77-20,489

MAROTTA, Rocco Francis, 1948-
PHARMACOLOGICAL MANIPULATIONS OF THE SEPTAL IRRITABILITY SYNDROME: ROLE OF DOPAMINERGIC MECHANISMS IN RECOVERY OF FUNCTION.

City University of New York, Ph.D., 1977
Psychology, physiology

PHARMACOLOGICAL MANIPULATIONS OF THE SEPTAL IRRITABILITY SYNDROME: ROLE OF DOPAMINERGIC MECHANISMS IN RECOVERY OF FUNCTION

by

ROCCO FRANCIS MAROTTA

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

1977

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

5/13/77
date

H P Zeigler
Chairman of Examining Committee

May 16 1977
date

Executive Officer

Eliot L. Gardner

Fernando Alverez

Stanley Novak

Saul Steiner

Mike Potegal

Supervisory Committee

The City University of New York

Abstract

PHARMACOLOGICAL MANIPULATIONS OF THE SEPTAL IRRITABILITY SYNDROME: ROLE OF DOPAMINERGIC MECHANISMS IN RECOVERY OF FUNCTION

by

Rocco Francis Marotta

Advisers: Professors H. Phillip Zeigler and Eliot Gardner

Irritable behavior resulting from destruction of the rat septal area (SpA) was used as a behavioral model for recovery of normal function after brain damage. Septal irritability lasts between 7 and 30 days depending on lesion placement and handling, and forms a convenient baseline for comparing effects of pharmacological agents on rate of recovery of affective behavior. The position of catecholaminergic (CA) neurotransmitter pathways, in and around the SpA, and the ability of brain CA depletion by 6-hydroxydopamine (6-OHDA) to induce irritability, lead to the formulation of four hypotheses:

1. Depletion of CAs in the SpA by micro-injections of 6-OHDA into septal tissue induces an irritability syndrome.

2. Stimulation of CA systems by peripheral injections of CA agents after radio frequency (RF) lesions of the SpA results in significant decreases in the intensity and duration of the syndrome, compared to controls.

3. Blockade of post-synaptic CA receptors in rats recovered from septal irritability results in a temporary return of irritability.

4. Chronic (7 day) presurgical treatment with CA blocking agents affects the development of irritability after subsequent SpA destruction.

To test these hypotheses, the following experiments were performed:

1. Micro-injections of 6-OHDA directly into the SpA bilaterally, in a dose sufficient to destroy both dopamine (DA) and noradrenaline nerve terminals and DA cell bodies, but not damage serotonergic, cholinergic and gabaminergic systems, induced irritability indistinguishable from that seen with large RF lesions of the SpA. A group pretreated with desmethylimipramine to protect noradrenergic neurotransmitter systems from damage by 6-OHDA also exhibited an irritability syndrome indistinguishable from RF lesioned animals. Micro-injections of saline vehicle had no effect. To control for the possible non-specific effects, rats receiving small RF lesions at coordinates used for injection of 6-OHDA did not exhibit increased irritability. These experiments implicate the CA systems, and DA subsystems, in septal irritability.

2. Rats subjected to stereotaxic RF lesions of the SpA were rated for irritability 24 hours later and received one of the following agents: L-DOPA, apomorphine, piribedil, amphetamine, theophylline, imipramine, methohexital, or saline. Experimenters, blind as to drug treatment, rated the rats after injection at various times. L-DOPA, apomorphine and amphetamine lead to complete and irreversible

dissipation of the syndrome within 7 hours. Methohexital and piribedil resulted in a transient decrease in irritability but did not affect the overall duration of the irritability syndrome. Theophylline and imipramine induced transient increases in irritability but had no effect on syndrome duration. Saline control animals showed gradual decreases in irritability, with the syndrome lasting at least 7 days. DA stimulating agents induced complete and irreversible recovery of normal affect within hours of administration. Dose response curves were plotted for all drugs studied. The mosaic of drug actions implicate DA systems in recovery from the septal syndrome.

3. Since stimulating DA receptors leads to decreased irritability, DA receptor blockade was attempted to reinduce the syndrome in recovered septal rats. The DA blocking agent pimozide was found ineffective at several dosages. However, the general stimulatory agent theophylline reinduced irritability in recovered septal animals at dosages one-half that necessary to induce irritability in normal rats. Therefore, DA stimulation may be necessary but not sufficient for recovery.

4. A final study examined the effect of chronic treatment with pharmacological agents on subsequent septal ablation. Haloperidol, apomorphine, alpha-methyl-para-tyrosine, morphine or saline was administered for seven days before septal surgery. Rats receiving chronic haloperidol exhibited little irritability, while all other groups ex-

hibited irritability syndromes comparable to controls in intensity and duration. Haloperidol only on the day of surgery induced the regular septal syndrome.

It is suggested that many of the effects of brain damage might be due to "diaschitic" processes, or to transient dysfunction in adjacent or connected areas. These findings implicate that the rate of recovery, in a number of experimental and clinical syndromes, is possibly modifiable by pharmacological agents.

ACKNOWLEDGEMENTS

I would first like to thank the members of my dissertation committee and the readers: Eliot Gardner, Mike Potegal, Phil Zeigler, Sol Steiner, Stan Novak and Nando Alvarez. These gentlemen put up gracefully with my *ad hoc* style and last minute madness.

Secondly, my deepest appreciation goes to those friends and colleagues who helped me over the years with this work. Some, like Nancy Logan, must look upon me as a mixed blessing. Others, like Stella Marie Riverso, still don't know any better. To Tom Seeger, Doug Wilkening, Stan Schiff, Gordon Barr, John Kruez, Judy Gibbons, Stan Walczak and others—thanks for your advice, presence, company, tea and fellowship.

I especially thank two very important women—Laurie Gordon and JoAnn Marotta. This work is Laurie's as much as mine—any excellence in form is hers. JoAnn took it upon herself to facilitate this work—*Ad Maiorem Gloriam Familiae*.

This dissertation is for Lily Snaredoff—she knows why—and all those who really do the work.

Also, I am taking this opportunity to write the names of some teachers—"*insegnanti*" or inseminators in the language of my fathers. I thank: Sam J. Korn, who I have come to love, for the warm office, advice and fatherliness; Mike for being a great "big brother"; Lucy and Charlie Marotta—what can I say without getting emotional;

Fr. Reynold Borzaga, Rev. Brother Charles James, Fr. Bruno Talzig and others at Manhattan College for the sense of history and hope for the future; Tony Summo, Jim Romano, Patrick Mullahy, Don Dillon and John Miele for being so very real as human beings; Eliot for being an ideal; Saul for being a "bubbalah"; Phil for being infused with the sacred madness, even if he won't admit it.

Finally and humbly I would like to dedicate this dissertation to the memory of both my intellectual grandparent Hans Lucas Teuber and my natural grandparents Frank and Francis Petrelli, and Rocco and Josephine Marotta.

A.M.G.D.

Dabit Tibi Dominus In Omnibus Intellectum

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

RECOVERY OF FUNCTION FOLLOWING DAMAGE TO THE CENTRAL NERVOUS SYSTEM

The understanding that the integrity of the central nervous system (CNS) is necessary for cognitive and motor functions developed over several hundred years (see Rosner, 1974 for review). That the changes in behavior resulting from insult to the CNS often attenuate with time was recorded early in the history of western medicine, and the recovery of sensory (Teuber, 1972), motor (Goldberger, 1974), and cognitive (Lenneberg, 1967) functioning after brain damage have all been well documented. However, except for the events that transpire following transection of a peripheral nerve, the processes involved in recovery of function after nervous system damage are still not fully understood.

The original clinical observations of recovery from the effects of CNS damage were made in the motor system (Jackson, 1882). Since there can be no real experimentation with humans in this area, research has progressed through the development of experimental animal model systems for the study of recovery. These have included peripheral nerve transection (Wolf, 1940), spinal cord damage (Garth, 1972; Matinian & Andreasian, 1976), and lesions in brain areas important for both homeostatic (e.g., eating) and emotional (e.g., rage) behaviors

(Teitelbaum & Epstein, 1962; Yutzey, Meyer & Meyer, 1967).

Although the mechanisms subserving recovery of function are not understood, the experimental and theoretical literature on the subject over the last century has been rich with explanatory theories for recovery, most of which reflect a few basic themes (Goldberger, 1974; Rosner, 1974).

Multiple Representation

Hughlings Jackson (1882) proposed that a given behavioral function may be represented several times at different levels of the CNS. This position assumed that higher brain regions are more finely organized than, easier to excite than, and exert an inhibitory influence over the lower levels from which they gradually assume behavioral control in the course of phylogenetic and ontogenetic development. When higher levels are destroyed, the lower levels are "released" from inhibition and perform in a new "dynamic organization" which accounts for the return of behavioral function.

Mass Action—Equipotentiality

Lashley (1938) proposed that all cortical tissue is equally capable of subserving behavioral functions relating to learning and memory, and that the sparing of behavioral function following cortical damage is an inverse function of the total mass of tissue lost.

Vicarious Functioning—Functional Reorganization

It is also possible to postulate that those systems

remaining after brain damage may alter their mode of operation, so as to functionally replace the subsystem which has been destroyed. This type of recovery mechanism has usually been called vicarious functioning. It can operate in one of two ways. Either a system can possess a latent capacity to mediate certain functions not usually attributed to it, or several systems may so overlap in their properties as to be interchangeable, or at least indistinguishable, a position not unlike Lashley's theory of cortical equipotentiality (see above). Similar to the notion of vicarious functioning is Kennard's (1938) theory of functional reorganization in which a number of subsystems are postulated to interact in a new way following brain damage. This view differs from vicarious functioning only in the implication of a permanent change in the functioning of the undamaged tissues, rather than the expression of latent functions.

Behavioral Substitution

The theory of behavioral substitution holds that after brain damage the organism learns new tricks, or maneuvers, to solve problems. An organism may begin to attend to new cues, or, to paraphrase Lashley, to roll through a maze instead of running. In a conceptually analogous position Luria (Luria, Nayden, Tsvatkova & Vinarskaya, 1969) stresses the importance of retraining in the recovery of brain damaged humans.

Diaschisis

Von Monakow (cited in Luria et al., 1969) argued that damage to the brain deprives other regions of normal afferent input. This sudden change produces a particular type of shock called diaschisis. Every local brain lesion arising as a result of hemorrhage, trauma, or tumor is complex. Some nerve cells are destroyed while others are preserved in a state of physiological inactivity. According to this position, the recovery seen after CNS damage is due to the return of inactivated tissue to a functional state. Luria and his associates (1969) feel that the main method of reactivation is deblocking or the restoration of synaptic conduction.

It should be noted that none of the above theories of the underlying mechanisms of recovery of function after brain damage are logical rivals. Each could apply in varying degrees to a given situation. Geschwind (1974) has stressed that the general question of the mechanisms subserving recovery of function is, "What is the sequence of events which occurs after a lesion is placed in the CNS?" This sequence may involve alterations in anatomical connections as well as physiological and biochemical functions in the vicinity of the damaged tissue. Other intact areas of the CNS may change their mode of responding as a consequence of such changes.

Factors Affecting Recovery of Function

Whether or not a given individual shows recovery of

function after brain damage depends on a number of factors.

The site, the size, and the type of lesion as well as the species, age, and sex of the subject are all important pre-insult determinants of recovery, while the environment of the post-insult subject is another important factor (Teuber, 1974).

The site of damage will not only determine the signs and symptoms resulting from the insult, but is also predictive of recovery. Damage to an area necessary for a critical function such as respiration may not allow for recovery. Less vital tissues, on the other hand, may show subtle differences in vulnerability. In the motor systems of primates, for example, certain topographically organized subsystems, such as cortical area 4, seem less capable of functional recovery than other motor subsystems. Destruction of only a small part of the projection region for the hand, for instance, leads to relatively permanent loss of tactile placing in the contralateral hand. But damage to a loosely or non-topologically organized motor system, such as the reticulospinal system, leads to permanent deficits only when extremely large amounts of tissue are destroyed. Differences in vulnerability are also evident when association cortex is compared to primary sensory projection cortex. Lesions of association cortex, when not all-encompassing, often permit nearly complete recovery (Rosen, Stein & Butters, 1971) while

comparable lesions in the visual cortex result in some permanent impairment (Teuber, 1974).

That the size of a lesion is another important factor affecting recovery of function was recognized very early. In 1888, Jackson stated, "I would suggest (I cannot speak definitely) that if a small quantity of a nervous organ be destroyed, there is recovery; if a large quantity, there is some recovery; if a very large quantity, scarcely any" (Jackson, 1888). A similar view was reiterated by Lashley (1938), who suggested on the basis of many years of research that, at least for learned movements, the mass of tissue removed, not its location, was the critical factor in CNS recovery.

Although the general principle of increased deficit with increased total mass of tissue destruction seems accurate, there are a number of instances of a second lesion leading to increased recovery of some specific movement pattern or function that was impaired by a first lesion. These include destruction of the pulvinar to relieve dystonia musculorum deformans (Cooper, 1975), ventrolateral thalectomy to relieve Parkinsonian tremor (Cooper, 1975), and amygdalectomy to attenuate septal irritability in rodents (King & Meyer, 1958). On the other hand, some secondary lesions that appear to facilitate recovery have been shown, on further analysis, to yield deficits of their own (Goldberger, 1974). For example,

lesions of the dentate-interpositus nuclei of the cerebellum result in an ataxic tremor of intent which can be "abolished" by secondary lesions produced anywhere along the corticospinal pathway (Aring & Fulton, 1936; Carpenter & Correll, 1961; Carrea & Mettler, 1955) as well as in the thalamocortical-pyramidal system (Carpenter & Hanna, 1962). However, if monkeys are trained preoperatively in motor tasks and then retested, the tremor persists (Growden, Chambers & Liu, 1967), indicating that the lack of tremor and ataxia resulting from the secondary lesion was due to a general lack of movement. Bowen (1975) has also noted that Parkinsonian patients subjected to thalamic lesions show greater cognitive and motor deterioration than non-lesioned control patients.

The type of lesion is also an important determinant of recovery. It has, for example, been noted that the behavioral result of CNS damage due to tumor is usually different from that seen when the same area is involved in a stroke (Escourolle & Poirier, 1973); the stroke usually results in greater deficits. This has been explained in terms of speed of onset, edema, and the time available for remaining tissue and blood flow to be reorganized in the presence of a comparatively slow growing tumor. Similar phenomena have been noted in the experimental use of the serial lesion technique in neuropsychological investigations of structural-functional brain

relationships. In this technique, bilateral destruction of homotopic brain areas is carried out serially, with only one side at a time being damaged, or larger concentric areas of tissue are serially removed, with the duration of the interoperative interval an important variable. With such serial lesion techniques, relative sparing of function has often been reported. For example, several workers have found visual functioning less impaired by serial lesions of visual cortex in rats than by comparable one-stage lesions (Cole, Sullins & Isaac, 1967; Isaac, 1964; Meyer, Isaac & Maher, 1958). Similarly, Stein (1974) has studied the effect of serial and one-stage bilateral lesions of frontal cortex, amygdala, hippocampus, and caudate nucleus on acquisition of successive discrimination learning and reversal, passive avoidance, simultaneous pattern discrimination and delayed spatial alternation tasks, and has found that although the two-stage animals showed some impairment on these tasks, the impairment was much less than that shown by the one-stage animals. In fact, rats with two-stage damage to the frontal cortex, hippocampus, and caudate did nearly as well as control animals (Stein, 1974).

Even more impressive sparing of function has been reported with serial lesions of various subcortical areas. Adametz (1959), for example, destroyed the brain stem tegmentum of adult cats, comparing serial and one-stage

operative procedures. For the most part, extensive single-stage bilateral lesions led to coma from which the animals did not recover. When the same size lesions were made, one at a time, with a three week recovery period between operations, the cats made a quick recovery from coma and regained motor functioning. Another subcortical system studied has been the lateral hypothalamus (LH). Lesions in the LH typically result in adipsia and aphagia (Arnand & Brobeck, 1951). To survive this procedure, rats must usually be force-fed for seven or more days. Stein (1974) studied the LH syndrome using the serial lesion technique and found that serial bilateral animals lost less weight than one-stage bilateral LH rats. The serial lesion technique has also been used to study the irritability syndrome that results from septal lesions; it has been found that with a one week interval between operative procedures, no excessive emotionality results (Marotta, unpublished observations).

One of the more detailed studies of functional recovery following serial ablation was that of Gleese and Cole (1950). They taught monkeys a fine motor task calling for the use of a hand and fingers. After training, the cortex of these animals was exposed and the tissue responsible for the control of the muscles necessary for the task was found using brain stimulation. This tissue was then destroyed and the monkeys were retested at the

motor task. The animals were found to be capable of relearning the task and were therefore operated on again, with the discovery that tissue on the border of the lesion was now capable of producing finger muscle contraction upon stimulation. This tissue was then lesioned and the animals retested. The procedure was repeated three times for one animal. These experiments indicate the possibility for much recovery, even in areas of cortex with highly organized topological projections.

Recent evidence suggests that the sex of a victim of brain damage also affects the extent of recovery. Teitelbaum (1973) performed one- and two-stage lesions on frontal cortex of male and female albino rats and examined their subsequent performance on a delayed spatial alternation task. She found that sham operated females learned the task faster than males. Also, there was no difference in the speed of learning between one- and two-stage operated females. The male rats, on the other hand, presented a different pattern. The one-stage bilateral frontal animals showed a deficit relative to the sham controls, while the two-stage animals did not. In another investigation of the effect of sex on recovery, Goldman and her associates (Goldman, Grawford, Stokes, Galkin & Rosvold, 1974) compared male and female, infant and juvenile (18 to 24 months) monkeys. The animals were tested on object discrimination reversal and delayed-response tasks at $2\frac{1}{2}$,

12 and 18 months after bilateral prefrontal orbital lesions. When the infants were tested at $2\frac{1}{2}$ months, only the males showed a deficit on the object reversal task. At 12 months postoperative, only the males showed delayed response deficits. By 18 months postoperative, both sexes were equally impaired.

With respect to the effects of age on recovery of function, it has been recognized since at least the beginning of this century that children often show nearly complete recovery after brain damage which would have incapacitated an adult (Lashley, 1938). Kennard (1938, 1940, 1942), in a series of experimental studies on this topic, demonstrated that lesions in young animals have different effects from those in more mature subjects. She noted that large lesions of Brodmann's areas 4 and 6 in animals operated upon in infancy led to considerably less severe impairments in posture and locomotion than in monkeys who underwent the same surgery later in life, and proposed that, in general, the behavioral consequences of motor cortex damage are directly related to age at time of damage. Other evidence in this regard comes from studies showing that young monkeys with dorsolateral frontal cortex lesions do not show the delayed response deficits exhibited by older monkeys with similar lesions (Akert, Orth, Harlow & Schiltz, 1960; Harlow, Thompson, Blomquist & Schiltz, 1970; Tucker & Kling, 1967). Other

more recent studies by Schneider and his colleagues (Schneider & Jhaveri, 1974), using young hamsters, have documented both the sparing of behavior in the young and some of the possible neuronal mechanisms involved.

