT is not yet clearly understood, but in many respects is similar to the effect of 6-OHDA. Several mechanisms have been proposed, all of which invoke rapid oxidation of the compound to form an initial oxidation product, the highly reactive quinone species (see figure 10). These mechanisms involve either formation of toxic agents, such as hydrogen peroxide, superoxide, and hydroxyl radical, or extensive cross-linking of quinones with intraneuronal proteins.

Before undertaking any other investigation, the effectiveness and selectivity of 5,7-DHT were further examined in the closely related NA LC-hippocampal and 5-HT MR-hippocampal systems.

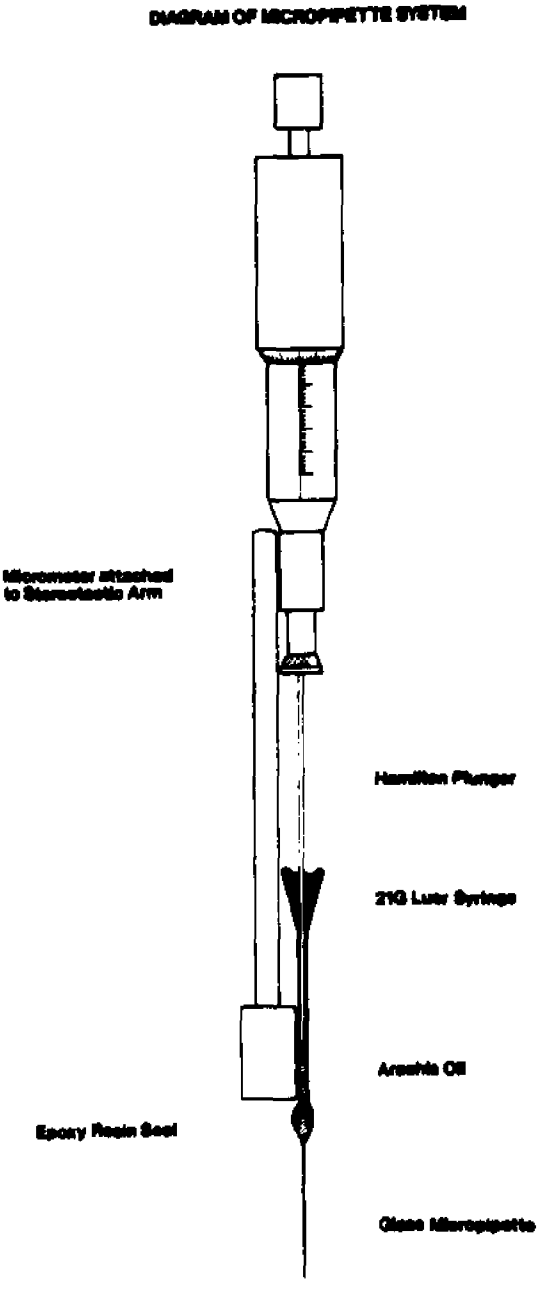
B. Microinjection of 5,7-DHT

Adult female Sprague-Dawley rats were injected intraperitoneally (i.p.) with desipramine (DMI, a norepinephrine uptake blocker) (1mg/100g body weight) and 30 minutes later were anesthetized with chloropent ( intraperitoneal injection, 0.25 ml per 100 gm body weight). The animals were then placed in a stereotaxic apparatus with the earbars positioned so as to penetrate the animal's eardrums, and the incisor bar set to 3.2 mm below the interaural line. The skin overlying the skull was shaved free of fur, and an incision was made in

the skin after applying alcohol. The underlying cranial muscle and fascia was removed using a pair of surgical tweezers. An opening in the skull was made using a dental drill, and the underlying dura was pierced with a fine dental hook under the operating microscope.

The pipette was sterilized utilizing 70% alcohol and washed with saline before use. The microinjections of 5,7-DHT were all made unilaterally through a glass micropipette (figure 11) (tip diameter 80-100 um) into the CB (5 ug in 400 nl ascorbic saline, coordinates with respect to bregma: 15°, AP=1.0 mm posterior, L=1.3 mm lateral, V=2.4 mm ventral to skull surface) and/or the FF (6.25 ug in 500 nl, coordinates: 15°, AP=1.0-1.2 mm depending on lambda-bregma distance, L=1.3 mm V=4.5 mm) at the rate of 50 nl/min by step injection. The micropipette was left in place for an additional minute before being slowly removed. The skin incision is closed with wound clips, swabbed with iodine, and sprayed with Neosporin Aerosol. The animal is left to recover from anaesthesia under the warmth of a bright light. The exact location and spread of the 5,7-DHT lesion in the fornix and CB have been discussed (Williams and Azmitia, 1981). Shams received unilateral injections of vehicle during identical operations. For lesioning the 5-HT neurons in the mesencephalon, 5,7-DHT (25 ug in 2ul ascorbic saline) was microinjected into the midline raphe (coordinates: 90° with respect to lambda, midline, AP=0.2 mm, V=7.8 mm) with the surgical procedures discribed above.

Figure 11: A diagram of the micropipette system used to deliver nanoliter quantities of solution by pressure. The pipette is used both to take-up and deliver precise amount of solution under visual inspection. The small space between the plunger and the glass tip is filled with oil and produces a smooth and sensitive ejection of fluid.



## 2. Horseradish Peroxidase Neurohistochemistry

### A. The Enzyme Marker and Its Mechanism

The enzyme horseradish peroxidase (HRP) has a wide range of applications for tracing neural connections. HRP was used as a marker in our studies to 1) observe the connections of the raphe-hippocampus and LC-hippocampus systems, 2) quantify the terminal distribution of raphe cells in the hippocampus, and 3) observe the reorganization of MRN 5-HT cell population in response to specific neurotoxic lesion to their fibers in the connecting paths.

HRP is taken up into neurons through endocytosis in the fiber terminals. The membrane-delimited endocytotic vesicles which contain the enzyme are then transported along neural processes that emanate from the site of administration. While the HRP molecule is not itself visible, a readily detectable reaction product is obtained by enzymatic action at the site of administration, as well as at site to which it has been transported.

The enzymatic activity of HRP arises from the cyclic reduction and oxidation of the iron atom in the hematin group, which is then covalently bound to the glycoprotein apoenzyme. In suitable medium, tissue-bound HRP readily combines with its substrate, hydrogen peroxide, and the resultant [HRP.$H_2O_2$] complex can oxidize a wide variety of chromogenic hydrogen donors. These chromogens (CHROM) assume a dense color in the oxidized state and precipitate as readily detectable markers for HRP activity. The general reaction is shown as

follows:

$$HRP + H_2O_2 = [HRP.H_2O_2]$$

$$[HRP.H_2O_2] + (CHROM)H_2 = (CHROM)_{precip.} + HRP + 2H_2O$$

The uptake, the transport, the fixative used, the reaction parameters, the choice of chromgens and the post-reaction stabilization of HRP were excellently and completely discussed by M. Mesulam (1982).

B. Microinjection of HRP

The rats of various experimental conditions were anesthetized, positioned, and surgically manipulated as described earlier. The HRP (Sigma type VI, 100nl of 10% in normal saline) was unilaterally microinjected into the DHipp according to the same surgical procedures described above (coordinates with respect to lambda: 90°, AP=4.5 mm anterior, L=1.5 mm, V=3.7 mm) at rate of 15 nl/min. The micropipette was left in situ for an extra minute before being slowly pulled out.

C. Histochemical Procedures

Twenty-four hours after HRP injection, the rats were deeply

anesthetized with chloropent and perfused with the following procedures through the ascending aorta: (1) rapid perfusion with 150-200ml normal saline until effluent clear (about 2 -5 minutes), and (2) 20-30 minutes with 300 ml of sodium phosphate buffer (PB, 0.1 M, pH=7.4) containing 2.5% glutaraldehyde fixative and 0.1% $MgSO_4$ . The brains were removed, cut into hippocampal cortex and brain stem blocks, and post-fixed in the same solution for 2 hours before being transfered to 3% sucrose-phosphate buffer for three hours. The blocks were then transfered to phosphate buffer (at 5°C) and left overnight. Frozen coronal sections (50 um) were cut on a Leitz freezing microtome and subsequently processed for HRP activity according to Mesulam's methods (1978) using the TMB chromogen. The sections were preincubated with TMB (0.005%) for 20 minutes, and then $H_2O_2$ (0.003%) was added. The reaction was allowed to run for 20 minutes at 19°-22°C under a dimmed light. The sections were then transfered to a 5% acetate buffer, collected on clean slides, and dried in a 37°C oven overnight before counterstaining with 0.5% acetate-buffered methyl green.

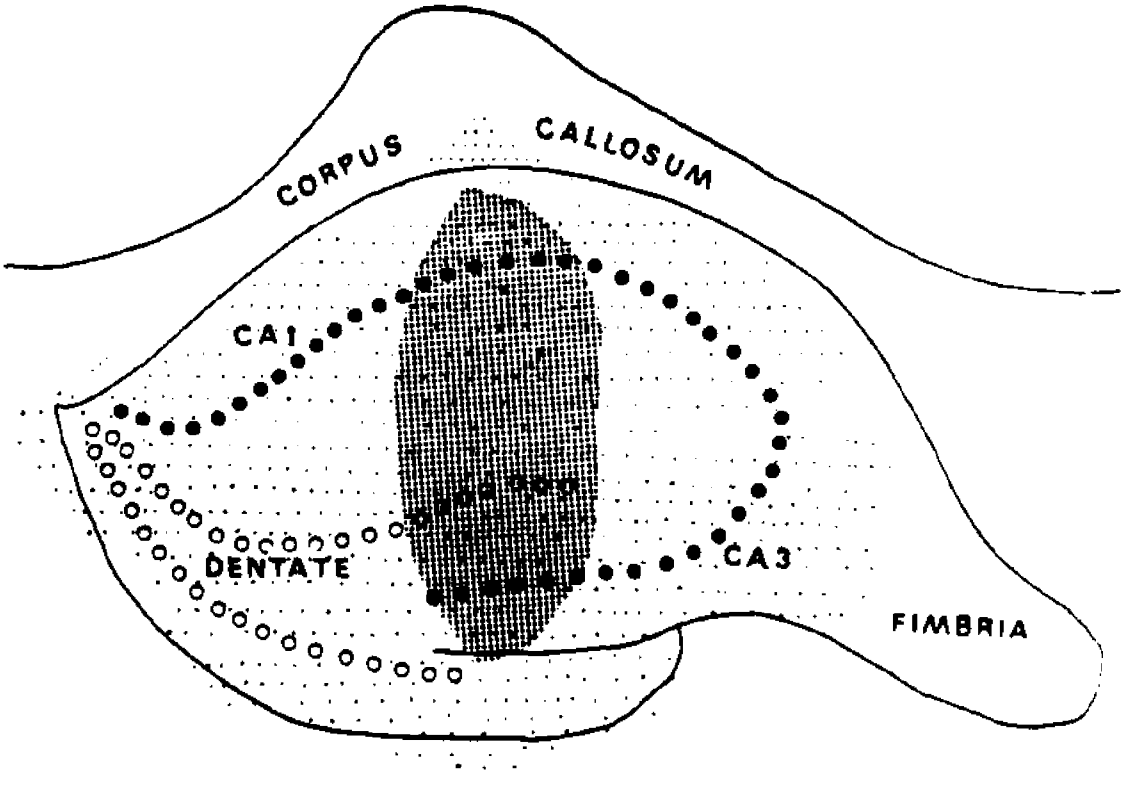
D. Time Course of Retrograde Transport In The Raphe-Hippocampal System

Initial experiments were performed to establish the time course for optimal labeling of midbrain raphe cells. Rats were injected with 100nl of 10% HRP into DHipp and killed at various times. The number of labeled cells counted (correction factor included, see below) was 0 at 2 h (n=2), 165 and 198 at 12 h (n=2), 223±37 at 18 h (n=4), 294±46 at 24 h (n=10), 324±58 at 36 h (n=3) and 156±27 at 48 h (n=4). In all

subsequent experiments the animals were perfused 24 h after the HRP injection, since it was convenient for our experimental design.

The HRP injection sites in the hippocampus were processed with the same method to verify that the injection center was located between CA1 and the dentate gyrus of the dorsal hippocampus (figure 12).

Figure 12: Schematic drawing of coronal section of injection of 100 nl 10% of HRP into the dorsal hippocampus. closed circles represent pyramidal cells, open circles represnt granule cells. Two hours after HRP injection into the dorsal hippocamopus, most of the dorsal hippocampus was covered with HRP shown by small dots. This HRP distribution extended rostrally to near the hippocampal commissure and caudally to the isthmus of the dorsal and ventral hippocampus. Twenty-four hours after injection, the HRP was localized to a small area shown by the dense dots. There was no diffusion of HRP into the contralateral side of the dorsal hippocampus.

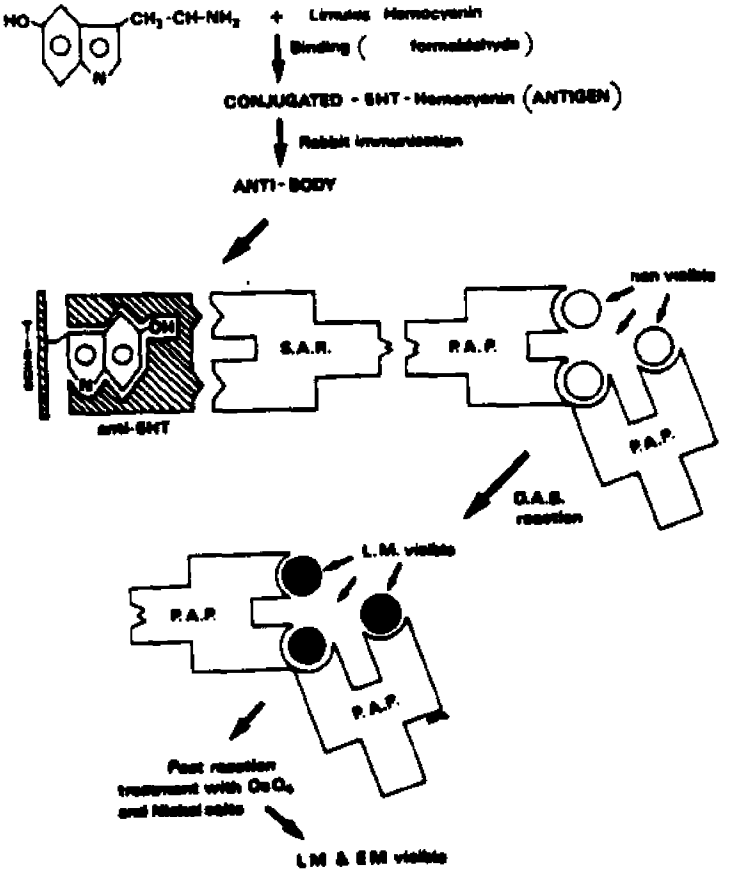


## 3. 5-HT Immunocytochemistry

### A. Anti-5HT Antiserum

5-HT immunocytochemical staining was performed using the indirect antibody enzyme method of Sternberger (1970) (figure 13), with minor modifications (Frankfurt and Azmitia, 1983). The anti-5HT antiserum used in this study was a generous gift of Dr. J. Lauder. This antiserum was made from rabbits that were sensitized with limulus hemocyanin conjugated 5-HT antigen (figure 13), and was characterized by Frankfurt and Azmitia as follows: Sections of dorsal raphe nucleus (DRN) were incubated for 18 hr with 5-HT antiserum (1:2000) that had been preabsorbed with various concentrations ranging from $10^{-5}$ to $10^{-2}$M, of 5-HT, norepinephrine (NA), dopamine (DA), and tryptamine. Increasing concentrations of 5-HT resulted in decreased staining and no staining was apparent above $10^{-3}$M 5-HT in the DRN. This was also true for tryptamine. DA reduced the intensity, but not the number, of stained cells in the dorsal raphe at high concentrations. NA did not have any effect on 5-HT staining. Areas known to contain dopaminergic cells, such as the substantia nigra and hypothalamic nucleus were also used as 5-HT immunostaining controls. In the substantia nigra, there was moderate staining of cell bodies in animals that were pretreated with pargyline and L-tryptophan. However, the intensity of staining was low and in no way resembled that observed in cells of the DRN. In the arcuate nucleus of the hypothalamus, no staining was observed.

Figure 13: Diagram of 5-HT immunocytochemical method. The 5-HT antibody is produsedagainst hemocyanin conjugated 5-HT antigen in rabbits. Tissue is incubated with this 5-HT antibody, sheep anti-rabbit antibody (S.A.R.), and peroxidase anti-peroxidase (P.A.P.) seqentially. The linked P.A.P. is visualized after reaction with diamino-benzedine (D.A.B.) in the light microscopic level or with $OsO_4$ and nickel salts in the ultrastructural level.



B. Drug Pretreatment

Two pretreatments were used to enhance staining of 5-HT fibers, to compensate for the low endogenous levels of 5-HT. Pargyline (200mg/Kg), an inhibitor of monoamine oxidase, the major degradation enzyme of 5-HT and L-tryptophan (200 mg/Kg), a precusor of 5-HT biosynthesis, were injected (i.p.) 1.5 and 1 h, respectively, prior to perfusion.

C. Immunocytochemical Procedures

All the animals processed for immunohistochemical staining were pretreated with L-tryptophan and pargyline. Each animal was then perfused intracardially for 3 min with 0.9% saline ($4^{o}C$) containing 0.1% $MgSO_4$, followed immediately by a 30 min perfusion with 250 ml of 4% formaldehyde (freshly prepared and filtered, purified grade) in a 0.1 M phosphate buffer (ph 7.4, $4^{o}C$) containing 0.1% $MgSO_4$ and 3% sucrose. The brain was removed, and the hippocampal cortex and brain stem were cut into blocks. These blocks were post-fixed in the fixative (same as above) for 2 hr at $4^{o}C$, and then rinsed several times in 0.1M phosphate buffer containing 3% sucrose and 0.1% $MgSO_4$ ($4^{o}C$).

The tissue blocks were coronally sectioned at 30 um with a Vibratome (Oxford). Sections were collected in phosphate buffer containing .9% NaCl (PBS) at $4^{o}C$. and rinsed 3 times (5 min per rinse) in ice cold PBS, and 3 times in ice cold o.1M, pH 7.4, Tris Buffer containing 0.9% Nacl (TBS). Alternate sections were incubated

with 5-HT antiserum at 1/1500 or 1/2000 dilutions. The antiserum and all subsequent reagents were made in TBS containing 0.2% Triton-X-100 and 1% normal sheep serum. All sections were incubated in the following sequence: 1) in primary antiserum at 4°C for 18 hr. 2) in sheep anti-rabbit antibodies (1:100, Antibodies Inc., Davis, CA) at 22°C for 30 min, 3) in peroxidase-anti-peroxidase (PAP) complex (1:100, Miles, Elkhart, IN) at 4°C for an hr, and 4) in 0.05% 3,3-diaminobenzidine and 0.003% $H_2O_2$ at 22 °C for 4-8 min. Between incubations sections were rinsed 3 times (5 min per rinse) in TBS. Following the immunocytochemical staining, the sections were mounted onto albumin coated glass slides. The slides were then dried overnight in a 45°C oven, dehydrated, counterstained with methyl green, coverslipped, and viewed with a Leitz Orthoplan microscope with light and dark field illumination.

## 4. Simultaneous Labeling of 5-HT and Retrogradely Transported HRP

In order to identify the chemical character of the neurons in the MRN, which project to the dorsal hippocampus, the tracing enzyme HRP and the 5-HT of the labeled cells were stained simultaneously.

### A. Perfusion

The animals were deeply anesthetized with chloropent and perfused intracardially by the following procedure: 1) 150-200 ml normal saline until the effluent became clear (about 2 min), 2) 3.6-4% formaldehyde fixative containing 0.1% $MgSO_4$ in phosphate buffer, with the

perfusion rate adjusted so that the infusion of 1000 ml was completed within 40-50 min, 3) cold 10% sucrose phosphate buffer for 60 min (about a 1200 ml perfusion).

Each brain was then removed, placed in the same sucrose solution, and sectioned through the brain stem (thickness 30 um) on a vibratome (Oxford).

B. Histochemical Procedures

The sections were collected in cold phosphate buffer and processed as follows: 1) rinse three times in PB, 2) rinse three times in Tris buffer (TB), 3) incubate in 0.5% $CoCl_2$ TB for 10 min, 4) rinse three times in TB, 5) rinse three times in PB, 6) incubate in 0.02% DAB in PB solution for 10 min, 7) incubate in same solution above with 0.01% $H_2O_2$ for 10 min, and 8) rinse three time in PB. Each rinse in the above buffers was about 5 min. The sections were then transfered to PBS for immunocytochemical staining of 5-HT according to the method described before.

## 5. Bilateral Adrenalectomies

Bilateral adrenalectomies and sham operations were performed immediately following 5,7-DHT or sham lesions in the lab while the animals were still under deep anesthesia. Animal were shaved free of fur on the lower dorsal trunk. Longitudinal incisions were made bilaterally, through the skin and underlying muscles, 2 cm from the vertebrae and below the ribs, using a pair of sterilized surgical

scissors. The adrenal glands were then localized, stablized with a pair of tweezers and carefully removed bilaterally with fine surgical scissors. The incision was sutured closed, and the animal was injected with $10^6$ units of penicillin intramuscularly 1, 2, and 7 days postoperatively. Sham operations consisted of the same surgical procedures except for removal of the adrenal glands.

## 6. Implantation of Corticosterone Pellets

Solid corticosterone pellets were subcutaneously implanted in bilaterally adrenalectomized rats according to the method of Meyer, et al (1979). 100% pellets were made by melting pure corticosterone in a 25 cg vial over a low gas flame. The melted steroid was then poured into a 6 mm diameter hole in a paraffin block. After the pellets had solidified, they were removed from the paraffin and trimmed to the correct weight (approximately 100 mg). Following adrenalectomy, the pellets were implanted in the nape of the neck of anesthetized animals. After the skin in the area had been shaved and a small incision made, the underlying fascia was spread, and the pellet was placed under the skin at least 1.5 cm caudal to the incision. The incision was then closed with wound clips. Pellets were replaced every 7 days to insure the maintenance of stable circulating levels of corticosterone. With this method of replacement therapy, the large variability in plasma hormone levels associated with corticosterone injection were avoided (Meyer et al, 1979).

## 7. Measurement and Statistics

All the brain sections for HRP activity were cut through the brain stem, each section at 50 um. The labeled cells were counted in serial section, and the size of cells was measured using a light microscope fitted with an ocular micrometer. The long and short axes of the cell with nucleus were averaged to determine the diameter (d). The tissue thickness (t) was 50 microns as indicated on a Leitz freezing microtome during sectioning. The modified thickness after processing was measured by interpreting the focus distance of a oil lens (64X) light microscope objective. Cells were designated as labeled cells when the number of HRP reaction granules exceeded 15 within perikaryon. The nuclei of these cells were frequently masked by reaction granules. The number of cells counted (ni) was obtained by manually counting all the labeled cells in each serial sections. In order to correct for counting the same cell twice in adjacent sections, a correction factor of 0.67 for MRN counts and 0.55 for LC count was calculated and used according to the Abercrombie's formula. The corrected number of labeled cells (N) is calculated as below:

$$N = ni \times [t / (t + d)]$$

The conditions for the quantitative HRP studies were constant for all animals. In all cases, experimental and control animals were processed at the same time.

The significance of the differences between mean values for control and experimental groups are assessed using Student's t-test and analysis of vaiance and levels of significance.

### 8. Photography

A Leitz light microscope with 10X, 25X, 40X, and 63X planapochromatic objective lense was used with a 35mm photographic attaghment and Panatomic-X film (32 ASA, fine grain panchromatic). All black and white photographs were printed on Ilfospeed paper using Dektol (Kodak) and Kodak rapid fixer.

## Experimental Design

### 1. The Lesioning Effect of 5,7-DHT

Twenty-two rats were microinjected in the CB and the FF with 5,7-DHT as previously described (figure 13). At various post-lesion times (zero hour, 1 hr, 1 day, 2 days, and 3 days, each with 4 animals) the animals were ipsilaterally injected with 100nl of 10% HRP into the dorsal hippocampus as previously described. Two gontrol groups were used: a sham control (n=3), in which animals were injected with ascorbic saline in the CB and FF, and a DMI control (n=4), in which

animals were pretreated with DMI before receiving an injection of ascorbic saline in the CB and FF.

All the animals were perfused twenty-four hours after the HRP injection. The brainstems were sectioned, and incubated for the HRP activity as described before. All the labeled cells in the MRN and the LC were counted in serial sections. The number of labeled cells was then adjusted with Abercrombie's formula based on the thickness of the section and the size of the cells.

## 2. Differentiation of Two Raphe-Hippocampal Cell Groups

Twenty-seven female rats were separated into five groups. Microinjections of 5,7-DHT were made into the CB (n|6), the FF (n=5) or both the CB and the FF (n=5). Normal controls (n=6) and vehicle injected controls (n=5) were used. Forty-eight hours after the 5,7-DHT-DMI injections, all rats were injected with HRP into the DHipp, and were perfused 24 hours after that. The HRP activity in the brainstem was stained using the same method. The labeled cells were then counted in the MR.

## 3. Serotonergic and Non-serotonergic raphe-DHipp neurons

Four animals were microinjected with 2 ul (25 ug) 5,7-DHT into MR (coordinates: $90^{o}$ with respect to lambda, midline, AP=0.2 mm, V=7.8 mm). Two of the animals were injected with HRP into the DHipp two days after lesion. The animals were perfused 24 hours after injection and

processed for HRP reaction. The other two animals were perfused two days after lesion and processed for 5-HT immunocytochemistry.

A group of three animals was injected with HRP into DHipp and processed for simutaneously staining of 5-HT and HRP in the MR by the method previously described. The stained cells were examined in the MR.

4. Homotypic Collateral Sprouting: An HRP Study

Animals were separated into four groups, each group containing sub-groups of various times post-lesion. The normal controls group were the animals injected with HRP only (n=6). The sham control animals were injected with vehicle alone, and then subsequently with HRP either 3, 21, or 42 days later (each with 3 animals). The experimental animals were lesioned in the cingulum bundle with 5,7-DHT, and HRP injections were made at 3 (n=6), 7 (n=4), 14 (n=5), 21 (n=6), or 42 (n=6) days post-lesion. The double lesioned control group was treated identically to the experimental group, but a second lesion was made in the fornix two days before HRP injections at 1, 19, and 40 days (each with 3 animals). All the animals were perfused 24 hr after HRP injection and processed for HRP activity in the brain stem. The number of labeled cells in MRN and IFN of various group animals were counted and adjusted with Abercrombie's formula.

A separate group of animals was largely denervated of 5-HT fibers from the DHipp. Animals were lesioned in the CB and the FF on the same day. These animals were injected with HRP 3 (n=4), 14 (n=3), and 42

(n=3) days post-CB-FF-lesion, and perfused 24 hr after the HRP injection. The brains were coronally sectioned through the brain stem, and processed for HRP activity. The labeled cells were counted in the MR and adjusted as before.

## 5. Homotypic Collateral Sprouting: An Immunocytochemical Study

Animals were unilaterally microinjected with 5,7-DHT (dose, coordinates, and procedures as before) in the CB, and subsequently sacrified 3 (n=4), 14 (n=3), and 42 days (n=3) post-lesion. These animals were pretreated with pargyline and L-tryptophan prior to perfusion with cold 4% formaldehyde in phosphate buffer solution. Their brains were coronally sectioned through the hippocampal area to immuno-stain the 5-HT fibers with 1:2000 anti-5HT antiserum. The procedures were as previously described.

## 6. The Glucocorticoid Effect On Sprouting

Animals were separated into 5 groups which include a sham injected control (n=7), an ADX control (n=3), a 5 ,7-DHT lesion in the CB (n=6), 5,7-DHT lesion in the CB of ADX rats (n=6), and a 5,7-DHT lesion in the CB of ADX rats with subcutanous implantation of corticosterone pellets (n=5). Animals were microinjected with HRP into the ipsilateral DHipp either at 3 or 21 days post-lesion, and processed for HRP activity in the brainstem area. The number of labeled cells were counted in the MRN and adjusted.

# RESULTS

## 1. Effect and Specificity of 5,7-DHT Lesioning

### Introduction

In order to make a specific lesion of 5-HT fibers, to study their homotypic sprouting, an efficient and specific neurotoxin, such as 5,7-DHT, is crucial. The specificity of the 5,7-DHT depends upon its uptake by neurons as an analogue of transmitter. Both 5-HT and NA neurons selectively take up 5,7-DHT. The 5-HT neuronal uptake mechanism has a 16 fold higher affinity than that of NA for 5,7-DHT (Baumgarten et al., 1978a). Nevertheless, 5,7-DHT causes a 40-50% reduction of NA in brain and spinal cord (Baumgarten et al., 1974; Gerson et al., 1974). However, the decrease in the level of NA by even 100 ug of 5,7-DHT can be prevented by pretreatment of rats with desipramine (DMI)(Gerson et al., 1975, Breese et al., 1978). DMI has been found to block the uptake mechanism of NA neurons (Baumgarten et al., 1978b; Lidbrink et al., 1971). The present study provides morphological evidence of the specificity of 5,7-DHT after pretreatment with DMI, in blocking 5-HT versus NA fibers in the raphe-hippocampal and LC-hippocampal systems. Furthermore, although the use of 5,7-DHT has been gaining popularity as a technique for producing specific lesions of the 5-HT system, little is known about how efficiently it works or how quickly it blocks 5-HT fibers. The time course of 5,7-DHT lesions was studied.

Microinjections of 5,7-DHT were made into the CB and the FF. The

neuronal connection tracing marker, HRP, was injected into the DHipp at various times. The surviving connections were counted by the HRP labeled cells in the MRN and the LC. The HRP labeled cells in the MRN and the LC were counted and considered to represent the surviving connections (figure 14).

Results

Injecting HRP into the dorsal hippocampus immediately after the injection of 5,7-DHT reduced the MRN labeling (normal=273+26) by half. A delay of two days before the injection of HRP reduced the number of labeled cells to 6.5% of the total number seen in controls (figure 15). A similar number of HRP labeled cells was seen after a delay of 3 days. DMI, a noradrenergic uptake blocker, had no significant effect on the retrograde transport of HRP by cells located within the MRN and the LC. The specificity of the 5,7-DHT intracerebral microinjections is shown by comparing neurons counted in the LC with the number in the MRN. This noradrenergic monoamine system also uses the CB and the FF pathways to innervate the hippocampus (Jones and Moore, 1977; Lindvall and Bjorklund, 1974). The number of labeled neurons in the LC was not significantly decreased from that found in the two control groups (see Figure 15).

Figure 14: Diagram shows designing of HRP method to measure the 5,7-DHT neurotoxic effect on 5-HT raphe-hippocampal system and NA locus ceruleus-hippocampal system. After the CB and FF pathway lesioned with 5,7-DHT, the connections of these two 5-HT and NA system are examined by HRP retrograde transport from hippocampus to raphe nuclei and locus ceruleus (LC).

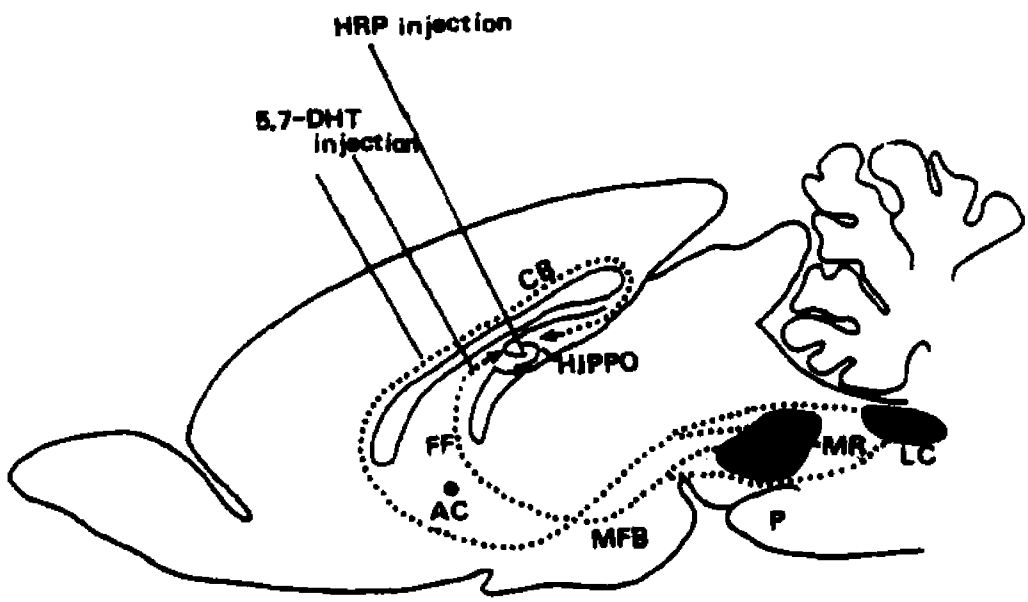
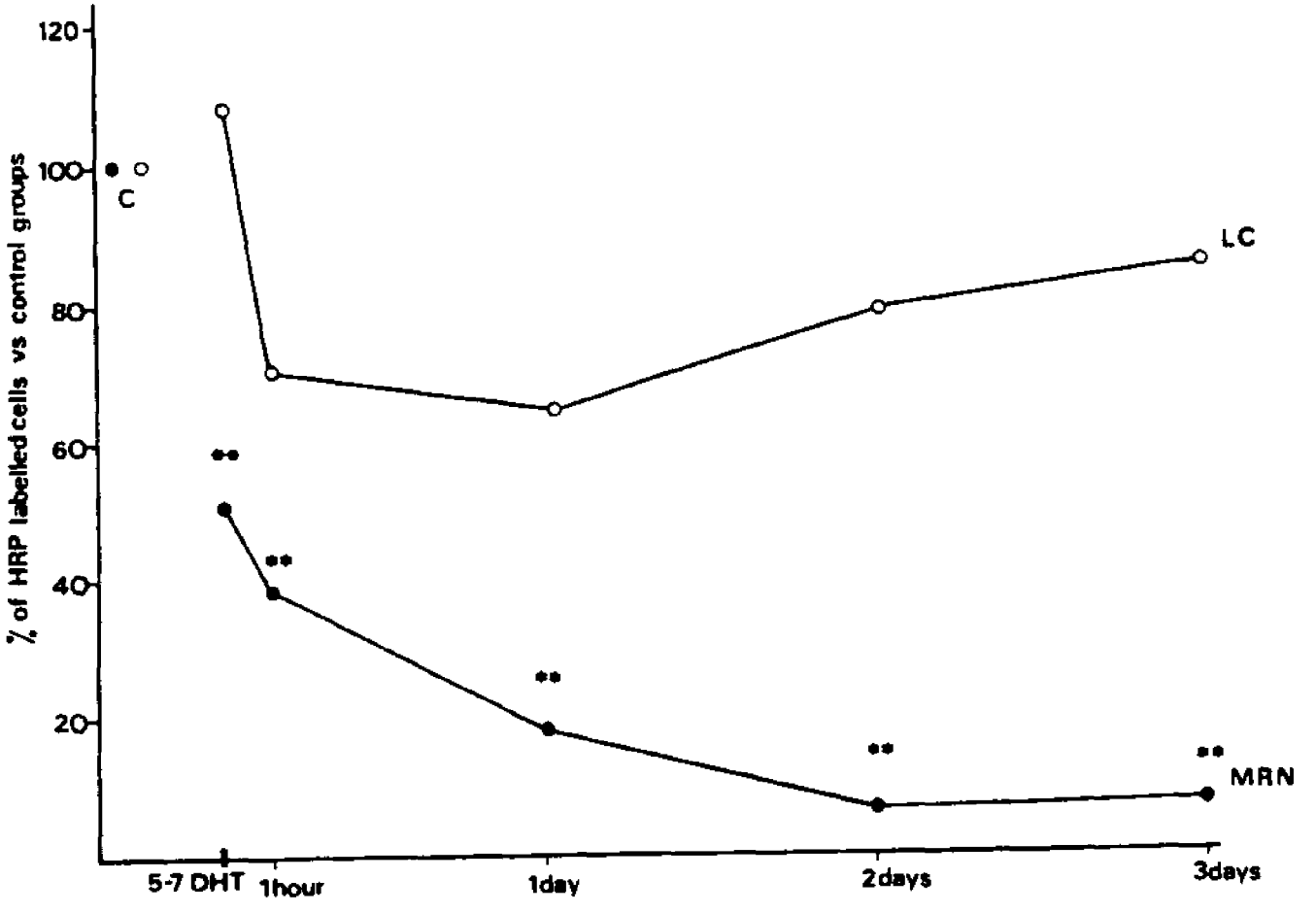


Figure 15: Time course showing effect of 5.7-DHT on 5-HT fibers and NA fibers. The percentage of the total number of HRP labeled cells in LC and MRN are compared to their controls. The sham + DMI groups (C) were taken as 100% controls. The control values were 273±24 neurons for MRN and 94±24 neurons for LC. Statistics shows significant differences from the normal controls (student's t-test):*$p<0.05$; **$p<0.001$.



Discussion

Studies have shown that HRP-retrograde labeling of neurons can be quantified and used to study dynamic changes in an afferent system. HRP quantitative studies in the peripheral nervous system demonstrated enhanced labeling of cells by physiological stimulation (Eiserman and Azmitia, 1982) and established the time course of regeneration after neuronal injury (Yu and Srinivasan, 1981). In the CNS, HRP quantitative studies compared various modifications of the original diaminobenzidine-HRP method (Mesulam and Rosene, 1979; Morrell et al., 1981), and established the time course of degeneration and sprouting after lesion (Goldschmidt and Steward, 1980).

The relative number and nuclear location of the labeled neurons after HRP microinjection into dorsal hippocampus were compared in normal rats and in rats injected with 5,7-DHT into either the CB or the FF. 5,7-DHT produced an immediate, selective, and nearly complete disruption of the raphe neurons projecting to the dorsal hippocampus (>93%). The time course study of the effect of 5,7-DHT on the neurons of MRN and LC suggests that: (1) 5,7-DHT can significantly block retrograde transport in the 5-HT fibers almost immediately after injection (50% reduction in labeled cells within one hour). (2) 5,7-DHT is selective for lesioning 5-HT fibers while leaving the NA fibers largely undamaged when the animals are pretreated with DMI. This provides direct morphological evidence of the selectivity of 5,7-DHT within these two monoaminergic systems, and extends previous biochemical studies which showed no decrease in high affinity $^{3}$H-NA

in hippocampal slices after intracerebral injections of 5,7-DHT (Azmitia et al., 1978; McNaughton et al., 1980). (3) The maximal blocking effect of the 5-HT fibers in the CB and the FF fiber pathways is achieved within 2 days after lesioning by 5,7-DHT (93.5% of labeled cells were eliminated). Immunocytochemical studies support the present finding that 5,7-DHT injections into CB and FF removes all the 5-HT immunoreactive fibers in these two pathways within 2 to 3 days. A further study was performed to investigate the survived cells after 5,7-DHT lesion. The present report agrees with Baumgarten et al (Baumgarten et al., 1981), who reported that the 5,7-DHT effect on 5-HT is substantially decreased by 24 h and there is little further reduction in the 5-HT level by the eighth day.

## 2. Differentiation of Two Raphe-Hippocampal Cell Groups

### Introduction

In order to observe the plasticity among the two groups of 5-HT cells which project through the CB and/or the FF to innervate the DHipp, the population, morphology and location of the two cell groups were studied at the light microscopic level. The 5-HT fibers in the CB or the FF were selectively lesioned to observe the projecting neurons in the other pathway. The labeled neurons were counted after HRP was injected into the DHipp, their topographic distribution was mapped, and their general morphology was studied in the raphe-CB-hippocampal and raphe-FF-hippocampal systems.

Results

Among the approximately 3000 neurons in the MRN, It is found that about 300 neurons innervate the dorsal hippocampus. They generally occupy the entire length of the MRN (about 2.5 mm). The cells are spindle-shaped, with diameters of approximately 12 um x 18 um. The cell bodies are usually oriented with their long axes along the same axis as the nucleus itself.

Raphe-CB-hippocampal neurons and raphe-FF-hippocampal neurons have a very similar distribution pattern in the MRN (figure 16). The shape and general characteristics of the cells in these two groups are indistinguishable (see figures 16 and 17). However, in these two MRN-hippocampal pathways, there were more neurons projecting through the CB (55% of the total MRN-hippocampal projection) than through the FF (21% of the total) (table 1). The remainder of the fibers (23% of the total MRN-hippocampal neurons) appear to have branched into both pathways.

Interestingly, labeled cells were seen in an interfascicular group of cells (IFN), located between the two medial longitudinal fasciculares, in the control and the FF lesion groups. These IFN cells were not seen after a lesion was made in the CB (table 1 and figure 18). Thus, the labeled IFN cells project exclusively through the CB to innervate the dorsal hippocampus (Figure 19).

Figure 16: The photomicrographs show coronal sections of the MRN along the midline of brainstem. (a) labeled neurons in MRN of normal animal, where bridged neurons (indicated by crossed arrow) are frequently observed. (b) neurons in MRN projecting through CB (after 5,7-DHT microinjection in to the FF , and (c) neurons projecting through FF (after 5,7-DHT injection into the CB). The HRP was injected into the dorsal hippocampus. The arrows indicate the HRP-lableled neuron. These MRN-hippocampal neurons are surrouned by a network of median raphe blood vessels (indicated by arrow head).

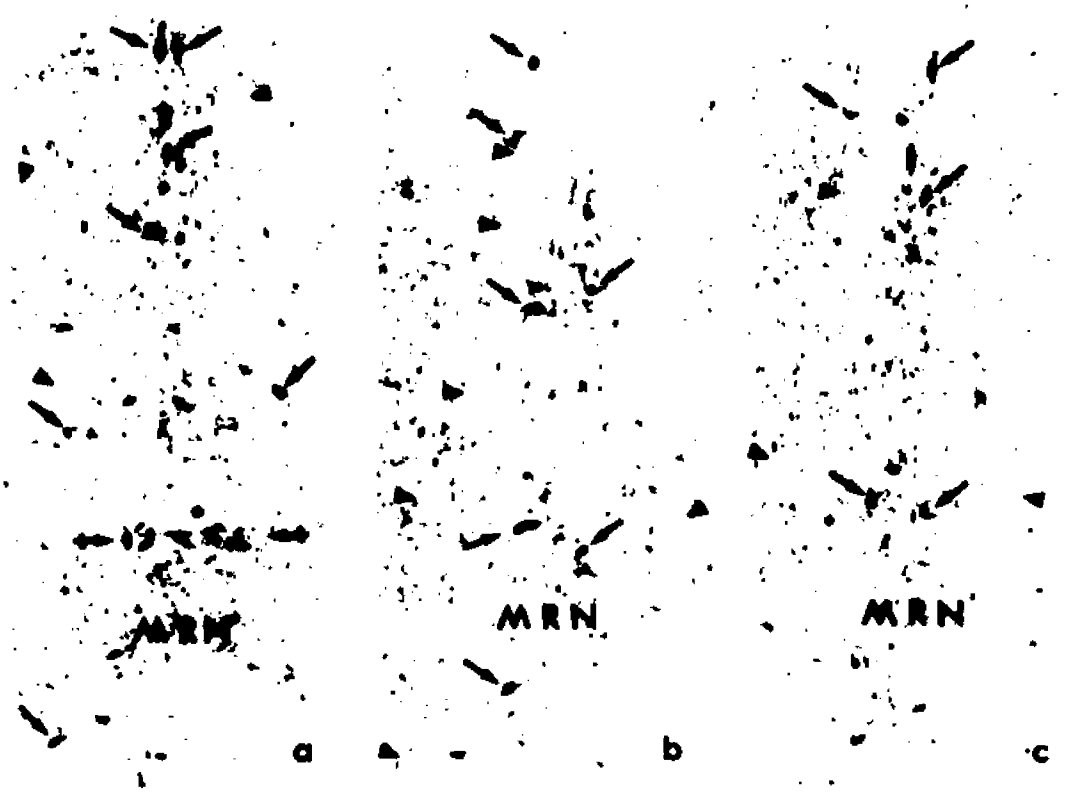


Figure 17: Mapping of median raphe neurons which innervate the dorsal hippocampus. The schematic drawings are representative coronal sections (50 um) of the rat brainstem, approximately 400 um apart. Each circle represents a single neuron labeled by retrogradely transported HRP from the dorsal hdippocampus. Closed circle represents neuron projecting through CB. Open circle represents neuron projecting thorugh FF.

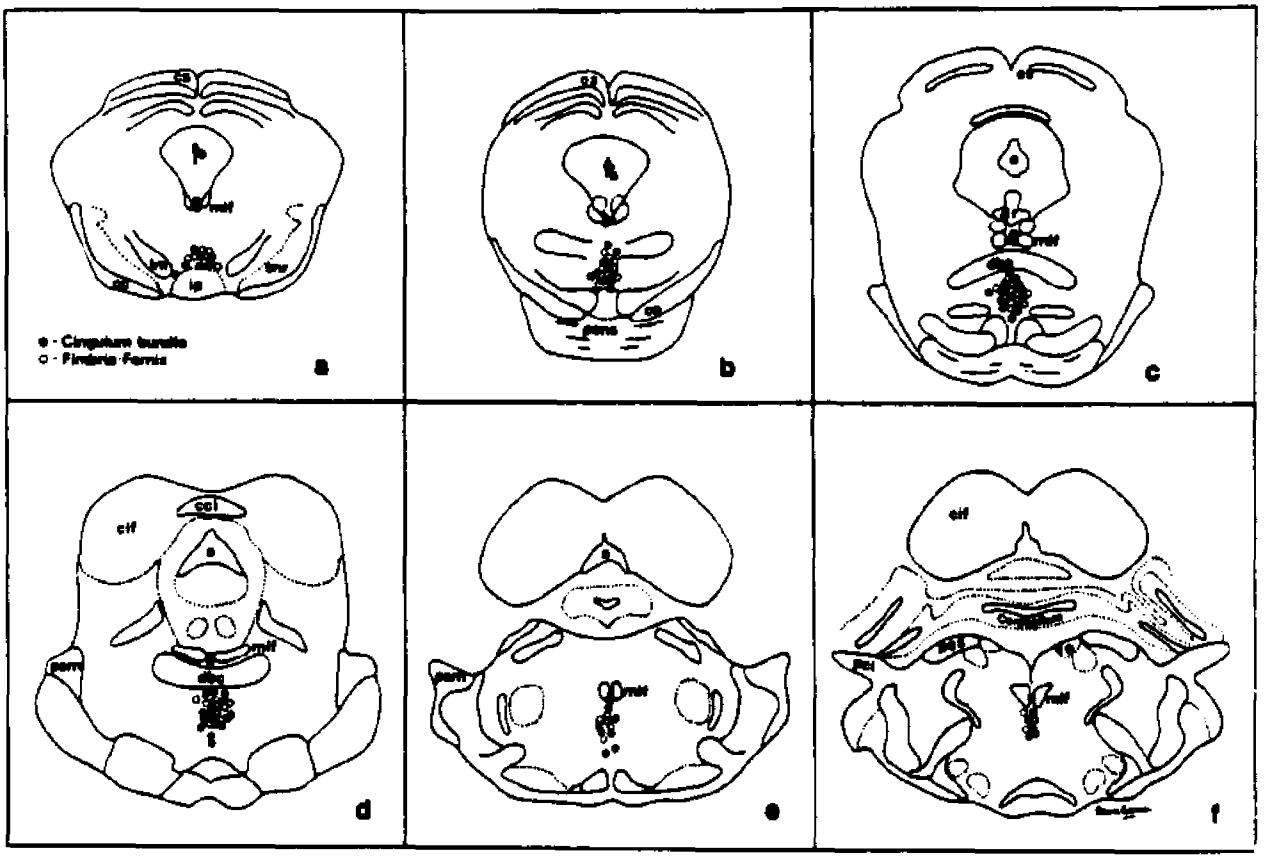


Figure 18: The photomicrographs show coronal sections in medial longitudinal fascicularis (mlf) area of the brainstem. Labeled neurons which innervate hippocampus from the interfascicular nucleus (IFN) are only seen in the normal (a) and FF-lesioned (b) animals, but not in the CB-lesioned animals (c).

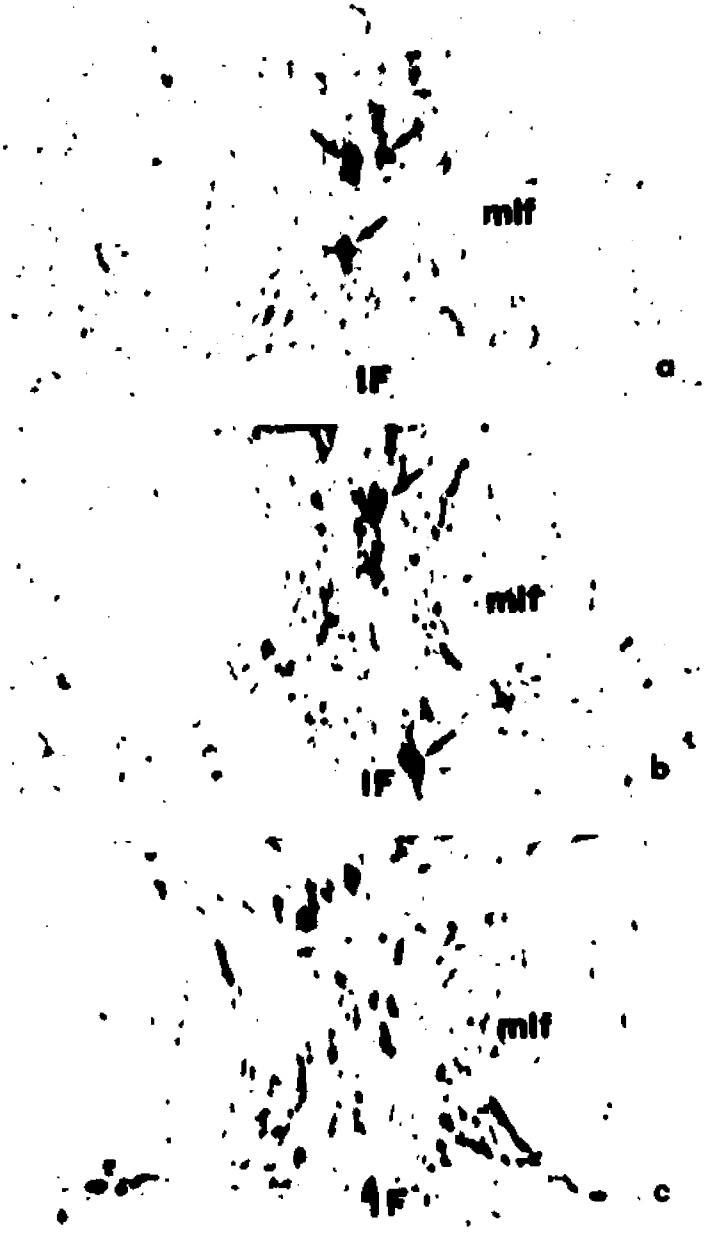


Table 1. The number of HRP labeled cells (mean ± SD) in MRN and IFN aft selective 5,7-DHT lesions of CB/FF.

| | N | MR | | IFN | |
|---|---|---|---|---|---|
| Normal | 6 | 295±39 | | 40±18 | |
| Sham CB | 3 | 312±23 | | 38±4 | |
| Sham FF | 2 | 296±23 | | 43±2 | |
| Lesion CB | 6 | 132±14 | *** | 2±1 | *** |
| Lesion FF | 5 | 232±33 | * | 30±10 | |
| Lesion CB & FF | 5 | 32±9 | *** | 3±2 | ** |

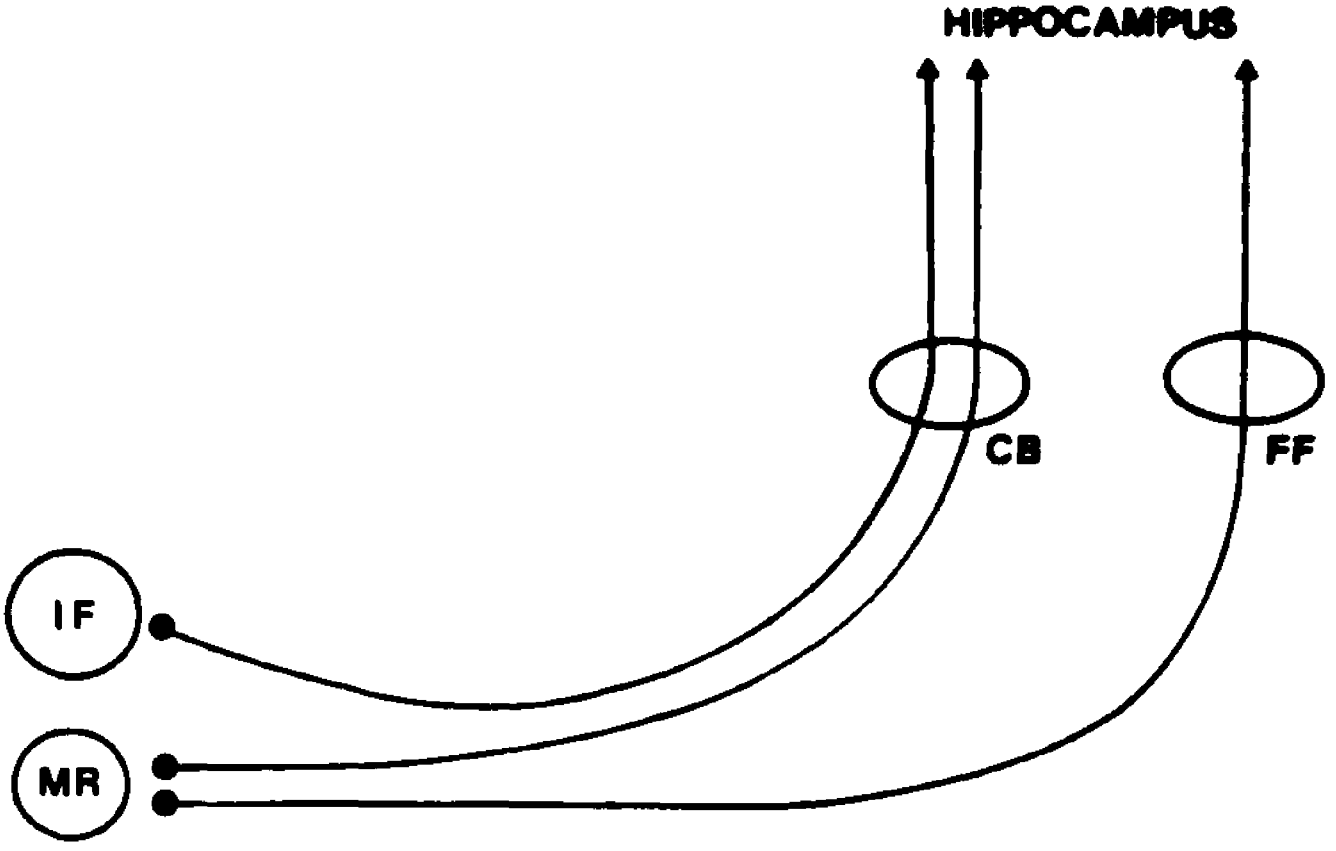
Student's t-test, significant difference from normal control:

* $p<0.05$

** $p<0.02$

*** $p<0.001$

Figure 19: Serotonergic innervation of the dorsal hippocampus. MRN takes the CB and the FF pathways to innervate mainly the dorsal hippocampus. IFN projects only through the CB to reach the dorsal hippocampus.



[illegible] suggest that more neurons project through the CB than the FF, although as many as a quarter of the cells may have branches in both pathways. The finding that the CB is the major MRN pathway is consistent with the observation that cingulum bundle transection reduces 5-HT by 50% in the dorso-medial aspect of the hippocampus, while combined lesions CB and FF produced a decrease of between 85-90% in total 5-HT hippocampal levels (Storm-Mathisen and Woldberg, 1974). Furthermore, measurement of tryptophan hydroxylase after neurotoxin injection (1ug of 5,7-DHT into the CB showed a 63% decrease in enzymatic activity within 6 days (Clewans and Azmitia, 1982). Finally, microinjection into the FF decrease the high affinity uptake of $^{3}$H-5-HT less than 50% (Williams and Azmitia, 1981). These biochemical studies taken together with our morphological findings strongly suggest that the CB is the predominant 5-HT route to the dorsal hippocampus.

The labeled interfascicular neurons project exclusively through this main-route CB to the dorsal hippocampus. Although this group of cells is traditionally classified as part of dorsal raphe, it projects to dorsal hippocampus along with cells in the MRN. This group of cells, along with cells in the lateral wings of the DRN, also innervate the ventral hippocampus. Thus, this area may represent a transitional zone between the MRN and the DRN. This is of interest since these two nuclei subserve different behaviors (Azmitia, 1978).

The MRN-CB-Hippocampal and the MRN-FF-Hippocampal neurons project

through anatomically distinct and separate pathways. Yet they appear to be largely a homotypic group of cells having a number of characteristics in common. They carry the same neurotransmitter and are both blocked by the serotonergic neurotoxin, 5,7-DHT. They have a similar general morphology in that they are both spindle-shaped cells with a diameter of approximately 12 um x 18 um and have overlapping distributions throughout the MRN. These cells share similar hippocampal terminal territories. They produce the same disruption of the septal driving of hippocampal theta rhythm and additive results are obtained after combined injections (McNaughton et al., 1980). Finally, and most important, long term studies after 5,7-DHT microinjections into the CB showed that FF 5-HT fibers could restore both the normal anatomical and functional patterns of the destroyed serotonergic system within the hippocampus. (Azmitia et al., 1978; Zhou and Azmitia, 1981). It may be concluded that these heterogeneous serotonergic pathways are functionally homogeneous within the hippocampus.