On the other hand, the question of altered recovery of function in different age groups is clearly a complex one, with much evidence that, for some behavioral functions, the effects of the damage are as devastating to the young organism as to the mature. For example, the behavioral effects of dorsolateral frontal cortex damage (Goldman & Rosvold, 1970), frontal eye field damage (Kennard, 1938), pyramidal damage (Lawrence & Hopkins, 1970) and certain types of motor cortex damage in monkeys (Kennard, 1940) are as severe in very young animals as in older ones. In the rat, Hicks and D'Amato (1970) found that when neonatal and adult motor system lesions were compared, some behavioral components were spared in the neonate while others were impaired.

In an overall consideration of the effects of age on recovery of function, Goldberger (1974) stresses a number of points. First, an infant cannot "lose" functions which it has not yet developed. In fact, Goldberger (1974) feels that there is little in common between sparing in the infant and recovery in the adult. Second, the sparing of function in the infant CNS is less impressive when behaviors are very strictly tested. What seems to be sparing may in fact be the development of new behavioral maneuvers.

Finally, there is a great deal of evidence for a variety of experiential and environmental influences on recovery from brain damage. For example, Luria and his colleagues (Luria et al., 1969) have stressed the importance of retraining in patients with brain damage. They feel that proper choice of a rehabilitation program can lead to extensive recovery of ability. In this regard, Lashley (1938) pointed out that the study of recovery after CNS lesion is complicated by the organism's initial experience. If the organism has difficulty using a limb contralateral to a corticospinal lesion, a disuse atrophy may set in which is secondary to the ablation of tissue.

In contrast to the human clinical literature, the animal experimental literature has a number of interesting instances of recovery due to retraining. For example, Gazzaniga (1974) increased the survival rate of rats with lesion-induced adipsia and aphagia by making the availability of a running wheel contingent upon the drinking of water. Since these animals had an apparent "need" to run, they were thus induced to drink immediately after surgery (Gazzaniga, 1974). Gazzaniga (1974) also studied monkeys with bilateral lesions of the inferotemporal lobe. Such animals normally fail at visual discrimination tasks when food is used as reinforcement. If, however, the monkeys are preoperatively trained so that errors in the visual discrimination task lead to "destabilization" of the test-

ing chamber, these animals did not show the usual post-operative visual discrimination deficits to either food reward or restabilization reward. It seems that training in two modes protected the monkeys from the expected deficits.

Other experimental influences on recovery after CNS damage have been studied in rodents with lesions in both the visual and limbic systems. For example, Meyer and his colleagues (Meyer et al., 1958) as well as subsequent investigators (Cole et al., 1967; Isaac, 1964) have studied the effect on visual performance of alterations in the animal's environment in the interval between surgery and behavioral testing. For these studies, a two-choice shock avoidance visual discrimination paradigm was used. Rats were extensively studied preoperatively to produce stable baselines of behavior. The animals were then subjected to large bilateral visual cortex lesions. For twelve days after ablation, the animals were housed under one of four conditions: (1) light and noise, (2) dark and noise, (3) light and quiet, or (4) dark and quiet. All the conditions of increased stimulation led to faster recovery of the visual discrimination response relative to the dark and quiet condition.

Finally, the effects of handling on the savage irritability that results from lesions of the olfactory bulb (Bernstein & Moyer, 1970; Cain, 1974), ventral

medial hypothalamic nucleus (Fulton & Ingraham, 1929; Reeves & Plum, 1969) and septal area (Brady & Nauta, 1953) have been studied (Brady & Nauta, 1955; Malick, 1970). Each of these irritability syndromes shows marked attenuation when the animals are extensively handled (Brady & Nauta, 1955; Malick, 1970). For the decrease in affect to occur, handling must take place over an extended period of days; the passage of time alone, or extensive handling over very few days is not sufficient for attenuation.

Pharmacological Manipulation of Recovery of Function

Some of the most provocative work on the problem of CNS recovery after brain insult involves the use of pharmacological agents known to affect neural transmission.

During the 1930's and 1940's, cholinergic compounds enjoyed a vogue among European clinical investigators in the treatment of a variety of nervous system damage syndromes (see Ward & Kennard, 1942 for review). Interestingly enough, experimental support for such use was provided by Wolf (1940) who surgically severed or damaged the sciatic nerves of cats with injections of alcohol and then evaluated the effects of methacholine, a long lasting cholinergic stimulator (Koelle, 1975b), or neostigmine, an anticholinesterase agent (Koelle, 1975a), in daily oral doses of 10 to 20 mg or 1 to 3 mg, respectively, on recovery. All the medicated cats showed clinical signs of recovery in 32 to 80 days, and complete recovery in 71 to

144 days. None of the untreated group showed any signs of recovery 200 days after the operation. Wolf then repeated this study using rats, with essentially the same results (although the time course of recovery for the rats was much quicker than for the cats). The treated rats were able to use their paralyzed limb to scratch at the end of the first postoperative week, while the untreated animals required 26 days or more to attain a similar satisfaction. Ten of eleven treated rats showed complete restoration of function in 87 to 93 days, while only 3 out of 13 controls exhibited such a rapid recovery. Histological examination revealed less muscle atrophy and better axonal and myelin regeneration in the medicated animals.

In another study of cholinergically active compounds on recovery of function, Ward and Kennard (1942) evaluated drug effects on recovery of motor function following unilateral destruction of Brodmann's areas 4 and 6 in monkeys. The animals were injected twice a day for several weeks and tested on a number of movement and coordination tasks. The experimental findings can be summarized as follows: a combination of strychnine, which blocks post synaptic inhibition and may act on glycine (Franz, 1975), and thiamine, which is concerned with the intermediate action of carbohydrate metabolism (Greengard, 1975), seemed to slightly accelerate recovery; carbachol, an acetylcholine agonist (Koelle, 1975b) which

does not pass the blood-brain barrier, seemed to greatly increase the rate of recovery; carbachol plus thiamine, and carbachol plus atropine, which blocks muscarinic cholinergic receptors (Koelle, 1975b), also greatly increased the speed of recovery; thiamine alone produced results no different from those of control animals. Although the authors did not suggest a mechanism for these drug actions and although some modern authorities consider the results puzzling and problematic, it seems conceivable that the lesions may have altered the blood-brain barrier sufficiently to allow carbachol to act on CNS tissue, or that the recovery effect was due to secondary effects mediated by the adrenals through cholinergic stimulation of ganglionic receptors. In 1945, Watson and Kennard published further work extending these initial studies. Brodmann's areas 4 and 6 were again unilaterally ablated in monkeys. She confirmed the speeding of motor recovery after injections of carbachol and atropine, and also showed that repeated injections of phenobarbital, a barbituate, slowed rates of recovery (Watson & Kennard, 1945). Diphenylhydantoin (dilantin) administered alone had no measurable effect on the rate of recovery, but was found to interfere with the facilitation of recovery usually seen with carbachol and atropine.

Amphetamine is believed to have a number of pharmacological actions including: inhibition of the enzyme

monoamine oxidase (MAO; Sourkes, 1972); release of catecholamines (CA) from presynaptic elements (Sourkes, 1972); and blockage of reuptake of CA into presynaptic terminals (Coyle & Snyder, 1969; Harris & Baldessarini, 1973). The two optical isomers and the racemic modification of amphetamine are all felt to act on CA systems, although with differential potency on either noradrenergic (NA) or dopaminergic (DA) neurons (Coyle & Snyder, 1969; Harris & Baldessarini, 1973).

Mailing and Acheson (1946) investigated the effect on recovery of function of d-amphetamine administered to decerebrate cats one to two hours following the surgical transection. The animals were then placed on their sides and righting attempts were observed. The drug seemed to have the same effect upon the "lower righting centers" as did survival for a period of days or weeks, i.e., the amphetamine sped up the recovery of the ability of these animals to right themselves. The authors postulated that either time or d-amphetamine permitted neural centers to develop spontaneous activity after loss of tonic input. Amphetamine was also used to study functional recovery in neodecorticate cats (Meyer, Horel & Meyer, 1963). All the cats in this study were examined for a number of weeks after lesioning, and both visual and tactile placing responses were observed under normal and drug (10 mg/kg amphetamine, i.p.) conditions. Within 20 minutes of the

injection, autonomic effects were noted, including increased respiration, piloerection and pupillary dilation. In addition, reinstatement of both the tactile and visual placing responses was seen. All these effects disappeared as the drug action subsided. The authors postulated an increase in arousal as responsible for the return of the placing responses.

As noted on page 14, the level of environmental stimulation during the interval between brain damage and behavioral assessment has been found to be an important variable in recovery of function by several different investigators (Isaac, 1964; Meyer et al., 1958). In a pharmacological extension of such studies, Cole et al. (1967) trained rats on a light-cues shock avoidance task until a stable baseline was reached and then subjected them to unilateral occipital cortex lesions. For several days after surgery, the animals were housed under one of the following four conditions: (1) in dark and quiet quarters; (2) in dark and quiet quarters with 2 mg/kg of amphetamine per day administered in the drinking water; (3) in well lit and noisy quarters; and, (4) in well lit and noisy quarters with 30 mg/kg of phenobarbital per day, again administered in the drinking water. On the eighth day, contralateral occipital cortex lesions were made. A six day normal recovery period for all groups was followed by a retest on the visual task. Both the group housed in light and noise and the group given amphetamine in the dark and

quiet showed complete sparing of the task. The rats housed in the dark and those in the light which were given phenobarbital showed great deficits on the task, with the drug group showing the greater losses. These results were interpreted as implicating a role for non-specific neural stimulation in functional recovery, with such stimulation able to be provided pharmacologically as well as sensorily. In an additional study, Braun, Meyer and Meyer (1966) also reported pharmacological facilitation of recovery of a visual task after visual cortex ablation in the rat. The animals were given amphetamine 15 minutes before retraining sessions postoperatively; doses of both 0.5 and 1.0 mg/kg were found to facilitate reacquisition of performance.

It is difficult to distinguish the possible mechanism of drug action in these studies. It may be that direct pharmacological action is sufficient for the acceleration of recovery, or that the drugs act by facilitating the action of sensory input on neuronal systems.

Luria and his Russian coworkers have recently published a review of their clinical experiences using cholinergically active compounds in the treatment of brain damaged patients (Luria et al., 1969). They report that the quarternary cholinergic compound neostigmine greatly facilitates recovery of sensory, motor and cognitive functioning. At times, the effects appear within two hours of the first injection of the compound. They also report that

eserine (physostigmine) and galanthamine (a tertiary compound), both acetylcholinesterase inhibitors, are also effective in inducing restoration of function. In fact, galanthamine is reported to be the fastest and most effective of the compounds used, a result felt to be due to its faster and greater penetration of the CNS. Luria has postulated that these drugs work by a process of "de-blocking" or disinhibition of tissue around the borders of, or distal to, a lesion. Luria invokes von Monakow's concept of "diaschisis" to explain the CNS dysfunction and proposes that as a result of the insult to the CNS, a functional depression is both the substrate of dysfunction and the process acted upon by the compounds that facilitate recovery. Luria stresses that much of the recovery seen with the use of drugs would have occurred in time anyway, and that there are usually residual deficits which are drug resistant.

Berger, Wise and Stein (1971) have reported that injection of noradrenaline (NA) directly into the lateral ventricles results in immediate feeding, even overeating, in rats previously rendered adipsic and aphagic from bilateral LH lesions. Administration of the alpha-NA blocking agent phentolamine suppressed feeding in both normal rats and rats who had recovered from the LH syndrome. Although relevant to the present topic, this study seems less a demonstration of pharmacological facilitation of recovery than it is an example of direct pharmacological elicitation of lost function by stimulation of receptors

deafferented by brain damage.

Ljungberg and Ungerstedt (1976) made rats aphagic and adipsic either by 6-hydroxydopamine (6-OHDA) lesions of the ascending dopamine (DA) systems or by electrocoagulation of the LH region. They found that injections of L-DOPA, the CA amino acid precursor, apomorphine, a DA stimulator (Ernst, 1967), or ET495, a possible DA stimulator (Corrodi, Fuxe & Ungerstedt, 1971), in small dosages could reinstate eating for a short time in the 6-OHDA lesioned rats but not in the LH lesioned animals. On the other hand, O'Laughlin and Feldman (1976) have reported that apomorphine injected directly into the LH regions 48 hours after LH lesioning could permanently reinstate eating and drinking. Data unpublished by the author indicate that peripheral administration of apomorphine can induce a permanent return of eating and drinking in LH lesioned rats, but the size of the lesion is a critical variable (Marotta, unpublished observations). Animals with large lesions of the LH area encroaching on the zona incerta and other areas are not likely to show pharmacologically induced recovery. Ljungberg and Ungerstedt do not present histology, but O'Laughlin (personal communication) makes LH lesions which do not destroy the zona incerta. This may be a critical variable since Zeigler and Karten (1974) describe the trigeminal system as a projection through this area, and Zeigler and his associates describe an aphagia and adipsia

syndrome associated with the trigeminal system.

In another pharmacological study using the LH model of recovery, Glick, Greenstein and Zimmerberg (1972) pretreated rats with alpha-methylparatyrosine (α-MPT), a blocker of the rate limiting enzyme in CA production (Moore & Dominic, 1971), for three days prior to bilateral LH lesions and then measured recovery from the lesion induced adipsia and aphagia. All saline control animals and those receiving 10 mg/kg of α-MPT died of adipsia and aphagia withing seven and eight days, respectively. The animals receiving 75 or 100 mg/kg of α-MPT recovered from the adipsia and aphagia in two to six days. These authors postulate that the recovery seen in the LH syndrome is due to the gradual development of supersensitivity in the NA system. Supersensitivity is felt to be an increase in responsiveness of denervated muscle, gland, or nervous tissue caused by its lack of transmitter substance. The tissue is believed to develop more post-synaptic receptors, and, over time, to become up to several thousand times more sensitive to its natural transmitter or to the transmitter's agonist (Sharpless, 1964). The pretreatment with α-MPT allowed the NA supersensitivity processes to begin before the lesions were made.

This observation has been replicated and extended. Hynes and his associates (Hynes, Anderson, Gianutsos & Lal, 1975) have shown that in addition to chronic presurgical treatment with α-MPT, the rate of recovery from the LH

syndrome could also be modified with haloperidol (4 mg/kg/day) and morphine (60 mg/kg/day). Both haloperidol and morphine have been found to interfere with normal transmission in CA systems (Puri & Lal, 1973). In still another study, Glick and Greenstein (1972) have presented additional evidence that recovery from the LH syndrome is facilitated if the frontal poles of the rat are ablated 30 days prior to the bilateral LH surgery. A supersensitivity mechanism is postulated in this situation also. Björklund and Stenevi (1972) transplanted iris tissue into the CNS and transected NA axons in the caudal hypothalamus. At the time of surgery, nerve growth factor (NGF) was injected into the ventricles. Seven days after surgery, rats with NGF were compared to untreated surgical controls by histofluorescent microscopic examination and the animals that had received NGF were found to show striking increases in the number of newly-formed sprouts in and around the transplant. In an extension of this work, Berger, Wise and Stein (1973) injected NGF into the ventricles of rats subjected to bilateral LH lesions. These animals showed facilitated recovery from the second stage (anorexia) of the LH syndrome. In the second and third week after lesioning, the NGF rats ate more food, gained more weight, ate more vigorously in response to intraventricular administration of NA, and were more resistant to reinstatement of the LH

syndrome by injection of 6-OHDA than animals that had not received NGF.

The LH syndrome in rats has also been modified by injection of insulin for five days prior to surgery (Balagura, Harrell & Ralph, 1973). The recovery period is shortened if insulin is administered, but lengthened if glucagon is given during the preoperative period (Balagura et al., 1973). It was also reported that rats kept at reduced body weight prior to surgery show facilitated recovery (Powley & Keesey, 1970).

Another interesting example of pharmacologically induced recovery of behavioral function has been supplied by Zis and his associates (Zis, Fibiger & Phillips, 1974). They found that destruction of the nigro-striatal DA projection system by microinjection of 6-OHDA directly into the substantia nigra of rats produced deficits in active avoidance performance, but that injections of L-DOPA for several days produced a decrease in active avoidance error scores.

Finally, Mark and his group in Boston have reported that microinjections of adrenalin or NA bilaterally into the amygdalae of cats with lesions of the ventromedial hypothalamus (VMH) lead to reversible attenuation of the VMH rage syndrome (Mark, Takada, Tsutumi, Takamatsu, Toth & Mark, 1975). The drug effect begins approximately 20 minutes after infusion of the drug and can be observed for

nearly an hour. Because of this time course, a simple receptor stimulation mechanism seems unlikely unless a critical level of drug is reached or a number of fibers have been affected before the behavioral result is seen.

De-blocking of Neurotransmission as an Element in Recovery of Function

As noted in the preceding section, evidence exists that a variety of quite different pharmacological agents can accelerate recovery of behavioral function following damage to the nervous system. Although it is extremely improbable that all such compounds act in a similar fashion, it is nevertheless not implausible that the majority achieve their effect by acting on a variety of somewhat disparate mechanisms and processes that can all be subsumed under the general rubric of diaschisis. As noted on page 4, diaschisis is a descriptive term for a type of neuronal shock state in which intact brain tissue is deprived, either anatomically or functionally, of normal afferent input. Thus, depending upon the exact nature of the diaschisis, a variety of processes could act to ameliorate the deafferentation and restore behavioral function. Included among such processes might be: (1) dimunition of edema, (2) development of denervation supersensitivity, (3) axonal sprouting and regrowth, and (4) deblocking of synaptic transmission processes inactivated by trauma.

Diminution of Edema

Many forms of CNS insult lead to swelling or edema, which tends to exert its maximal effects within a few days and then to gradually subside. Such sequelae of brain trauma as coma and cranial nerve compression are often attributable to edema. In addition to the swelling itself, there are correlated biochemical alterations (Katzman, 1975). The gradual diminution of edema with time may account for a significant percentage of the observed instances of behavioral recovery following brain damage, and pharmacological acceleration of such diminution would seem theoretically possible.

Denervation Supersensitivity

Denervated neurons develop increased sensitivity both to stimulation over remaining afferent pathways (Sharpless, 1964) and to pharmacological challenge (Ungerstedt, 1971b). There is evidence that some of these changes in sensitivity may be due to modification of post-synaptic receptor mechanisms (Mishra, Gardner, Katzman & Makman, 1974). This phenomenon of "denervation supersensitivity" (Trendelenburg, 1963) has been suggested as responsible for some instances of functional recovery after brain damage, especially in view of the prolonged time course of the supersensitivity process, a time course not often unlike that of behavioral recovery. It is not inconceivable that pharmacological agents could alter

recovery of function by acting to prime or accelerate the processes of denervation supersensitivity. Indeed, at least one of the studies cited in the previous section (see page 23; Glick et al., 1972) showing facilitated recovery from LH aphagia syndrome by α-MPT pretreatments, appears to fit this category.

Axonal Sprouting and Regrowth

The ability of central axons to generate collateral sprouts or teleodendritic processes was first demonstrated by Liu and Chambers (1958). These processes which have been visualized using both histofluorescence (Katzman, Björklund, Owman,Stenevi & West, 1971) and electronmicroscopy (Lynch, Deadwyler & Cotman, 1973; Lynch, Mosko, Parks & Cotman, 1973; Raisman, 1969a) have demonstrated the formation of new functional synapses in the hippocampal region after destruction of the entorhinal cortex, with consequent recovery of spontaneous alternation behavior. Similarly, Schneider and Jhaveri (1974) have presented evidence for changes in innervation of the visual system of hamsters which may be responsible for the sparing of function seen after lesions in young animals, but not observed in adults of that species. Thus, the phenomena of axonal sprouting and regrowth following CNS lesion seem clearly established, with considerable suggestive evidence that such processes can be involved in some instances of recovery of function. And it is not unlikely

that, in some of the reported instances of pharmacological augmentation of recovery from brain damage, the compounds used exert their effects by acting upon sprouting and regrowth processes. Indeed, this seems very likely in the reported facilitation of recovery from the LH syndrome by NGF (Berger et al., 1973), and also possible in the use of insulin by Balagura et al. (1973), in view of the chemical similarities between insulin and NGF (Berger et al., 1973).

Deblocking of Synaptic Transmission Processes

Although, as suggested above, some pharmacological agents may affect functional recovery by acting on edema, denervation supersensitivity, or axonal sprouting and regrowth, it seems likely that the majority of instances of pharmacological augmentation of recovery require another explanatory framework. Thus, the uses of cholinomimetics and cholinesterase inhibitors (Luria et al., 1969; Ward & Kennard, 1942; Watson & Kennard, 1945), amphetamine (Braun et al., 1966; Cole et al., 1967; Maling & Acheson, 1946) and catecholamine precursors (Zis et al., 1974) to facilitate behavioral recovery seem most amenable to an explanation based upon reactivation or deblocking of neurotransmission processes (Luria et al., 1969). Such deblocking might include reactivation of axoplasmic flow, reactivation of enzymatic processes necessary for neurotransmitter synthesis, or reactivation of enzyme processes

involved in second messenger mechanisms (Theonen, 1975). Of these, perhaps the most likely is an effect on neurotransmitter synthetic processes, especially in view of Luria's report (Luria et al., 1969) of occasional restoration of motor and sensory functions in brain damaged patients following a single injection of a cholinergically active compound.

The "Septal Syndrome" as a Model for Studying Recovery of Function

The papers reviewed in this report indicate that it is possible to modify the rate of recovery after certain types of brain damage with pharmacological agents. However, none of the works cited have made a detailed study of a given syndrome. Therefore, little is known of the underlying neural disorganization responsible for the appearance of a lesion-induced syndrome, nor is there any understanding of the mechanisms whereby reorganization or recovery might take place. What is needed for further progress in this area is the development of a model system; this will allow a pharmacological and physiological dissection of the processes involved in neural recovery. It is suggested that the septal irritability syndrome may be a convenient model.

The septal syndrome is an affective rage or irritability syndrome resulting from large bilateral lesions of the rodent septal forebrain area. The syndrome, first described by Brady and Nauta (1953, 1955), is characterized

by an explosive increase in emotionality followed by a gradual diminishing emotional intensity over a time course of approximately 14 days. The syndrome is operationally defined in terms of a list of behaviors with weighted scores (King, 1958). Among these behaviors are: reaction to probing on the animal's ventral surface, visual following behavior, height jumped following stimulation, vigor and frequency of biting a probe, difficulty in capture, muscle tension, vocalization, and mouse-killing. In addition to the transient emotional components, recovered septal animals exhibit long-term passive avoidance deficits and deficits in behaviors associated with differential reinforcement-of-low-rates; septal animals also show facilitation in active avoidance tasks (see Fried, 1972 for review).

Rationale for the Use of the Septal Model

There are a number of characteristics which make the study of the septal syndrome a useful experimental model for the study of recovery of function after CNS damage. First, the syndrome can be readily elicited by well-placed lesions (Olton & Gage, 1974; Schnuir, 1972; Turner, 1970). Second, the syndrome can be observed in a number of species, including humans (Zeman & King, 1958), with appropriate lesion. Third, the behavioral form and the time course of the syndrome are constant if the experimental conditions are appropriately controlled. Fourth, the

syndrome is easy to measure and quantify in rodents, there being little ground for confusion between a normal animal and a septal animal. Behavior rating scales for the syndrome have been worked out in detail. Fifth, since the syndrome has a stable and characteristic time course, the effect of manipulation which alter this time course are easy to measure. In addition, the time course, although transient, is not so ephemeral that a wide variety of manipulations cannot be performed. At the same time, the relative shortness of the time course allows many experiments to be performed without excessive logistical problems. Sixth, the anatomical relationships, neurochemical levels and distributions, both before (Brownstein, Saavedra & Palkovits, 1974; Lewis & Shute, 1967; Ungerstedt, 1971c) and after lesions (Bernard, Berchek & Yutzey, 1975; Harvey, 1965; Heller & Moore, 1965; Poncy, Bernard & Chernov, 1972) have been researched in detail. Seventh, many of the non-emotive behavioral effects of septal lesions have been extensively reviewed (Caplan, 1973; Fried, 1972). Finally, of course, the septal syndrome is an instance of behavioral dysfunction following brain damage which exhibits a clear pattern of recovery of function, against which drug-induced alterations in recovery can be readily compared. In fact, in terms of recovery of function, the septal preparation has been the most extensively studied of any model of affective behavior (Glass & Thomas, 1970; Gotsick & Marshall,

1972; King, 1958; King & Meyer, 1958; Marotta, Potegal, Gardner & Glusman, 1975; Montgomery & Christian, 1973; Yutzey, Meyer & Meyer, 1964, 1967). In addition, a number of papers have specifically addressed the problem of recovery of normal affect after septal destruction. King (1958) showed that subsequent amygdalectomy attenuates the irritability syndrome. Yutzey et al. (1967) implicated cortex in the recovery process by showing that the removal of as little as 50% of neocortex simultaneously with septal lesioning prevented attenuation of the syndrome. In a conceptually related study, Cytawa & Teitelbaum (1967) used spreading depression to reinstate the syndrome, although Glass and Thomas (1970) were unable to replicate the effect.

Classical Septal Neuroanatomy

The septal nuclei are a telencephalic structure in the limbic system. Its neuroanatomy has been examined by a number of methods. Both degeneration (Nauta, 1956; Raisman, 1966, 1969b) and evoked potential (Green & Arduini, 1954; Petsche, Stumpf & Gogolak, 1962; Powell, 1963) studies have indicated that septal nuclei receive connections from a great variety of structures, including the subdivisions of the hippocampus, amygdala, hypothalamus and midbrain. Septal afferents have been traced from the hippocampus through the fimbria and dorsal fornix, from pyriform cortex and amygdala through the stria terminalis, and from the

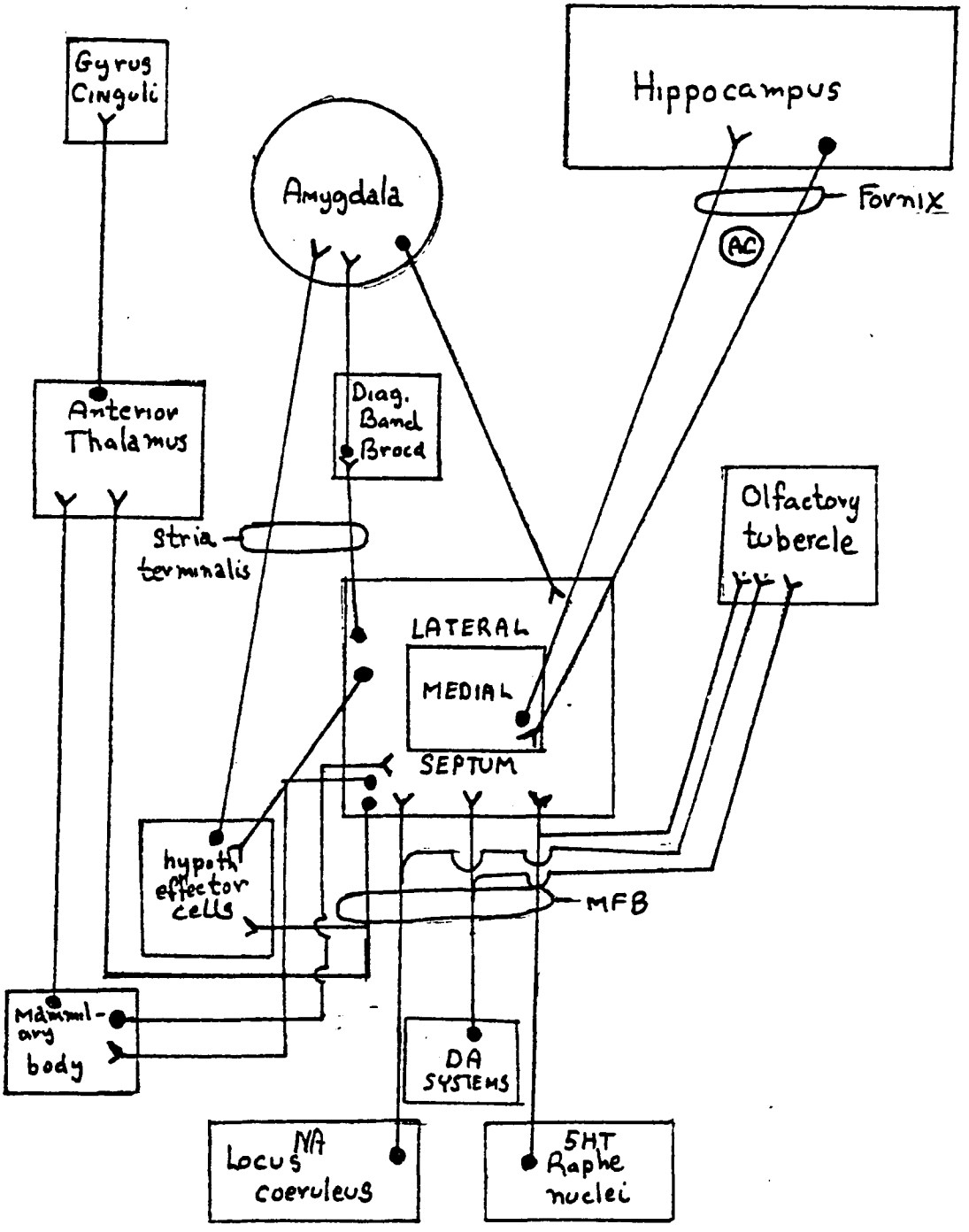


Figure 1: Schematic of the Major Anatomical Connections of the Medial and Lateral Septal Nuclei.

olfactory tubercle via the medial aspect of the medial forebrain bundle (MFB). Diencephalic septal afferents are contained primarily in the MFB and are derived from the rostro-caudal extent of the hypothalamus, and more caudally, from regions of the rostral midbrain.

The tracing of efferents by fiber degeneration is complicated by the passage of the fornix, fimbria, stria terminalis and MFB in or near the septum (Raisman, 1966). Still, fibers have been traced to the olfactory tubercle and degeneration has been found in the rostrum of the corpus callosum after septal lesions. Caudal fibers travel to the fimbria of the hippocampus. They are ventrally coursing fibers projecting through the MFB which terminate at various levels in the hypothalamus, primarily the preoptic and LH areas. Other major projections are found from the septal area to the habenula and from the medial septal nuclei to the hippocampus via the fornix.

A major comparative study was performed by Valenstein and Nauta (1969). Species differences in the projections of the fornix to and from the septum are described. Interspecific differences in the fornix projections of rats, cats, guinea pigs and monkeys seem to be in the number and location of synapses interconnecting the various parts of the limbic system. For example, in the rat and guinea pig, hippocampal connections to the lateral hypothalamus are made through septal and lateral preoptic nuclei, while

in the cat the projection is more direct.

It is interesting to note that the relatively new technique of horseradish peroxidase marking to trace neural connections has yielded information not in complete correspondence with the classical methods. After application of radioactive horseradish peroxidase to the septum, Siegel and Landis (1975) discovered no labeling in the hippocampus except for the area just rostral to the genu of the corpus callosum. Labeled cells were observed in the amygdala, olfactory tubercle, cingulate and frontal cortex, the periventricular area of hypothalamus—extending from the level of the ventromedial nuclei to the mammilary bodies. Evidence of retrograde transport was also found in the diencephalic habenular, medial raphe, ventral tegmentum, and with particularly high density in various thalamic nuclei. In the mesencephalon, DA neurons along the anterior extent of the ventral tegmental area, just dorsal to the interpeduncular nuclei, were labeled. Horseradish peroxidase was also found in the medial (but not dorsal) raphe, and scattered through the locus coeruleus.

Septal Neurochemistry

While light microscopic observation of the septal nuclei indicate a heterogeneous cell population (Fox, 1940), the new histofluorescent and biochemical techniques yield colorful and provocative results.

A series of papers (Brownstein et al., 1974, Palkovitz, Brownstein, Saavedra & Axelrod, 1974; Saavedra, Brownstein &

Palkovitz, 1974) by members of Axelrod's laboratory of Clinical Science at NIMH present levels of DA, NA, serotonin (5HT) and choline acetyltransferase (CHAS) concentration in the limbic system of rats. They used a sensitive enzymatic isotopic method with an ingenious micro-dissection technique (Saavedra, Brownstein & Axelrod, 1973) to yield very precise results for over 34 different limbic nuclear areas. The medial septum and nucleus septalis fimbrialis contain relatively high levels of CHAS. The amygdaloid nuclei also have relatively high concentrations while the nucleus interpeduncularis has the highest levels by a factor of six over the anterior amygdala. NA concentration in the septal nuclei are highest in the triangular nucleus. Comparable values are found in nuclei such as the lateral mammillary body, and the nucleus interstitialis striae terminalis dorsal. The highest limbic NA concentrations are found in the nucleus interstitialis striae terminalis ventral. The lateral and intermediate septal nuclei have the highest DA levels, while the highest limbic DA levels are found in the olfactory tubercle. It is interesting to note that the olfactory tubercle DA levels are higher than the DA levels found in the caudate nucleus. Their data show that all the classic putative neural transmitters have relatively high concentration in the septal nuclei and its adjacent structures. It is difficult to state that one nucleus is

more cholinergic or serotonergic, but comparing medial to lateral nuclei for each transmitter indicates that there is relatively equal amounts of NA and 5HT, while the medial is higher in CHAS than the lateral, and the lateral is higher in DA concentrations than the medial.

The histochemical technique of Falck, Hillarp, Thieme and Torp (1962) is one of the more powerful and elegant methods to be developed in this decade. The histofluorescent microscopy allows the visualization of NA, DA and 5HT in cell bodies and nerve terminals. When this technique is added to the methods for staining cholinesterases, first developed by Koelle and Friedenwald (1949), some of the neurochemical profile of the brain becomes clearer. Ungerstedt (1971c) both reviews the literature on monoamine pathways in the brain and presents new experimental data. A detailed analysis of the NA pathways is difficult because of the critical areas where lesions must be made. It seems that the cell groups in the medulla oblongata and the pons, the A1, A2, A5 and A7 groups give off axons that ascend medially in the reticular formation and continue rostrally mainly within the MFB. This ventral NA pathway innervates the whole hypothalamus, preoptic and the ventral interstitial striae terminalis. The DA system which involves the septal area is called the meso-limbic DA system. These axons arise from cell bodies dorsal to the nucleus interpeduncularis and ascend with the axons of the

nigro-striatal DA system. They follow a more medial route and never enter the crus cerebri but ascend just dorsal to the MFB. At the level of the anterior commissure one branch enters the nucleus accumbens septi and the nucleus interstitialis striae terminalis dorsal. The other branch enters the olfactory tubercle. The 5HT cell bodies in the raphe nuclei dorsalis and medianus give rise to an ascending system of axons. These axons occupy the most ventral part of the MFB and may be separated into a medial and lateral component. The medial axons ascend in the septum and travel to the cingulum. The lateral axons move through the hypothalamus and enter the amygdala.

Lewis and Shute (1967) studied the acetylcholinesterase (AChE) system in the limbic system of the rat. They showed that the ascending reticular pathways from the brainstem contain AChE along their length and are probably cholinergic. They also found cholinergic cells in the septum which project to the hippocampus. Specifically, the medial nucleus projects to the dentate gyrus and the dorsal and ventral hippocampus, as does the nucleus of the diagonal band. The nucleus accumbens sends cholinergic axons to the olfactory tubercle. Finally, the highly vascularized subfornical organ was found to be innervated by cholinergic axons from the dorsal fornix and the midline raphe of the upper septum.

Nauta (1960) considers the septal area to be a critical

site for the organization and integration of the limbic telencephalic and diencephalic influences. Nauta also stresses that neural circuits, by themselves, often fail to explain the influence of a neural component upon the various effector mechanisms involved.

Factors Affecting Recovery of Function in the Septal Syndrome Model

Recovery of normal affect after destruction of the septal nuclei seems to result from an interaction of time and handling (Brady & Nauta, 1953, 1955; King, 1958; Nielson, McIver & Boswell, 1965; Reynolds, 1965). The usual time course is approximately 14 days if the rats are handled daily, but if the rats are maximally handled, it may last as little as 7 days (Gotsick & Marshall, 1972; Singh, 1969). If, on the other hand, the handling is minimized, the rats will still be extremely irritable 60 days after lesioning (Brady & Nauta, 1955). Most importantly, for any given handling schedule, holding animal size and lesion constant, a fairly regular time course of return to normal affect can be expected (Gotsick & Marshall, 1972). Changing the stimulus conditions under which the rats are housed or stimulating the animals by "bouncing" them in a pail, does not take the place of handling or decrease the time course of the syndrome (Gotsick & Marshall, 1972). In addition, while the manner in which an animal is approached can affect the extent of

irritability, it does not affect the overall time course of the syndrome (Max, Cohen & Lieblich, 1974). For the septal rage syndrome, then, a specific class of stimulation is needed to facilitate recovery of normal affect.