## 3. Serotonergic and Nonserotonergic Neurons in Raphe-DHipp System

### Introduction

Are all the raphe neurons which innervate the hippocampus serotonergic? Today, more evidence suggests that nuclei or neuronal regions are not necessary comprised of chemically homogeneous neurons. Non-serotonergic neurons were observed among the serotonergic neurons in the DRN-caudate-putamen system (Steinbusch, 1980), in the 5-HT

bulbospinal system of rats (Bowker, 1981), and in raphe-striatal system in monkeys (Pasik and Pasik, 1983).

Two additional efforts were made to identify the chemical character of raphe-hippocampal neurons in addition to specific lesions of their 5-HT axons towards the hippocampus: 1) Study of the MRN-hippocampal connections using HRP tracing following neurotoxic destruction of 5-HT neurons in the MR and 2) Simultaneous labeling of immuno-reactive 5-HT cells and HRP-retrograde labeled cells in the same section.

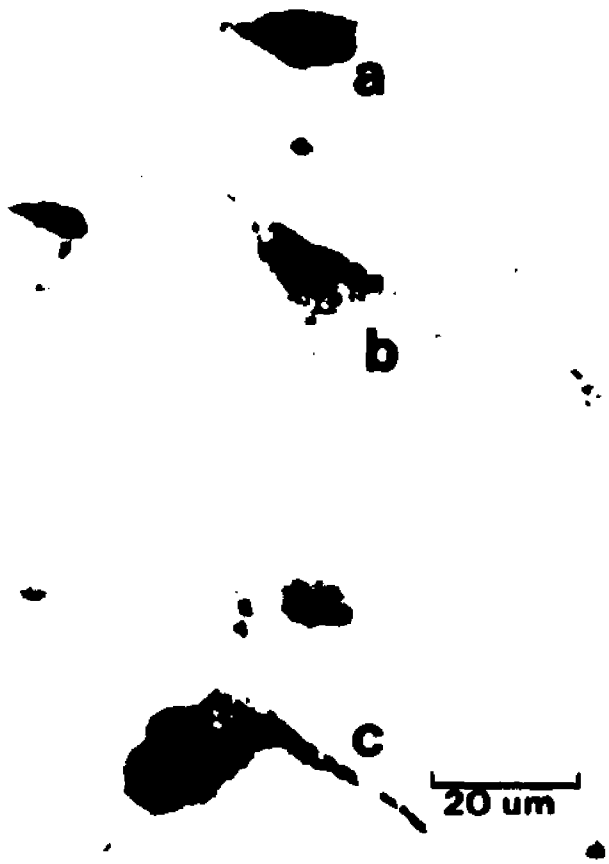
Results

Five days after a 2 ul 5,7-DHT injection into the MR, the 5-HT-IR neurons in the DRN, MRN, and B9 were totally removed. Degenerating 5-HT-IR debris was observed in these raphe areas. In such neurotoxin treated animals, 36 and 22 HRP labeled neurons of each animal were still observed in the MRN after HRP was injected into the DHipp. There were no labeled cells in either the IFN or the DRN (figure 20).

After simultaneous injection of HRP into the DHipp, and immunocytochemical staining, three categories of stained cells were observed in the MRN (figure 21). The majority were 5-HT-IR without HRP. The 5-HT-IR cells labeled with HRP were difficult to visualize, since the $CoCl_2$ enhanced dark HRP labeled granules were frequently masked by dark brown 5-HT-IR staining. Finally, 34±8 cells were HRP labeled without 5-HT-IR staining.

Figure 20: The 5-HT and non-5-HT neurons in the MRN-DHipp system. The 5-HT immunoreactive (5-HT-IR) cells are observed in the median (MRN), dorsal (DRN) raphe and B9 of the brain stem (a). After 2 ul injection of 5,7-DHT into midbrain raphe area, the 5-HT-IR cells were totally degenerated and gradually removed. The degenerated debris is shown by arrow in (b) and (c). However, after HRP injection into DHipp of such treated animals, a few HRP labeled cells are still observed in the MRN (d).

Figure 21: After HRP injection into dorsal hippocampus, three groups of cells were observed distinctly by anti-5HT antiserum reaction and HRP-diaminobenzedine staining: 5-HT immunoreactive (5-HT-IR) cell (a), HRP reactive cell (b), and 5-HT-IR and HRP double labeled cell (c).



Discussion

Previous data have shown less than 7% of the MRN-hippocampal cells were still labeled after both the CB and FF pathways were chemically lesioned. Several possibilities exist to explain these remaining MRN cells. 1. Myelinated Fibers: The efficiency of 5,7-DHT may not be equal in all 5-HT containing fibers. For example, myelinated fibers immunoreactive to a 5-HT antibody have been seen in the medial forebrain bundle of the rat (Azmitia and Gannon, 1983). 2. Alternate Pathways : Additional routes to the dorsal hippocampus outside of the CB and FF may not be damaged by the injections. The existence of several minor pathways have been observed using both radioautography with $^{3}$H-5-HT and immunocytochemistry with a 5-HT antibody (Azmitia, unpublished observation). 3. Non-Serotonergic cells: The existence of non-serotonergic neurons in the midbrain raphe projecting to the dorsal hippocampus has been reported. In a previous study from our laboratory, 15% of the raphe neurons retrogradely labeled by HRP were not labeled by $^{3}$H-5-HT injections into the dorsal hippocampus (Azmitia, 1978). Kohler and Steinbusch believe that a quarter of the MRN-hippocampal cells are non-serotonergic since they found that after injection of granular blue into the hippocampus 25% of the retrogradely labeled cells in the MRN were not 5-HT immunoreactive (Kohler and Steinbusch, 1982). Finally, the transport of HRP from the hippocampus to the MRN was still detected in about 29 of 300 MRN-hippocampal neurons following 5,7-DHT lesions directly into the MRN and DRN, While similar portion of neurons (about 10%) were HRP labeled in MRN by injection in DHipp but not 5-HT immunocytochemically stained. The

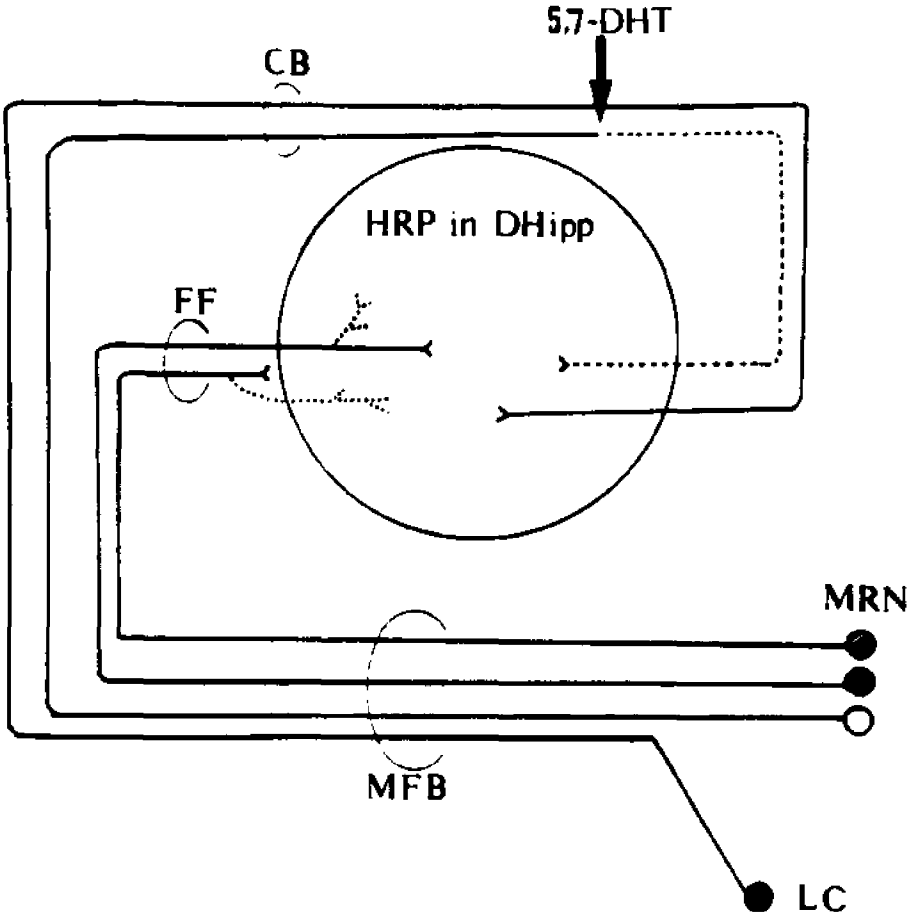
resolution of the discrepancies in the percentage of nonserotonergic neurons (7%-25%) requires further work, especially since electrophysiological studies have found that the vast majority of midbrain raphe cells with long axons can be pharmacologically identified as serotonergic (Aghajanian et al., 1978).

## 4. Homotypic Sprouting: An HRP Study

### Introduction

The dynamic change of 5-HT fibers in the hippocampus after a 5,7-DHT lesion in the CB was studied using HRP technique. The ability of raphe terminal fibers in the DHipp to take up HRP and transport it in a retrograde fashion to their neurons in the MR was used as a parameter to study the dynamic change after lesioning. The reorganization of the population, the location and the morphology of the labeled cells in MR were studied after damage to their 5-HT axons in the CB using the neurotoxin-HRP combination method (figure 22).

Figure 22: Normally the 5-HT and NA terminal fibers within the HRP injection site of dorsal hippocampus (DHipp) will take-up HRP which will be retrogradely transported to their neurons in the median raphe nucleus (MRN) and locus ceruleus (LC) (shaded circles). After 5,7-DHT lesion in the CB, the 5-HT fibers are degenerated (shown in dash line) and thois results in an unlabeled cell in the MRN (open circle). When sprouting occurs in the 5-HT fibers projecting through FF, the sprouting fibers (shown in dotted line) will take-up extra HRP and result in newly labeled cells or more intensive labeled cells in the MRN (solid circle).



The 5-HT fibers in the CB were lesioned using 5,7-DHT. The reorganization of labeled cells 3, 7, 14, 21, and 42 days post CB-lesion were compared with animals sham-lesioned at several of these time points, as well as normals. Sequential 5,7-DHT lesions were made in the FF to identify the character of the regrowing fibers.

A futher study of the reorganization of the raphe-hippocampal system was performed by lesioning of both major MRN-hippocampal connections. The reorganization of labeled cells in the MR was subseqently observed 3, 14, and 42 days post CB-FF-lesion.

Results

The dorsal hippocampi were sectioned and stained with diaminobenzidine (DAB) to assure the consistency of injection coordinates. A 100 nl injection of a 10% HRP-saline solution was confined to the ipsilateral side of the dorsal hippocampus. The stained neurons in the brain stem area were observed mainly in the MRN, locus ceruleus, and the interfascicular nucleus (IFN).

(A) Reorganization of Cell Population

(I) Median raphe nucleus

The number of labeled cells in MRN (see figure 23) of normal

control (303±37) and sham control (296±33 ) were not statistically different from each other. 5,7-DHT has an immediate, specific, and almost complete blocking effect on serotonergic fibers of DMI pretreated rats. Three days after 5,7-DHT lesion in CB, the number of labeled cells was significantly decreased about 54% from normal (132±14). The number of labeled cells in MRN remained significantly low at 7 days (136±46) and 14 days (144±24) post-lesion. These cells were mostly spindle-shaped with diameters of approximately 12um x 18um.

21 days post-lesion, the number of HRP labeled cells in the MRN returned to a normal level (2% lower than normal control and 7% lower than corresponding sham control). 42 days post-lesion the number of labeled cells increased to 38% above the corresponding sham control.

Although the number of labeled cells in long-term CB-lesioned (21 and 42 days post-lesion), morphologically and topographically, the labeled cells of long-term CB-lesioned animals are indistinguishable from those seen 3 days post-lesion or normal (figure 24). The density of HRP-reactive granules is appreciablely increased in the labeled cells in the MRN of long-term CB-lesioned animals.

To determine whether the newly labeled cells traveled in the CB (regenerating fibers) or in the FF (collateral sprouting fibers), a second 5,7-DHT lesion in the FF was made 2 days before HRP injection in previously CB-lesioned animals. The number of labeled neurons in MRN was almost totally eliminated in 3, 21 and 42-day CB-lesioned animals (8% 7% and 10% left respectively). The number of labeled cells left after the FF lesion of long-term CB-lesioned rats was similar to the

number of labeled cells seen after combined CB-FF neurotoxin lesions (32±9) but was much lower than the number of cells seen after FF lesions in normal rats (232±33).

(II) Interfascicular nucleus

A group of HRP labeled cells is seen clustered between the MLF after injection into the DHipp of normal rats. These cells are spindle shaped and have a vertical orientation which differs from labeled cells in either the MRN or DRN, which are oriented horizontally or obliquely (figure 18). A previous study has shown that cells in this group, the interfascicular nucleus (IFN), travel only through the CB to innervate dorsal hippocampus (Zhou and Azmitia, 1983). Nearly all of the cells in the IFN were eliminated by the 5,7-DHT lesion in the CB. The number of labeled cells in IFN remained low even at 42 days post-lesion (Table 2), although at these long term survival times the number of HRP labeled cells in the MRN had significantly increased.

(III) The labeled cells in DRN after the CB and the FF lesion

Normally, the labeled cells after HRP injected into DHipp, were observed in the MRN (about 300), the IFN (35±15) and few, if any, in the DRN near the junction of IFN. Three and fourteen days after 5,7-DHT injection into the CB and the FF, the number of labeled cells in MRN after HRP injected into DHipp was reduced about 91% and 93% respectively. The labeled cells in IFN were eliminated ,while DRN remained unlabeled. However, at 42 days CB-FF lesion, the number of

labeled cells in MRN (31±9) and IFN (2±1) remained low. But a group of HRP labeled cells (82±13) were observed appeared in the ipsilateral wing of DRN near the aqueduct in all three animals (figure 25).

Figure 23: Diagram shows the number of HRP labeled cells in the CB-lesioned animals (solid square) after HRP injection in the DHipp at various post-lesion time. The number of labeled cells is substantially decreased 3-day post-CB lesion as compared to normal (open circle) (P<-002) and corresponding sham (open square) (P<.002). The number of HRP labeled cells remains low in 7 and 14 day post-CB lesion. However, the number of HRP labeled cells is significantly increased at 21-day and 42-day post-CB lesion. (3-day vs 21-day: P<.001; 21-day vs 42-day:P<.002). However, when the FF of the CB-lesioned groups were lesioned 2 days before HRP injection, the number of labeled cells (solid circle) in MRN are mostly removed in 3-day, 21-day, and 42-day post-CB lesioned.
Solid squares: CB-lesion groups
Solid circles: CB-FF-lesion groups
Open squares: corresponding sham
Open circle: normal

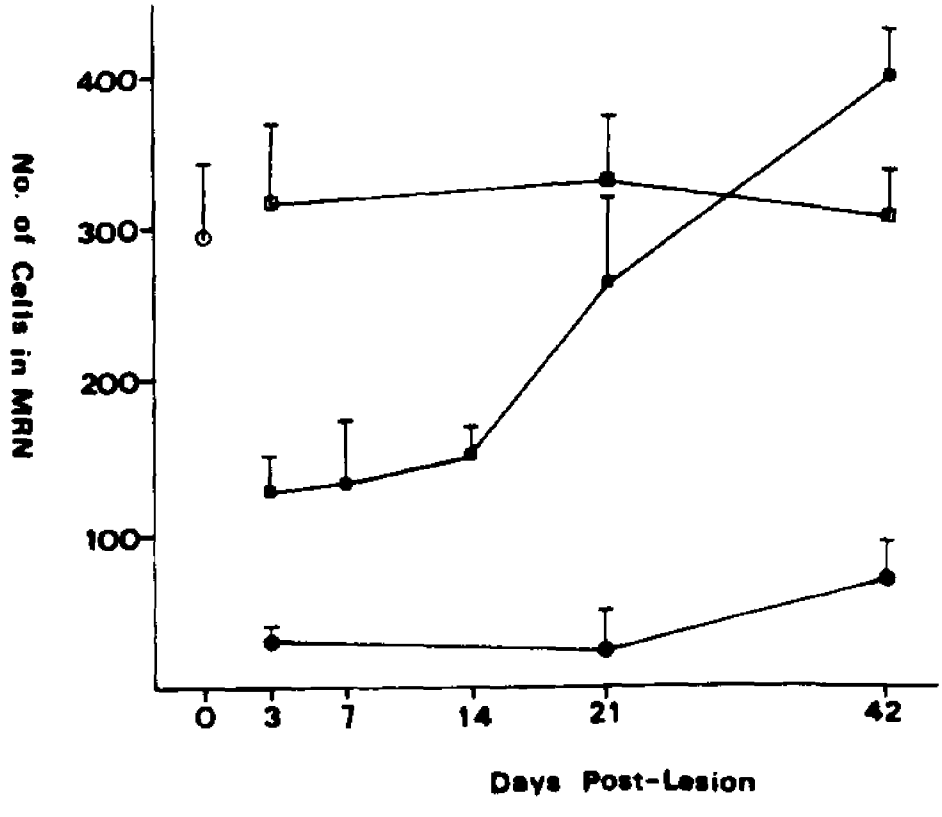


Figure 24: The photomicrographs show coronal section of the MRN in the brainstem. The labeled neurons after HRP injection in DHipp are shown in the normal (a), 3-day (b), 21-day (c), and 42-day (d) post-CB lesion animals. The number of HRP labeled cells were significantly decreased at 3-day post-lesion, but increased to about normal level at 21-day and above normal at 42-day post-lesion. The arrows indicate labeled cells.

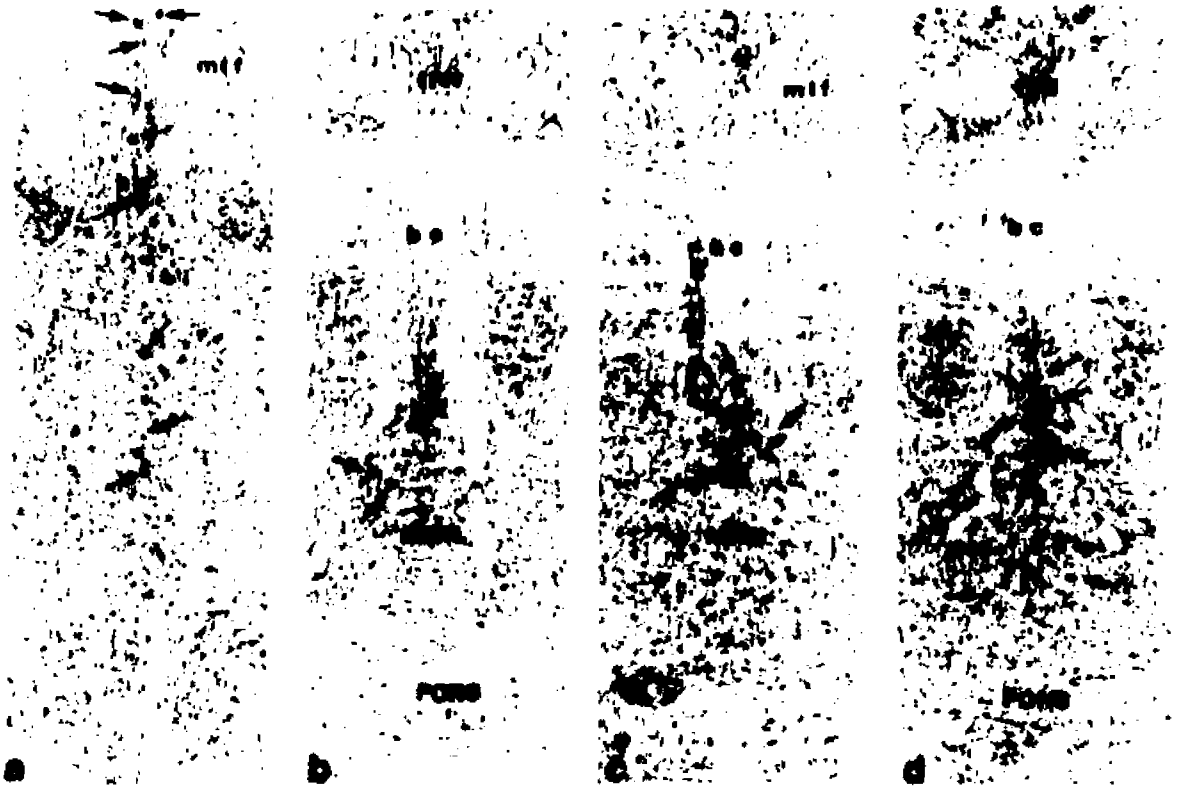
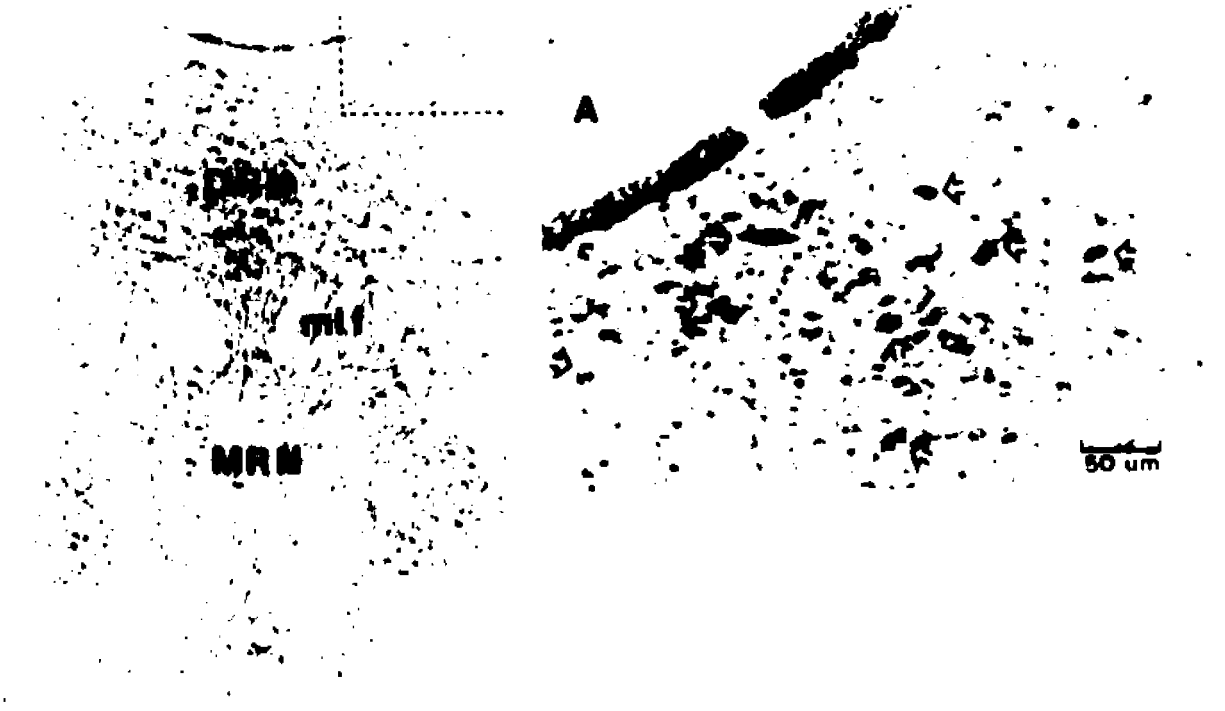


Table 2: The number of labeled cells in the interfasciular nucleus throughout the time course. Note that nearly all of the cells in the IFN were eliminated by a 5,7-DHT lesion in the CB. These cells did not regenerate back into the hippocampus even at the 42 days post lesion.

| group | number | days post-lesion | number of cells in IFN |
|---|---|---|---|
| normal | 6 | — | 35±15 |
| sham | 3 | 3 | 38±3 |
| | 3 | 21 | 32±5 |
| | 3 | 42 | 39±18 |
| CB lesion | 6 | 3 | 2±1 |
| | 6 | 21 | 2±1 |
| | 5 | 42 | 3±1 |
| CB+FF lesion | 3 | 3 | 2±1 |
| | 3 | 21 | 1±2 |
| | 3 | 42 | 2±2 |

Figure 25: Photomicrographs show coronal section in the midbrain level. The labeled cells after HRP injection in the DHipp at 42-day post-CB and FF lesion. the labeled cells are mostly removed in the MRN. However, a group of labeled cells appears in the ipsilateral wing of the DRN. This group of cells is not normally labeled by HRP injection in the DHipp but by HRP injection into the ventral hippocampus. Arrows indicate the HRP labeled cells. A: aquiduct.



Discussion

Normally there are 303±37 HRP labeled cells detected in the MRN after injection of 100nl of 10% HRP into DHipp. Three days after 5,7-DHT injection into CB, approximately 158 cells (54%) lost their connections with the hippocampus as a result of damage to their fibers. At this stage, the labeled cells which have survived after CB lesion are predominately projecting through the FF because a second lesion 3 days after the first injection into the CB in this pathway reduced the number of labeled neurons to 28±24. The cells in the CB-lesioned group have no particular topographic distribution to distinguish them from neurons in the FF lesioned group (except for IFN, see below). The size of these labeled cells after CB injections are similar to that of adjacent unlabeled neurons.

The number of labeled cells in MRN remained significantly low 7 and 14 days post-lesion. However, by 21 days after CB lesion, the number of labeled cells significantly increased as compared to 3 days post-lesion and approached the normal level. Approximately 155 more neurons in MRN were now labeled by HRP retrograde transport from the dorsal hippocampus. These results suggest that the fibers of the MRN cells in hippocampus spared by the CB lesion had increased the amount of HRP transported presumably by expansion of their terminals within the denervated area. Tryptophan hydroxylase activity in the hippocampus of CB lesioned animals dropped at 3, 7, and 14 days post-lesion and started to increase by 28 days post-lesion (Clewans and Azmitia, 1982). Thus, the biochemical studies show a similar temporal

pattern.

The increased number of labeled cells in 42-days lesioned animals compared to normal animals (Figure 23) suggests hyperinneration by MRN fibers in the hippocampus. An average of 90 (29.7%) more cells in MRN are innervating the hippocampus. A similar hyperinnervation in the dentate gyrus was observed by anterograde transport of $H^3$-proline from the MRN 90 days after CB lesion (Azmitia et al., 1978). Evidence for hyperinnervation by regenerating 5-HT fibers after long-term 5,7-DHT lesion has also been reported in the hypothalamus (Frankfurt and Azmitia, 1982) and in the medulla (Wiklund et al., 1980).

Two additional explanations of the increased cellular labeling in the long-term CB-lesioned rats is that the amount of HRP retrogradely transported is increased in existing fibers due to loss of competition from other fibers in the injection area or to changes in uptake and transport rates. Surviving fibers in the terminal area would have a greater opportunity to sequester HRP molecules if a major input was removed, such as the entorhinal afferents (see discussion by Stewart and Vinsant, 1978; Goldschmidt and Stewart, 1980). However, the serotonergic innervation density in the hippocampus is approximately 100 boutons/10,000 $u^2$ which represents about 0.5% of the total bouton density in the hippocampus (Koda and Bloom, 1977). Similarly, in the cerebral cortex, the percentage of boutons containing 5-HT is 0.1% (Beaudet and Descarries, 1976).

The other alternative explanation is that the HRP uptake reflects a increase in uptake by undamaged axons. Electrical (Litchy, 1973;

Nishino et al., 1979) and chemical (Eiseman and Azmitia, 1982; Dolivo et al., 1977) stimulation of peripheral nerves increases either the uptake and retrograde transport of HRP. Axotomy of the optic nerve reduces the uptake of HRP in the first four hours and then increases the uptake of HRP for the next fourteen hours after lesion (Halperin et al.,1975). The relevance, if any, of these observations to studies of long-term sprouting is not known.

(A) Sprouting versus regeneration

Is the increase in the number of HRP labeled cells due to the regeneration of raphe-hippocampal fibers thrcugh the CB or to collateral sprouting fibers through the FF? A second neurotoxin lesion in the FF pathway of previously CB lesioned animals was found to eliminate the labeled cells in the MRN (Figure 23). The second lesion was made in the FF rather than the CB because performing two temporally separated lesions in the same site (CB) might be confounded by a "conditional lesioning effect" - axonal regeneration following an axotomy is altered as a result of axon having undergone a previous injury (Mcquarrie et al. , 1973, 1978; Forman et al., 1980). Our results, therefore, indicate that the increase in the number of labeled cells in the MRN of long-term animals is due to the growth of undamaged 5-HT fibers traveling within the FF. It is a compensatory growth of intact fibers in response to the selective and localized damage of 5-HT fiber in CB.

The second fact in favor of collateral sprouting is the

observation that cells in the IFN did not reappear. The IFN is a group of neurons situated between the MLF, which only takes the CB route to innervate hippocampus (Zhou and Azmitia, 1983). HRP labeled neurons in the IFN were eliminated by 5,7-DHT lesion in CB, but not affected by 5,7-DHT lesion in the FF. Label in this group of neurons never returns even after 42 days post-CB-lesion. This observation is consistent with the interpretation that the HRP labeled cells which appear after 21 days are not regenerating damaged fibers in the CB.

In the CB and FF lesioned animals after HRP injection in the DHipp, labeled cells in MRN were mostly removed 3 days post-lesion. This low level of HRP labeling have remained up to 42 days. Without significantly increase. Since the MRN is the major resource of 5-HT projection to DHipp, obviously there were no regeneration from MRN or the regenerating fibers can not reach the DHipp in 42 day post-lesion.

Interestingly, at this stage, a group of HRP labeled cells were observed in the lateral wing of DRN which usually send 5-HT fibers to ventral hippocampus (VHipp). The 82 DRN cells obviously sprouts new fibers from the VHipp to innervate the denervated DHipp, when regenerating fibers from MRN fail to do so at 42 days post-lesion. The competition of these two different regrowth is further discussed in the general discussion section.

(B) Homotypic versus heterotypic sprouting

Homotypic sprouting is a compensatory growth of undamaged fibers which carry the same transmitter as the damaged fibers, while

heterotypic sprouting is the growth of a different chemical fiber type in response to damage of a neighboring fiber. In the present study using the raphe-hippocampal dual innervation model, the microinjection of 5,7-DHT limits the damage to a single fiber type (5-HT) in the CB pathway. Furthermore, a testing lesion using 5,7-DHT injection into the sporuting tract in the FF of long-term CB lesioned animals eliminated most of the increased labeling. This supports the hypothesis that the sprouting fibers in the FF are also serotonergic. Thus, sprouting of 5-HT fibers traveling in the FF is a response to the removal of a single type 5-HT fibers in the CB, a homotypic sprouting.

## 5. Homotypic Sprouting: An Immunocytochemical Study

### Introduction

The organization of 5-HT fibers in the hippocampal terminal field after partial denervation was also studied. In the 1970's, fluorescent histochemistry provided a detailed profile of degeneration and regrowth of reactive fibers after damage. However, this technique has two major drawbacks: the fluorescent product of 5-HT, the beta-carboline, is extremely sensitive to ultraviolet light. This results in a rapid decomposition and fading of the yellow fluorescent product. Secondary, recent methodological modifications of fluorescence staining remarkably increase its sensitivity for catecholamine, but do not seem to provide any clear improvement in visualization of indolamine (IA).

Furthermore, it is often difficult to distinguish between the green and yellow fluorescence.

Immunocytochemical staining provided direct visualization of 5-HT containing fibers with a high degree of sensitivity and specificity. The distribution and the morphology of the 5-HT fibers in the CB, the FF, and the DHipp were examined using immunocytochemical staining.

Results

In normal animals, the thick, straight 5-HT immunoreactive (5-HT-IR) axons in the CB were observed to be accumulated in the medial portion of this bundle, throughout its length, projecting backwards to enter the dorsal hippocampus, while in the fornix-fimbria they were scattered evenly in the whole bundle before entering the hippocampus. In the dorsal hippocampus, a restricted laminar pattern characterizes the 5-HT innervation of the hippocampus (figure 26). Tortuous, fine 5-HT-IR fibers with large varicosities are densely distributed in the stratum lacunosom-moleculare and stratum oriens of the cornu Ammonis, and in the infra-granular layer of the dentate gyrus. The rest of the hippocampus had fewer of these fibers, in varying amounts. Super-dense 5-HT-IR fibers with abundant varicosities were evident in the fasciola cinerea (FC, between CA1 and subiculum).

Three days after a CB lesion, the 5-HT-IR fibers in the CB distal to and around the lesion were degenerated and mostly absent, with dark dropletlike stumps (DDS) scattered around, while on the contralateral

side, the 5-HT-IR fibers in the CB were normally distributed with slight degeneration near the midline. The 5-HT-IR fibers in the CB-lesioned dorsal hippocampus were distributed in a pattern similar to the normal, but the density was reduced, especially in CA1 area and the infragranular layer of the low arm in the dentate gyrus (figure 27). DDS were detected, and were most prominent in the area of the FC.

At 14 days post-lesion, no evidence of increase in density of the 5-HT-IR fibers was shown, nor was the distribution pattern changed in the dorsal hippocampus as compared with that of a 3-day CB-lesion. DDS gradually disappeared. However, a few fine 5-HT-IR fibers were observed budding from the remaining DDS (figure 28).

At 42 days post-lesion, the 5-HT-IR fibers were densely distributed in the dorsal hippocampus ipsilaterally as compared to those in contralateral side and normals (figure 29). They were more dense ipsilaterally than contralaterally in the FC. No restoration of 5-HT-IR fibers in the ipsilateral CB was shown, but a dramatic increase of 5-HT-IR fibers in the ipsilateral fimbria adjacent to the hippocampus was observed as compared with the contralateral side and normals (figure 30).

Figure 26: Dark field photomicrographs show the normal distribution of 5-HT fibers in the dorsal hippocampus. 5-HT immunoreactive (5-HT-IR) fibers attached with large varicosities are densely distributed in the infra-granular layer of the dentate gyrus (DG) (B), and in the stratum lacunosom-moleculare (S.L-M) (C) of cornu Ammonis (CA). The rest of the area is sparsely distributed in variant degree. A super-dense 5-HT-IR fibers with abundant varicosities is observed in the fasciola cinerea (FC) (A). CC: corpus callosum; GR: granule layer.

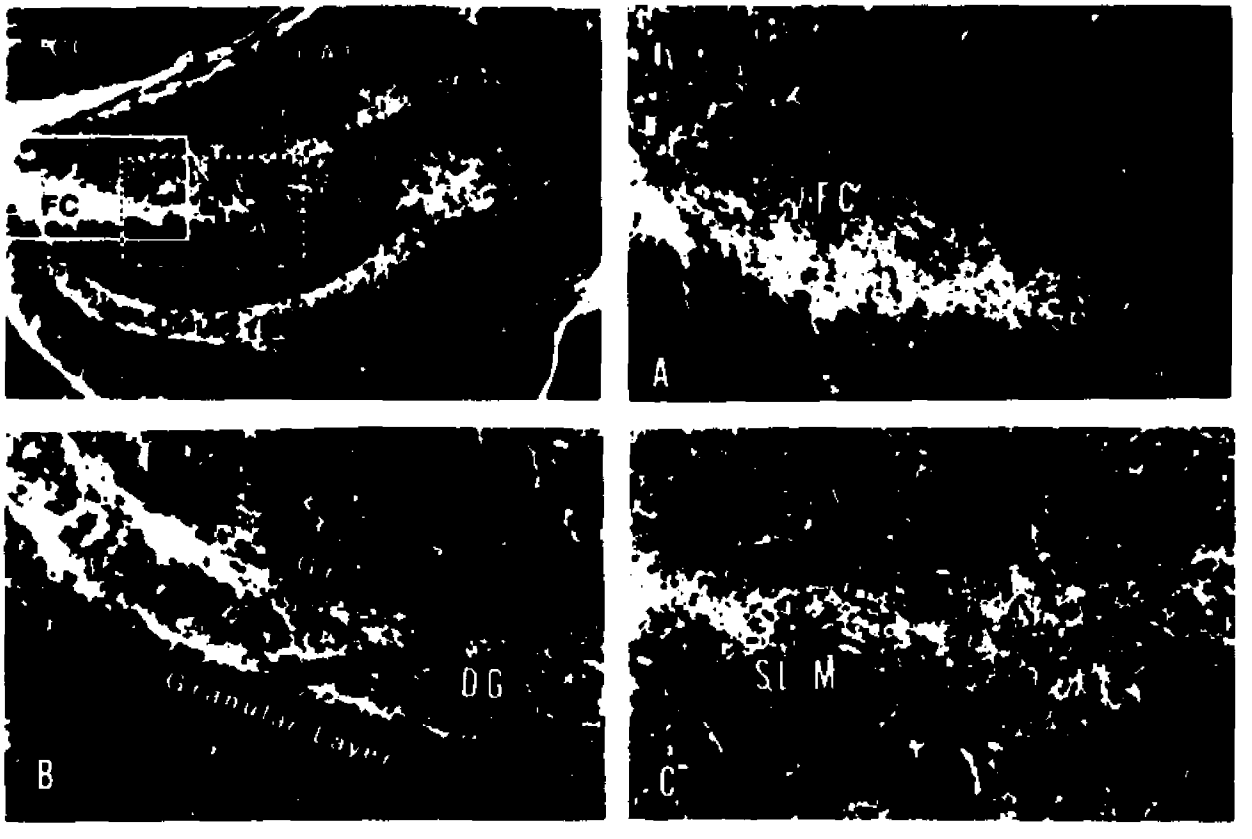


Figure 27: 5-HT immunoreactive (5HT-IR) fibers in dorsal hippocampus of 3-day post-CB lesion animals. The 5-HT-IR fibers in the dorsal hippocampus are distributed in a pattern similar to the normal, but the density is reduced in all the area, especially in CA1 area and infragranular layer in the dentate gyrus (DG). CC: corpus callosum, CA1: cornu Ammonis area 1, FC: fasiola cinereum, ipsi: ipsilateral side of the lesion, Pr: pyramidal cells layer.

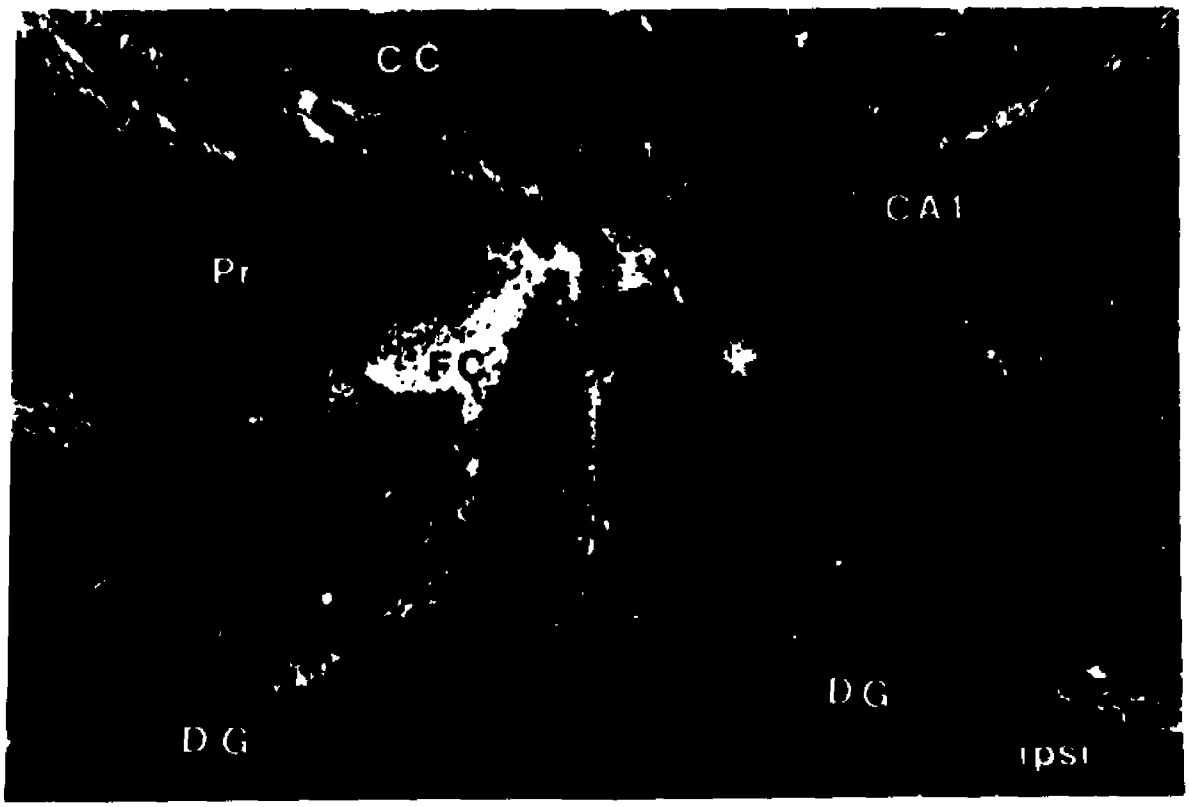


Figure 28: Regenerating fibers (shown by arrow) observed budding from a stump of degenerating fiber in the cingulum bundle 14 days after 5,7-DHT lesion.

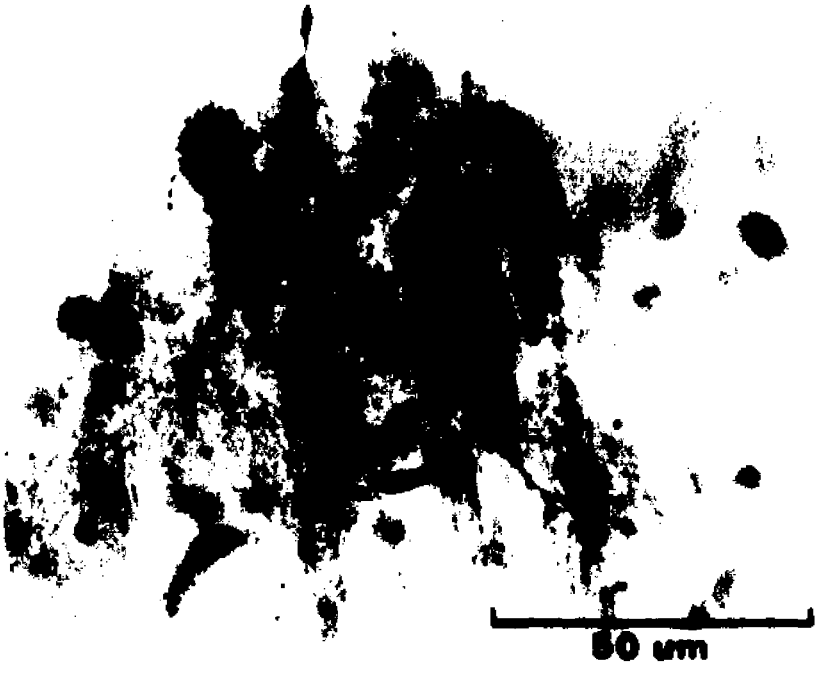


Figure 29: 5-HT immunoreactive (5-HT-IR) fibers in dorsal hippocampus of 42-day post-CB lesioned animals. The density of 5-HT-IR fibers is greatly increase in the fasiola cinereum (FC), the infragranular layer of the dentate gyrus (DG), and the stratum lacunosom-moleculare of CA1 to CA3. A supra-normal density of the 5-HT-IR fibers is observed in the FC as compared with its contralateral side. CC: corpus callosum, CA: cornu Ammonis, DF: dors fornix, GR: granule cells layer, ipsi: ipsilateral side of the lesion.

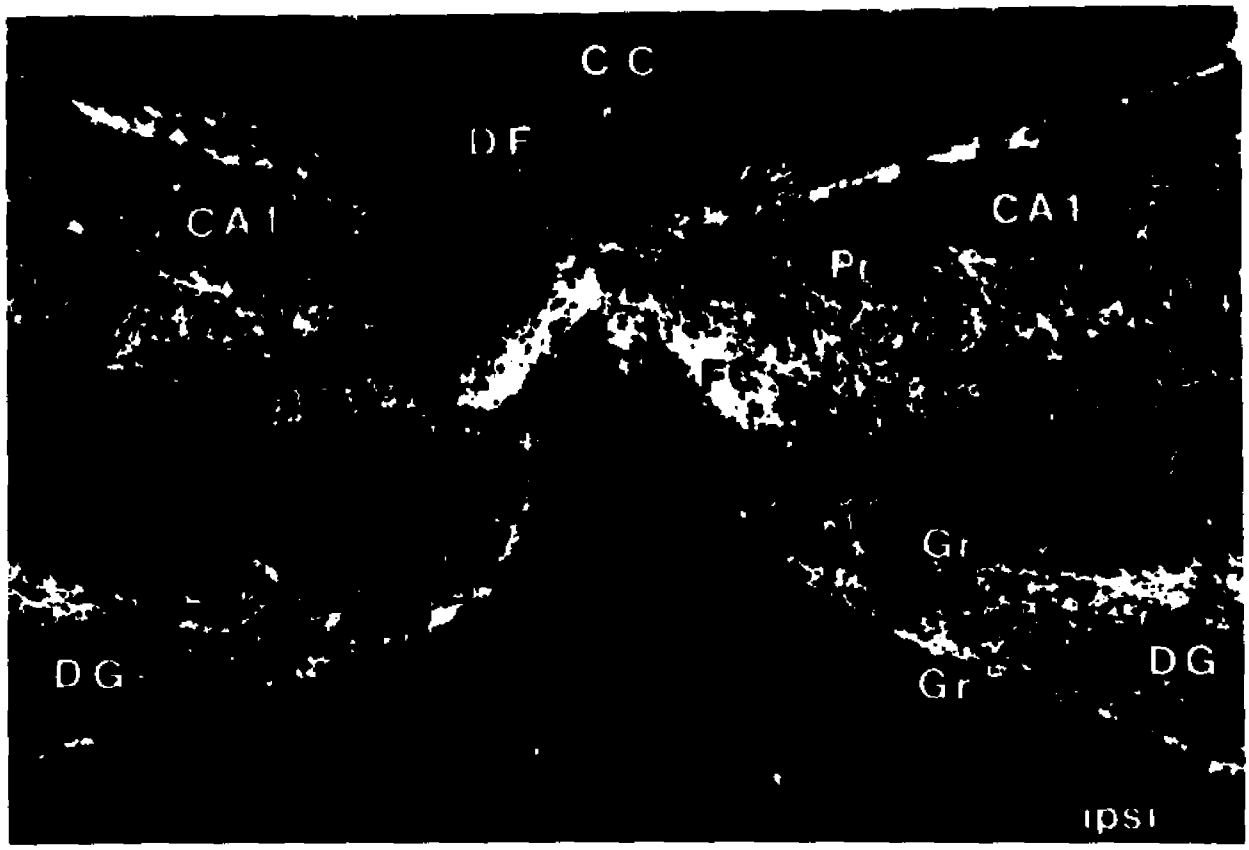
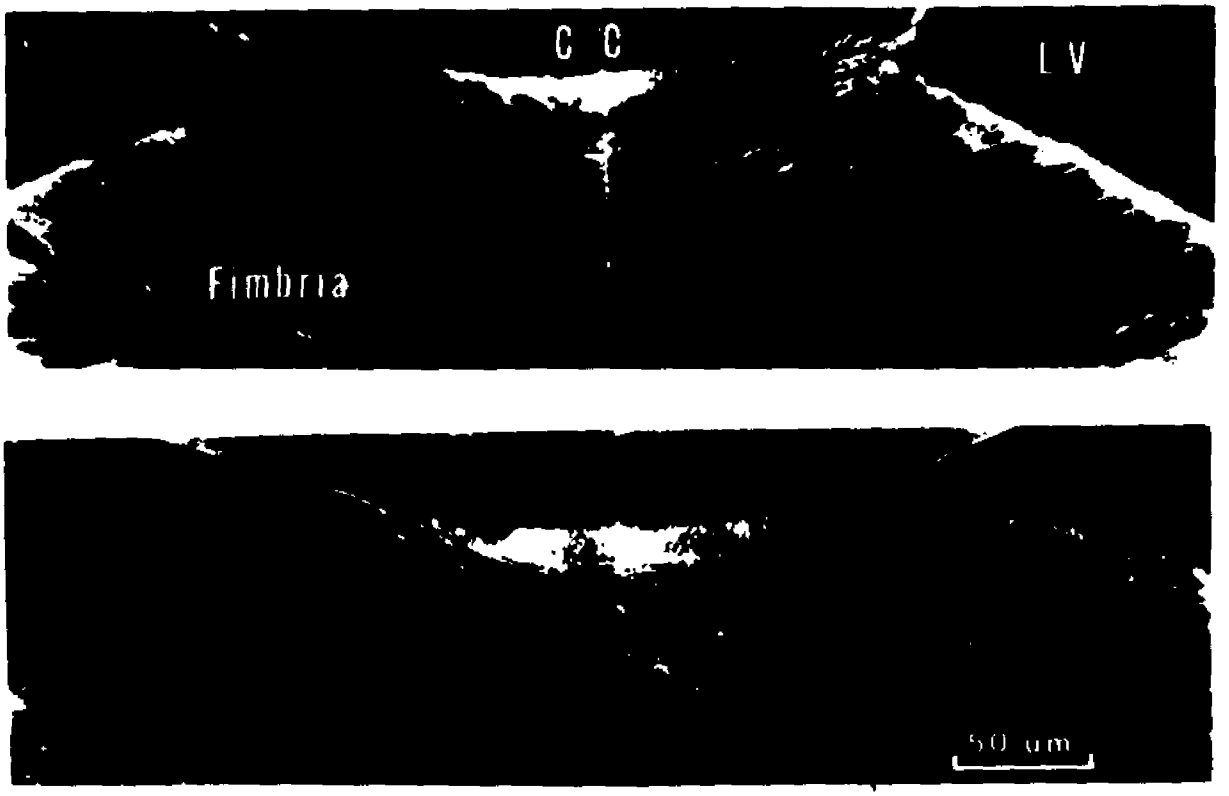


Figure 30: The 5-HT immunoreactive (5-HT-IR) fibers in the fimbria of 42-day post-CB lesion animals (upper) are more dense in the ipsilateral side (right side) than in the contralateral side and in the normals (lower). LV: lateral ventricle.



Discussion

The 5-HT-IR terminal fibers were clearly observed only after pretreatment with L-tryptophan and pargyline. The 5-HT-IR terminal fibers in the DHipp were observed distributed in a similar laminar pattern to that previously reported by Azmitia (1980), who used autoradiographic localization of $^{3}$H-5-HT reuptake, and by Lidov et al (1980), who used anti serotonin-BSA immunohistochemical staining. Abundant 5-HT-IR fibers with extremely dense varicosties in the FC (figure 26, A) are reported here for the first time. It will be interesting to investigate the electrophysiological pattern and functional significance of 5-HT synapses in this area.

At 3 days post CB-lesion, the degeneration profile of 5-HT fibers in the DHipp and the CB were similar to that reported in the spinal cord and medulla as elucidated by histofluorescence staining (Wiklund and Bjorklund, 1980). The most prominent features of this period is the occurrence of swollen and distorted densely stained axons, and DDS on the proximal side of lesioned 5-HT fibers. These thick 5-HT-IR axons and DDS are presumably dependent on the accumulation of 5-HT by continued anterograde transport in the lesioned neurons. The DDS were most frequently observed in the FC, the CA1 area and the infragranular layer of the dentate gyrus, especially in the CB. A large number of lesioned terminal 5-HT-IR fibers disappeared in the DHipp. This is consistent with the low number of labeled cells in the MRN after HRP injection into the DHipp at this stage.

At 14 days post CB-lesion, no apparent difference in the density

of 5-HT-IR terminal fibers was observed as compared with that of 3 days post CB-lesioned, except that the amount of DDS and of swollen axons in the CB were decreased. A number of fine regenerating fibers were evident budding from DDS among the thick axons in the medial portion of the CB. The clearance of degenerating debris, the onset of regeneration from lesioned stumps, and the preparation of homotypic sprouting from surviving 5-HT fibers in the DHipp characterized this stage.

However, at 42 days post CB-lesion, the density of 5-HT-IR fibers was greatly increased in the FC, the infragranular layer of the dentate gyrus, and the stratum lacunosom-moleculare of CA1 to CA3. The density of the 5-HT-IR fibers was greater in the ipsilateral FC as compared with the contralateral. This hyperinnervation is temporally coincident with an abnormally high number of labeled cells in the MRN after HRP injection into the DHipp of 42 days post CB-lesioned animals.

These high density 5-HT-IR fibers were followed along their pathways to the CB and the FF. There was no restoration of 5-HT-IR fibers distal to the lesion in the CB. However, a high density of 5-HT-IR fibers was observed in the ipsilateral FF, near the entrance to the DHipp, in these long-term CB-lesioned animals, as compared with those of contralateral and of normals.

These dense 5-HT fibers in the DHipp were traced back to their origin by retrograde transport of HRP from DHipp. An average of 90 more neurons than in normals were labeled in the MRN at this stage. When the 5-HT fibers in the FF were lesioned by 5,7-DHT, only 32±9

labeled neurons in the MRN remained. Thus, the specific damage of 5-HT fibers in the CB is capable of inducing a proliferation of 5-HT fibers from the undamaged FF, an example of compensatory homotypic sprouting.

## 6. The Glucocorticoid Effect On Sprouting

### Introduction

The glucocorticoid effects on growth in the adult nervous system are interesting. They have been reported to enhance axonal regeneration (by corticotropin) in the severed olfactory bulb (Fertig et al., 1971) and in the spinal cord (McMaster et al., 1962), to have no effect on regeneration of peripheral nerve transplanted in the cerebrum (Knowles and Berry, 1978), to retard heterotypic sprouting of commissural-associational fibers in the dentate gyrus after ipsilateral entorhinal lesion in the rat (Scheff et al, 1980; 1982) and to retard sprouting of vasopressin-containing fibers in the zona interna of the median eminence after electrical lesion of mixed fibers of the paraventricular nucleus (Silverman and Zimmerman, 1982). The present study provids evidence that corticosterone is a necessity for the "homotypic sprouting" of 5-HT system in the dorsal hippocampus.

### Results

The total number of neurons in the MRN projecting to the dorsal hippocampus decreased by 56% at 3 days post CB-lesion as compared with

sham injected control (sham: 295±48 neurons labeled with 100 nl of 10% HRP injected into the DHipp). This reduction returned to close to normal levels by 21 days post-lesion. However, at this time the number of labeled neurons in the MRN remained significantly reduced (64% lower than sham injected control) in CB-lesioned ADX animals. The ADX control group, sham injected control group, and normal controls showed no differences in cell labeling.

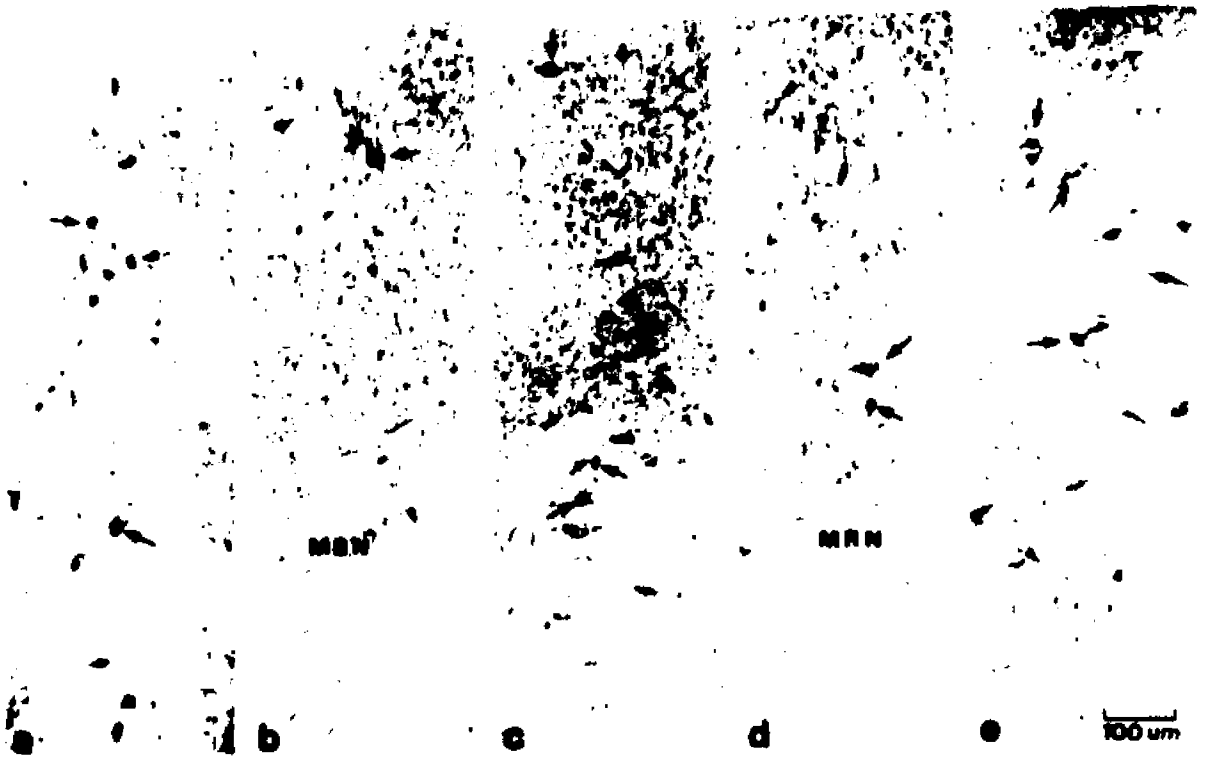
However, the reduction of labeled cells in the MRN of 21-day post CB-lesioned ADX animals is reversed by subcutaneous implantation of corticosterone pellets (288±53 neurons labeled) (see table 3 and figure 31).