The septal syndrome can be modified in several ways by a second lesion. If an amygdaloid lesion precedes a septal lesion, the irritability has been reported to be either partially reduced (Jonason, Enloe, Contrucci & Meyer, 1973; King, 1958) or enhanced (Kleiner & Meyer, 1967). If an amygdaloid lesion follows a septal lesion, the irritability is reduced to baseline (King & Meyer, 1958; Schwartzbaum & Gay, 1966). If an animal which has recovered from the irritability syndrome receives an amygdalectomy, the syndrome may reappear (Jonason et al., 1973; Kleiner & Meyer, 1967). If septal and amygdaloid lesions are produced simultaneously, an irritability syndrome of varying intensity results (Harvey, Heller, Moore, Hunt & Roth, 1964; Jonason et al., 1974, Kleiner & Meyer, 1967).

Cingulate or neocortical lesions raise the level of septal irritability if these lesions are made 21 days before the septal lesions (Yutzey et al., 1967), but cingulate lesions following septal lesions seem to have no effect on irritability (Schwartzbaum & Gay, 1966).

Destruction of the fornix pathways nine days before a septal lesion diminishes the septal irritability. The degree of attenuation of the syndrome seems to be re-

lated to the size of the fornix lesion; the greater the fornix damage, the more the attenuation of rage (Olton & Gage, 1974). These authors also report that fornix lesions made simultaneously with septal lesions attenuate the septal rage, although these data differ from those reported by Brady and Nauta (1953, 1955).

In recent years, a number of investigators have turned their attention to the possibility of pharmocological manipulation of functional recovery in the septal syndrome. At the same time, other workers have attempted to study the biochemical correlates of both septal rage and its subsequent functional recovery pattern.

Pharmacological studies. A number of pharmacological compounds have been found to cause transient decreases in septal irritability. These include chlorpromazine (Cytawa & Kutulas, 1972; Raitt, Nelson & Tye, 1961), haloperidol (Marotta et al., 1975), chlordiazepoxide (Quenzer, Feldman & Moore, 1974; Schalleck, Koehn & Jew, 1962) physostigmine (Stark & Henderson, 1972), and barbiturate (Harvey et al., 1964). Since many of these compounds yielding a transient effect on septal irritability have sedative properties in common, at least some of the effect on irritability may be simply a sedative one. On the other hand, Loizzo and Massotti (1973) have reported that a number of non-narcotic analgesic agents, such as aspirin, also have a transient suppressive effect on septal

irritability, so the question apparently remains an open one. In any event, since the effects of these compounds are so transient, they would appear to have little relevance for the overall question of augmented recovery of function.

Of more relevance, seemingly, is the work of Dominguez and Longo (1969, 1970), who found that injections of 300 mg/kg of para-chlorophenylalanine (pCPA), a tryptophan hydroxylase inhibitor (Koe & Weissman, 1966), given to irritable septal animals two days after surgery resulted in complete and irreversible attenuation of the syndrome within approximately 120 minutes. Lower doses of pCPA (200 mg/kg) produced a 40% dimunition in irritability, while injections of α-MPT, the tyrosine hydroxylase inhibitor (Moore & Dominic, 1971), or disulfiram, the dopamine-β-hydroxylase inhibitor (Goldstein & Nakajima, 1967) were without effect on the syndrome. Dominguez and Longo (1969, 1970) also gave injections of 300 mg/kg of pCPA to animals during the septal surgery itself, rather than two days after surgery, but found no effect on the emergent syndrome.

In a somewhat related study, Harrell and Balagura (1975) gave doses of pCPA (200 mg/kg) for three days prior to septal lesion surgery and found a "mitigation" of the resulting irritability. Control animals given saline for three days prior to septal surgery exhibited typical

irritability for at least 10 days.

While attempts to reinstate the septal syndrome by stimulus manipulation (Hammond & Thomas, 1971) or by a second lesion (Jonason et al., 1973; Schwartzbaum & Gay, 1966; Yutzey et al., 1967) have either failed completely or produced confusing results, there is one report of pharmacological reinduction of the syndrome. Pirch and Norton (1965) administered the monoamine-oxidase inhibitor phenylisoproplyhydrazine (JB-516) to formerly irritable rats and found that injections of 20 mg/kg produced a transient reappearance of irritability approximately five hours later. This effect disappeared by 14 hours after the injection.

Biochemical studies. To date, studies of biochemical changes accompanying the septal syndrome have produced a mosaic of contradictory results. The one consistent finding seems to be that septal lesions lead to a decrease in ACh content of whole brain tissue (Sorensen & Harvey, 1971). In one of the few biochemical studies to employ the technique of serial assays at various postsurgical times, Pepeu, Mulas and Mulas (1973) compared whole brain ACh content of irritable septal animals to that of non-irritable rats showed a significant decrease in ACh, with a 10% decrease ten hours post-lesion, a 20% decrease three days post-lesion and a 21% decrease at 21 days post-lesion (interestingly, the ACh content of the brains of the

irritable animals remained 20% below control animals even after the animals had recovered from the irritability). Additional research has implicated the hippocampus in at least some of the ACh changes that follow septal lesions (Dudar, 1975; Mellgren & Srebro, 1973; Sethy, Kuhar, Roth, Van Woert & Aghajanian, 1973; Srebro & Mellgren, 1974).

With respect to NA levels following septal lesions, some workers have reported them unchanged (Heller & Moore, 1965; Poncy et al., 1972), some have reported them decreased (Bernard et al., 1975; Montgomery & Christian, 1973), and some have reported them increased (Salama & Goldberg, 1973). Of these investigations, only the one by Bernard and his colleagues (Bernard et al., 1975) was performed specifically during the period of irritability and the decreased NA levels found by these investigators were localized to the hypothalamus. These workers also studied DA levels in the septal syndrome and found regional decreases in both hypothalamic and limbic areas.

Although several theoretical reviews (e.g., Caplan, 1973; Fried, 1973) have suggested a causal role for 5HT in the septal syndrome, the biochemical evidence for changes in 5HT levels following septal lesions is problematic. Harvey (1965) and coworkers (Lints & Harvey, 1969) reported decreases in 5HT of approximately 13% following septal lesions, but other investigators (Bernard et al., 1975; Salama & Goldberg, 1973) have reported that levels are unaffected after septal lesions. Pirch and Norton

(1965), in their investigation of reinduction of irritability by MAO inhibition in recovered septally lesioned animals measured both NA and 5HT levels and found a decrease in 5HT concentration concomitant with the reinduction of irritability. They suggested that the irritability might, therefore, have a serotonergic basis. However, there are reports which strongly suggest that other neurotransmitter systems have a critical role in septal irritability. The most recent studies attempting to correlate brain amine levels with septal irritability indicate a relationship between ACh (Pepeu et al., 1973), NA, DA (Bernard et al., 1975) and septal irritability. Further evidence comes from the induction of irritability states by intraventricular administration of the CA damaging agent 6-OHDA (Coscina, Seggie, Godse & Stancer, 1973; Nakamura & Thoenen, 1972). The experiments reported in this study will further elaborate on the possible underlying neurochemical substrate of the syndrome. The emphasis will be on catecholaminergic systems as the possible critical links in both the production and dissapation of septal irritability, and attempts will be made to differentiate between DA and NA function (Antelman & Caggiula, 1977).

The Present Experiments

It can be seen that destruction of the septal nuclei not only disrupts a major neural integrating center,

where influences from hippocampal, amygdaloid, olfactory, hypothalamic and midbrain regions converge (Raisman, 1966), but also interferes with the ascending monoamine neurotransmitter systems so elegantly described by the fluorescent microscopists (Lindvall & Björklund, 1974). Therefore, it is not surprising that ablation of the septal nuclei results in such dramatic behavioral alterations as extreme irritability (Brady & Nauta, 1953) and disruption of performance in standard avoidance and operant paradigms (Fried, 1972). In general, interruption of the CA systems of the brain, either through lesioning the source nuclei in the pons and midbrain with heat or neurotoxins, or pharmacological manipulations with enzyme or transmitter blockers, results in behaviors like those seen with septal lesions. For example, interference with NA systems with either receptor blockers or disruption by 6-OHDA facilitates active avoidance behavior in rats (Merlo & Izquierdo, 1965). Active avoidance is also facilitated by septal lesions (King, 1958). Also both septal lesions and 6-OHDA administration can induce irritability. The high levels of CAs found in and around the septal nuclei (Brownstein et al., 1974) as well as the correspondence between the effects of septal lesions and interference with CA action, led to the postulation that a pharmacological understanding of the septal irritability syndrome was possible. The experimental design includes exploration

of a subset of neural elements by making specific neurotoxic lesions of the septal area with 6-OHDA. The neurochemical substrate of septal irritability will be further studied by evaluating the effect of various pharmacological agents active on CA systems on the time course and intensity of septal irritability. Other studies will attempt to reinstate the syndrome in recovered septal rats by pharmacological means. Finally, the effect of chronic treatment of rats with CA agents before surgery will be observed.

The following hypotheses have been entertained: (1) The underlying neurochemical substrate of septal irritability is catecholaminergic. Therefore damage to the CA cells, fibers and nerve terminals in and around the septal nuclei by intraseptal microinjections of 6-OHDA should induce an irritability syndrome essentially indistinguishable from the irritability syndrome induced by large radiofrequency (RF) lesions of the septal area. (2) Since irritability induced by septal lesions is felt to be due to disruption of CA function in and around the septal area and in distal structures connected to the septal area, administration of CA facilitating agents should accelerate the rate of recovery. This will be accomplished by injection of drugs which cross the blood-brain-barrier into rats 24 hours after lesioning the septal area, and comparing these animals to lesioned

animals receiving saline or barbiturate. (3) Since a return of CA function is felt to be responsible for recovery of normal affect in septally lesioned rats, it is postulated that blockade of CA receptors with a neuroleptic drug will lead to a transient return of irritability. (4) It is postulated that chronic presurgical treatment of rats with CA stimulating and blocking agents will affect the appearance of septal irritability after subsequent lesioning. CA blocking agents which increase CA production and turnover should prevent the appearance of septal irritability.

The objectives of these experiments are twofold. First, to indicate a possible underlying catecholaminergic substrate of septal lesion-induced irritability and to attempt to delineate the specific contribution of either DA or NA to the syndrome. Second, to demonstrate that drug induced facilitation of recovery is possible in a model system following brain damage, and to distinguish pharmacologically some of the possible neuronal systems involved.

CHAPTER 2

GENERAL METHODS

Subjects

Six hundred adult male Long-Evans hooded rats, approximately 120 days of age, purchased from the Blue Spruce Company (Altamont, N.Y.) were used. All the animals were ad libitum fed and watered and were individually housed in steel cages (20 x 18 x 34 cm) at a constant temperature (22 $\pm$ 1^{o}C) on a 12 hour on- 12 hour-off light schedule.

Behavioral Methods

Destruction of the septal nuclei in rats results in the appearance of an intense explosive irritability syndrome with a duration of at least seven days (Brady & Nauta, 1953); the syndrome may persist for as long as 60 days (Brady & Nauta, 1955). The dependent measures included the intensity and duration of this irritability syndrome.

Individual subjects were moved in their own enclosed steel living cages to a large (55 x 45 x 85 cm) plexiglass testing chamber. Two arm holes in the chamber allowed an experimenter to release a rat from its cage into the chamber and, then, remove the cage over the top of the chamber. After a two minute pause, the following seven stimuli were presented in random order by an experimenter wearing heavy steel-studded leather gloves. The responses of the rats to each of the stimuli were assessed using the

rating scale described below:

Bite

A blunt pencil was placed just in front of the snout of a rat. If the animal:

a) ignored or sniffed, a point score of 0 was assigned.

b) bit and nibbled, a point score of 1 was assigned.

c) bit vigorously and repeatedly, a point score of 2 was assigned.

d) bit vigorously and repeatedly, and attacked and pursued the pencil, a point score of 3 was assigned.

Probe

A blunt pencil was touched to the flank of a rat. If the animal:

a) ignored the pencil, a point score of 0 was assigned.

b) turned briskly and oriented to the probe, a point score of 1 was assigned.

c) turned and fled, a point score of 2 was assigned.

d) turned, fled, jumped and made repeated attempts to escape from the cage, a point score of 3 was assigned.

Magnet Reaction

The point of a blunt pencil was held 5 cm above and in front of the head of a rat and then moved slowly along the anterior-posterior axis several times. If the animal:

a) ignored or just glanced at the pencil, a point score of 0 was assigned.

b) oriented and reared up to follow the probe, a point

score of 1 was assigned.

c) reared up, followed the probe and nearly fell over, a point score of 2 was assigned.

d) reared up and fell repeatedly while following the probe, a point score of 3 was assigned.

Capture

The experimenter attempted to confine the rat and lift him off the ground using one gloved hand. If the animal:

a) was easily picked up and held, a point score of 0 was assigned.

b) was only picked up by using both hands, a point score of 1 was assigned.

c) was only picked up using both hands after repeated attempts, a point score of 2 was assigned.

Handle

The experimenter attempted to hold the rat in one hand. If the animal:

a) was easily held, a point score of 0 was assigned.

b) struggled, but could still be held using two hands, a point score of 1 was assigned.

c) struggled and could only be held using two hands, a point score of 2 was assigned.

d) continued to vigorously and persistently struggle, even when held with two hands, and exhibited pronounced muscle tension, a point score of 3 was assigned.

Jump

The experimenter slapped the rat on the flank. If the animal:

a) did not leave the ground, a point score of 0 was assigned.

b) jumped to 6 cm, a point score of 1 was assigned.

c) jumped to 12 cm, a point score of 2 was assigned.

d) jumped to 20 cm, a point score of 3 was assigned.

Vocalization

Any high pitched vocalization or squeal which was audible to the experimenter was rated. If the animal:

a) did not vocalize, a point score of 0 was assigned.

b) vocalized up to two discrete times, a point score of 1 was assigned.

c) vocalized from 3 to 8 times, a point score of 2 was assigned.

d) vocalized repeatedly and intensely throughout the session, a point score of 3 was assigned.

These seven behaviors allow for a maximum score of 21; unoperated animals characteristically were rated 0 on the above scale. Some rats were handled by more than one experimenter so that inter-rater reliability coefficients could be computed. A Spearman rank correlation of .92 was found. The specific handling and rating schedules for each experimental group is presented in the separate method sections.

Surgical Procedures

In the course of these experiments, two types of stereotaxic procedures were employed: (1) destruction of the septal forebrain region with RF current; and, (2) damage to the catecholaminergic systems in and around the septal nuclei by injection of micro-amounts of the neurotoxin 6-OHDA directly into the septal area.

General Procedure

Rats were anesthetized using Nembutal (approximately 50 mg/kg) following atropine sulfate pretreatment. The animals' heads were then shaved and placed securely into a stereotaxic instrument (Scientific Prototype). This apparatus fixes the skull of a rat with reference to an external coordinate system. Three dimensional histological maps (stereotaxic atlases) are available which allow an experimenter to compute the position of a deep brain structure with reference to the external coordinate system of the stereotaxic instrument and certain landmarks on the animal's skull. Thus, once the scalp of a rat is incised and retracted, a burr hole may be drilled into the skull and a probe lowered to a specific site in the brain itself. The technique allows a high degree of accuracy in placement. Coordinates for RF electrode and cannulae placements were taken from Pellegrino and Cushman (1967). Lesions and cannulae placements were verified histologically.

6-hydroxydopamine. In order to restrict damage to catecholamine neurotransmitter systems in and around the septal nuclei, the neurotoxin 6-OHDA was injected directly into neuronal tissue. This was accomplished by stereotaxic placement of a guide cannula (diameter 20 gage) into two points of each septal nucleus, for a total of four points. Once in position, the inner stylette was removed and replaced by an inner injection cannula. This cannula was connected to a Hamilton micro-injection syringe by polyethylene tubing. The syringe was positioned in a variable speed Harvard micro-injection pump. With this apparatus, it was possible to slowly infuse small amounts of fluid directly into brain tissue. For all micro-injection procedures, fluid was infused at a rate of 0.5μ1/min. Exact coordinates for injection were +7.2 and +8.4 mm AP, +1.5 mm DV and $\pm$0.8 mm ML. The 6-OHDA-HBr was dissolved in cold sterile saline containing 0.2 mg/ml of ascorbic acid. Sixteenμg of 6-OHDA-HBr was dissolved in 8μ1 of the saline-ascorbic acid vehicle. The rate of infusion of 6-OHDA into brain tissue was therefore 1μg/min. In this acute surgical procedure, 64 minutes were necessary for the injection of the neurotoxin. Slow infusion minimized possible tissue damage due to pressure effects.

Two control procedures were also performed for this study. First, the saline-ascorbic vehicle alone was injected using the same procedure. Second, small RF lesions

were made at the same stereotaxic coordinates. The lesion maker was set at 57°C for 60 sec.

Radio frequency. All RF lesions were made with a Radionex (Model RF 4) lesion maker. This model allows a surgeon to monitor the actual temperature of the brain tissue by means of a thermocouple in the electrode tip. Since the actual lesion is produced by heat generated by vibration of the tissue due to the RF output, this apparatus permits consistent production of lesions of a regular size and shape. For bilateral destruction of the septal area, the electrode tip was placed bilaterally at coordinates +7.6 mm AP; +1.4 mm DV; and, ±0.7 mm ML. The tip was maintained at 66°C for 60 sec.

Histology

After completion of the experiments, rats were sacrificed under Nembutal anesthesia and perfused intracardially with saline followed by formalin (10% solution). The brains were then carefully dissected out of the skull and stored in 10% formalin for at least two weeks. The brains were then blocked and frozen 40 micron sections were cut on a microtome and stained with cresyl violet to examine cell bodies or with a modified Kluver-Barrera method for visualization of fibers (Wolf, 1971). Selected brains were prepared in celloidin before staining to facilitate examination of cells; celloidin fixation permits tissue to be cut quite thin.

Size and position of lesions, cannulae tracts and cell necrosis were carefully examined under projection and light microscopes. Composite reconstructions of the largest and smallest septal lesions were prepared.

Drugs

The following drugs were used in the pharmacological studies:

Indirectly Acting Catecholamine Agonists

a) L-DOPA

b) amphetamine

References: Ernst, 1967; Fuxe, Agnati, Corrodi, Everitt, Hökfelt, Löfström and Ungerstedt, 1975.

Dopamine Receptor Agonists

a) apomorphine

b) piribedil

References: Ernst, 1967; McDowell and Sweet, 1975.

Ultra-short-acting Barbiturate

a) methohexital

Reference; Harvey, 1975.

Phosphodiesterase Inhibitor; Catecholamine Facilitator

a) theophylline

Reference: Fuxe and Ungerstedt, 1974.

Noradrenaline Reuptake Inhibitors

a) desmethylimipramine

b) imipramine

Reference: Breese and Traylor, 1971.

Dopamine Receptor Blockers

a) haloperidol

b) pimozide

Reference: Sedvall, Fyrö, Nyback and Wiesel, 1975.

All of the above drugs were dissolved in sterile water or saline except for the L-DOPA. The catecholamine precursor, L-DOPA, could only be dissolved by first heating in an acidic medium and then bringing the pH to 6.8 by titrating with a base.