Table 3: The effect of ADX and corticosterone replacement on number of HRP-labeled cells (mean±SD) in MRN after HRP injection into dorsal hippocampus of CB-lesioned animals.

| Treatment groups | Days post-lesion | | |
|---|---|---|---|
| | 0 | 3 | 21 |
| Normal/CB lesioned | 295±48 n=7 | 132±14 n=6# | 280±41 n=6 |
| ADX/CB lesioned | 286±13 n=3 | 121±18 n=3# | 106±19 n=6#* |
| ADX/CB lesioned /Replacement | - | - | 288±53 n=5 |

0 day gorups represent sham-lesioned groups. ANOVA, *= P < .001 with respect to effect of ADX, the various ADX groups are compared with normal ( without ADX) gorups at either 0, 3 or 21 days correspondingly. #= P < .001: with respect to time course, 3 and 21 days post-lesion groups is compared with 0 day groups (sham-lesioned control) in either CB lesioned or ADX/CB-lesioned animals.

Figure 31: Photomicrographs show labeled cells in the MRN after HRP injection in the dorsal hippocampus. The number of labeled cells are significantly reduced at 3-day post-CB lesion (b) as compared to normal (a), but returns to normal level at 21-day post-lesion. However, when animals are adrenalectomized (ADX) during the CB lesion, the number of HRP labeled cells remains low at 21-day post-CB lesion (d). When corticosterone pellets are implanted in ADX rats, the number of labeled cells again increases at 21-day post-CB lesion (e).



Discussion

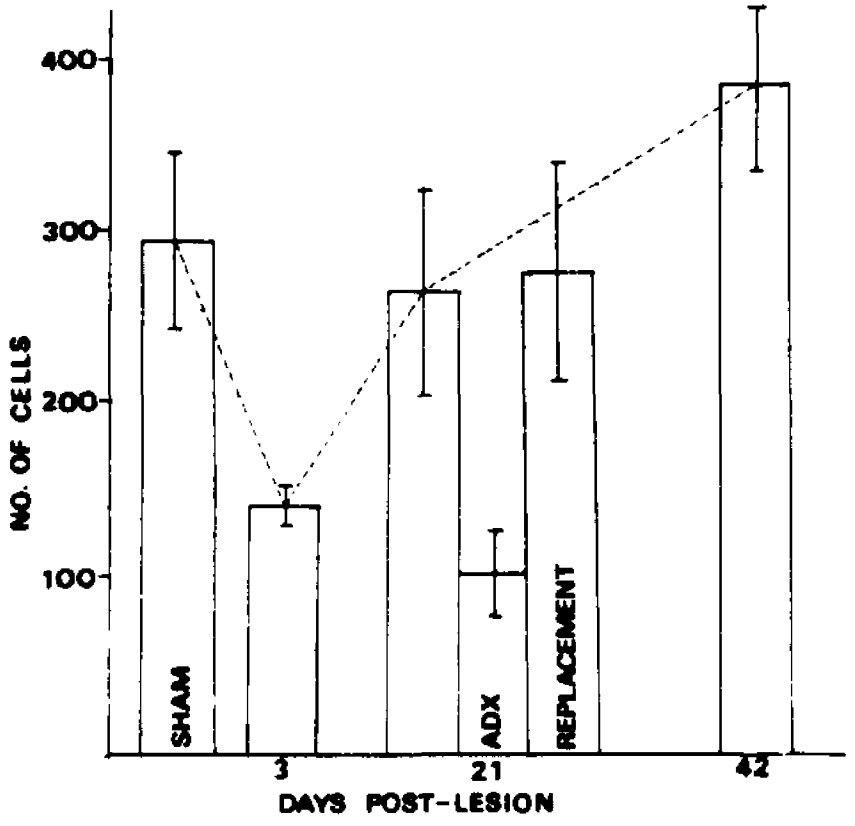
The sprouting of homotypic fibers in the FF after the 5,7-DHT lesion in the CB has been studied. There were more cells projecting into the DHipp as a result of homotypic sprouting in 21-day post CB-lesion animals than in 3-day post CB-lesion animals. However, this sprouting was retarded by ADX. The number of cells projecting to the DHipp remained low in 21-day post CB-lesion rats which had been ADXed. A subsequent replenishment of corticostorone by subcutaneous implantation of solid corticosterone pellets after ADX restored the number of labeled cells (see figure 31) which project to the DHipp in 21-day post CB-lesion ADX rats.

Scheff and his colleagues (1980) reported that injection of additional corticosterone retarded sprouting of commissure-associate fibers in the dentate gyrus after entorhinol lesion. These authors later agreed that both lower and higher doses of corticosterone (relative to physiological level) inhibit the sprouting (1982). The present observation has shown that the ADX which removes corticosterone from the circulation, totally blocks the sprouting. On the other hand, replenishment of corticosterone in ADX rats back to physiological levels releases the suppression on sprouting. Thus, corticosterone plays a permissive role on sprouting after brain damage in the adult rat.

Interestingly, the level of circulating corticosterone may play a crucial role in regulating different neuronal sprouting. The extremely

low level of corticosterone (by ADX) in the circulation may increase (Silvermen and Zimmermen, et al 1982) or have no effect (Scheff et al, 1982) on heterotypic sprouting of vasopressin fibers in the median eminence and pyramidal fibers in the dentate gyrus, but suppresses homotypic sprouting of 5-HT fibers in the hippocampus. The "permissive level" in different type of sprouting need to be studied further.

Figure 32: The number of HRP labeled cells in MRN at 3, 21 and 42 days after 5,7-DHT microinjections into the CB. In the CB-lesioned animals , there is a significant decline in the number of labeled cells at 3 days (-56% P<.001 as compared to sham-lesion). The number of labeled cells significantly increases by 21 days and 42 days post-lesion (3 days' vs 21 days': P<.001; 21 days' vs 42 days': P<.002). However, in the CB-lesioned ADX-animals the number of labeled neurons remains significantly reduced (-64% P<.001 as compared to sham-lesion) at 21 days. However, corticosterone replacement in ADX rats restores the number of labeled cells in MRN to that seen in normal rats and significantly greater than seen in ADX rats (CB-lesioned ADX vs replacement: P<.001).



# GENERAL DISCUSSION

## 1. Model

As regards the plasticity after damage in the CNS, attempts at regeneration back to their original targets have frequently failed because of glial scar formation or some unfavoralbe condition. Heterotypic sprouting conducts chemically wrong signals. "Homotypic reactive synaptogenesis", the response of intact fibers carrying the same chemical as fibers in the denervated zone, is theoretically the ideal model for reinnervating the disconnected targets and making functional contacts.

Reactive synaptogenesis has been shown to result in functional recovery. As described, most of these successful cases involve lesions of ipsilateral projections, and functional restoration resulted from sprouting of alternative homologous fibers from the contralateral side. During evolution systems in which bilateral projection are received from chemically identical neurons of each side developed in many brain regions. "Bilateral innervation" may not be physiologically significant, but its development may have preserved the capability of a neuronal system to recover and survive after damage of its projections to brain regions. The undamaged fibers originating from the contralateral side carry the same transmitter and make homologous connections as the damaged fibers. Furthermore, already present in the denervated area, they do not have to travel far from distal lesion site. In contrast, regenerating fibers may have to travel millimeters

or centimeters and with difficulty of necrosis. Geographically, these sprouting fibers are in a better position and should be more efficient than regeneration in resulting in reinnervation. However, most of the bilateral innervation cases involved lesions of more than one type of fibers. The questions of whether homotypic sprouting can be triggered by damage to the same type fibers remains to be tested. Unfortunately, functional recovery by contralateral compensation sometimes results in abnormal lateralization, such as in motor-visual system (Schneider, 1973). In this case abnormal lateralization could be overcome by homotypic sprouting to a brain region of the same side.

There are several advantages of the raphe-hippocampal model for analysis of the reaction of the CNS to injury: 1. It is a homotypic reactive synaptogenesis model. The hippocampus receives 5-HT fibers via the CB and the FF from two homologous group of neurons. Selective lesioning of 5-HT fibers in the one pathway induces homotypic sprouting from the other. 2. Homotypic and heterotypic sprouting in response to removal of a single chemically identified fiber type can be studied. Three different chemical systems afferent to dorsal hippocampus use the CB and the FF: 1)cholinergic system (Shute and Lewis, 1961; Cotman et al., 1973, Mosko et al., 1973), 2) noreadrenergic system (Blackstad et al., 1967; Moore and Halaris, 1975; Jones and Mooore, 1977), and 3) serotonergic system (Ungerstedt, 1971; Azmitia and Segal, 1978; Lidov et al., 1980; Zhou and Azmitia, 1983). 3. The HRP method can identify the cells of origin of the sprouting fibers during the period in which sprouting is actually occurring, while immunocytochemical staining of 5-HT fibers directly provides evidence of dynamic changes in 5-HT

termination patterns in the hippocampus after lesion.

## 2. Homotypic Sprouting

Homotypic sprouting should meet several criteria: Reinnervating fibers should 1) originate from an undamaged neuronal population which is homologous to that of the damage fibers, 2) carry the same chemical transmitter as that of the denervated fibers, and 3) subserve the same function as those of the lesioned fibers. Several lines of evidence support the idea that neurons of the Raphe-CB-Hippocampal system and Raphe-FF-Hippogampal system are morphologically (Zhou and Azmitia, 1983) , biochemically (Azmitia et al, 1978, McNaughton et al, 1980) and electrophysiologically (McNaughton et. al., 1980) homologous. 5,7-DHT microinjection into the FF of long-term CB-lesioned animals eliminated almost all the increased HRP labeling seen in the MRN suggesting that the sprouting fibers in the FF are serotonergic. Direct evidence of sprouting also comes from immunocytochemical studies. 5-HT fibers were diminished in the CB-lesioned animals, but near normal in long-term CB-lesioned animals. The functional recovery of asymmetrical turning after unilateral CB lesions has been demonstrated to be due to sprouting of 5-HT fibers from the FF (see next section). These data support the idea that removal of serotonergic fibers is sufficient and necessary for the induction of homotypic collateral sprouting of the hippocampal serotonergic fibers and these new sprouts restore both normal morphology and function of the raphe-hippocampal system.

3. Functional Restoration

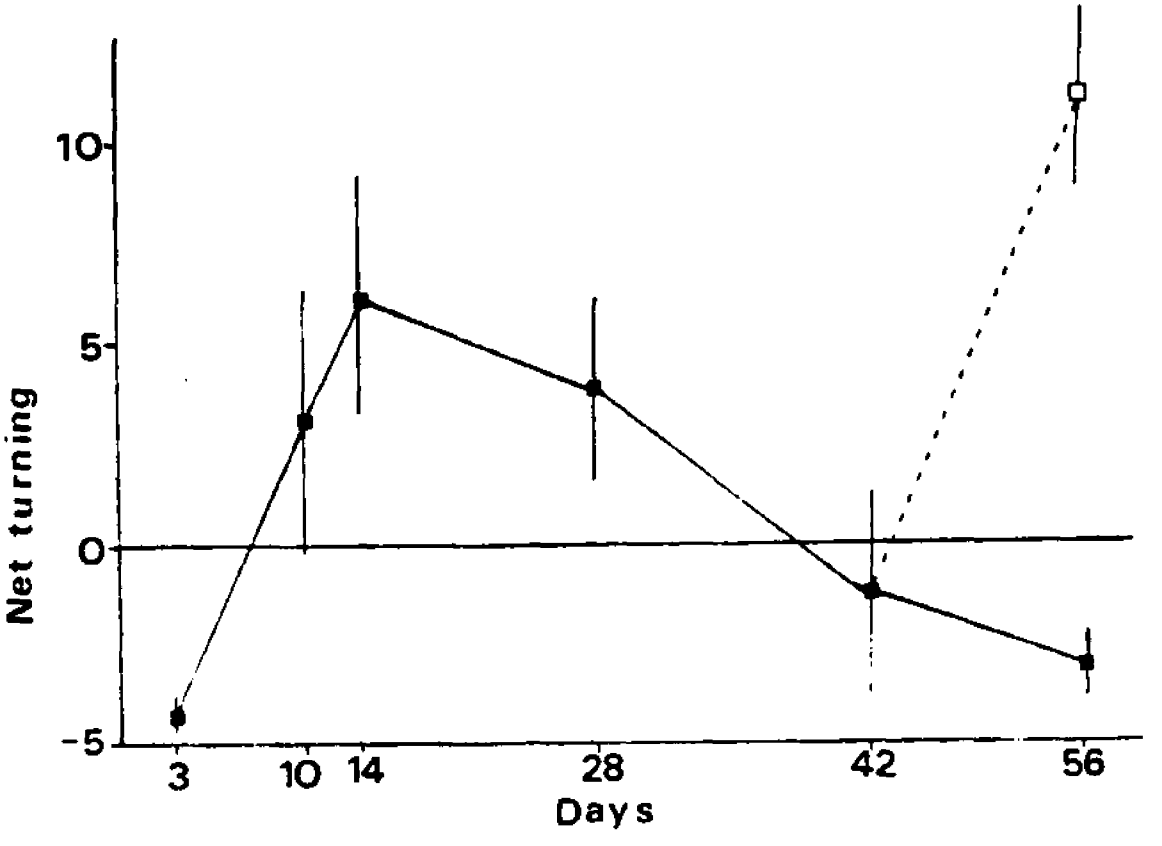
A functional correlate of sprouting in the raphe-hippocampal system has been observed by Azmitia et al. (1978). A behavior which reveals a unilateral hippocampal dysfunction due to 5-HT inbalance was chosen for this study. The turning of animals in an unfamiliar environment was considered to be such a behavior, as the hippocampus has been implicated in novelty detection.

Rats were placed in a circular bowl (30 cm in diameter) and observed for 20 minuts before and 30 minuts after the injection of L-5-hydroxytryptophan (5-HTP, a precusor of 5-HT). This treatment resulted in a specific stimulation of 5-HT receptors (Jacobs et al., 1977). Three days after unilateral CB-lesion, the rats turned to the contralateral side before 5-HTP and to the ipsilateral side after 5-HTP (figure 33). Sham injected rats showed no differences in turning in the two directions before or after 5-HTP. At 14-28 days after microinjection, there were no differences in turning before 5-HTP, but a marked contralateral turning after injection. These observations suggest that 5-HT receptors had become supersensitive to 5-HT on the lesioned side by day 14. This is similar to changs observed in on 5-HT receptors in the spinal cord (Nygren et al., 1974) and acetylcholine receptors in muscle fibers (Miledi and Potter, 1971). At 42-56 days after CB lesion, the rats no longer displayed any consistent direction of turning before or after 5-HTP administration (figure 33). However, a strong contralateral turning reappeared with 5,7-DHT injection into the midline of the FF in the long term animals.

The change in turning response is not due to generalized motor dysfunction because the animals' total activity remained unimpaired after unilateral CB lesion and the asymmetrical turning behavior continued to habituate rapidly over the 20 min test period (75% occurring in the first 4 min).

At present, functional restoration is thought to result from homotypic reinnervation (sprouting or regeneration). However, functional restoration does not necessarily always reflect morphological reconstruction. Denervation supersensitivity (for recent review, see Thesleff and Sellin, 1980), unmasking of physiologically silent innervation (Dostrovsky et al., 1976; Basbaum and Wall,1976; Wall and Eggar, 1971), and rearrangement of cortex presentation (sensory-motor) through a foreign reinnervating fibers to a particular body region (Brinkman et al., 1983) have been reported to reverse functional abnormalities from neuronal damage in the absence of substantial reinnervation. The definition of functional restoration depends on the task that animals perform. For example, in the process of remodeling of central visual circuitry following lesions of the superior colliculus (SC) of the developing hamster, retinal axons that would normally innervate the damaged SC, are grow into the contralateral SC (Schneider, 1973; Schneider and Jhaveri, 1974). The consequenge of this reorganization is that the animals regain the ability to visualize but exhibit abnormal orientation to stimuli presented in the visual field of the eye that was deprived of its central targets. Animals may not catch food from the affected side, but they can perceive the approaching predator and escape.

Figure 33: The net turning (TN) at various times after unilateral injection of 5,7-DHT into the right CB is expressed as the difference in 180° turns before (od) and after (d) 5-hydroxytryptophan (5-HTP) [TN = (R - L)od - (R-L)d]. At 3 day the rats showed increase left (contralateral) turing before and right (ipsilateral) turning after 5-HTP. However at 10, 14, and 28 days after lesion, the situation is reversed with rats displaying no differensce in turining before, but a markedly increase left turning after 5-HTP injection. The net turning at 42, and 56 days after CB lesion demonstrated that the rats are no longer showing any significant asymmetrical turing behavior. However if a midline injection of 5,7-DHT is made into FF of 42-day CB-lesion rats, then the net turning (dotted line) is similar to that observed 14-day CB-lesion alone, but significantly different from a comparable group of rats at 56-day CB-lesion. Adapted form Azmitia et al., 1978.



4. Mechanism

The Mechanism Of the Regrowth Of the Nervous system

At present, there is no single mechanism which can apply to all phenomena of regrowth. Most attempts to elucidate mechanisms of regrowth succeed only partially. Perhaps different forms of regrowth have distinct mechanisms.

Most of the brain's molecular structure, as in other parts of the body, is in a state of catabolic and anabolic balance. In the axon, this balance results in elongation, retraction and maintenance of the axon. Axonal skeleton proteins essential for axonal growth , such as actin and tubulin and components of neurofilaments, are transported down to axon. The assembly and disassembly of microtubules and neurofilaments is balanced by continuous transport of these proteins and by a calcium dependent disassembling enzyme, protease, in the axon terminal (Hoffman and Lasek, 1975). It is assumed that other elements, glycoproteins, lipids, etc, are modulated in a similar manner. Manipulation of protease activity, by the concentration of $Ca^{++}$ in the axon terminal, might alter the equilibrum between polymerized microtubules and free tubulin, and in turn affect axonal elongation. Lasek and Hoffman (1976) propose that axonal sprouting is normally prevented by continual disassembly of neurofilaments and microtubules in the axonal terminals due to entry of $Ca^{++}$ into the nerve terminal that normally occurs as a prelude to transmitter release.

Most regrowth has been observed after a brain region was lesioned. Lesioning has multiple effects, which include degeneration, inactivity of target receptors, the release of trophic or inhibiting factors, and increased glial activity. Each phenomenon has been proposed as the stimulus for initiating regrowth in one or more forms, and has been individually tested.

A. Neurotrophic Interaction

All lesions result in degeneration of neurites. Release of humoral neurotrophic factors can be induced by degeneration of presynaptic elements. Ramon y Cajal wrote in 1928 that "The degenerated nerves of the peripheral stump give out into the scar some substance—an enzyme, nutritive substance, or other material—which stimulates the assimilation and growth of the sprouts....." .

Hoffman (1950) found that extracts of degeneration products induce sprouting of fibers innervating intact muscles. This extract was subsequently identified as an unsaturated fatty acid in the myelin sheath and named "neurocletin". The products of nerve degeneration can also cause supersensitivity of post-synaptic receptors similar to that caused by deneravation. (Jones and Vyskocil, 1975; Lomo and Westgaard, 1975; Jones and Vrbova, 1974; Vrbova, 1967).

The effects of degeneration products are probably nonspecific with respect to regrowth. This hypothesis may apply to regeneration and reactive synaptogenesis but not to pruning. Furthermore, this mechanism contradicts sprouting demonstrated in the absence of

degeneration as described below.

Denervated targets gan also induce nerve growth by the release of similar trophic factors. Inactive muscles gan cause sprouting of their nerve supply, and show receptor hypersensitivity to them (Fambrough, 1979; Edwards, 1979; Burt, 1978; Purves and Lichtman, 1978; Pestronk and Drachman, 1976; Lavoie and Collier, 1976; Lomo and Westgaard, 1975). A soluble humoral agent extracted from denervated muscles, has been shown to work as a neurotrophic factor, inducing sprouting (van Harreveld, 1947). Tetrodotoxin, causing muscle inactivity by blocking impulse propagation (Brown and Ironton, 3977); botulinum toxin, blocking neuromuscular transmission by preventing acetyl choline release (Ironton et al., 1978); D-tubocurarine (Wernig and Stover, 1979) and alpha-bungarotoxin (Holland amd Brown, 1980; Pestronk and Draghman, 1978), blocking acetylgholine binding on muscle receptors, were all reported to induce sprouting within 3 to 5 days without damaging nerve fibers. These experiments indicate that target disuse gan cause sprouting in neuro-muscular junctions. Muscle inactivity per se cannot be a direct stimulus, as suggested by Cotman et al, (1981). What is implicit in the hypothesis is that inactive muscles secrete a substance that induces sprouting or that inactive muscles cease to secrete a substance that prevents sprouting. Similarly a trophic factors may be involved in regeneration and reactive synaptogenesis, but is probably not involved in pruning.

On the other hand, neuronal regrowth can be a result of release from normal inhibition. In other words, neurons may normally secrete humoral factors inhibiting the regrowth of the other neurons. In the

salamander, lesion of spinal nerve 16 induced collateral sprouting of the two adjacent spinal nerves (15 and 17) into the denervated territory of nerve 16 in the hindlimb. Identical results were oberved after treatment of nerve 16 with colchicine, without interrupting nerve impulses nor causing degeneration (Cooper et al, 1977; Diamond et al., 1976; and Aguilar et al., 1973). These results were expanded upon by Diamond et al. (1976), who demonstrated that a factor inhibiting sprouting travels to the nerve endings by axonal transport, and is liberated there to counteract a growth-promoting stimulus continuously secreted by the target tissue. Similar findings were reported in mammalian neuromuscular junction (Guth et al., 1980) and hippocampus (Goldowitz and Cotman, et al., 1980).

Again, this hypothesis may account for reactive synaptogenesis, but it cannot explain pruning.

B. Principle of Conservation

Many hypotheses regarding the mechanism of regrowth do not adequately address the sprouting responsive to pruning.

The so-called principle of conservation, articulated dy Dever and Schneider (1975), applies to such phenomenon. According to this principal, neurons are intrinsically programmed to elaborate a defined quantity of terminal arborizations during development. Lesions preventing the growth of one branch of the neuron result in a compensatory overgrowth from the other branches. This has also been

called the "pruning effect" (Schneider, 1973).

The principle also holds true with respect to the converse situation. Excessive arborization during development (references see introducion, p. 15) and after a lesion in the adult (Murray, 1982) occurrs but innervation eventually returns to the normal level as programmed. However, this principal seems contradictory to reactive synaptogenesis and other types of collateral sprouting that generate extra arborizations to a denervated territory.

C. Glial Interaction

A role for the glial cells in neuronal repair and regeneration, although still inconclusive, has long been proposed. Ramon y Cajal proposed in his studies of regeneration that glial cells play a fundamental role by stimulating axon endings to form new branches. Glial cells have been reported to proliferate in the denervated area, migrate to the vicinity of the intact fibers (Gall et al., 1979) and associate themselves with newly formed sprouts (Speidel, 1941).

Dividing glia induce axons to grow. This was first observed by Speidel in the living tadpole (1933; 1941). Dividing Schwann cells cause the axon to sprout collaterals, with concomitant formation of a node of Ranvier (Edds, 1953; Speidel, 1941). The negative side is that X-ray irradiation to inhibit glial cell division did not seem to influence regrowing fibers (Edds, 1953). Futhermore, glial activity declines before regrowth is initiated in the CNS (Gall and Lynch, 1979, McWilliams and Lynch, 1978; and Lee et al., 1977). However, the

degenerating terminals are rapidly removed by glia from post-synaptic site. This may indicate that the glial activity prepares a suitable environment for growth by various afferents.

Glia may also secrete neurotrophic factors (Varon and Somjen, 1979; Varon and Bunge, 1978). Trophic substances released by glia such as NGF (Levi-Montalcini and Hamburger, 1951; Levi-Montalcini, 1976; Bradshaw and Young, 1976) and glial-released protein (GRP) (Arenander and DeVellis 1981a,b) have been proposed to initiate regrowth.

## The Hypothetical Mechanism of Glucocorticoid Effect on Homotypic Sprouting

There are three possible ways which glucocorticoids can influence homotypic sprouting of 5-HT fibers in the hippocampus.

A. Hippocampal trophic factor

The hippocampus is the preferential uptake site for endogenous corticosterone as well as for exogenous $^{3}$H-corticosterone (McEwen and Micco, 1980) in the CNS. Both pyramidal and granular neurons have a high affinity uptake mechanism for glucocorticoids. The binding of glucocorticoid occurs first in the cytosol, and then the molecule is translocated to the necleus (McEwen and Wallach, 1973; Rhees et al., 1975; Turner and McEwen, 1980). By this route, glucocorticoids may influence transcription of hippocampal trophic factor which in turn reprograms the metablic balance in presynaptic neurons.

B. Glial interaction

Glucocorticoids have been reported to increase activity of astrocytes (Scheff et al., 1980). Astrocytes are an essential element in maintaining the neuronal activity. On the other hand, glycerol-3-phosphate dehydrogenase (GPDH), a phospholipid synthetic enzyme essential for the myelination, is regulated throughout the brain by glucocorticoids (De Vellis and Inglish, 1968). In adult rats, ADX cause a 60% decrease in GPDH activity. Administration of ACTH or glucocorticoid prevents this decrease in enzyme activity. GPDH was later confirmed as being located in oligodendrocytes (Leveille et al., 1980). Myelination by oligodendrocytes provides guidance to the growing axons (Reference see above). Interestingly, on the other hand, glucocorticoid administration to developing rats was reported to inhibit myelination (Friedrich and Bohn, 1980). The diversity of glucocorticoid effects is also exemplified by the observation that these steroids regulate homotypic sprouting of 5-HT fibers (present studies) and heterotypic sprouting of cholinergic and pyramidal fibers (Scheff et al., 1980, 1982). The concentrations of glucocorticoid required in the regulation of these various processes might be the key to the variation.

Futhermore, The existence of glucocorticoid receptors in glial cells, although little, has been identified. Autoradiographic studies with $^{3}$H-dexamethasone reveal a rather uniform distribution of radioactivity across brain regions and point to a labeling of neuropil. Localization to cell nuclei typical of glial cells was detected (Rees et al., 1973, Rhees et al., 1975; Warembourg, 1975). Bindings in glial

cells is also both cytosolic and nuclear. Glucocoticoids may also play a role in regulating transcription of glial trophic factor.

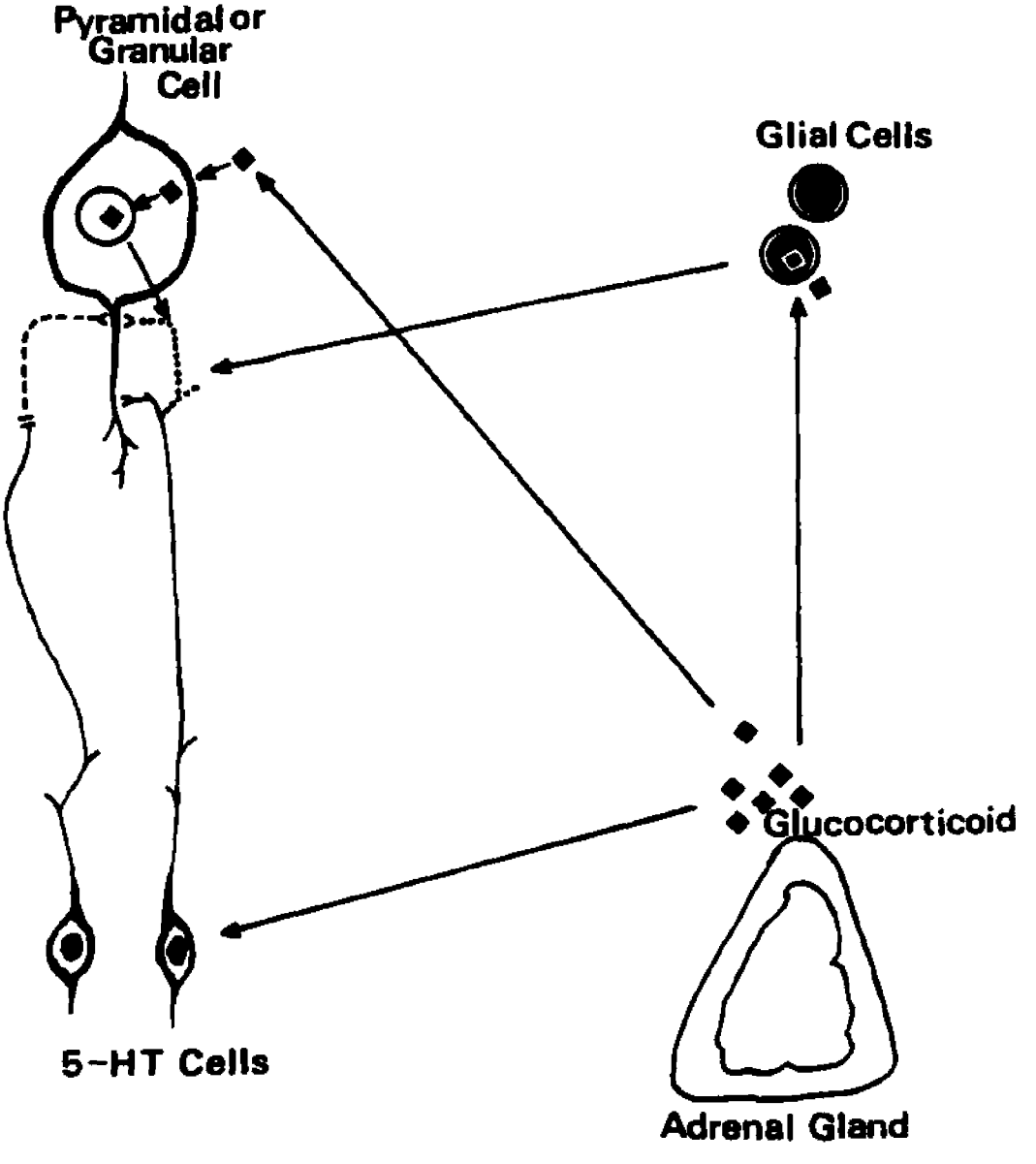
C. Effect on transmitter development

A peculiar character of glucocorticoid makes it a suitable candidate for regulating homotypic sprouting. The glucocorticoid has a selective effect on transmitter development. It is possible to experimentally control the type of transmitter in sympathetic neurons through manipulation of the hormonal environment surrounding them (Bunge et al., 1978; Patterson, 1978; LeDouarin, 1980). Normally all neurons in the neonatal rat superior cervical ganglion (SCG) display catecholamine fluorescence (Eranko, 1972). Biochemical assays demonstrate that the neurons are capable of synthesizing and accumulating catecholamines, but not acetylcholine (Patterson and Chun, 1977b). Interestingly, these same sympathetic neurons can become cholinergic through the influence of some factors of non-neuronal cells (Patterson and Chun 1977a, Landis, 1980; Hawrot, 1980). The neurons develop the capacity to produce acetylcholine and form functional cholinergic synapses (Ko et al., 1976; O'Lague et al., 1978; Furshpan et al., 1976; Nurse and O'Lague, 1975). However, corticosterone inhibits the development of cholinergic properties (McLennan et al., 1980), and facilitates the development of catecholamines in the SCG. Normally after 14 days in culture, choline acetyltransferase (an acetylcholine synthetic enzyme) activity is high and tyrosine hydroxylase (TH, catecholamine synthetic enzyme) levels have fallen; $10^{-6}$M corticosterone in the medium prevents this change and decreases the choline acetyl-transferase/TH activity ratio tenfold

(Mclennan et al, 1980). Thus, glucocorticoids preferentially facilitate catecholamine and inhibit acetylcholine development in the SCG.

As described, glucocorticoids are also involved in the development of brain tryptophan hydroxylase (TrH). It plays a permissive role in TrH maturation during development (Sze, 1976; 1980) as well as in maintaining TrH activity in the adult rat (Azmitia and McEwen, 1969). Thus, glucocorticoids may provide a favorable environment specifically for the growth of homotypic 5-HT fibers, while supressing heterotypic growth of pyramidal and cholinergic fibers in the hippocampus.

Figure 34: The three posible ways that Glucocorticoids can influence growth after damage in the hippocampus. The glucocorticoids may bind to the nuclei of the pyramidal or granular cells, and influence transcription of hippocampal trophic factor. They may effect glial cells which provide trophic factors or physio-chemical guidence for growing fibers. Or, they may direct influence the development of the transmitter in the growing 5-HT fibers.



## 5. Competition

After injury to a brain region, many types of regrowth rather than a single form may occur.

Regeneration, regenerative sprouting, reactive synaptogenesis, and pruning may occur at the same time. Different fibers, heterotypic or homotypic, may grow. They may coexist or compete, or there may be a hierarchy or priority among them.

Only one system of fibers seems to be able to stablize and make permanent connections with disconnected targets. Those failing to make contacts eventually die. How these different fibers compete or compromise one another remains a mystery.

Sprouting and regenerating fibers quite often seem to be competing with each other. In the peripheral nervous system (PNS), reinnervation by sprouts occurs sooner (from hours to days) than reinnervation by regenerating fibers (from weeks to months) after partial dennervation (Grafstein and McQuarrie, 1978). When the regenerating fibers reach their muscle targets, the end plates formed by "foreign nerve" sprouts are displaced by regenerating fibers (Bennett et al., 1979; Stephenson, 1979; Dennis and Yip, 1978; Thompson, 1978; Brown and Ironton, 1978; Denburg et al, 1977; Kuffler et al., 1977; Haimann et al, 1976; Fangboner and Vanable,1974; and Cass et al., 1973).

In the CNS, destruction of the serotonergic innervation of the rat subcommissural organ results in heterotypic reinnervation of this

region by non-5-HT fibers. 5-HT fibers can be seen regenerating but appear not to reach their target organ to replace the heterotypic sprouts by 8 months (Wiklund and Mollgard, 1979). In our case, regenerative sprouting occurs in the CB earlier (14 days) than homotypic sprouting (21 days). By the time (42-day CB-lesion) homotypic sprouts had reinnervated the DHipp, regenerating fibers were perhaps still on their way. Most sprouting occurs proximal to the disconnected targets, whereas regeneration usually starts in a distal lesion site. This might not be a fair competition based on the distance to be traveled by growing fibers. After the 5-HT fibers in the CB were lesioned, homotypic fibers sprouted through the FF and reinnervated the DHipp. If these homotypic fibers in the FF were not available, more distant homotypic fibers from the ventral hippocampus might serve this function. On the other hand, the growth of new fibers does occur in the lesioned CB, but it may take much longer for the new growing fibers to reach their destination from the damaged ends, if they reach it at all. Thus, when HRP was injected in the DHipp of 42-day CB-FF-lesioned animals, there was no return of labeling in the MRN. Instead, a group of cells were labeled in the DRN. The DRN normally projects to the ventral hippocampus. In this instance, it extends its innervation to the dorsal hippocampus after lesioning of 5-HT fibers in the CB and the FF. However, in some cases when regenerating fibers are the only homotypic source of reinnervation and homotypic fibers in the denervated region are totally removed, they are able to grow long distances and reinnervate their targets. 5-HT fibers grow at least 5 cm from the point of lesion in the medulla to lumbar-sacral spinal cord (Wiklund and Bjorklund, 1980, Bjorklund and

Wiklund, 1980).

The first cycle of synaptic turnover may not have "chemical preference", but rather have a "proximity preference". Fibers near the denervated zone receive trophic factors or are released from inhibition and grow into the zone on a "first come, first served" basis, regardless of chemical type. When a second cycle arrives, the target cell seems to prefer homotypic regenerating fibers for a permanent relationship (Proctor et al., 1979; Roper and Ko, 1978; Nja and Purves, 1978; and Guth and Bernstein, 1961).

With respect to homotypic sprouting competing with regeneration of homotypic fibers, the first comer to the denervated targets area may suppress the regrowth of the late comer, in which case the regenerating fibers are usually at disadvantage. A longer observation is required for this study of the present model. Ongoing experiments are progressing to test homotypic competition. A group of animals had fetal raphe transplanted into the DHipp. The relationship of the established host 5-HT fibers and the newly transplanted growing 5-HT fibers in the DHipp will be assessed.

Regeneration and collateral sprouting obviously occur in very different ways. Their mechanisms remain unknown. Several lesion-induced neurotrophic factors have been isolated: neurocletin (Hoffman and Springell, 1952), hippocampal growth factor (Crutcher and Collins, 1982, Bjorklund et al, 1981), neurotrophic factor (Nieto-Sampedro et al., 1982). The presence of nerve growth factors has an effect on regeneration and/or different types of sprouting, but

the means by which the neurite recognizes these factors as a stimulus to regenerate or to sprout is uncertain.

Wiklund has recently hypothesized that the mode of axonal termination may also play a role in determining the nature of axonal regrowth. He proposes that non-juctional 5-HT innervation (lack of typical membranous specialization in synapsis) tends to favor regeneration, while junctional 5-HT innervation (with typical dense plate in the synapsis) favors collateral sprouting. This is due to the loose or rigid relationship between the pre- and the post-synaptic elements in the synaptic junction. It will be interesting to investigate the type of synaptic contact of raphe-pyramidal connection.

For multiple fiber systems to coexist in a terminal field, They must compromise morphologically. Lamination is very often established among various afferents (in the cingular gyrus, hippocampus and many other cortical areas). Damage of one fiber system in a terminal field disturbs the established balance. The plasticity of afferents to the dentate gyrus has been extensively studied. After unilateral destruction of the entorhinal cortex in the adult, heterotypic sprouting of cholinergic fibers from the septum, and association-commissural fibers from CA4 pyramidal cells was observed. Homotypic sprouting was observed from the contralateral entorhinal cortex. Regeneration of cholinergic fibers was reported after transection of the fornix. How do these regrowing fiber systems compete for targets or share their new territory? It appears that one system can manipulate the response of another. CA4 fibers appear to

serve as the critical afferent that establishes the pattern of septal growth and reorganization (Lewis and Cotman, 1980; Cotman, 1979). In the dentate gyrus, the major inputs to the molecular layer are from entorhinal and CA4 hippocampal neurons. Fibers from the ipsilateral entorhinal cortex (with a few from contralateral side) project to the outer 3/4 of the molecular layer of the dentate gyrus, while CA4 neurons project to the inner 1/4. A supragranular band of septal fibers is located just beneath CA4 fibers, while a moderately dense projection of septal fibers co-exists along with entorhinal fibers in the outer zone. The CA4 zone contains very few if any septal fibers.

When entorhinal cortex is lesioned, the CA4 fiber in the inner zone expand toward the outer. The septal and contralateral entorhinal fibers in the outer zone replace the ipsilateral entorhinal territory but avoid the area of expanded CA4 territory. The inner dense band of septal fibers under the suppression of CA4 fibers does not expand. When CA4 neruons are totally removed bilaterally, septal fibers in the dense inner band are then able to proliferate into the CA4 fiber area. Incomplete destruction of CA4 fibers prohibits such proliferation of septal fibers. Thus, a hirearchy of interactions exists among the fiber systems. This has been called the "critical afferent theory" of lamination (Cotman et al., 1981). It is a special type of competitive mechanism where the critical afferent enjoys a special advantage. The authors also hypothesized that entorhinal fibers may selectively suppress the proliferation of CA4 fibers but they do not affect the growth of septohippocampal fibers. In a multiinnervated system, a mechanism must exist to select the different inputs individually. A

critical afferent could provide the selection mechanism.

## 6. Regulation

Many factors have been proposed to influence neuronal plasticity, including type of damage, distance of reinnervation, age and sex of the animals, and neurotrophic factors. At present, how to regulate neuronal plasticity is still in its infant stage.

Hormonal regulation, such as by glucocorticoids, has been reported to play a permissive role (the present study), retard, have no effect on regrowth (references see p. 96) and reduce the rate of axonal sprouting (Cotman and Scheff, 1979). Thyroxine (Diaz-Guerrero et al., 1947) and triiodothyronine (Heinicke, 1977; McIsaac and Kiernan, 1975; Cockett and Kiernan, 1973 and Fertig et al., 1971) have been reported to stimulate regeneration. Estrogen has been demonstrated to facilitate axonal sprouting and synaptic regeneration (Matsumoto and Arai, 1979). Testosterone was observed to promote axonal regeneration (Yu, 1982; Yu and Srinivasan, 1981).

In addition to hormonal regulation, a mechanical stimulation was found to increase regrowth. Electrical stimulation of undamaged nerves is able to enhance their sprouting to partial denervation in the nerve innervation of muscles (Maehlen and Nja, 1979; Rutledge, 1978a,b; Rutledge et al., 1974).

Furthermore, the conditioning lesion effect is used as a strategem to enhance neuronal regrowth. A minor first lesion increases the rate of sprouting following a second lesion (McQuarrie et al., 1977;

McQuarrie and Grafstein, 1973; Scheff et al., 1978). It appears that a initial minor lesion can prepare the neuron for the subsequent regrowth in response to a future damage.

## CONCLUSIONS

In the present 5-HT raphe-hippocampal model, after lesioning of 5-HT fibers in the CB of a group of MRN cells, compensatory sprouting is triggered. The sprouting fibers originate from a functionally and morphologically homologous and chemically identical neuronal group. This group of 5-HT cells project through the FF to innervate the similar territory in the dorsal hippocampus — a homotypic reactive synaptogenesis.

Regenerative sprouting of lesioned 5-HT fibers in the CB does occur, but does not reach the denervated area in the dorsal hippocampus at the longest time point (42 days) of present study. A pruning effect might have occurred, since some portion of MRN 5-HT cells have collaterals that project via both the CB and the FF to innervate the dorsal hippocampus. Thus, the growth of 5-HT fibers in the FF may be a response (in a part) to the pruning of their collaterals in the CB. However, these growing sprouts in response to pruning also project to the denervated area. Thus, it is a pruning as well as reactive

synaptogenesis.

If both major 5-HT raphe-DHipp connections (the CB and FF) are lesioned, MRN cells fail to reinnervate the dorsal hippocampus, but a sprouting fibers from DRN are triggered to innervate the dorsal hippocampus. Normally these DRN cells innervate the ventral hippocampus through the perforant path.

Corticosterone is shown to regulate the homotypic sprouting of 5-HT fibers in the dorsal hippocampus. ADX suppresses this sprouting. However, the suppression is reversed by replacement of the corticosterone. This hormone appears to play a permisive role in homotypic sprouting of 5-HT fibers in the dorsal hippocampus.

## SIGNIFICANCE

Homotypic collateral sprouting is one of the natural recovery processes after an injury has occurred in the central nervous system. In the present work, this phenomenum is studied in two anatomically distinct serotonergic projections to the hippocampus. By focusing on the serotonergic fibers within the hippocampus, and using chemo-neurosurgical technique (5,7-DHT) lesioning in one pathway while observing homotypic collateral sprouting from the other pathway, insight into specific growth is gained. Finally, the hormonal regulation of homotypic and heterotypic sprouting by corticosteroids

can be exploited to maximize homotypic (functional) sprouting relative to heterotypic (non-functional) sprouting.

## REFERENCES


Abercrombie, M., Estimation of nuclear population from microtome sections, Anat.Rec., 94(1946)239-247.

Aghajanian, G.K., Wang, R.Y., and Baraban, J.. Serotonergic and non-serotonergic neurons of the dorsal raphe: reciprocal changes in firing induced by peripheral nerve stimulation. Brain Res. 153(1978)169-175.

Aguilar, C.E., Bisby, M.A., Cooper, E., and Diamond, J., Evidence that axoplasmic transport of trophic factors is involved in the regulation of peripheral nerve fields in salamanders, J. Physiol. London, 234(1973)449-464.

Arenander, A.T., and DeVellis, J.D., Glial-released proteins: II. two-dimensional electrophoretic identification of proteins regulated by hydrocortisone, Brain Res., 224(1981)105-116.

Arenander, A.T., and DeVellis, J.D., Glial-released proteins: III. Influence on neuronal morphological differentiation, Brain Res., 224(1981)117-127.

Assaf, S.Y., Miller, J.J., Neuronal transmission in the dentate gyrus: role of inhibitory mechanisms, Brain Res., 151(1978)587-592.

Assaf, S.Y., Crunelli, V., and Kelly, J.S., Action of 5-Hydroxytryptamine on granule cells in the rat hippocampal slice, J. Physiol., Paris, 77(1981)377-380.

Azmitia, E.C., The visualization and characterization of 5HT reuptake sites in the rodent and primate hippocampus. A preliminary study, J. Physiol., Paris, 77(1981)175-182.

Azmitia, E.C.,The serotonin-producing neurons of the midbrain median and dorsal raphe nuclei. In: Handbook of Psychopharmacology, vol. 9, edited by Iversen, L.L., Iversen, S.D., and Snyder, S.H., New York:Plenum Publishing (1978).

Azmitia, E.C. Buchan, A.M. and Williams, J.H., Structural and functional restoration by collateral sprouting of hippocampal 5-HT axons, Nature, 274 (1978) 374-376.

Azmitia, E.C., and Gannon, P.J., Myelinated 5-HT immunoreactive axons in monkey and rat MFB: nickel enhancement, Soc. Neurosci. Abstr., 8(1982)134.

Azmitia, E.C., Hess, P., and Reis, D., Tryptophan Hydroxylase Changes in Midbrain of the Rat after Chronic Morphine Administration, Life Sci., 9(1970)633-637.

Azmitia, E.C., and Marovitz, W.F., In vitro hippocampal uptake of tritiated serotonin (H-5HT): A morphological biochemical and pharmacological approach to specificity, J. Histochem Cytochem., 28(1980)636-644.

Azmitia, E.C., and McEwen, B.S., Corticosterone regulation of tryptophan hydroxylase in the midbrain of the rat, Science, 166(1969)1274-1276.

Azmitia, E.C., Perlow, M.J., Brennan, M.J., and Lauder, J.M., Fetal raphe and hippocampal transplants into adult and aged C57BL/6N Mice: A preliminary immunocytochemical study, Brain Res. Bull., 7(1981)703-710.

Azmitia, E.C. and Segal, M., An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat, J. Comp. Neurol., 179(1978)641-667.

Basbaum, A.I., and Wall, P.D., Chronic changes in the response of cells in adult cat dorsal horn following partial deafferentation: the appearance of responding cells in a previously non-responsive region, Brain Res., 116(1976)181-204.

Baumgarten, H.G., Bjorklund, A., Holstein, A.F., and Hobin, A., Organization and ultrastructural identification of the catecholamine nerve terminals in the neural lobe and pars intermedia of the rat pituitary, Z. Zellforsch. Mikrosk. Anat., 126(1972)483.

Baumgarten, H.G., Bjorklund, A., Lachenmayer, L., and Nobin, A., Evaluation of the effects of 5,7-dihydroxytryptamine on serotonin and catecholamine neurons in the rat CNS, Acta physiol. scand., Suppl., 391(1974)1-19.

Baumgarten, H.G., Bjorklund, A., Nobin, A., Rosengren, E., and Schlossberger, H.G., Neurotoxicity of hydroxylated tryptamines: structure-activity relationships. 1. Long-term effects on monoamine content and fluorescence morphology of central monoamine neurons. Acta Physiol. Scand. Suppl., 429(1975)1-28.

Baumgarten, H.G., Jenner, S., and Klemin, H.P., Serotonin neurotoxins: recent advances in the mode of administration and molecular mechanism of action, J. Physiol., 77(1981)309-314.

Baumgarten, H.G., Klemm, H.P., Lachenmayer, L., Bjorklund, A., Lovenberg, W., and Schlossberger, H.G., Model of mechanism of action of neurotoxic indoleamines: A review and a progress report. Serotonin Neuroxins, Ann. N. Y. Acad. Sci., 305(1978b)3-24.

Baumgarten, H.G., Klemm, H.P., Lachenmayer, L., and Schlossberger, H.G., Effect of drug on the distribution of [$^{14}$C]-5,6- and [$^{14}$C]-5,7-dihydroxytryptamine in rat brain. Ann N.Y. Acad. Sci., 305(1978a) 107-118.

Baumgarten, H.G., Lachenmayer, L., and Bjorklund, A., Chemical lesioning of indolamine pathways, In: Methods of psychobiology, edited by Myers, R.D., Academic Press, New York, 3(1977)47-98.

Baumgarten, H.G., and Schlossberger, H.G., Effects of 5,6 dihydroxytryptamine on brain monoamine neurons in the rat, In: Serotonin and Behavior, edited by Barchas, J.D., and Usdin, E., Academic Press, New York, (1973)209-224.

Beaudet, A., and Descarries, L., Quantitative data on serotonin nerve terminals in adult rat neocortex, Brain Res., 111(1976)301-309.

Bennett, M.R., McGrath, P.A., and Davey, D.F., The regression of synapses formed by a foreign nerve in a mature axolotl striated muscle, Brain Res., 173(1979)451-469.

Bernstein, J.J., and Bernstien, M.E., Axonal regeneration and formation of synapses proximal to the site of lesion following hemisection of the rat spinal cord, Exp. Neurol., 30(1971)336-351.

Berry, M., and Riches, A.C., An immunological approach to regeneration in the central nervous system, Br. Med. Bull., 30(1974)441-476.

Blackstad, T.W., Fuxe, K., and Hokfelt, T., Noradrenaline nerve terminals in the hippocampal region of the rat and the guinea pig. Zellforschung, 78(1967)463-473.

Bjorklund, A., Baumgarten, H.G., Lachenmayer, L., and Rosengren, E., Recovery of brain noradrenaline after 5,7-dihydroxytryptamine-induced axonal lesions in the rat, Cell Tissue Res., 161(1975)145-155.

Bjorklund, A., Katzman, R., Stenevi, U., and West, K.A., Development and growth of axonal sprouts from noradrenaline and 5-hydroxytryptamine neurons in the rat spinal cord, Brain Res., 31(1971)21-33.

Bjorklund, A., and Lindvall, O., Reformation of normal terminal innervation pattern by central nor adrenergic neurons after 5,7-dihydroxytryptamine induced axotomy, Brain Res., 171(1979)275-293.

Bjorklund, A., Nobin, A., and Stenevi, U., Regeneration of central serotonin enurons after axonal degeneration induced by 5,6-dihydroxytryptamine, Brain Res., 50(1973a)214-220.

Bjorklund, A., Nobin, A., and Stenevi, U., The use of neurotoxic dihydroxytryptamines as tools for morphological studies and localized lesioning of central indolamine neurons, Z. Zellforsch, 145(1973b)479-501.

Bjorklund, A., and Stenevi, U., In vivo evidence for a hippocampal adrenergic neuronotrophic factor specifically released on septal deafferentation, Brain Res., 229(1981b)403-428.

Bjorklund, A., and Stenevi, U., Reformation of the severed

septohippocampal cholinergic pathway in the adult rat by transplanted septal neurons. Cell Tissue Res., 185(1977)289-302.

Bjorklund, A., and Stenevi, U., Regeneration of monoaminergic and cholinergic neurons in the mammalian central nervous system, Physiol. Rev., 59(1979)62-100.

Bjorklund, A., Wiklund, L., and Descarries, L., Regeneration and plasticity of central serotoninergic neurons: a review. J. Physiol. (Paris) 77(1981)247-256.

Bjorklund, A. Stenevi, U., and Svendgaard, N.A., Growth of monoaminergic neurons into the adult hippocampus along the perforant path, Nature London, 262(1976)787-790.

Bjorklund, A., Stenevi, U., Dunnett, S.B., and Iversen, S.C., Functional reactivation of the deafferented neostriatum by nigral transplants, Nature, 289(1981)497-499.

Bjorklund, A., and Wiklund, L., Mechanisms of regrowth of the bulbospinal serotonin system following 5,6-dihydroxytryptamine induced axotomy I, Biochemical correlates, Brain Res., 191(1980)109-127.

Bobillier, P., Seguin, S., Deguerce, A., Lewis, B.D., and Pujol, J.F., The efferent connections of the nucleus raphe centralis superior in the rat as revealed by radiography, Brain Res., 166(1979)1-8.

Bobillier, P., Seguin, S., Petiltjean, F., Salvert, D., Touret, M. and Jouvet, M., The raphe nuclei of the cat brain stem: A topographic atlas of their efferent projections as revealed by autoradiography, Brain Res., 113(1976)449-486.

Bogdansky, D.F., Pletcher, A., Brodie, B.B., and Udenfriend, S., Identification and assay of serotonin in brain, J. Pharmacol. Exp. Ther., 117(1956)82-88.

Bowker, R.M., Steinbusch, H.W.M., and Coulter, J.D., Serotonergic and peptidergic projections to the spinal cord demonstrated by a combined retrograde HRP histochemical and immunocytochemical staining method, Brain Res., 211(1981)412-417.

Bradshaw, R.A., and Young M., Nerve growth factor-recent developments and perspectives, Biochem. Pharmacol., 25(1976)1445-1449.

Breese, G.R., and Mueller, R.A., Alterations in the neurocytotoxicity of 5,7-dihydroxytryptamine by pharmacologic agents in adult and developing rats, Ann. N. Y. Acad. of Sci., (1978)160-174.

Breese, G.R., Traylor, T.D., Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons, J. Pharmacology and Therapeutics, 174(1970)413-420.

Brinkman, C., and Porter, R., Plasticity of motor behavior in monkeys with crossed forelimb nerves, Science, 220(1983)438-440.

Brown, M., and Ironton, R., sprouting and regression of neuromuscular synapses in partially denervated mammalian muscles, J. Physiol. London, 278(1978)325-348.

Bunge, R.P., Johnson, M., and Ross, C.D., Nature and nurture in development of the autonomic neuron, Science: 199(1978)1409-1416.

Burt, D.R., Denervation effects in sympathetic ganglia, Brain Res., 143(1978)573-579.

Cajal, S.R., Degeneration and Regeneration of the Nervous System, London: Oxford Univ. Press, (1928).

Cass, D.T., Sutton, T. J., and Mark, R.F., Competition between nerves for functional connexions with axolotl muscles, Nature Lodon, 243(1973)201-203.

Chu-Wang, I.W., and Oppenheim, R.W., Cell death of motoneurones in the chick embryo spinal cord: I. A light and electron microscopic study of naturally occurring and induced cell loss during development, J. Comp. Neurol., 177(1978a)33-58.

Chu-Wang, I.W., and Oppenheim, R.W., cell death of motoneurones in the chick spinal cord: II. A quantitative and qualitative analysis of degeneration in the ventral root, including evidence for axon outgrowth and limb innervation prior to cell death, J. Comp. Neurol., 177(1978b)59-86.

Clarke, P.G.H., Rogers, L.A., and Cowan, W.M., The time of origin and the pattern of survival of neurons in the isthmo-optic nucleus of the chick, J. Comp. Neurol., 167(1976)125-142.

Clemente, C.D., Regeneration in the vertebrate central nervous system, Int. Rev. Neurobiol., 6(1964)257-301.

Clemente, C.D., Structural regeneration in the mammalian central nervous system and the role of neuroglia and connective tissue, In: Regeneration inthe Central Nervous System, edited by Windle, W.F., Springfield, Ill: Thomas, (1955)147-161.

Clemente, C.D., and Windle, W.F., Regeneration of severed nerve fibres in the spinal cord of the adult cat., J. Comp. Neurol., 101(1954)691-731.

Clewans, C. and E.C. Azmitia, Tryptophan hydroxylase in the hippocampus and midbrain following 5.7-DHT injections into the cingulum bundle, Soc. Neurosci Abstr., 7(1982)744.

Cockett, S.A., and Kiernan, J.A., Acceleration of peripheral nervous regeneration in the rat by exogenous triiodothyronine, Exp. Neurol.,

39(1973)389-394.

Conrad, L.C., Leonard, C.M., and Pfaff, D.W., Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study, J. Comp. Neurol., 156(1974)179-206.

Cooper, E., Diamond J., and Turner, The effects of nerve section and of colchicine treatment of the density of mechanosensory nerve endings in salamander skin, J. Physiol. London, 264(1977)725-749.

Costa, M., Eranko, O., and Eranko, L., Hydrocortisone-induced increase in the histochemically demonstrable catecholamine content of sympathetic neurons of the newborn rat, Brain Res., 67(1974)457-466.

Cotman, C.W., Specificity of synaptic growth in brain: remodeling induced by kainic acid lesions, In: Progress in Brain Research, Development and Chemical Specificity of Neurons, edited by Cuenod, M., Amsterdam: Elsevier, 51(1979)203-215.

Cotman, C., Gentry, C., and Steward, O, Synaptic replacement in the dentate gyrus after unilateral entorhinal lesion: electron microscopic analysis of the extent of replacement of synapses by the remaining entorhinal cortex, J. Neurocytol., 6(1977)455-464.

Cotman, C.W., Lewis, E.R., and Hand D., The critical afferent theory: a mechanism to account for septohippocampal development and plasticity, In: Lesion-Induced Neuronal Plasticity in Sensorimotor System, edited by Flohr, H., and Precht, W., Springer-Verlag, New York, (1981)13-26.

Cotman, C.W., Lynch, G.S., Reactive synaptogenesis in the adult nervous system, In: Neuronal Recognition, edited by Barondes, S., Plenum Press, New York, (1976)69-108.

Cotman, C.W., Matthews, D.A., Taylor, D., and Lynch, G., Synaptic rearrangement in the dentate gyrus: Histochemical evidence of adjustments after lesions in immature and adult rats, Proc. Natl. Acad. Sci. USA, 70(1973)3473-3477.

Cotman, C.M. and Nadler, V., Reactive synaptogenesis in the hippocampus. Neuronal Plasticity, Raven Press, N. Y. , (1978)227-272.

Cotman, C.W., Nieto-Sampedro, M., and Harris, E.W., Synapse replacement in the nervous system of adult vertebrates, Physiol. Rev., 61(1981)684-784.