Statistical Analyses

The irritability scores which are presented in all of the experiments are ordinal data. Therefore, all of the statistics used are nonparametric (Siegel, 1956). The measure of central tendency in all cases is the median. Both the Friedman nonparametric analysis of variance (χ^2_r) and the Mann-Whitney rank test for independent samples (U) were used in analyzing the data.

CHAPTER 3

EFFECT OF 6-HYDROXYDOPAMINE LESIONS OF THE SEPTAL NUCLEI ON IRRITABLE BEHAVIOR

The neural substrate of irritable behavior has been investigated by the placement of lesions in a number of structures including the septal area (Brady and Nauta, 1953; 1955), ventral medial hypothalamus (Glusman, 1974), olfactory bulbs and tubercles (Nurimoto, Ogawa & Ueki, 1974; Thorne, Aaron & Latham, 1974). All these areas of the limbic system are rich in the biogenic amines as shown by sensitive radio enzymatic assay techniques (Brownstein et al., 1974; Saavedra et al., 1974). Ascending aminergic systems, as described by fluorescent microscopy also appear to be involved (Lindvall & Björklund, 1974; Ungerstedt, 1971c). In fact, destruction of the septal nuclei, in particular, results in significant reduction of whole brain NA and DA (Bernard et al., 1975) and 5HT (Lints & Harvey, 1969). Specific regional decreases of DA are found in the hypothalamus and limbic system (Bernard et al., 1975). These alterations have been cited as evidence supporting an important role for the biogenic amines in the expression of irritable behavior.

Further evidence for this position comes from studies on the effects of depletion of catecholamines by 6-OHDA. This neurotoxin destroys both NA and DA terminals, as well as DA cell bodies (Ungerstedt, 1971a), while doing little

or no damage to serotonergic, cholinergic or gabaminergic neuronal systems. The infusion of the ventricular system of the rat with high doses of 6-OHDA through either the lateral ventricles (Nakamura & Thoenen, 1972) or the cisterna magna (Coscina et al., 1973) results in an irritability syndrome which persists for several weeks. This irritability, like the syndrome induced by large lesions of the septal nuclei can be attenuated by repeated handling, so that the animal will return to a baseline level of affect within two weeks. Biochemical assays show decreased levels of both NA and DA (Stricker & Zigmond, 1976) after this procedure.

Although the induction of an irritability syndrome in rats by intraventricular 6-OHDA clearly implicates catecholamine mechanisms in certain irritability states, the actual locus of action remains obscure. 6-OHDA action on neural tissue after intraventricular infusion is due to absorption of the substance through the ependyma into the parencyma. Histofluorescence analyses indicate that this diffusion extends no more than several millimeters from the ventricular wall into brain tissue (Ungerstedt, 1971a). The structures involved in the intraventricular 6-OHDA induced irritability, therefore, are likely to lie close to the ventricular surface. This raises the intriguing possibility that the high levels of 6-OHDA injected into the cerebral spinal fluid of these animals

could damage catecholaminergic cells and fibers in the septal nuclei, a mid-line structure bordered by the lateral ventricles. It was therefore hypothesized that infusion into the septal nuclei would result in an irritability syndrome indistinguishable from that shown by rats with large RF lesions of the septal nuclei.

Method

Subjects

Adult male Long Evans hooded rats weighing 470 to 510 grams were used. They were individually housed, and were fed and watered ad libitum. The animals were handled and rated for irritability (see pages 50-53) on at least three pre-operative days.

Surgical Procedures

The surgical procedure is the same as the general procedure (see pages 54-56) except that repeated supplemental dosages of Nembutal were used to maintain long lasting anesthesia.

Ten mg of 6-OHDA-hydro-bromide was dissolved in 5 ml of saline (with 0.2 mg/ml ascorbic acid to retard oxidation). The 6-OHDA was administered stereotaxically into two points of both the left and right septal nuclei (AP 7.2 and 8.4; DV +1.5; ML $\pm$0.8 mm) using a Hamilton micro-syringe driven by a Harvard infusion pump, and a specially made cannula (20 gage) and cannular guide (25 gage). The fluid was delivered at a speed of 0.5μl per min. Rats were

assigned to one of four conditions: (1) 16μg in 8μl was injected into four points of the septal nuclei in 8 rats; (2) 16μg in 8μl was injected into four points of the septal nuclei in 8 rats who had been administered a 25 mg/kg intraperitoneal injection of desmethylimipramine (DMI) 30 minutes before surgery; (3) 8μl of saline with ascorbic acid (vehicle) was injected into four points of the septal nuclei in 8 rats; and, (4) small RF lesions were made at the four analogous points (57°C for 60 sec) in 5 rats to control for non-specific damage by 6-OHDA.

Histology

Following completion of behavioral testing, the rats were killed with an overdose of Nebutal and perfused intracardially with isotonic saline followed by 10% formalin solution. Every tenth 40 micron thick section was stained with cresyl violet. Brains of animals were prepared with celloiden and by frozen section.

Results

Histological

Microscopic analyses revealed no gross histological abnormalities in the 6-OHDA rats. Cannulae tracts ended in the ventral area of the lateral septal nuclei. Rats in the small RF lesion control group had lesions with an average diameter of 0.35 mm in the lateral septal area. Composite reconstructions of the cannulae placements are presented in Figure 2.

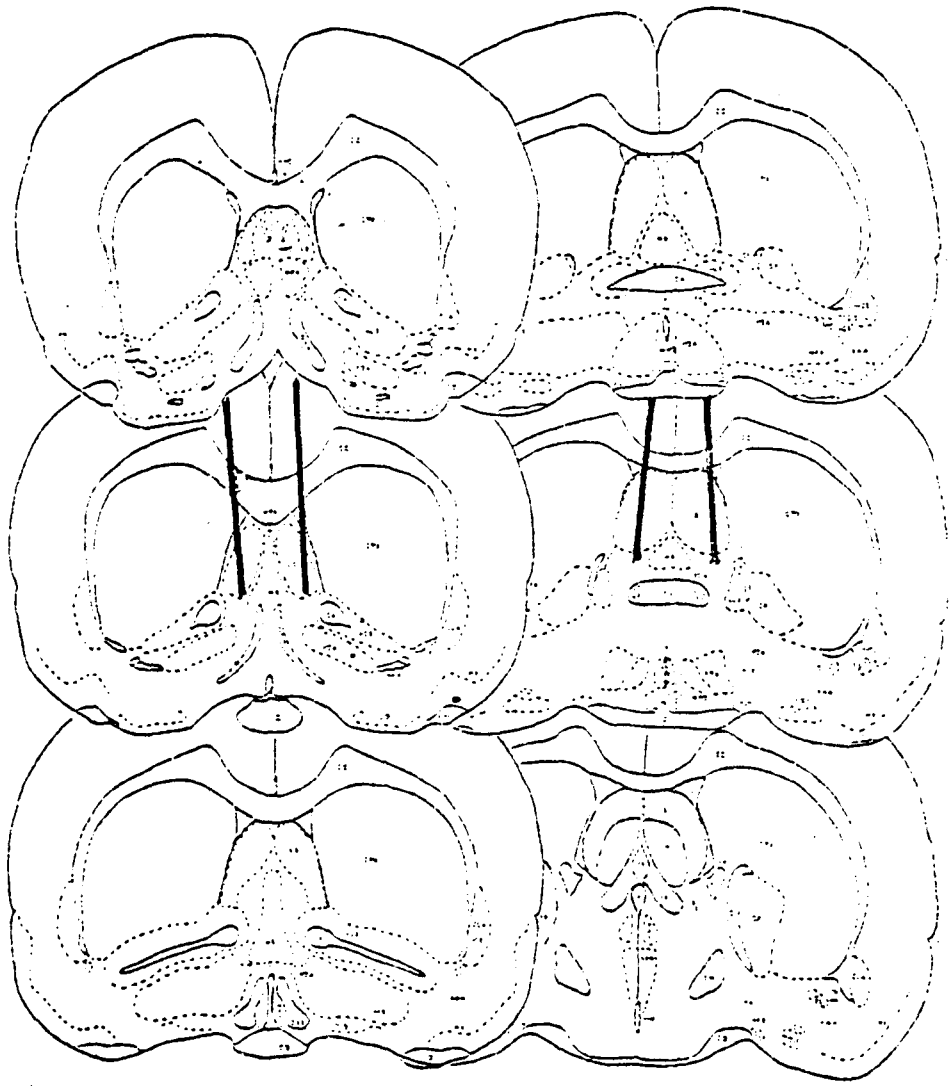


Figure 2: Coronal Sections Passing Through Various Anterior-Posterior Levels of the Septal Nuclei Taken from the Atlas of Pellegrino and Cushman (1967). The casts left by typical cannulae penetrations are indicated.

Figure 3 presents the median irritability scores for 6-OHDA, DMI/6-OHDA and saline rats. Twenty-four hours postoperatively all animals were handled and rated for irritability. Intracerebral 6-OHDA rats exhibited a significant increase in irritability over preoperative levels (there was no overlap in irritability scores between the preoperative and postoperative tests). This increase in irritability gradually diminished over the ensuing ten days ($\chi^2_r = 24.9$, $p < .05$). The rats pretreated with DMI and then intracerebrally injected with 6-OHDA also exhibited an irritability syndrome. Like the non-pretreated rats, these median irritability scores were significantly higher than preoperative levels, and the irritability scores gradually returned to baseline over the next ten days ($\chi^2_r = 167$, $p < .001$). However, the median irritability scores of the DMI/6-OHDA rats were consistently less intense than the 6-OHDA animals. Still, median irritability scores of rats in both the 6-OHDA and DMI/6-OHDA groups were significantly higher than those of the rats receiving intracerebral injections of saline and ascorbic acid vehicle. Rats in the two control groups showed no increase in irritability scores over preoperative levels, and the median irritability scores of the 6-OHDA and DMI/6-OHDA rats did not equal those of the controls until at least eight days postoperatively.

The behavioral form of the irritability syndrome

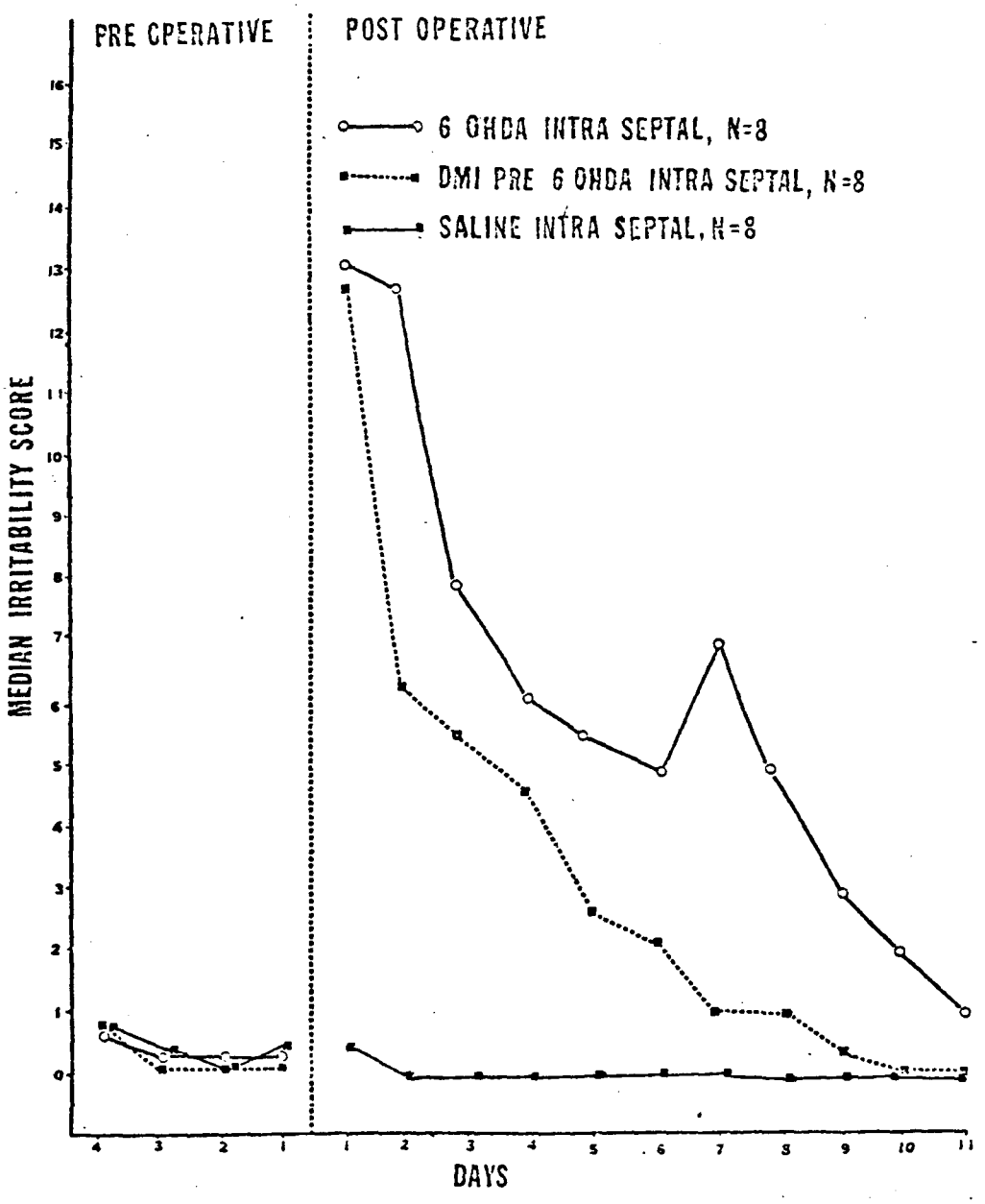


Figure 3: Pre- and Postoperative Irritability Ratings for Rats Receiving Intraseptal Micro-Injections of either 6-OHDA, 6-OHDA preceded by DMI, or Saline.

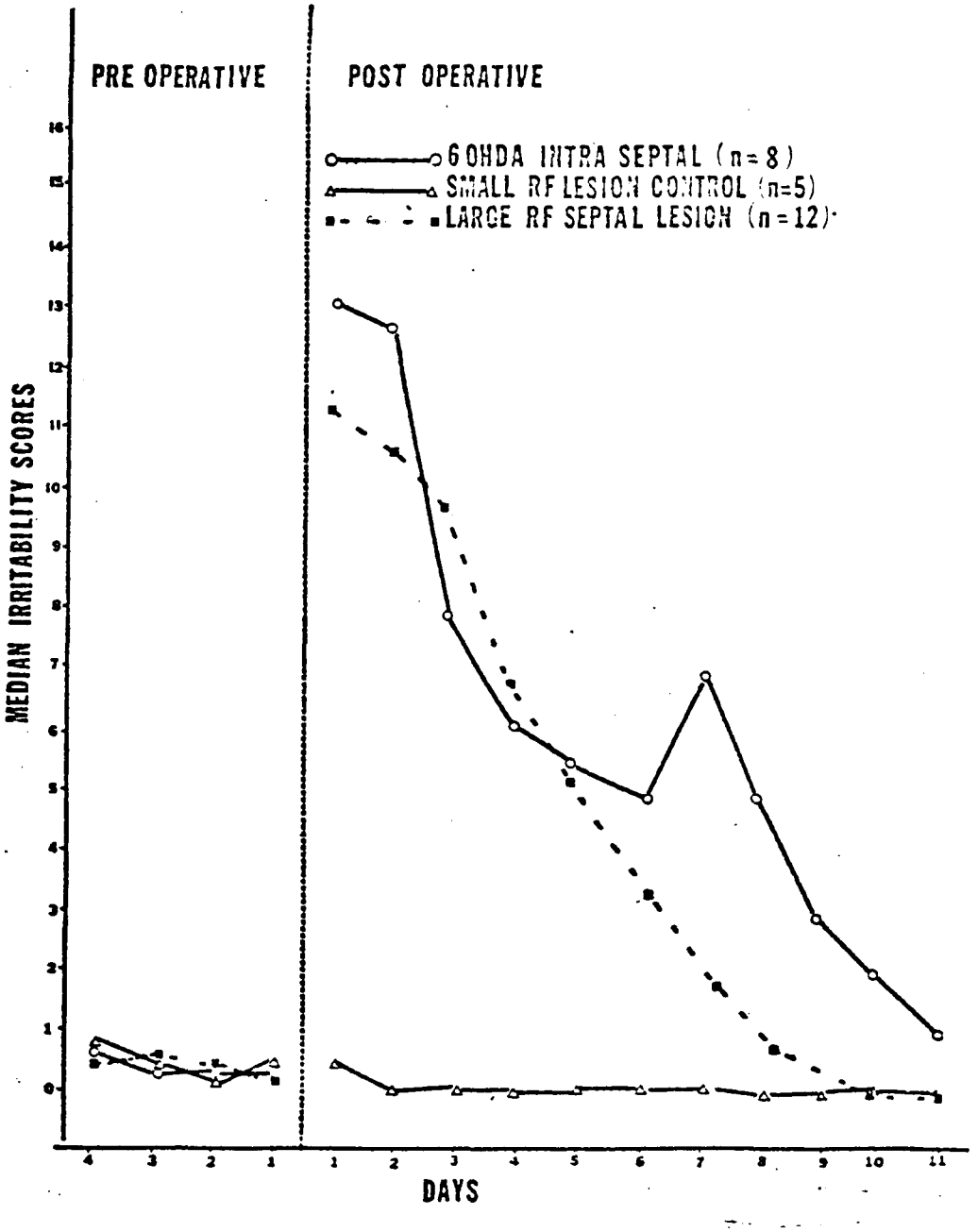


Figure 4: Pre- and Postoperative Irritability Ratings for Rats Receiving Intraseptal Micro-Injections of 6-OHDA, Small Radio Frequency Lesions at Stereotaxic Coordinates Used for Micro-Injection Placement, or Large Radio Frequency Lesions of the Septal Nuclei.

exhibited by rats receiving intracerebral injections of 6-OHDA did not differ in form or intensity from animals receiving large RF lesions of the septal nuclei (Figure 4).

Discussion

These experiments strongly implicate a catecholaminergic mechanism in the development of the septal irritability syndrome. The dose of 6-OHDA applied (16μg in 8μl) although high does not destroy non-catecholaminergic cell bodies and axons (Ungerstedt, 1971a). Micro-injections directly into brain parenchyma results in a small area of nonspecific damage directly surrounding the cannula tip of approximately 0.3 mm. This was controlled for by making small RF lesions. The area of depletion of catecholamines as visualized by fluorescent microscopy has a diameter of 1.5 to 2.5 mm (Ungerstedt, 1971a). Since the septal nuclei has an anterior-posterior extent of more than 4 mm, a dorsal-ventral height of 4.8 mm and a medial-lateral extent of 4 mm, micro-injection of 16μg into two points of each side of the septal nuclei should cause catecholamine depletion in much of the nuclei, with maximal damage at the cannulae tips and in the medial septal nucleus.

6-OHDA treatment, either intracerebrally or intraventricularly induced degeneration in axon terminals of both NA and DA fibers. It does not result, however, in NA cell body degeneration (Ungerstedt, 1971a). Treatment of rats with tricyclic antidepressant desmethylimipramine (Breese & Traylor, 1971) had the effect of disrupting the

uptake of 6-OHDA into NA elements thereby protecting these terminals from destruction by 6-OHDA. If animals are pretreated with DMI before injection with 6-OHDA, the neurotoxin induced degeneration of NA elements is retarded, while that of DA elements, both cells and fibers, goes on unabated. Since animals receiving intraseptal 6-OHDA with and without DMI pretreatment show the irritability syndrome, it may be inferred that damage to DA subsystems in and around the septal nuclei are critical in the development of the septal syndrome, since protection of NA systems does not prevent the development of irritability. Since the total weight of 6-OHDA injected is only 64μg, it is difficult to ascribe the irritability syndrome to diffusion through the ventricular system. The weight of 6-OHDA injected by investigators (Coscina et al., 1973; Nakamura & Thoenen, 1972) into the ventricles to induce an irritability syndrome is as high as two injections of 300μg of 6-OHDA 24 hours apart. Such a high does of the neurotoxin is capable of also inducing adipsia and aphagia as well as depletion of DA and NA (Stricker & Zigmond, 1976).

Both intraseptal 6-OHDA and DMI/6-OHDA rats exhibited postoperative irritability, although the DMI/6-OHDA irritability was less intense. This result might have been due to either an interaction of DA and NA on the induction of the syndrome, or the differential protection of some DA processes by DMI. Antelman and Caggiula (1977) have recently stressed the possible interactions

between NA and DA transmitter systems.

Some researchers (Poirier, Langelier, Roberge, Boucher & Kitsikis, 1972) have criticized the apparent specificity of the 6-OHDA technique. They believe that other investigators (Jacks, De Champlain & Cardeau, 1972; Ungerstedt, 1971a) have overstated the case for 6-OHDA specific toxicity in the CA systems. However, Ungerstedt (1971a) does indicate that intracerebral micro-injections of 6-OHDA directly into brain tissue results in an area 0.1 mm in diameter around the cannula tip of nonspecific damage. He believes that in a large volume of tissue this damage is not critical. In the present experiment nonspecific damage was controlled for by making small RF lesions at the cannular placement coordinates. This technique, of course, does not preclude the possibility of more extensive nonspecific damage having been induced by 6-OHDA. The question of specificity within the parameters of this study can only be answered by the utilization of electronmicroscopy and careful postadministration monitoring of putative neurotransmitter levels. Still, the present experiment is highly suggestive of an important role for CAs in the septal irritability syndrome. It also implies that further examination of the model syndrome using pharmacological agents acting on CA systems should yield provocative results.

CHAPTER 4

EFFECT OF CATECHOLAMINERGIC DRUGS ON THE INTENSITY AND RATE OF RECOVERY FROM IRRITABILITY IN SEPTALLY LESIONED RATS

The first set of experiments demonstrated that depletion of catecholamines in the septal area by direct application of 6-OHDA could induce irritability. This implicated these neurotransmitter systems in the development of the irritability syndrome. It, therefore, seemed possible that pharmacological stimulation of the catecholamine system would, in some way, alter the duration and intensity of the septal syndrome.

That the administration of pharmacological agents might modify rates of recovery after brain damage has recently become the focus of extensive research (Dominguez & Longo, 1970; Gage & Olton, 1976; Ljungberg & Ungerstedt, 1976; Luria et al., 1969). Both the clinical and experimental use of pharmacological agents to accelerate recovery from brain damage has revealed facts pertinent not only to the treatment of human victims of cerebral insult but also has suggested critical factors that must be accounted for in any general theory of neural function. The septal syndrome, as a general model for studying recovery after experimentally-induced brain damage, has also served as a convenient test system to evaluate the possible effect of a number of pharmacological agents

on the duration and extent of recovery. The general paradigm for analysis of drug action in this model consists of measuring irritability in rats postoperatively and comparing animals receiving a given compound to those receiving saline. Usually, the rats receive only one administration of drug. Most studies, therefore, are of an acute nature.

A number of pharmacological agents have been found to have transitory effects on the irritability syndrome characteristic of septal lesioned animals. These include the catecholamine blocking agent, chlorpromazine (Cytawa & Kutulas, 1972; Raitt et al, 1961) chlordiazepoxide (Quenzer et al., 1974; Schalleck et al., 1962), the acetylcholinesterase inhibitor, physostigmine (Stark & Henderson, 1972) and glycine (Stern & Catovic, 1975). These results may not be due to the direct putative pharmacologic action of some of these drugs since it has also been reported that septally lesioned rats are more susceptible to the sedative effects of barbiturates (Harvey et al., 1964). Since both chlorpromazine and chlordiazepoxide are sedative agents, any transient decrease in irritability seen when these agents are administered to septal rats may be due to their general sedative properties and, therefore, not necessarily due to any action on specific irritability mechanisms. The glycine effect is rendered problematic by the failure of nalorphine, an

agent which can induce increased levels of glycine in the brain, to have any effect on septally irritable rats (Stern & Catovic, 1975). It has also been reported that administration of non-narcotic analgesic agents, including aspirin, can result in transient decreases in irritability (Loizzo & Massotti, 1973). It must be stressed that all these effects are transitory, lasting only for the time of pharmacological action of the particular drug.

There exists one study in the literature which concerns the effect of multiple injections of a drug in septal animals. Cytawa and Kutulas (1972) injected sedative levels of chlorpromazine daily for the full duration of the irritability syndrome. Their animals returned to high levels of irritability each day as the drug action subsided. The total time necessary for recovery did not differ significantly between drug and control groups. A number of agents have been found to have no effect on the septal syndrome. These include 5-hydroxytryptophan and methysergide (Dalhouse, 1974), nalorphine, mephenesin (Stern & Catovic, 1975), ∝MPT, alpha-methylmetatyrosine, alpha-methyldopa and disulfiram (Dominguez & Longo, 1969, 1970). All these findings suggest that the agents discussed do not act upon systems critical in the induction or facilitation of recovery in septal rats.

In marked contrast to the above reports is the finding by Dominguez and Longo (1969, 1970) that 300 mg/kg of pCPA administered 48 hours after septal surgery to rats exhibit-

ing clear and persistent irritability, resulted in complete attenuation of the syndrome. A distinct decrease in irritability was evident 30 minutes after injection of pCPA. After two hours, the rats were at their preoperative baseline levels of irritability and they never returned to pre-drug levels of affect. A single administration of an agent, therefore, was observed to have a permanent effect on the syndrome. The irritable rats receiving saline continued to maintain their irritability for at least ten subsequent days. The amazing effect of pCPA was also shown to be dose dependent. The Italian researchers felt that the above effect was due to the depletion of serotonin via pCPA inhibition of tryptophan hydroxylase (Koe & Weissman, 1966). Several threads of evidence weigh against an interpretation such as the one above. Most directly, serotonergic blocking drugs such as methysergide (Dalhouse, 1974), do not duplicate the effect of pCPA on septal rats, although it must be admitted that the action of these two drugs might be different on the serotonergic neuron. Secondly, septal surgery results in a significant decrease in the whole brain levels of serotonin (Lints & Harvey, 1969). It is hard to understand why even further decreases in serotonin should decrease the severity of the syndrome. Finally, pCPA has effects on catecholamine systems, in addition to those on serotonin (Brody, 1970; Gal, 1973; Welch & Welch, 1967). Tyrosine hydroxylase and dopamine-beta-hydroxylase are inhibited shortly after injection of pCPA, while the effect of tryptophan hydroxylase inhibition

is not seen for several hours. The attenuating effect of pCPA on the septal syndrome had a latency of approximately 90 minutes (Dominguez & Longo, 1970).

The induction of irritability by depletion of catecholamines with intraseptal injections of 6-OHDA implicates NA and DA in the septal syndrome (see Chapter 3). The pharmacological data cited above further suggests a role for catecholaminergic systems in both the production and reduction of irritability in these animals. It was thought, therefore, that a series of experiments should be undertaken to clarify the role of the catecholamine systems in both the expression of septal lesion-induced irritable behavior and in its eventual attenuation. The basic paradigm called for inducing irritability via septal lesions and by subsequently administering drugs 24 hours postoperatively. The duration of intensity of the syndrome in rats receiving various drugs could then be compared to those receiving saline. It was hypothesized that the pharmacological stimulation of catecholamine receptors by peripherally-injected drugs would significantly decrease the intensity and duration of the septal irritability syndrome.

Method

Subjects

Long-Evans hooded male rats, individually housed, weighing approximately 300 gm each, fed and watered ad

libitum, were used in this study.

Surgery

These animals were subjected to bilateral RF lesions of the septal nuclei using standard surgical and stereotaxic techniques (see Chapter 2).

Procedure

Twenty-four hours postoperatively, these animals were moved in their home cages to a specially designed plexiglass observation chamber and rated for irritability. If two raters agreed on an irritability score of ten points or higher, the rat was assigned to a drug group. The raters were unaware of what drug an animal was to receive. Each animal was then administered an intraperitoneal injecetion of one of the following: L-DOPA (100 mg/kg, n = 8; 60 mg/kg, n = 5; 40 mg/kg, n = 3; 30 mg/kg, n = 4; or, 10 mg/kg, n = 4), apomorphine (20 mg/kg, n = 12; 15 mg/kg, n = 5; 10 mg/kg, n = 6; or, 5 mg/kg, n = 5), amphetamine (4 mg/kg, n = 10; or, 2 mg/kg, n = 6), piribedil (300 mg/kg, n = 7; 200 mg/kg, n = 3), theophylline (25 mg/kg, n = 10; 20 mg/kg, n = 8), imipramine (10 mg/kg, n = 6), methohexital (40 mg/kg, n = 14) or saline (n = 15). Following these injections, each animal was returned to its home cage and then removed to the observation chamber and rated for hyperirritability at $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4, 24, 48, 72, 96, and 120 hours post-injection. The amphetamine animals were additionally tested at 5 and 6 hours post-

injection, while the methohexital animals were also rated at $\frac{1}{4}$ and 3/4 hours after injection of drug. These additional observations were made on amphetamine and methohexital animals in order to monitor the longer time course of the amphetamine action, and the quick latency of the methohexital effect.

Histology

Tissue was prepared for histological verification in the usual fashion (see Chapter 2).

Results

Projection microscope examination of histology for rats in all groups reveals a common pattern of destruction. The lesions destroyed both the medial and lateral septal nuclei, and damaged the adjacent caudate nuclei, nucleus accumbens septi, stria terminalis, anterior commissure, corpus callosum and fornix column. Size or position of lesions did not differ significantly when the pattern of destruction found in the separate groups was compared. The lesions could be most completely classified as extensive. Figure 5 presents composite histological reconstructions for this experiment, expressing the largest and smallest lesion extents.

The animals injected with saline (Figure 6) exhibited a constant level of irritability for the first 24 hours, followed by a gradual dimunition in irritability over the ensuing five days ($\chi^2_r = 47.8$, $p < .001$). This slow and

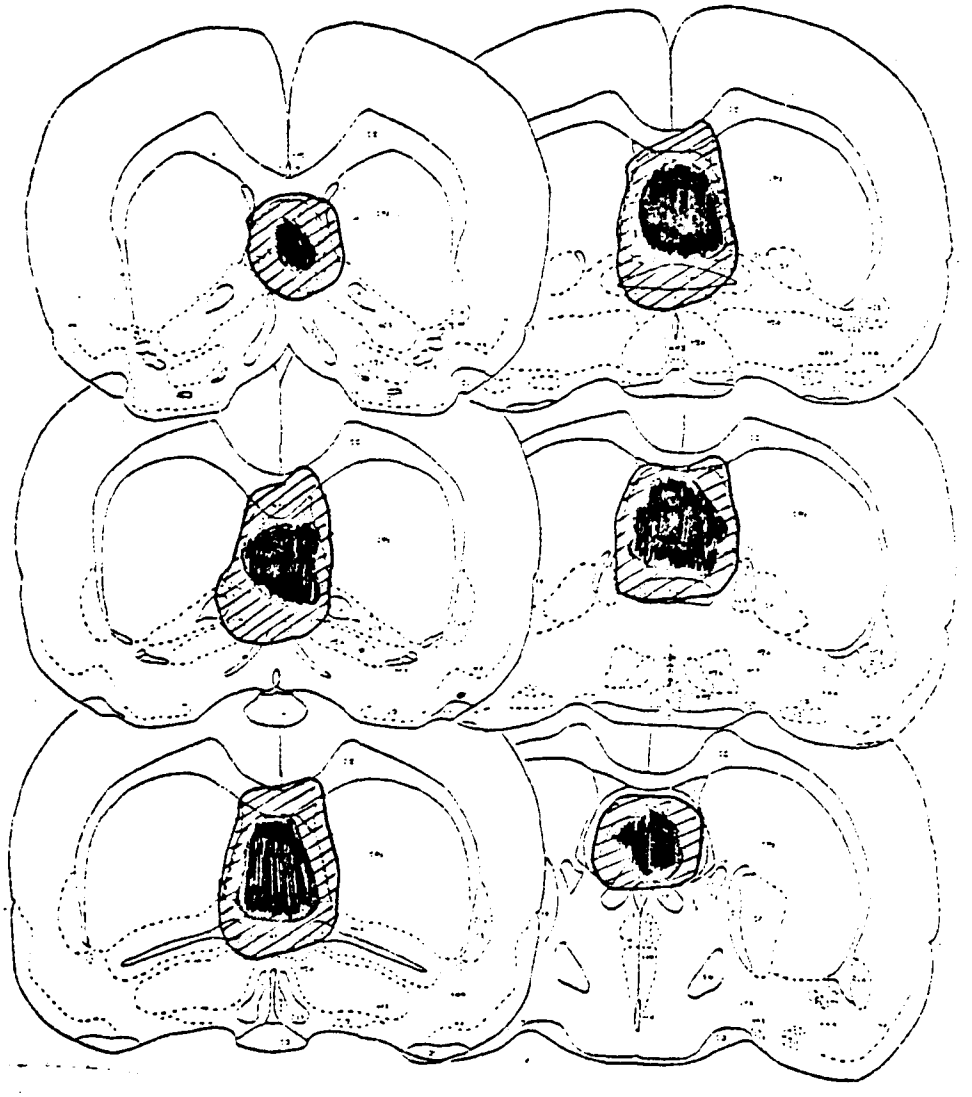


Figure 5: Coronal Reconstructions of Histology of Large Radio Frequency Lesions of the Septal Nuclei. The area of minimal damage is in black; the largest lesion is cross-hatched. Most lesions totally destroyed the septal area from the corpus callosum dorsally, to the anterior commissure ventrally, and from the wall of each caudate nucleus laterally. In 30% of the brains, there was some dilation of the third ventricle.

gradual recovery of normal emotional function agrees well with that seen in totally non-injected animals (Brady & Nauta, 1955; Schnuir, 1972). In marked contrast to the saline injected or to the non-injected animals, animals given sufficiently high doses of L-DOPA (100 mg/kg) showed a dramatic and prompt return to preoperative baseline levels of irritability (Figure 7; for the 0-4 hour post-injection period, $\chi^2_r = 43.8$, $p < .001$). The latency for a measurable effect was 90 minutes ($T = 0$, $p < .01$), and by 120 minutes after injection, the animals' emotional behavior was essentially indistinguishable from that of non-lesioned controls. The difference between the 100 mg/kg L-DOPA animals and the saline controls reached highly significant levels by 120 minutes after injection ($U = 1.5$, $p < .001$). The injected animals showed normal righting, placing and balance reflexes, and it should be emphasized that these animals gave no appearance whatever of being sedated; they continued to eat and drink, and remained normally responsive to environmental stimuli. This drug-induced abolition of the lesion-induced irritability symptoms was also noteworthy for its permanence; none of the 100 mg/kg L-DOPA animals showed any return of irritable behavior, even though some were tested for as long as one month post-injection. At 60 mg/kg of L-DOPA, a less marked, but still prompt and clear, attenuation of the lesion-induced symptoms occurred ($\chi^2_r = 19.8$, $p < .01$).

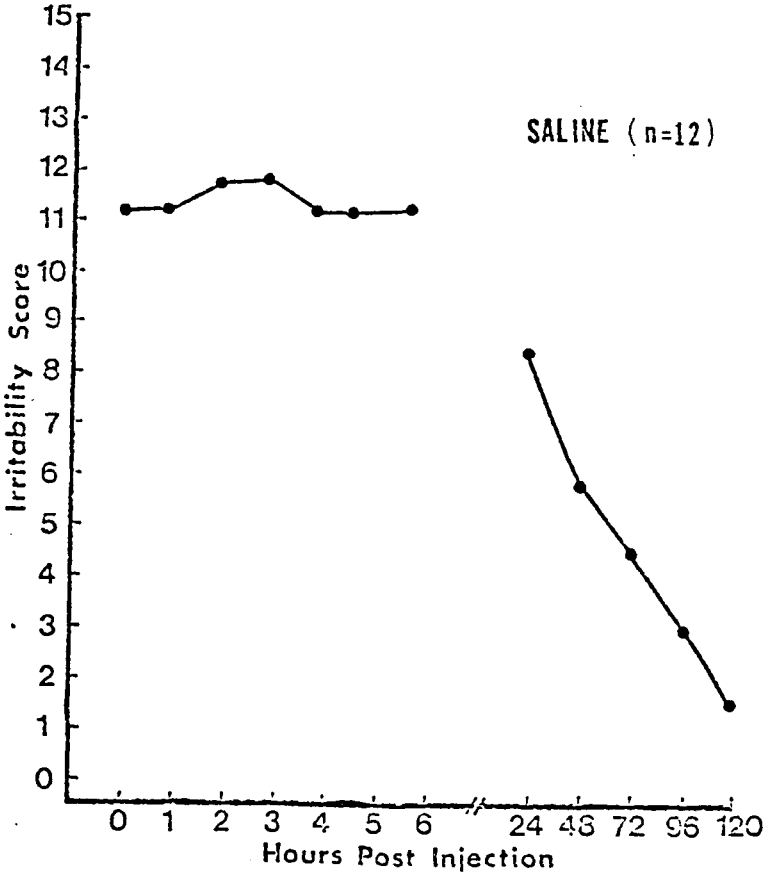


Figure 6: Pre- and Post-Administration Irritability Ratings for Rats Administered a Single Intraperitoneal Injection of Saline 24 Hours after RF Lesioning of the Septal Nuclei.