Cotman, C.W., and Scheff, S.W., Compensatory synapse growth in aged animals after neuronal death, In: Mechanisms of Aging and Development, edited by Strehler, B.L., Lausanne: Elsevier Sequoia S.A., (1979)103-117.

Cousin, M.A., Lando, D., and Moguilewsky, M., Ornithine Decarboxylase Induction by Glucocorticoids in Brain and Liver of Adrenalectomized

Rats, J. Neurochem., 38(1982)1296-1304.

Cowan, W.M., and Wenger, E., Degeneration in the nucleus of origin of the preganglionic fibers to the chick ciliary ganglion following early removal of the optic vesicle, J. Exp. Zool., 168(1968)105-123.

Cowan, W.M., and Wenger, E., Cell loss in the trochlear nucleus of the chick during normal development and after radical extirpation of the optic vesicle, J. Exp. Zool., 164(1967)267-280.

Crutcher, K.A., An attempt to identify the suprasegmental source of spinal cord monoamines in the opossum (Didelphis marsupialis), Anat. Res., 187(1977)559.

Crutcher, K.A., and Collins, F., In vitro evidence for two distinct hippocampal growth factors: basis of neuronal plasticity? Science, 217(1982)67-68.

Curzon, G., and Green, A.R., Effect of Hydrocortisone on Rat Brain 5-Hydroxytryptamine, Life Sci. 7(1968)657-663.

Dahlstrom, A., and Fuxe, K., Evidence for the existence of monoamine neurons in the central nervous system II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neurons, Acta Physiol. Scand., Suppl., 247(1965)1-36.

Dahlstrom, A., and Fuxe, K., Evidence for the existence of monoamine containing neurons in the central nervous system. 1. Demonstration of monoamines in brain stem neurons, Acta physiol. Scand., 62, Suppl., 232(1964)1-55.

Denburg, J.S., Seecof, R.L., and Horridge, G.A., The path and rate of growth of regenerating motor neurons in the cockroach, Brain Res., 125(1977)149-153.

Dennis, M.J., and Yip, J.W., Formation and elimination of foreign synapses on adult salamander muscle, J. Physiol. London, 274(1978)299-310.

DeVellis, J., and Inglish, D., Hormonal control of gylcerol phosphate dehydrogenase in the rat brain, J. Neurochem., 15(1968)1061-1070.

Devor, M., Schneider, G.E., Neuroanatomical plasticity: the principle of conservation of total axonal arborization, In: Aspects of neural plasticity, edited by Vital-Durand, F., and Jeannerod, M., Paris: Inserm, (1970)191-201.

Diamond, J., Cooper E., Turner, C., and MacIntyre, L., Trophic regulation of nerve sprouting, Science, 193(1976)371-377.

Diaz-Guerrero, R., Thomson, J.D., and Hines, H.M., Effect of thymectomy hyperthyroidism and hypothyrodism on neuromuscular atrophy and regeneration, Am. J. Physiol., 151(1947)91-95.

Dieringer, N., and Precht, W., Modification of synaptic input following unilateral labyrinthectomy, Nature, 269(1977)431-433.

Dieringer, N., and Precht, W., Mechanism of compensation for vestibular deficits in the frog. I. Modification of the excitatory commissural system, Exp. Brain Res. 36(1979a)311-328.

Dieringer, N., and Precht, W., Mechanism of compensation for vestibular deficits in the frog. II. Modification of the inhibitory pathways, Exp. Brain Res., 36(1979b)329-341.

Dolivo, M., Meurant,C., and Verdan C.I., The retrograde axonal flow depends on neuronal activity, Experientia, 33(1977)778.

Dostrovsky, J.O., Millar, J., and Wall, P.D., The immediate shift of afferent drive of dorsal column nucleus cells following deafferentation: a comparison of acute and chronic deafferentation in gracile nucleus and spinal cord, Exp. Neurol., 52(1976)480-495.

Druckman, R., Review of structural evidence of regeneration of nerve fibres in injury to the human spinal cord, In: Regeneration in the Central Nervous System, edited by Windle, W.F., Springfield, III: Thomas, (1955)241-246.

Dunnett, S.B., Bjorklund, A., Stenevi, U., and Iversen, S.B., Behavioral recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. 1. Unilateral lesions, Brain Res., 215(1981a)147-161.

Dunnett, S.B., Bjorklund, A., Stenevi, U., and Iversen, S.B., Behavioral recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. II Bilateral lesions, Brain Res., 229(1981b)457-470.

Edds, M.V., Jr., Collateral nerve regeneration, Q. Rev. Biol., 206(1953)260-276.

Edds, M.V., Jr., Schneider, G.E., Gaze, R.M., and Irwin, L.N.(editors), Neurosci. Res. Program Bull., 17(1979)245-275.

Edwards, C., The effect of innervation on the properties of acetylcholine receptors in muscle, Neuroscience, 4(1979)565-584.

Eiserman, J.S., and Azmitia, E.C., Physiological stimulation enhances HRP marking of salivary neurons in rats, Brain Res. Bull., 8(1982)73-78.

Emson, P, C., Bjorklund, A., and Stenevi, U., Evaluation of the regenerative capacity of central dopaminergic, noradrenergic and cholinergic neurons using iris implants as targets, Brain Res., 135(1977)87-105.

Eranko, L., Postnatal development of histochemically demonstrable

catecholamines in the superior cervical ganglion of the rat, Histochem. J., 4(1972)225-236.

Eranko, O., Heath, J., and Eranko, L., Effect of Hydrocortisone on the Ultrastructure of the Small, Intensely Fluorescent, Granule-Containing Cells in Cultures of Sympathetic Ganglia of Newborn Rats, Z. Zellforsch., 134(1972)297-310.

Ernst, M., Uber Untergang von zellen wahrend der normalen Entwicklung bei Wirbeltieren, Z.Anat. Entwicklungsgesch, 79(1926)228-262.

Erspamer, V., Asero, B., Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine, Nature, 169(1952)800-801.

Etgen, A.M., Lee, K.S., and Lynch, G., Glucocorticoid Modulation of Specific Protein Metabolism in Hippocampal Slices Maintained in Vitro, Brain Res., 165(1979)37-45.

Fambrough, D.M., Control of acetylcholine receptors in skeletal muscle, Physiol. Rev., 59(1979)166-227.

Fangboner, R.F., and Vanable, J.W., Jr., Formation and regression of inappropriate nerve sprouts during trochlear nerve regeneration in Xenopus lasvis, J. Comp. Neurol., 157(1974)391-406.

Feringa, E.R., Gurden, G.G., Strodel, W., Chandler, W., and Knake, J., Descending spinal motor tract regeneration after spinal cord transection, Neurol., 23(1973)599-608.

Feringa, E.R., Johnson, R.D., and Wendt, J.S., Spinal cord regeneration in rats after immunosuppressive treatment, Arch, Neurol., Chicago, 32(1975)676-683.

Fertig, A., Kiernan, J.A., and Seyan, S.S.A.S., Enhancement of Axonal Regeneration in the Brain of the Rat by Corticotrophin and Triiodothyronine, Exp. Neurol., 33(1971)372-385.

Forman, D.S., McQuarrie, I.G., Labore, F.W., Wood, D.K., Stone L.S., Braddock C.H., and Fuchs, D.A., Time course of the conditioning lesion effect on axonal regeneration, Brain Res. 182(1980)180-185.

Frankfurt, M., and Azmitia, E., The effect of intracerebral injections of 5,7-dihydroxytryptamine and 6-hydroxydopamine on the serotonin-immunoreactive cell bodies and fibers in the adult rat hypothalamus, Brain Res., 261(1983)91-99.

Frankfurt, M., and Azmitia, E.C., Regeneration of serotonergic fibers in the adult rat hypothalamus: an immunocytochemical study, Soc. Nuerosci. Abstr., 8(1982)758.

Frankfurt, M., Lauder, J.M., and Azmitia, E.C., The immunocytochemical localization of serotonergic neurons in the rat hypothalamus, Neurosc.

Lett., 24(1981)227-232.

Freed, W.J., Perlow, M., Karoum, F., Seiger, A., Olson, L, Hoffer, B.J., and Wyatt, R.J., Restoration of dopaminergic function by grafting of fetal rat substantia nigra to the caudate nucleus: long-term behavioral, biochemical, and histochemical studies, Ann. Neurol., 8(1980)510-519.

Friedrich, V.L., Jr., and Bohn, M.C., Glucocorticoids inhibit myelination in developing rat, Abstr. 1323, Soc. Neurosci. Ann. Mtg., Cincinnati, OH, (1980)380.

Furshpan, E.J., MacLeish, P.R., O'Lague, P.H., and Potter, D.D., Chemical transmission between rat sympathetic neurons and cardiac myocytes developing in microcultures: evidence for cholinergic, adrenergic and dual-function neurons, Proc. Natl. Acad. Sci. USA, 73(1976)4225-4229.

Fuxe, F., Evidence for the exixtence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system, Acta. Physiol. Scand. Suppl., 247(1965)37-85.

Fuxe, K., Distribution of monoamine nerve terminals in the central nervous system, Acta Physiol. Scand. Suppl., 247(1965)37-85.

Fuxe, K., and Jonsson, G., Further mapping of central 5-hydroxytryptamine neurons: studies with the neurotoxic dihydroxytrptamine, In: Advance Biochemical psychopharmacology, edited by Costa, E., Gessa, G.L., and Sandler, M., New York: Raven, 10(1974)1-10.

Gall, C., Rose, G. and Lynch G., Proliferative and migratory activity of glial cells in the partially deafferented hippocampus, J. Comp. Neur., 183(1979)539-550.

Gash, D., Sladek, J.R., Vasopressin neurons grafted into brattleboro rats -Viability and activity, Peptides, 1(1980)11-14.

Gerson, S., and Baldessarini, R.J., Selective destruction of serotonin terminals in rat forebrain by high doses of 5,7-dihydroxytryptamine, Brain Res., 85(1975)140-145.

Gerson, S., Baldessarini, R.J., and Wheeler, S.C., Biochemical effects of dihydroxylated tryptamines on central indoleamine neurones, Neuropharmacology, 13(1974)987-1004.

Gilad, G.M., Joh, T.H., Pickel, V.M.,, and Reis, D.J., Biochemical and immunocytochemical evidences for collateral sprouting in mesolimbic dopaminergic neurons in the rat brain, Soc. Neurosci. Abstr., 2(1976)813.

Gilad, G.M., and Reis, D.J., Collateral sprouting in cerebral

mesolimbic dopamine neurones: biochemical and immunocytochemical evidence of changes in the activity and distribution of tyrosine hydroxylase in terminal fields and in cell bodies of A10 neurons, Brain Res., 160(1979)17-36.

Glucksmann, A., Cell deaths in normal vertebrate ontogeny, Biol. Rev., 26(1951)59-86.

Goldberger, M.F. and Murry, M., Restitution of function and collateral sprouting in the rat spinal cord: The deafferented animal, J. Comp. Neurol., 158(1974)37-54.

Goldowitz, D., and Cotman, C.W., Do neurotrophic interactions control synapse formation in the adult rat brain? Brain Res., 181(1980)325-344.

Goldowitz, D., Vincent, S.R., Wu, J.Y., and Hokfelt, T., Immunohistochemical demonstration of plasticity in GABA neurons of the adult rat dentate gyrus, Brain Res., 238(1982)413-420.

Goldschmidt,, R. and Steward, O., Time course of increase in retrograde labeling and increase in cell size of entorhinal cortex neurons sprouting in response to unilateral entohrinal lesion, J. Comp. Neurol., 189(1980)359-179.

Goodman, D.C., and Horel, J.A., Sprouting of optic tract projections in the brain stem of the rat, J. Comp. Neurol., 127(1967)71-88.

Goodman, D.C., Bogdasarian, R.S. and Horel, J.A., Axonal sprouting of ipsilateral optic tract following opposite eye removal, Brain Behav. Evol., 8(1973)27-30.

Grafstein, B., and McQuarrie, I.G., Role of nerve cell body in axonal regeneration, In: Neuronal Plasticity, edited by Cotman, C.W., New York: Raven, (1978)155-195.

Gray, J.A., The Neuropsychology of Anxiety: An Inquiry into the Functions of the Septo-Hippocampal System, Oxford: Clarendon Press and New York Oxford University Press (1982).

Guth, L., Regeneration in the mammalian peripheral nervous system, Physiol. Rev., 36(1956)461-478.

Guth, L., and Bernstein, J.J., Selectivity in the re-establishment of synapses in the superior cervical ganglion of the cat, Exp. Neurol., 4(1961)59-69.

Guth, L., Smith, S., Donati, E.J., and Albuquerque, E.X., Induction of intramuscular collateral nerve sprouting by neurally applied colchicine, Exp. Neurol., 67(1980)513-525.

Haimann, C., Mallert, A., and Zilbergachelin, N.F., Competition between motor nerves in the establishment of neuromuscular junctions in

striated muscles of Xenopus laevis, Neurosci. Lett., 3(1976)15-20.

Halaris, A.E., Jones, B.E., Moore, R.Y., Axonal transport in serotonin neurons of the midbrain raphe, Brain Res., 107(1976)554-574.

Halperin, J.J., and LaVail, J.H., A study of the dynamics of retrograde transport and accumulation of horseradish peroxidase in injured axons, Brain Res., 100(1975)253-269.

Hamburger, V., and Levi-Montalcini, R., Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions, J. Exp. Zool., 111(1949)457-501.

Harrison, R.G., The outgrowth of the nerve fiber as a mode of protoplasmic movement, J. Exp. Zool., 9(1910)787-848.

Harrison, R.G., Observation on the living developing nerve fiber, Anat. Res., 1(1908)116-118.

Hawrot, E., Cultured sympathetic neurons: effects of cell-derived and synthetic substrata on survival and development, Dev. Biol., 74(1980)136-151.

Heinicke, E., Influence of exogenous triiodothyronine on axonal regeneration and wound healing in the brain of the rat, J. Neurol. Sci., 31(1977)293-305.

Hoffman, H., Local re-innervation in partially denervated muscle: a histophysiological study, Aust. J. Exp. Biol. Med. Sci., 28(1951)383-397.

Hoffman, P.N., Lasek, R.J., The slow component of axonal transport: identification of major structural polypeptides of the axon and their generality among mammalian neurons, J. Cell Biol., 66(1975)351-366.

Holland, R.L., and Brown, M.C., Postsynaptic transmission block can cause terminal sprouting of a motor nerve, Science, 207(1980)649-651.

Hughes, W.F., and LaVelle, A., The effects of early tectal lesions on development in the retinal ganglion cell layer of chick embryos, J. Comp. Neurol., 163(1975)265-284.

Humbertson, A.O., Jr., and Martin, G.F., The development of monoaminergic brainstem-spinal systems in the North American Opossum, Anat. Embryol., 156(1979)301-318.

Ironton, R., Brown, M.C., and Holland, R.L., Stimuli to intramuscular nerve growth, Brain Res., 156(1978)351-354.

Iversen, L.L., and Storm-Mathisen, J., Uptake of [$^3$H]GABA and [$^3$H]glycine acid in excitatory nerve endings in the hippocampal formation of the rat, Acta physiol. scand. 96(1976)22A-23A.

Jacobs, B., Trimbach, C., Eubanks, E.E., and Trulson, M, Hippocampal mediation of raphe lesion- and PCPA-induced hyperactivity in the rat, Brain Res., 94(1975)253-261.

Jacoby, J.H., Lytle, L.D., Nelson, M.F., Long-term effects of 5,7-dihydroxytryptamine on brain monoamines, Life Sci., 14(1974)909-919.

Jones, B.E. and Moore, R.Y., Ascending projections of the locus coeruleus in the rat: Autoradiographic analysis, Brain Res., 127(1977)25-53.

Jones, R., and Vrbova, G., Two factors responsible for the development of denervation hypersensitivity, J. Physiol., London, 236(1974)517-538.

Jones, R., and Vyskocil, F., An electrophysiological examination of the changes in skeletal muscle fibers in response to degenerating nerve tissue, Brain Res., 88(1975)309-317.

Kallius, E., Der Zelluntergang als Mechanismus bei der Histio-und Morphogenese, Verh. Anat. Ges. Suppl. Anat. Anz., 72(1931)10-22.

Kao, C.C., Chang, L.W., and Bloodworth, J.M.B., Jr., Axonal regeneration across transected mammalian spinal cords: an electron microscopic study of delayed microsurgical nerve grafting, Exp. Neurol., 54(1977)591-615.

Katzman, R., Bjorklund A., Owman, C.H., Stenevi, U., and West, K.H., Evidence for regenerative axon sprouting of central catecholamine neurons in the rat mesencephalon following electrolytic lesions, Brain Res., 25(1971)579-596.

Katzman, R., Broida, R., and Raine, C.S., Reinnervation, Myelination and organization of iris implanted into the midbrain— an ultrastructural study, Brain Res., 135(1977)112-123.

Knowles, J.F., and Berry, M., Effects of deoxycorticosterone acetate on regeneration of axons in the mammalian central nervous system, Exp. Neurol., 62(1978)1-15.

Knyihar E., Csillik, B., and Rakic, P., Transient synapses in the embryonic primate spinal cord, Science, 202(1978)1206-1209.

Ko, C-P., Burton, H., Johnson, M.I., and Bunge, R.P., Synaptic transmission between rat superior cervical ganglion neurons in dissociated cell cultures, Brain Res., 117(1976)461-485.

Koda, L.Y., and Bloom, F.E., A light and electron microscopic study of noradrenergic terminals in the rat dentate gyrus, Brain Res., 120(1977)327-335.

Kohler, C., and Steinbusch, H., Identification of serotonin and

non-serotonin-containing neurons of the mid-brain raphe projecting to entorhinal area and the hippocampal formation. A combined immunohistochemical and fluorescent retrograde tracing study in the rat brain, Neuroscience, 7(1982)951-975.

Kohler,C., Chan-Palay, V., and Steinbusch, H., The distribution and orientation of serotonin fibers in the entorhinal and other retrohippocampal areas: An immunohistochemical study with antiserotonin antibodies in the rat's brain, Anat. Embryol., 161(1981)237-264.

Kreiger, D.T., Perlow, M.J., Suson, M.J., Davies, T.F., Zimmerman, E.A., Ferin, M., and Charlton, E.M., Brain grafts reverse hypoganadism of gonadotropin releasing hormone deficiency, Nature, 298(1982)468-471.

Kuffler, S., Thompson, W., and Jansen, J.K.S., The elimination of synapses in multiple-innervated skeletal muscle fibers of the rat. Dependence on distance between end-plates, Brain Res., 138(1977)353-358.

Landmesser, L.T., The role of the periphery in cell death, Neurosci. Res. Prog. Bull., 14(1976)295-301.

Landmesser, L., and Pilar, G., Synapse formation during embryogenesis on ganglion cells lacking a periphery, J. Physiol. (London), 241(1974a)751-736.

Landis, S.C., Developmental changes in the neurotransmitter properties of dissociated sympathetic neurons: a cytochemical study of the effects of medium, Dev. Biol., 77(1980)349-361.

Landmesser, L.T., The role of the periphery in cell death, Neurosci. Res. Prog. Bull., 14(1976)295-301.

Landmesser, L., and Pilar, G., Synapse formation during embryogenesis on ganglion cells lacking a periphery, J. Physiol. (London), 241(1974a)751-736.

Landmesser, L., and Pilar, G., Synaptic transmission and cell death during normal ganglionic development, J. Physiol. (London), 241(1974b)737-749.

Lavoie, P.A., Collier, B., and Tenenhouse, A., Comparison of alpha-bungarotoxin binding to skeletal muscle after inactivity of denervation, Nature London, 260(1976)349-350.

LeDouarin, N.M., The ontogeny of the neural crest in avian embryo chimeras, Nature, 286(1980)663-669.

Lee, K.S., Stanford, E.J., Cotman, C.W., and Lynch, G.S., Ultrastructural evidence for bouton sprouting in the adult mammalian brain, Exp. Brain Res., 29(1977)475-485.

Leveille, P.J., McGinnis, J.F., Maxwell, D.S., and DeVellis, J.,

Immunocytochemical localization of glycerol-3-phosphate dehydrogenase in rat oligodendrocytes, Brain Res., 196(1980)287-305.

Levi-Montalcini, R., The nerve growth factor: its role in growth, differentiation and function of the sympathetic adrenergic neuron, In: Progress in Brain Research Perspectives in Brain Research, edited by Corner, M.A. and Swaab, D.F., Amsterdam: Elsevier, 45(1976)235-258.

Levi-Montalcini, R., and Hamburger, V., Selective growth stimulating effects of mouse sarcoma on sensory and sympathetic nervous of the chick embryo, J. Exp. Zool., 116(1951)321-362.

Lidbrink, P., Jonsson, G., and Fuxe, K., The effect of imipramine-like drugs and antihistamine drugs on uptake mechanisms in the central noradenaline and 5-hydroxytryptamine neurons. Neuropharmocol. 10(1971)521-536.

Lidov, H.G.W., Grzanna, R. and Molliver, M.E., The serotonin innervation of cerebral cortex in the rat: an immunohistochemical analysis, Neuroscience, 5(1980)207-223.

Lindvall, O., and Bjorklund, A., The organization of ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence technique, Acta. Physiol. Scand. Suppl., 412(1974)1-48.

Litchy, W.J., Uptake and retrograde transport of horseradish peroxidase of frog sartorius nerve in vitro, Brain Res., 56(1973)377-381.

Liu, C.M. and Chambers, W. W., Intraspinal sprouting of dorsal root axons, Arch. Neurol. (Chic), 79(1958)46-61.

Loesche, J. and Steward, O., Behavioral correlates of denervation and reinnerration of the hippocampal formation of the rat: recovery of alternation performance following unilateral entorhinal cortex lesion, Brain Res. Bull., 2(1977)31-39.

Lomo, T., and Westgaard, R.H., Control of ACh sensitivity in rat muscle fibers, Cold spring Harbor Symp. Quant. Biol., 40(1975)263-274.

Loy, R., and Milner, T.A., Sexual Dimorphism in Extent of Axonal Sprouting in Rat Hippocampus, Science, 208(1980)1282-1284.

Lund, R.D., Cunningham, T.J. and Lund, J.S., Modified optic projection after unilateral eye removal in young rats, Brain Behav. Evol., 8(1973)51-72.

Lund, R.D., and Lund, J.S. Synaptic adjustment after deafferentation of the superior colliculus of the rat, Science, 171(1971)804-807.

Lynch, G.S., Deadwyler, S.A., and Cotman, C., Postlesion axonal growth produces permanent functional connections, Science, 180(1973)1364-1366.

Lynch, G., Gall, C., Rose, G. and Cotman, C., Change in the distribution of the dentate gyrus associational system following unilateral or bilateral entorhinal lesion in adult rat, Brain Res., 110(1976)57-71.

Lynch, G.S., Matthews, D.A., Mosko, S. Parks, T., and Cotman, C.W., Induced acetylcholinesterase-rich layer in rat dentate gyrus following entorhinal lesions, Brain Res., 42(1972)311-318.

Lynch, G., Rose, G., Gall, C., and Cotman, C.W., The response of the dentate gyrus to partial deafferentation, In: Golgi Centennial Symposium Proceedings, edited by Santini, M., New York: Raven, (1975)505-517..

Maehlen, J., and Nja, A., Sprouting after partial denervation in the superior cervical ganglion: effect of preganglionic nerve stimulation, Acta Physiol. Scand., 105(1979)18A-19A.

Manthorpe, M., Nieto-Sampedro, M., Skaper, S.D., Lewis, E.R., Barbin, G. Longo, F.M., Cotman, C.W., and Varon, S., Neuronotrophic activity in brain wounds of the developing rat. Correlation with implant survival in the wound cavity, Brain Res., 267(1983)47-56.

Martin, G.F., Cabana, T., and Humbertson, A.O., Jr., The brainstem origin of monoaminergic projections to the spinal cord of the North American opossum: a study using fluorescent tracers and fluorescence histochemistry, Brain Res. Bull., 9(1982)217-225.

Matsumoto, A., and Arai, Y., Synaptogenic effect of estrogen on the hypothalamic arcuate nucleus of the adult female rat, Cell Tiss. Res., 198(1979)427-433.

McCouch, G.P., Austin, G.M., Liu, C.N. and Liu, C.Y., Sprout as a cause of spasticity, J. Neurophysiol., 21: (1958)205-216.

McEwen, B.S., and Micco, D.J., Jr., Toward an understanding of the multiplicity of glucocorticoid actions on brain function and behavior, In: The brain as an endocrine target organ in health and disease, editors: van Keep, P.A., and DeWied, D., (1980)11-28.

McEwen, B.S., and Wallach, G., Corticosterone binding to hippocampus: nuclear and cytosol binding in vitro, Brain Res., 57(1973)373-386.

McGeer, P.L., McGeer, E.G., and Nagai, T., GABAergic and cholinergic indices in various regions of rat brain after intracerebral injections of folic acid., Brain Res., 260(1983)107-116.

McLennan, I.S., Hill, C.E., and Hendry, I.A., Glucocorticosteroids modulate transmitter choice in developing superior cervical ganglion, Nature, 283(1980)206-207.

McMaster, R.E., Regeneration of the spinal cord in the rat: effects of

piromen and ACTH upon the regeneration capacity, J. Comp. Neurol. 119(1962)113-125.

McEwen, B.S., Weiss, J.M., and Schwartz, L.S., Retention of corticosterone by cell nuclei from brain regions of adrenalectomized rat, Brain Res., 17(1970)471-482.

McIssaac, G., and Kiernan, J.A., Acceleration of neuromuscular re-innervation by triiodothyronine, J. Anat., 120(1975)551-560.

McNaughton, N., Azmitia, E.C., Williams, J.H., Buchan, A., and Gray, J.A., Septal elicitation of hippocampal theta rhythm after localized de-afferentation of serotonergic fibers, Brain Res., 200(1980)259-269.

McQuarrie, I.G., The effect of a conditioning lesion on the regeneration of motor axons, Brain Res., 152(1978)597-602.

McQuarrie, I.G., Accelerated axonal sprouting after nerve transection, Brain Res. 167(1973)185-188.

McQuarrie, I.G., and Grafstein, Axon outgrowth, Enhanced by a previous nerve injury, Arch. Neurol. Chicago, 29(1973)53-55.

McQuarrie, I.G., Grafstein, B., and Gershon M.D., Axonal regeneration in the rat sciatic nerve: effect of a conditioning lesion and of dbcAMP, Brain Res., 132(1977)443-453.

McRae-Degueurce, A., Berod, A., Mermet, A., Keller, A., Chouvet, G., Joh, T.H., and Pujol, J.F., Alterations in tyrosine hydroxylase activity elicited by raphe nuclei lesions in the rat locus coeruleus: evidence for the involvement of serotonin afferents, Brain Res., 235(1982)285-301.

McWilliams, R., and Lynch, G., Terminal proliferation and synaptogenesis following partial deafferentation: the reinnervation of the inner molecular layer of the dentate gyrus following removal of its commissural afferents, J. Comp. Neurol., 180(1978)581-616.

Mesulam, M-M., Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivy for visalizing neural afferents and efferents, J. Histochem. Cytochem. 26(1978)106-117.

Mesulam, M-M., Tracing Neural Connection with Horseradish Peroxidase. John Wiley and Sons Publishing, N.Y. 1982.

Mesulam, M.M., and Rosene, D.L., Sensitivity in Horseradish Peroxidase Neurohistochemistry: A comparative and quantitative study of nine methods, J. Histochem. Cytochem., 27(1979)763-773.

Meyer, J.S., Micco, D.J., Stephenson, B.S., Krey, L.C., and McEwen, B.S., Subcutaneous implantation method for chronic glucocorticoid replacement therapy, Physiol. Behav., 22(1979)867-870.

Miledi, R., and Potter, L.T., Acetylcholine receptors in muscle fibers, Nature, 233(1971)599-603.

Millard, S.A., Costa, E., and Gal, E.M., On the control of brain serotonin turn over rate by end product inhibition, Brain Res., 40(1972)545-551.

Moore, R.Y., Bjorklund, A., and Stenevi, U., Growth and plasticity of adrenergic neurons, In: The Neurosciences, 3rd Study Program, edited by Schmitt, F.O., Cambridge: MIT Press, (1974)961-977.

Moore, R.Y., Growth of adrenergic neurons in the adult mammalian nervous system, In: Dynamics of Degeneration and Growth in Neurons, edited by Fuxe, K., Olson, L., and Zotterman, Y., Oxford: Pergamon, (1974)379-388.

Moore, R.Y., In: Plasticity and Recovery of Function in the Central Nervous System, edited by Stein, D.G., Rosen, J.J., and Butters, New York: Acad. Press, (1974b)111-128.

Moore, R.Y., Bjorklund, A., and Stenevi, U., Plastic changes in the adrenergic innervation of rat septal area in response to denervation, Brain Res., 33(1971)13-39.

Moore, R.Y. and Halaris A.E., Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat, J. Histochem. Cytochem., 164(1975)171-184.