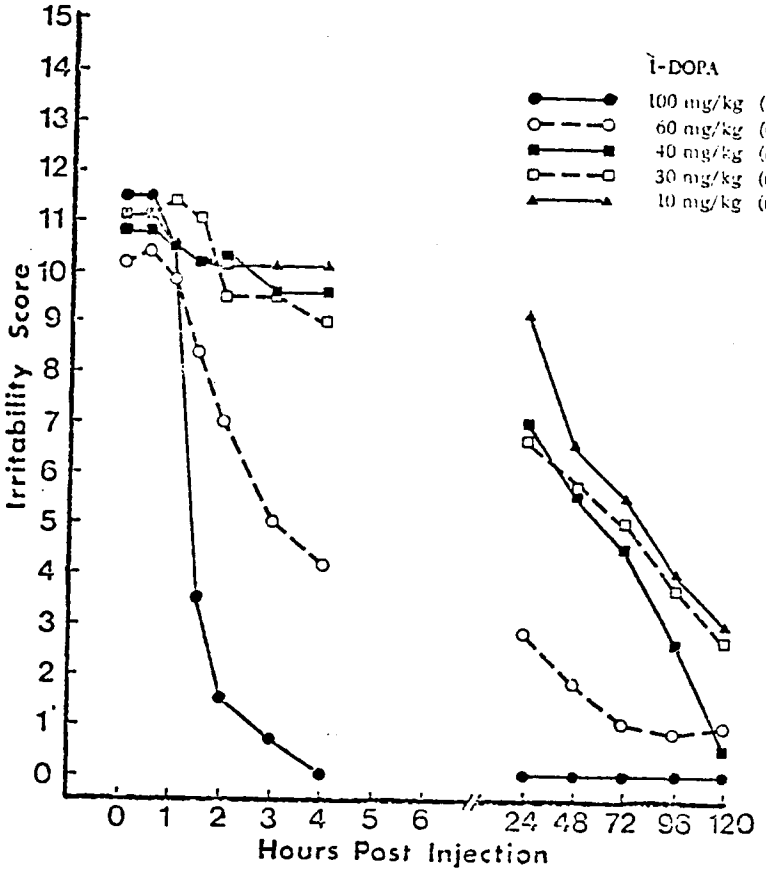


Figure 7: Pre- and Post-Administration Irritability Ratings for Rats Administered a Single Intraperitoneal Injection of L-DOPA 24 Hours after RF lesioning of the Septal Nuclei.

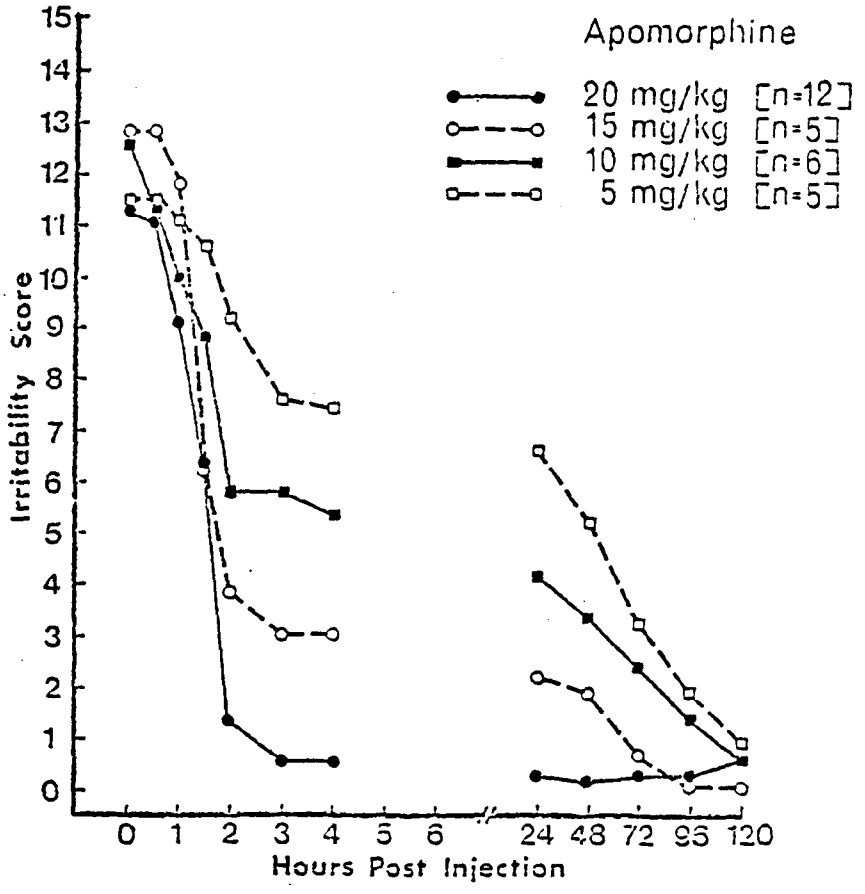


Figure 8: Pre- and Post-Administration Irritability Ratings for Rats Administered a Single Intraperitoneal Injection of Apomorphine 24 Hours after Lesioning of the Septal Nuclei.

At doses lower than 60 mg/kg, some septal amelioration occurred in individual animals, but the overall group means show little difference from saline-injected controls.

Animals injected with the dopamine agonist, apomorphine, also showed drug-induced septal irritability amelioration (Figure 8). As with L-DOPA, the apomorphine effect was dose-related. A dose of 20 mg/kg of apomorphine produced complete and permanent abolition of the lesion-induced symptoms within 120 minutes ($\chi^2_r = 34.5$, $p < .001$); lower doses produced quantitatively less effect on the time course of recovery. As with the L-DOPA effect, the animals were not merely sedated, and the drug-induced behavioral change was permanent. In fact, the effects of apomorphine and L-DOPA were essentially indistinguishable.

Injections of either the dopamine agonist piribedil (Figure 9) or amphetamine (Figure 10) produced effects on the time course of septal recovery that were, in only some respects, similar to those produced by L-DOPA and apomorphine (Figure 7, Figure 8). Piribedil produced the same initial amelioration of septal symptoms during the first four hours post-injection as did L-DOPA and apomorphine ($\chi^2_r = 15.1$, $p < .01$, for 200 mg/kg piribedil; $\chi^2_r = 26.7$, $p < .001$, for 300 mg/kg piribedil). However, this was followed by a subsequent increase in irritability at 24 and 48 hours post-injection (most noticeably in the 300 mg/kg animals, which had exhibited the most septal attenuation during the first four hours post-injection;

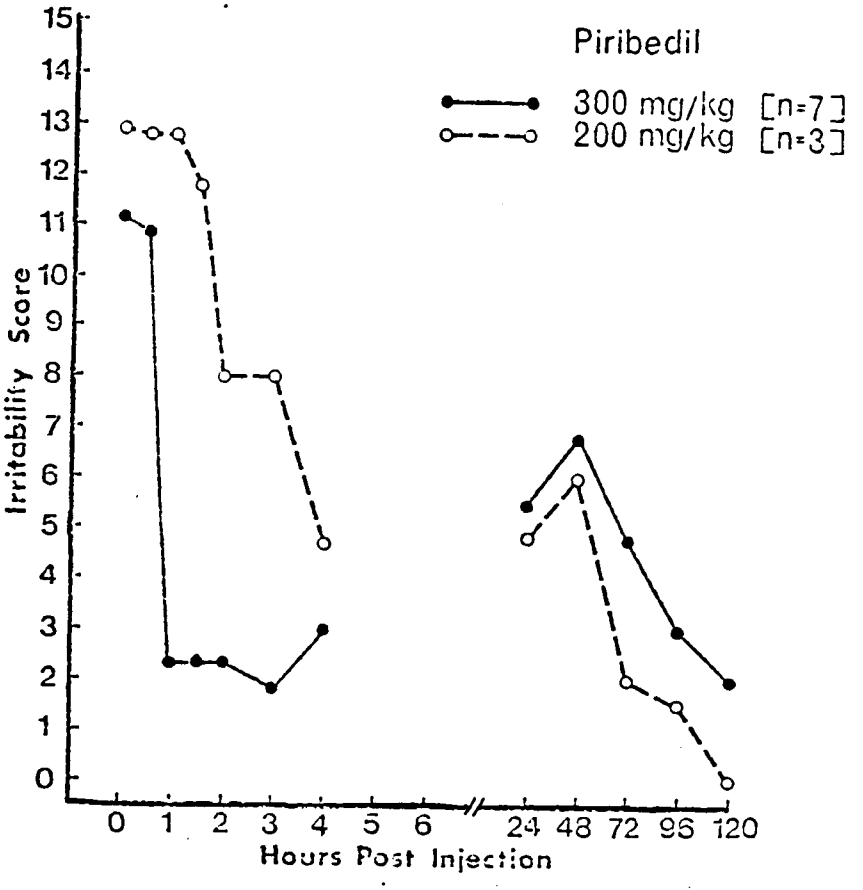


Figure 9: Pre- and Post-Administration Irritability Ratings for Rats Receiving a Single Intraperitoneal Injection of Piribedil 24 Hours after Lesioning of the Septal Nuclei.

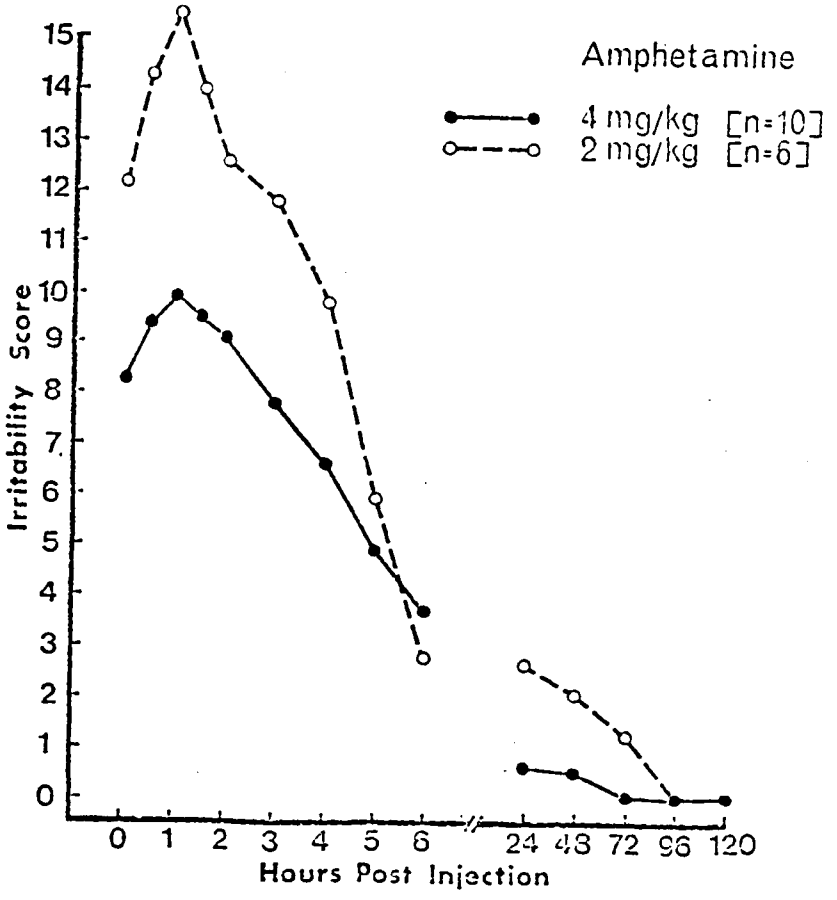


Figure 10: Pre- and Post-Administration Irritability Ratings for Rats Receiving a Single Intraperitoneal Injection of Amphetamine 24 Hours after Lesioning of the Septal Nuclei.

$\chi^2_r = 9.3$, $p < .05$). The irritability of the piribedil-injected rats then became attenuated over the next four days ($\chi^2_r = 8.5$, $p < .01$, for 200 mg/kg; $\chi^2_r = 13.0$, $p < .01$, for 300 mg/kg) at the same rate observed in the saline-injected or untreated controls. Amphetamine (Figure 10), on the other hand, produced an initial increase in irritability during the first 90 minutes after injection (with only the increase for the 2 mg/kg animals being significant; $\chi^2_r = 9.25$, $p < .01$), followed by a rapid and permanent abolition of the septal symptoms ($\chi^2_r = 44.4$, $p < .001$, for 2 mg/kg; $\chi^2_r = 43.6$, $p < .001$, for 4 mg/kg).

The phosphodiesterase inhibitor theophylline (Figure 11) at both dose levels evaluated, lead to a transient increase in irritability scores in all rats tested ($T = 0$, $p < .001$). The effect was measurable at $\frac{1}{4}$ hour and was maximal $1\frac{1}{2}$ hours after injection. At 3 hours post-injection, these rats had irritability scores below their initial level. However, at 24 hours post-injection these animals had irritability scores which were higher than their lowest drug day scores. The rest of the recovery function over days did not differ ($\chi^2_r = 10.1$, $p < .01$) from that shown by the saline controls (Figure 6).

Imipramine (Figure 12), a drug which potentiates the action of NA by preventing its re-uptake into presynaptic nerve terminals (Schildkraut, 1973), was also found to induce a transient increase in irritability ($T = 0$, $p < .001$).

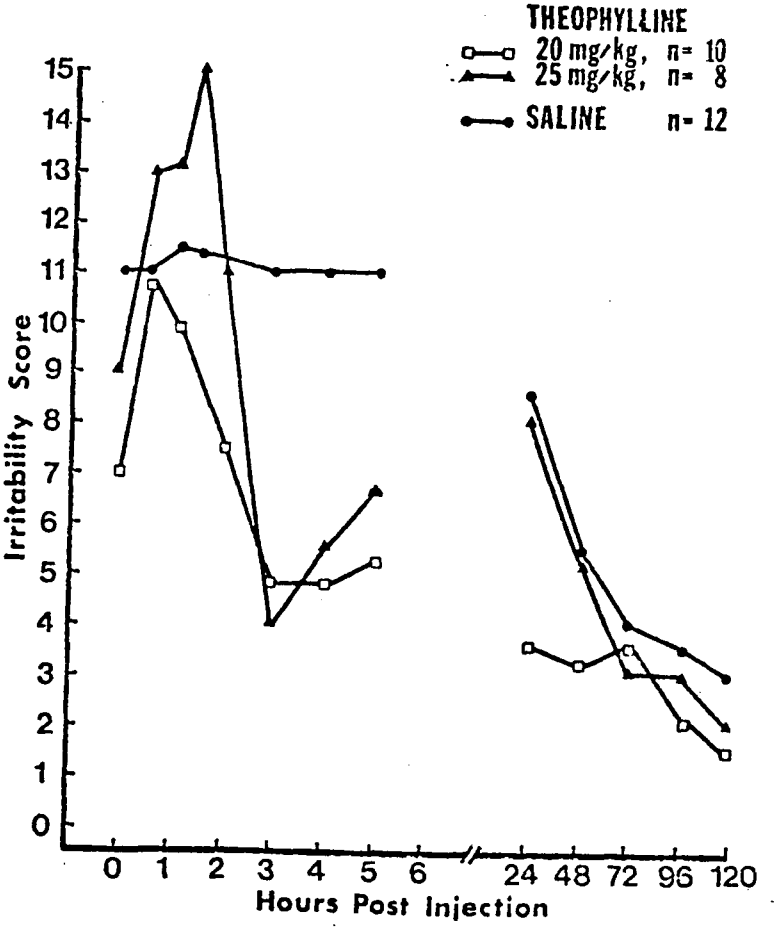


Figure 11: Pre- and Post-Administration Irritability Ratings for Rats Receiving a Single Intraperitoneal Injection of Theophylline or Saline 24 Hours after Lesioning of the Septal Nuclei.

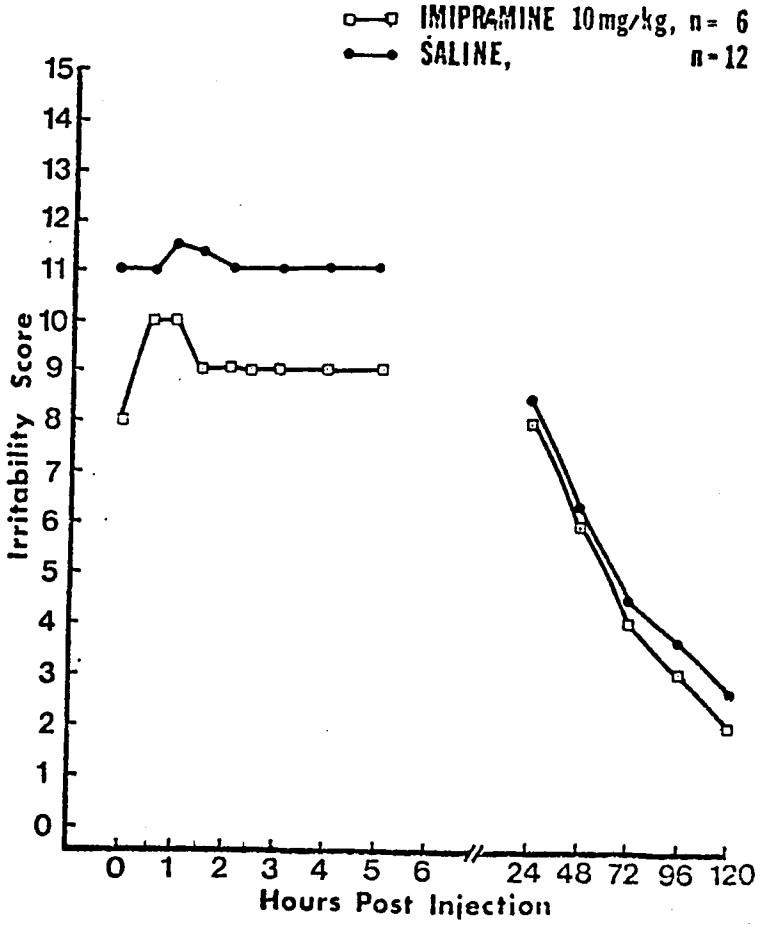


Figure 12: Pre- and Post-Administration Irritability Ratings for Rats Receiving a Single Intraperitoneal Injection of Imipramine or Saline 24 Hours after Lesioning of the Septal Nuclei.