Moore, R.Y., Halaris, H.E., and Jones, B.E., Serotonin neurons of the midbrain raphe: ascending projections, J. Comp. Neurol., 180(1978)417-438.

Morrell, J.I., Greenberger, L.M., and Pfaff, D.W., Comparison of horseradish peroxidase visualization methods: quantitative results and further technical specifics, J. Histochem. Cytochem., 29(1981)903-916.

Mosko, S., Lynch, G., and Cotman, C.W., Distribution of the septal projections to the hippocampal formation of the rat, J. Comp. Neurol., 152(1973)163-174.

Murakami, F., Fujito, Y. and Tsukuhara, N., Physiolo- gical properties of the newly formed corticorubral synapses of red nucleus neurons due to collateral sprouting, Brain Res., 103(1976)147-51.

Murray, M., A quantitative study of regenerative sprouting by optic axons in goldfish, J. Comp. Neurol., 209(1982)352-362.

Murray, M., and Edwards, M., A quantitative study of the reinnervation of the goldfish optic tectum following optic nerve crush, J. Comp. Neurol., 209(1982)363-373.

Murray, J.G., and Thompson, J.W., the occurence and function of

collateral sprouting in the sympathetic nervous system of the cat, J. Physiol., London, 135(1957)133-162.

Nadi, N.S., Head, R., Grillo, M., Hempstead, J., Grannot-Reisfeld, N., and Margolis, E.L., Chemical deafferentation of the olfactory bulb: plasticity of the levels of tyrosine hydroxylase, dopamine and norepinephrine, Brain Res., 213(1981)365-377.

Nadler, J.V., Paoletti, C., Cotman, C.W., and Lynch, G., Histochemical evidence of altered development of cholinergic fibers in the rat dentate gyrus following lesions. II, Effects of partial entorhinal and simultaneous multiple lesions, J. Comp. Neurol., 171(1977a)589-604.

Nadler, J.V., Vaca, K.W., and White, W.F., Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents. Nature 260(1976)538-540.

Nadler, J.V., White, W.F., Vaca, K.W., and Cotman, C.W., Calcium-depnedent y-aminobutyrate release by interneurons of rat hippocampal regions: lesion-induced plasticity, Brain Res., 131(1977b)214-258.

Neckers, L., and Sze, P.Y., Regulation of 5-hydroxytryptamine metabolism in mouse brain by adrenal glucocorticoids, Brain Res., 93(1975)123-132.

Nja, A., and Purves, D., Specificity of initial synaptic contacts made on guinea pig superior cervical ganglion cells during regeneration of the cervical sympathetic trunk, J. Physiol. London, 281(1978)45-62.

Nakamura, Y., Mizuno, N., Konishi, A. and Sato, M., Synaptic reorganization of the red nucleus after chronic deafferentation from cerebellorubral fibers: an electron microscope study in the cat, Brain Res., 82(1974)298-301.

Nieto-Sampedro, M., Lewis, E.R., and Cotman, C.W., Brain injury causes a time-dependent increase in neuronotrophic activity at the lesion site, Science, 217(1982)860-861.

Nishino, H., Ono, T., Sasaki, K., Nishino, A., and Muramoto, K., Retrograde transport of horseradish peroxidase in sciatic nerve of rats and dystrophy mice, Neurosci. Lett., 14(1979)1-6.

Nobin, A.H., Baumgarten, H.G., Bjorklund, A., Lachenmayer, L., and Stenevi, U., Axonal degeneration and regeneration of the bulbospinal indolamine neurons after 5,6-dihydroxytryptamine treatment, Brain Res., 56(1973)1-24.

Nurse, C.A., and O'Lague, P.H., Formation of cholinergic synapses between dissociated sympathetic neurons and skeletal myotubes of the rat in cell culture, Proc. Natl. Acad. Sci. USA, 72(1975)1955-1959.

Nygren, L.G., Fuxe, K., Jonsson, G., and Olson, L., Functional

regeneration of 5-hydroxytryptamine nerve terminals in the rat spinal cord following 5,6-dihydroxytryptamine induced degeneration, Brain Res., 78(1974)377-394.

Nygren, L.G., and Olson, L., On spinal noradrenaline receptor supersensitivity: correlation between nerve terminal dendrites and flexor reflexes at various times after intracisternal 6-hydroxydopamine. Brain Res., 116(1976)455-470.

Nygren, L.G., and Olson, L., Intracisternal neurotoxins and monoamine neurons innervating the spinal cord: acute and chronic effects on cell and axon counts and nerve terminal densities, Histochemistry, 306(1977)52-281.

O'Lague, P.H., Potter, D.D., and Furshpan, E.J., Studies on rat sympathetic neurons developing in cell culture.III. Cholinergic transmission, Dev. Biol., 67(1978)424-443.

Parent, A., Descarries, L., and Beaudet, A., Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of $^{3}$H5-HT, Neurosci., 6(1981)115-130.

Parvez, H., and Parvez, S., The effects of metopirone and adrenalectomy on the regulation of the enzymes monoamine oxidase and catechol-o-methyl transferase in different brain regions, J. Neurochem., 20(1973)1011-1020.

Pasik, T., and Pasik, P., Serotoninergic afferents in the monkey neostriatum, Acta biol. Acad. Sci. hung. 33(2-3)(1982)277-288.

Patterson, P.H., Environmental determination of autonomic neurotransmitter functions, Ann. Rev. Neurosci., 1(1978)1-17.

Patterson, P.H., and Chun, L.L.Y., The induction of acetylcholine synthesis in primary cultures of dissociated rat sympathetic neurons.I. Effects of conditioned medium. Develop Biol., 56(1977a)263-280.

Patterson, P.H., and Chun, L.L.Y., The induction of acetylcholine synthesis in primary cultures of dissociated rat sympathetic neurons.II. Developmental aspects, Dev. Biol., 60(1977b)473-481.

Pecci Saavedra, J., Pasik, T., and Pasik, P., Immunocytochemistry of serotoninergic neurons in the central nervous system of monkeys, In: Neural Transmission, Learning and Memory, editors: Caputto, R., and Ajmone Marsan, C., Raven Press, New York, (1983)81-96.

Perlow, M.J., Freed, W.J., Hoffer, B.J., Seiger, A., Olson, L., and Wyatt, R.J., Brain grafts reduce motor abnormalities produced by the destruction of the nigrostriatal dopamine system, Science, 204(1979)643-647.

Pestronk, A., and Drachman, D.B., Motor nerve sprouting and acetylcholine receptors, Science, 199(1978)1223-1225.

Pestronk, A., Drachman, D.B., and Griffin, J.W., Effect of muscle disuse on acetylcholine receptors, Nature London, 260(1976)352-353.

Piatt, J., The influence of the peripheral field on the development of the mesencephalic V nucleus in Amblystoma, J. Exp. Zool., 102(1946)109-141.

Pickel, V.M., Krebs, H., and Bloom, F.E., Proliferation of norepinephrine-containing axons in rat cerebellar cortex after penduncle lesions, Brain Res., 59(1973)169-179.

Pickel, V.M., Segal, M. and Bloom, F.E., Axonal proliferation following lesions of cerebellar peduncles: a combined fluorescence microscopic and radioautographic study, J. Comp. Neurol., 155(1974)43-50.

Precht, W., Shimazu, H., and Markham C.H., A mechanism of central compensation of vestibular function following hemilabyrinthectomy, J. Neurophysiol., 29(1966)996-1010.

Prestige, M.C., The control of cell number in the lumbar ventral horns during the development of Xenopus laevis tadpoles, J. Embryol. Exp. Morphol., 17(1967a)453-471.

Prestige, M.C., The control of cell number in the lumbar ventral horns during the development of Xenopus laevis tadpoles, J. Embryol.Exp. Morphol., 18(1967b)359-387.

Proctor, W., Frenk, S., Taylor, B., and Roper, S., "Hybrid" synapses formed by foreign innervation of parasympathetic neurons: a model for selectivity during competitive reinnervation, Proc. Natl. Acad. Sci. USA 76(1977)4695-4699.

Puchala, E., and Windle, W.F., The possibility of structural and functional restitution after spinal cord injury. A review, Exp. Neurol., 55(1977)1-42.

Purves, D., Lichtman, J.W., Formation and maintenance of synaptic connections in autonomic ganglia, Physiol. Rev., 58(1978)821-862. contralateral entorhinal cortex following ipsilateral entorhinal lesion, Exp. Brain Res., 20(1974)45-66.

Rager, G., Systems-matching, II. Interpretation of the generation and degeneration of retinal ganglion cells by a mathematical model, Exp. Brain Res., 33(1978)79-90.

Rager, G., The development of the retinotectal projection in the chicken, Adv. Anat. Embryol. Cell Biol., 63(1980).

Rager, G., The significance of neuronal cell death during the

development of the nervous system, In: "Lesion-Induced Neuronal Plasticityin Sensorimotor Systems" ,edited by Flohr, H., and Precht, W., Berlin Heidelberg: Springer-Verlag(1981).

Rager, G., and Rager U., Systems-matching by degeneration: I. A quantitative electronmicroscopic study of the generation and degeneration of retinal ganglion cells in the chicken, Exp. Brain Res., 33(1978)65-78.

Raisman, G., Neuronal plasticity in the septal nuclei of the adult rat, Brain Res., 14(1969)25-48.

Raisman, G., and Field, P.M., A quantitative investigation of the development of collateral reinnervation after partial deafferentation of the septal nuclei, Brain Res., 50(1973)241-264.

Ralston, H.J., III, and Chow, K.L., Synaptic reorganization in the degenerating lateral geniculate nucleus of the rabbit, J. Comp. Neurol., 147(1972)321-350.

Rand, M., and Reid, G., Source of serotonin in serum, Nature, 168(1951)385.

Rees, H.D., Stumpf, W.E., Sar, M., Autoradiographic studies with $^{3}$H-dexamethasone in the rat brain and pituitary, In: Anatomical neuroendocrinology, editors: Stumpf, W.E., and Grant, L.A., Karger, Basel, (1975)262-269.

Reid, G., and Rand, M., Pharmacological actions of synthetic 5-hydroxytryptamine (serotonin, thrombocytin), Nature, 169(1952)801-802.

Reis, D.J., Ross, R.A., Dynamic changes in brain dopamine-b-hydroxylase activity during anterograde and retrograde reactions to injury of central noradrenergic neurons, Brain Res., 57(1973)307-326.

Reis, D.J., Ross, R.A., and Joh, T.H., Reaction of central catecholaminergic neurons to injury: model systems for studying the neurobiology of central regeneration and sprouting. In: Neuronal Plasticity, edited by Cotman, C.W., New York: Raven, (1978)197-226.

Rhees, R.W., Grosser, B.I., Stevens, W., The autoradiographic localization of $^{3}$H-dexamethasone in the brain and pituitary of the rat, Brain Res., 100(1975)151-156.

Robinson, R.G., Bloom, F.E., and Battenberg, E.L.F., A fluorescent histochemical study of changes in noradrenergic neurons following experimental cerebral infarction in the rat, Brain Res., 132(1977)259-272.

Roper, S., and Ko, C.P., Synaptic remodeling in the partially denervated parasympathetic ganglion in the heart of the frog, In: Neuronal Plasticity, edited by Cotman, C.W., New York: Raven,

(1978)1-25.

Rutledge, L.T., Effects of cortical denervation and stimulation on axons, dentrites, and synapses, In: Neuronal Plasticity, editied by Cotman, C.W., New York: Raven, (1978)273-289.

Rutledge, L.T., The effects of denervation and stimulation upon synaptic ultrastructure, J. Comp. Neurol., 178(1978)117-128.

Rutledge, L.T., Wright, C., and Duncan, J., Morphological changes in pyramidal cells of mammalian neocortex associated with increased use, Exp. Neurol., 44(1974)209-228.

Scheff, S.W., Bernardo, L.S., and Cotman, C.W., Hydrocortisone administration retards axon sprouting in the rat dentate gyrus, Exp. Neurol., 68(1980)195-201.

Scheff, S.W., Benardo, L.S., Cotman, C.W., Effect of serial lesions on sprouting in the dentate gyrus: onset and decline of the catalytic effect, Brain Res., 150(1978)45-53.

Scheff, S.W. and Cotman, C.W., Recovery of spontaneus alternation following lesion of entorhinal cortex in adult rats: possible correlation to axon sprouting. Behav. Biol., 21(1977)286-293.

Scheff, S.W., and Cotman, C.W., Chronic Glucocorticoid therapy alters axon sprouting in the Hippocampal Dentate Gyrus, Exp. Neurol., 76(1982)644-654.

Schneider, G.E., Early lesion of superior colliculus: Factors affecting the formation of abnormal retinal protection, J. Comp. Neurol., 159(1973)149-176.

Schneider, G.E., and Jhaveri, S.R., Neuroanatomical correlates of spared or altered function after brain lesions in the newborn hamster, In: Plasticity and Recovery of Function in the Central Nervous System, edited by Stein, D.G., Rosen, J.J., and Butters, N., New York: Academic, (1974)65-109.

Schwartz, J.C., Histamine as a transmitter in brain. Life Sci. 17(1975)503-518.

Segal, M., Interactions of ACTH and norepinephrine on the activity of rat hippocampal cells, Neuropharmacology, 15(1976a)329-333.

Segal, M., 5-HT antagonists in rat hippocampus, Brain, Res., 103(1976)161-166.

Segal, M., Physiological and pharmacological evidence for a serotonergic projection to the hippocampus, Brain Res., 94(1975)115-131.

Shute, C.C.D., and Lewis, P.R., The use of cholinesterase techniques

combined with operative procedures to follow nervous pathways in the brain, Bibliotheca Anatomica, 2(1961)88-89.

Silverman, A.J., and Zimmerman, E.A., Adrenalectomy increases sprouting in a peptidergic neurosecretory system, Neurosc., 7(1982)2705-2714.

Speidel, C.C., Adjustment of nerve endings, Harvey Lect., 36(1941)126-158.

Speidel, C.C., Studies of living nerves: II. Activities of ameboid growth cones, sheath cells, and myelin segments, as revealed by prolonged observation of individual nerve fibers in frog tadpoles, Amer. J. Anat., 52(1933)1-80.

Srebro, B., Azmitia, E.C., and Winson, J., Effect of 5-HT depletion of the hippocampus on the neuronal transmission from perforant path through dentate gyrus, Brain Res., 235(1982)142-147.

Steinbusch, H.W.M., Distribution of serotonin-immunoreactivity in the central nervous system of the rat cell bodies and terminals, Neuroscience, 6(1981)557-618.

Steinbusch, H.W.M., Van Der Kooy, D., Verhofstad, A.A.J., and Pellegrino, A., Serotonergic and non-Serotonergic projections from the nucleus raphe dorsalis to the caudate-putamen complex in the rat, studied by a combined immunofluorescence and fluorescent retrograde axonal labeling technique, Neurosc. Letters, 19(1980)137-142.

Stenevi, U., Bjorklund, A., and Moore, R.Y., Morphological plasticity of central adrenergic neurons, Brain Behav. Evol., 8(1973)110-134.

Stenevi, U., Bjorklund, A., and Moore, R.Y., Growth of intact central adrenergic axons in the denervated lateral geniculate body. Exp. Neurol., 35(1972)290-299.

Stephenson, R.S., Axon reflexes in axolotl limbs: evidence that branched motor axons reinnervate muscle selectively, Exp. Neurol., 64(1979)174-189.

Steward, O., Cotman, C.W., and Lynch, G.S., Growth of a new fiber projection in the brain of adult rats: reinnervation of the dentate gyrus by the contralateral entorhinal cortex following ipsilateral entorhinal lesion, Exp. Brain Res., 20(1974)45-66.

Steward, O., Cotman, C.W., and Lynch, G., A quantitative autoradiographic and electrophysiological study of the reinnervation of the dentate gyrus by the contralateral entorhinal cortex following ipsilateral entorhinal lesion, Brain Res., 114(1976)181-200.

Steward, O., Cotman, C.W., and Lynch, G.S., Re-establishment of electrophysiologically functional entorhinal cortical input to the dentate gyrus deafferented by ipsilateral entorhinal lesions: innervation by the contralateral entorhinal cortex, Exp. Brain Res.,

18(1973)396-414.

Steward, O. and Loesche, J., Quantitive autoradiographic analysis of the time course of proliferation of contralateral entorhinal efferents in the dentate gyrus denervated by ipsilateral entorhinal lesions, Brain Res., 125(1977)11-21.

Steward, O., and Messenheimer, J.A., Histochemical evidence for a postlesion reorganization of cholinergic afferents in the hippocampal formation of the mature cat, J. Comp. Neurol., 178(1978)697-710.

Steward, O. and Vinsant, S., Identification of the cells of origin of a central pathway which sprouts following lesion in mature rats, Brain Res., 147(1978a)223-243.

Steward, O., and Vinsant, S.L., Collateral projections of cells in the surviving entorhinal area which reinnervate the dentate gyrus of the rat following unilateral entorhinal lesions, Brain Res., 149(1978b)216-222.

Storm-Mathisen, J., Choline acetyltransferase and acetylcholinesterase in fascia dentate following lesion of the entorhinal afferents, Brain Res., 80(1974)181-197.

Storm-Mathisen, J., and Guldberg, H.C., 5-Hydroxytryptamine and noradrenaline in the hippocampal region: effect of transection of afferent pathways on endogenous levels, high affinity uptake and some transmitter-related enzymes, J. Neurochem., 22(1974)793-803.

Swanson, L.W. and Hartman, B.K. The central adrenergic system. an immunofluorescence study of the location of cell bodies and their efferent connection in the rat utilizing dopamine-beta-hydroxylase as a marker, J. comp. neurol., 163(1975)467-505.

Sugar, O., and Gerard, R.W., Spinal cord regeneration in the rat, J. Neurophysiol., 3(1940)1-19.

Sze, P.Y., Glucocorticoids as a Regulatory factor for brain tryptophan hydroxylase during development, Dev. Neurosci., 3(1980)217-223.

Sze, P.Y., Glucocorticoid regulation of the serotonergic system of the brain, Adv. Biochem. Psychopharmacol., 15(1976)251-265.

Sze, P., and Neckers, L., Requirement for adrenal glucocorticoid in the ethanol-induced increase of tryptophan hydroxylase activity in mouse brain, Brain Res., 72(1974)375-378.

Taber, E., Brodal, A., Waldberg, F., The raphe nuclei of the brain stem in the cat, J. Comp. Neurol., 114(1960)161-188.

Tassin, J.P., Lavielle, S., Herve, D., Blanc, G., Thierry, A.M., Alvarez, C., Beger, B., and Golwinski, J., Collateral sprouting an

induced activity of the rat mesocortical dopaminergic neurons after selective destruction of the ascneding noradrenergic bundles. Neurosci., 4(1979)1569-82.

Taylor, A.N., Matheson, G.K., and Dafny, N., Modification of the responsiveness of components of the limbic-midbrain circuit by corticosteroids and ACTH, In: Steroid Hormones and Brain Function, Sawyer, C.H., and Gorski, R.A., Univ. of California Press, Berkeley, (1971)67-78.

Telegdy, G., and Vermes, I., Effects of adrenocortical Hormones on Activity of the serotoninergic system in limbic structures in rats, Neuroendocrinology, 18(1975)16-26.

Thelseff, S. andd Sellin, L.C., Trands Neurosci. 3(1980)122-126.

Thompson, W., Reinnervation of partially denervated rat soleus muscle, Acta Physiol. Scand., 103(1978)81-91.

Tranzer, J.P., Thoenen, H., Ultramorphologische veranderungen der sympatischen nervenendigungen der katzenach vorbehandling mit 5- and 6-hydroxy-dopamin, Naunyn-Schmiedebergs Arch, Exp. Pathol. Pharmakol., 257(1967)343.

Turner, B.B., and McEwen, B.S., Hippocampal cytosol binding capacity of corticosterone: no depletion with nuclear loading, Brain Res., 189(1980)169-182.

Ungerstedt, U., Sterotaxic of mapping of the monomaine pathways in the rat brain, Acta Physiol. Scand. Suppl. 367(1971)1-48.

Unsicker, K., Krisch, B., Otten, U., and Thoenen, H., Nerve growth factor-induced fiber outgrowth from isolated rat adrenal chromaffin cells: Impairment by glucocorticoids, Proc. Natl. Acad. Sci. U.S.A., 75(1978)3498-3502.

Van Harreveld, A., On the mechanism of the "spontaneous" reinnervation in paretic muscles, Am. J. Physiol., 150(1947)670-676.

Varon, S.S., and Bunge, R.P., Trophic mechanisms in the peripheral nervous system, Annu. Rev. Neurosci., 1(1978)327-361.

Varon, S.S., and Somjen, G.G., Neuron-glia interactions, Neurosci. Res., Program Bull., 17(1979)1-239.

Vermes, I., Smelik, P.G., and Mulder, A.H., Effects of hypophysectomy, adrenalectomy and corticosterone treatment on uptake and release of putative central neurotransmitters by rat hypothalamic tissue in vitro, Life Sciences, 19(1976)1719-1726.

Vrbova, G., Induction of an extrajunctional chemosensitive area in intact innervated muscle fibers, J. Physiol. London., 191(1967)20-21.

Wall, P.D., and Egger, M.D., Formation of new connexions in adult rat brains after partial deafferentation, Nature London, 232(1971)542-545.

Warembourg, M., Radioautographic study of the rat brain and pituitary after injection of $^{3}$H-dexamethasone, Cell Tissue Res., 161(1975)183-191.

Weddell, G., Guttmann, L.G., and Guttmann, E., The local extension of nerve fibers into denervated areas of skin, J. Neurol. Psychiatry, 4(1941)206-225.

Weiss, P., and Edds, M.V., Spontaneous recovery of muscle following partial denervation, Am. J. Physiol., 145(1946)587-607.

Wernig, A., and Stover, H., Sprouting and repression of the nerve at the frog neuromuscular junction, Pfluegers Arch., 379(1979)R38.

West, J.R., Deadwyler, S.A., Cotman, C.W., and Lynch, G., Time dependent changes in commissural field potentials in the dentate gyrus following lesions of the entorhinal cortex in adult rats, Brain Res., 97(1975)215-233.

Wiklund, L. and Bjorklund, A., Mechanisms of regrowth in the bulbospinal serotonin system following 5,6-dihydroxytryptamine studied with high resolution radioautography in the rat dorsal accessory olive, J. Neurocyt., 10(1981)1009-1027.

White, W.F., Nadler, J.V., Hamberger, A., Cotman, C.W., and Cummins, J.T., Glutamate as transmitter of hippocampal perforant path. Nature 270(1977)356-357.

Wiklund, L., and Bjorklund, A., Mechanisms of regrowth in the bulbospinal serotonin system following 5,6-dihydroxytryptamine induced axotomy. II. Fluorescence histochemical observations. Brain Res., 191(1980)129-155.

Wiklund, L., Bjorklund, A., and Nobin, A., Regeneration of serotonin neurons in the rat brain after 5,6-dihydroxytryptamine induced axotomy, Ann. NY Acad. Sci., 305(1978)370-384.

Wiklund, L., and K. Mollgard, Neurotoxin destruction of the serotonergic innervation of the rat subcommissural organ is followed by reinnervation through collateral sprouting of non-monoaminergic neurons, J. Neurocytol., 8 (1979)469-480

Williams, J.H. and Azmitia, E.C., Hippocampal serotonin re-uptake and nocturnal locomotor activity after microinjections of 5,7-DHT in the fornix-fimbria, Brain Res., 207(1981)95-107.

Windle, W.F., Regeneration of axons in the vertebrate central nervous system, Physiol. Rev., 36:1956(426-440).

Windle, W.F., and W.W. Chambers, Regeneration in the spinal cord of

the cat and dog., J. Comp. Neurol., 93(1950)241-257.

Windle, W.F., Clemente, C.D., and Chambers, W.W., Inhibition of formation of a glial barrier as a means of permitting a peripheral nerve to grow into the brain, J. Comp. Neurol., 96(1952)359-370.

Winson, J., and Abzug, C., Gating of neuconal transmission in the hippocampus: efficacy of transmission varies with behavioral state, Science, 196(1977)1223-1225.

Withrow, C.D., Woodbury, D.M., Some aspect of the pharmacology of adrenal steroids and the central nervous system, In: Steroids and Brain Edema, editors: Peulen, H.J., Shurmann, K., New York: Springer-Verlag, (1972)41-55.

Wong-Riley, M.T.T., Changes in the dorsal lateral geniculate nucleus of the squirrel monkey after unilateral ablation of the visual cortex, J. Comp. Neurol., 146(1972)519-548.

Wuttke, W., Bjorklund, A, Baumgarten, H.G., Lachenmayer, Lachenmayer, L., Fenske, M., and Klemm, H.P., De- and regeneration of brain serotonin neurons following 5,7-dihydroxytryptamine treatment: effects on serum LH, FSH and prolactin levels in male rats, Brain Res., 134(1977)317-331.

Yu, W. H., Sex difference in the regeneration of the hypoglossal nerve in rats, Brain Res., 238(1982)404-406.

Yu, W.H., Srinivasan, R., Effect of testosterone and 5-alpha-dihydrotestorone on regeneration of the hypoglossal nerve in rats, Exp. Neurol., 71(1981)31-35.

Zhou, F.C., and Azmitia, E., Effects of 5,7-dihydroxytryptamine on HRP retrograde transport from hippocampus to midbrain raphe nuclei in the rat, Brain Res. Bull., 10(1983)445-451.

Zhou, F.C., and Azmitia, E.C., Induced collateral sprouting of hippocampal 5-HT fibers; a quantitational HRP study in the rat, Soc. Neurosci. Abstracts, 7(1981)68.

Zimmer, J., Extended commissural and ipsilateral projections in postnatally deentorhinated hippocampus and fascia dentata demonstrated in rats by silver impregnation, Brain Res., 64(1973)293-311.

Zimmer,J., and Hjorth-Simonsen, A., Crossed pathways from the entorhinal area to the fascia dentata II. Provocable in rats, J. Comp. Neurol., 161(1975)71-102.

## ABBREVIATIONS

| | |
|---|---|
| A | aqueduct |
| AC | anterior commissure |
| ADX | adrenalectomy |
| ALV | alveus |
| BC | brachium conjunctiva |
| CA | catecholamine |
| CB | cingulem bundle |
| CC | Corpus callosum |
| cc | Crus cerebri |
| cci | inferior collicular commissure |
| CHROM | chromogen |
| cif | inferior colliculus |
| CNS | central nervous system |
| cs | superior colliculus |
| DA | dopamine |
| DAB | 3',3-diaminobenzidine |
| dbc | decussation of brachium conjunctiva |
| DDS | dark droplet stump |
| Deg | degeneration |
| DG | dentate gyrus |
| DHipp | dorsal hippocampus |
| 5,6-DHT | 5,6-dihydroxytryptamine |
| 5,7-DHT | 5,7-dihidrooxytrptamine |
| DMI | desipramine |
| DRN | dorsal raphe nucleus |
| FC | fasciola cinereum |
| FF | fimbria-fornix |
| GABA | gamma-aminobutyric acid |
| GPDH | glycerol-3-phosphate dehydrogenase |
| GRP | glial-released protein |
| 5-HIAA | 5-OH-indole acetic acid |
| Hipp | Hippocampus |
| HRP | horseradish peroxidase |
| 5-HT | serotonin |
| 5-HT-IR | serotonin immunoreactive |
| 5-HTP | 5-hydoxytryptophan |
| IA | indolamine |
| IFN | interfascicular nucleus |
| ip | interpeduncular nucleus |
| ipsi | ipsilateral |
| LC | locus ceruleus |
| lm | lateral lemniscus |
| ME | median eminance |
| MFB | medial forebrain bundle |
| mlf | medial longitudinal fascicularis |
| MR | midbrain raphe |
| MRN | medain raphe nucleus |
| NA | norepinephrine, noreadrenaline |
| nc 12 | hypoglossal nucllеus |

| | |
|---|---|
| nc 7 | facial nucleus |
| NMT | mesencephalic nucleus of trigeminal nerve |
| 6-OHDA | 6-hydoxydopamine |
| OI | inferior olive |
| P | pons |
| PAP | peroxidase anti-peroxidase antiserum |
| PB | phosphate buffer |
| PBS | phosphate buffer containing .9% NaCl |
| pci | inferior cerebellar peduncle |
| pcm | middle cerebellar peduncle |
| pcs | superior cerebellar peduncle |
| PF | perforant pathway |
| PNS | peripheral nervous system |
| precip | precipitation |
| Pr | pyramidal neuron |
| reg | regeneration |
| SAL | still alert activity |
| SAR | sheep anti-rabbit antiserum |
| S. L-M | stratum lacunosom-moleculare |
| snr | substantia nigra |
| 5 spin | spinal trigeminal nucleus |
| sub | subiculum |
| TH | tyrosine hydroxylase |
| TMB | 3',3,5',5-tetramethyl benzidine |
| TP | tryptophan |
| TrH | tryptophan hydroxylase |
| V | ventricle |