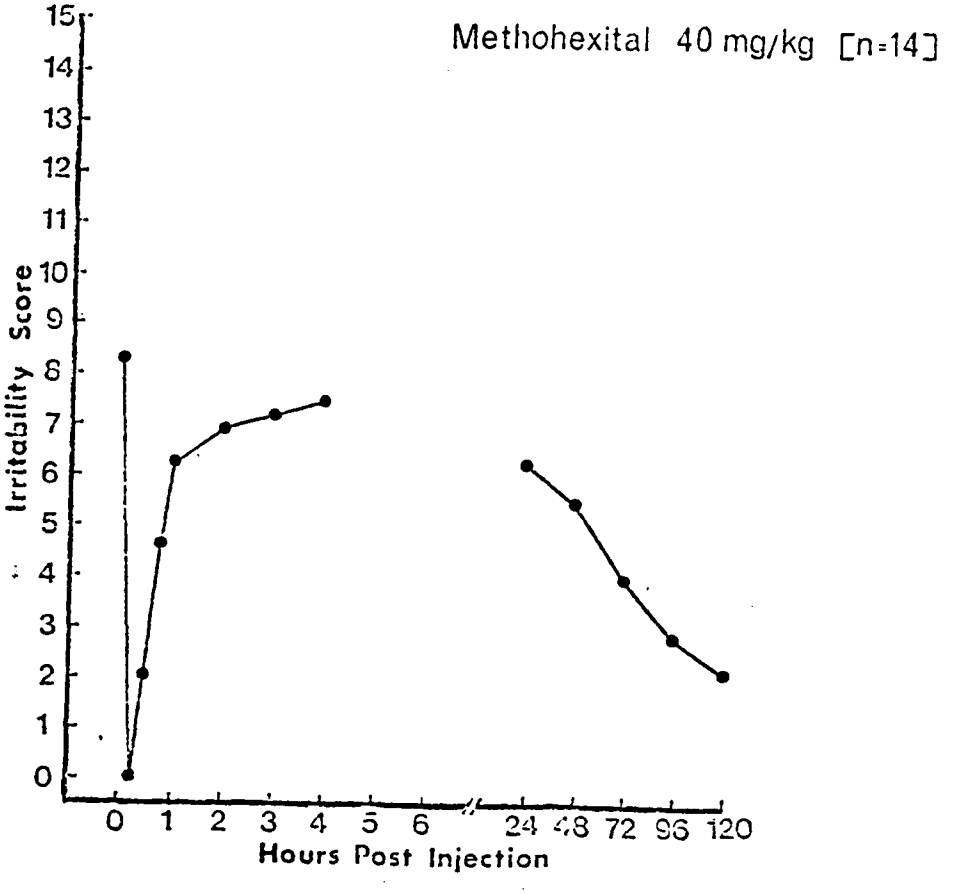


Figure 13: Pre- and Post-Administration Irritability Ratings for Rats Receiving a Single Intraperitoneal Injection of Methohexital 24 Hours after Lesioning of the Septal Nuclei.

The rats returned to baseline within the next two hours, and their irritability gradually became attenuated over the next five days ($\chi^2_r = 9.1$, $p < .01$) at a rate similar to that of control animals.

In marked contrast to the L-DOPA, apomorphine, piribedil or amphetamine effects, injection of the short-acting barbiturate methohexital produced immediate sedation ($T = 0$, $p < .001$) followed by a rapid return (within an hour) to full, lesion-induced, septal irritability ($\chi^2_r = 29.1$, $p < .001$), which then gradually became attenuated over the next five days ($\chi^2_r = 27.8$, $p < .001$) at a rate similar to control animals (Figure 13). Essentially, methohexital produced a brief and profound sedation, but had no effect whatever on the time course of recovery from the lesion-induced symptoms. Thus, the action of L-DOPA and apomorphine on the septal syndrome cannot be ascribed to the effect of handling during a transient period of tranquilization.

Discussion

The preceding pharmacological data are important for two reasons. First, they show that CA agonists, such as L-DOPA, apomorphine, and amphetamine, can induce the same complete and irreversible dissipation of the septal syndrome as pCPA. This indicates that the pCPA action, as seen in septal rats (Dominguez & Longo, 1970), may be due to its action on CA systems and not, as is usually suspected, by

its action on serotonergic systems. Second, since apomorphine is a relatively specific DA agonist (Ernst, 1967; Iversen & Creese, 1975), the data also strongly suggest that DA stimulation may be the critical factor in the drug induced recovery.

The latency of action for pCPA, L-DOPA and apomorphine on the irritability syndrome was approximately 90 minutes for effective dosages. The time lag between direct pharmacological action and the attenuation or dissipation of the behavioral syndrome suggests that the drugs do not have their action by direct receptor stimulation. The effect is, therefore, not due simply to replacement of reduced levels of endogenous neurotransmitter as is done in Parkinson's disease (Cotzias, Van Woert & Schiffer, 1967). The receptor stimulation by a single administration of the L-DOPA or apomorphine must set in motion processes which will eventually result in an irreversible effect. What these processes are can only be hypothesized.

Both theophylline and imipramine, when administered to septally irritable rats, induced a significant increase in irritable behavior which lasted several hours. Neither, however, led to a significant change in the overall rate of postsurgical recovery compared to animals receiving saline. Theophylline's postulated biochemical action is felt to be dual. First, it inhibits certain forms of cyclic nucleotide phosphodiesterase (Butcher & Sutherland, 1962). It could, therefore, potentiate the action of

catecholaminergic neurotransmitters. Theophylline also interferes with calcium dependent mechanisms (Rasmussen, 1970). In intact organisms the CNS is activated with increased motor activity, reflex excitability, and stimulation of cardiac muscle. The increased irritability seen in these animals might be due to all of the above effects. The lack of recovery would be due to theophylline's lack of direct DA stimulatory action, although it might be predicted that theophylline would potentiate the action of L-DOPA or apomorphine.

Imipramine acts by inhibiting the re-uptake of NA into the pre-synaptic side of CNS nerve endings (Schildkraut, 1973). Since this is the major mechanism whereby NA is deactivated, administration of imipramine leads to increased activation of noradrenergic systems, along with some partial potentiation of serotonergic systems. When injected into septally irritable animals, imipramine produces a significant transient increase in the measured syndrome. Again, there was no significant difference between the rate of recovery shown by imipramine animals and that shown by controls. We can therefore infer that indirect stimulation of NA systems does not induce recovery.

The catecholamine agonist amphetamine produced a biphasic effect. There was an initial exacerbation of the septal irritability syndrome lasting approximately two hours, followed by a gradual dimunition of the syndrome

over the next four hours. Since amphetamine stimulates both dopaminergic and noradrenergic neurotransmitter systems (Glowinski & Axelrod, 1965; Randrup & Munkvad, 1966) the biphasic response might be due to an initial NA stimulation inducing excitment (as seen in the effects of theophylline or imipramine on the syndrome), followed by the DA stimulation induced attenuation. The longer latency for attenuation of the irritability with amphetamine may be a result of the initial CNS excitation masking the presence of an ongoing decrease in the irritability induced by amphetamine's DA stimulation. Amphetamine, therefore, seems capable of inducing the complete and irreversible attenuation seen with L-DOPA and apomorphine.

The putative DA agonist, piribidil, induced an initial decrease in septal irritability, but on the second post-injection day the irritability scores were increased, and the overall rate of recovery did not differ from that of saline controls. Although this result seems contradictory to that found with other DA agonists, it is possible that piribedil is not a true DA post-synaptic receptor stimulator. Piribedil is not as effective in the treatment of Parkinsonism as the other putative DA agonists, nor does it elicit as intense a stereotypy in intact animals, or as intense a turning in rats with unilateral lesions in ascending DA systems. Also, the action of piribedil is blocked by pretreatment with the tyrosine hydroxylase

inhibitor αMPT (Cools & Van Rossum, 1976; Corrodi, Fuxe, Hokfeld, Lindbrink & Ungerstedt, 1973; Costall & Naylor, 1975). This point is critical since a true post-synaptic receptor stimulator should not be inhibited by enzyme blockage of this sort.

Methohexital, an ultra-short-action barbiturate, induced a transient decrease in septal irritability. The overall rate of recovery did not differ from that seen in the saline control animals. This drug was used to control for the possibility that the attenuation seen in some groups of animals was due to handling during a period of tranquilization. This hypothesis was not supported. Both saline and barbiturate control animals were still exhibiting higher than preoperative levels of irritability six days after surgery, even though they were subjected to extensive handling and behavioral testing.

Recently a paper has been published which has direct relevance to this work. It was reported that high dosages of L-DOPA lead to a decrease in septal irritability when compared to controls (Gage & Olton, 1976). These investigators did not mention the complete recovery found on the day of administration which is reported in this dissertation. A major procedural difference between their work and that reported here is that their animals were injected with L-DOPA and then returned to their home cages; the rats were neither observed nor rated until 24

hours later. Their control animals exhibited irritability for periods two or three times as long as the irritability duration reported in this study. Their lack of histology makes a comparison of lesion size and position impossible.

It therefore seems appropriate to stress that dopaminergic stimulation alone may not be sufficient to induce recovery in the septal syndrome. Both somatosensory stimulation and drug action may be necessary. It is interesting to note in this context that it has recently been reported that sensory stimulation in the rat can lead to release of dopamine in the corpus striatum (Antelman, Szechtma, Chin & Fisher, 1975). Sensory stimulation might therefore lead to increased release and turnover of DA. Extensive handling (stimulation) is normally necessary for recovery of rats after septal lesioning (Brady & Nauta, 1955). Injection of high dosages of a DA agonist might, therefore, act by accelerating normally occurring central or peripheral processes.

The series of experiments reported in this section strongly suggest that a DA mechanism figures importantly in recovery from septal lesion-induced irritability. Further pharmacological studies were therefore undertaken to attempt to clarify the problem.

CHAPTER 5

AN ATTEMPT TO REINSTATE THE SEPTAL IRRITABILITY SYNDROME IN RECOVERED RATS

The previous experiments presented in this study implicate dopaminergic mechanisms in the septal irritability syndrome. The ability of micro-injections of 6-OHDA directly into the septal area to induce irritability, as well as the ability of dopaminergic agonists, such as apomorphine, to abolish the irritability are strong supporting evidence for a DA dependent mechanism in this syndrome. In an attempt to further elucidate the underlying pharmacological mechanisms in the syndrome, the action of drugs on rats which recovered from the effects of lesions was studied. In the previous literature on the septal syndrome, there is only one published report using this design. Pirch and Norton (1965) administered beta-phenylisopropylhydrazine, a known MAO inhibitor (Horita, 1958) to "tame" septal rats. These animals had been tamed by repeated handling and exhibited the classic gradual decline in irritability presented by numerous other investigators. When beta-phenylisopropylhydrazine was injected into both recovered septals and normal rats, the septals showed a marked increase in irritability scores which lasted over seven hours, being maximal at five hours. The non-lesioned control rats also showed some increase in irritability, but to a much lesser extent than that seen in

the recovered septally lesioned rats.

Since the present work implicated DA stimulation in initiating the processes of recovery in septal rats, it was hypothesized that blockade of dopaminergic post-synaptic receptors would result in a return of the irritability syndrome. Unfortunately there is one major technical problem with such an experiment. Most of the known DA blocking agents are therapeutically active anti-psychotic drugs with extensive tranquilizing and CNS depressant side-effects. It was therefore necessary to choose a drug with minimal side-effects for this study. The test drug pimozide was felt to be the best possible agent, since recent Scandinavian clinical trials suggest that it has minimal depressant side-effects and maximal DA blockade (Anden, Corrodi, Fuxe & Ungerstedt, 1971). A second agent, theophylline, which was found in a previous experiment (see Chapter 4) to cause excitation in irritable septals was also evaluated in this paradigm.

Method

Rats which had been subjected to RF lesions in the usual fashion (Chapter 2), and who exhibited a complete septal irritability syndrome were assigned to this study. These animals had shown recovery due to either drug manipulation or control handling alone. Animals were not used in this study until they had been without measurable signs of irritability for at least two weeks. Unoperated rats of

approximately the same weight served as controls.

The drugs were suspended in sterile water gum arabic solution and injected intraperitoneally.

Animals were rated for irritability at ¼ hour pre-injection and ¼, ½, 3/4, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 6, and 24 hours post-injection.

Results

In Figure 14 the median irritability scores are presented for both recovered septal and control rats receiving either pimozide or theophylline. Administration of either 0.5 mg/kg or 1.0 mg/kg of pimozide had no effect on irritability scores; there was no return of any of the components of the irritability scale. The rats were rated as 0 both pre- and post-injection. Administration of 20 mg/kg of theophylline to recovered septal rats resulted in a significant increase in irritability ($T = 0$, $p < .01$) over both their own pre-administration levels of irritability, as well as, the pimozide animals' level. This transient effect began within minutes of injection and lasted approximately 3½ hours. Septally irritable rats receiving theophylline exhibited an increase in irritability with a similar latency and time course.

The median irritability scores for normal rats receiving theophylline are presented in Figure 15. An injection of 20 mg/kg of theophylline to a normal animal resulted in little increased irritability, while the same

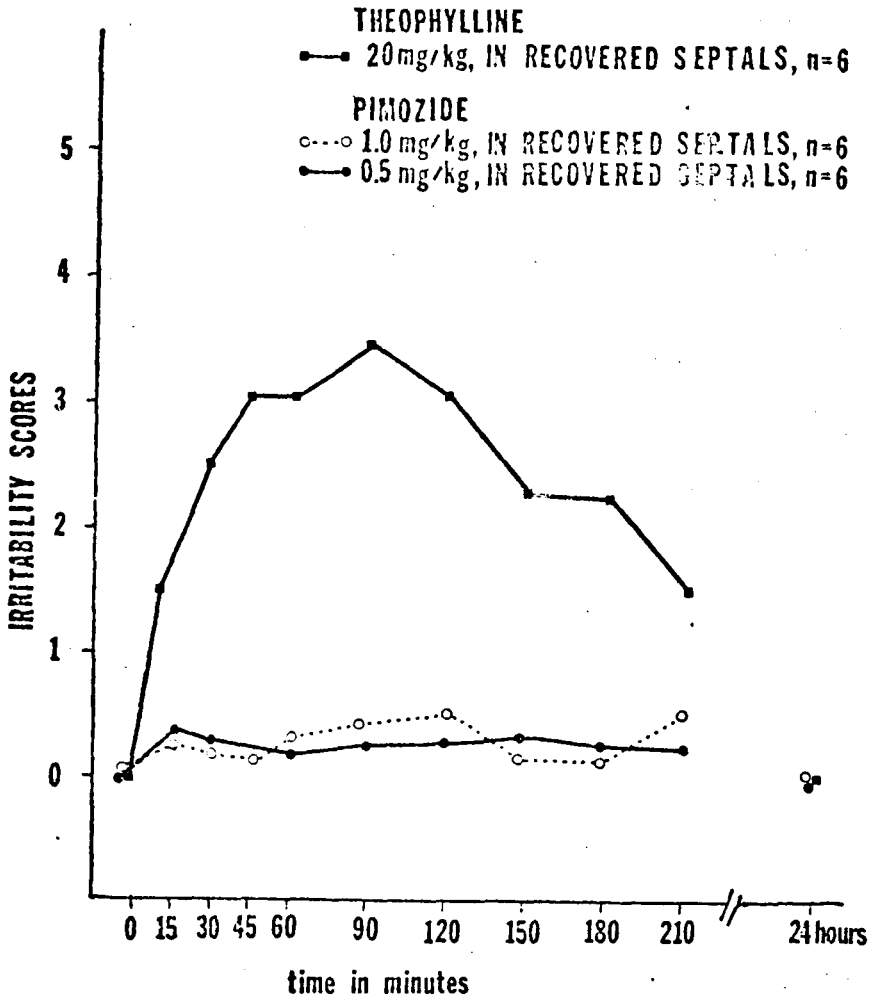


Figure 14: Pre- and Post-Administration Irritability Ratings for Rats Receiving a Single Intraperitoneal Injection of Pimozide or Theophylline. All rats had recovered from septal lesion-induced irritability and had not exhibited any signs of irritability for at least two weeks prior to drug administration.

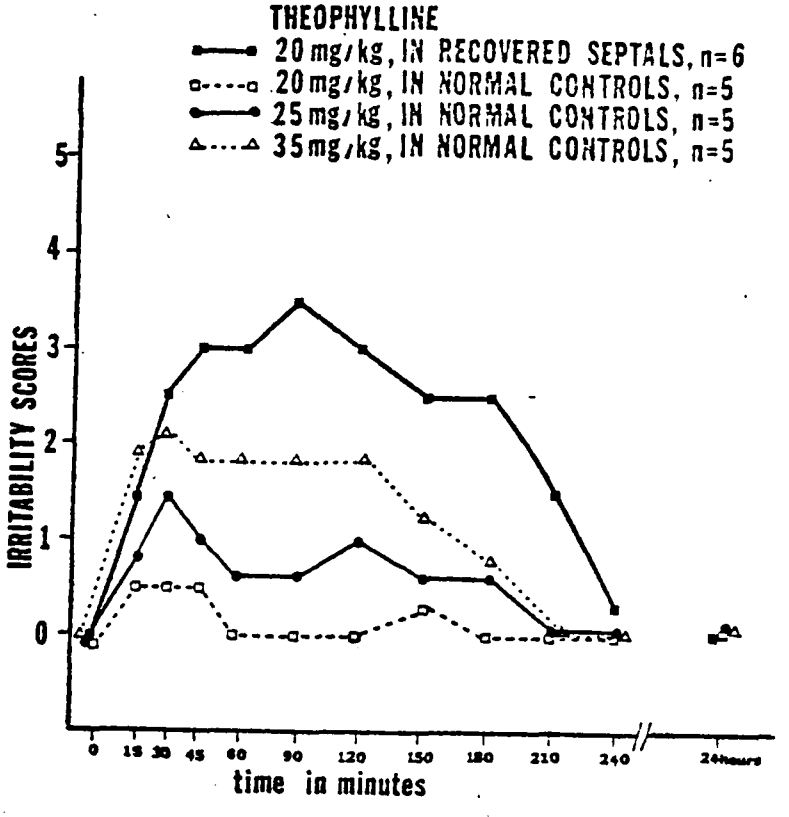


Figure 15: Pre- and Post-Administration Irritability Ratings for Rats Receiving a Single Intraperitoneal Injection of Theophylline after Recovery from Septal Lesion-Induced Irritability as Compared with Non-Lesion Control Animals.

dose in a recovered septal animal had a marked effect. An injection of 35 mg/kg of theophylline, although inducing a measurable increase in irritability in normal rats, still did not have as great an effect as 20 mg/kg in recovered septals. Septal animals, therefore, even weeks after the dissipation of the irritability syndrome are more sensitive to the action of a general stimulatory agent. No differences were found in the effect of theophylline on rats which recovered after extensive handling or rats which recovered after DA agonist drug treatment.

Discussion

There are two implications of these experiments. First, the failure of the dopamine antagonist pimozide to reinstitute the syndrome in recovered rats indicates that the mechanisms of recovery cannot be reversed by drugs having a converse action to those capable of inducing recovery. The simple logic of DA stimulation of irritable rats inducing recovery, with DA blockade in recovered rats inducing return of the syndrome is not validated. This fact in no way subtracts from the data supporting the hypothesis that dopaminergic stimulation may be sufficient to induce recovery from the emotional components of the septal syndrome. There is no a priori reason to expect a simple blockade to work; the results of the experiments do not necessarily follow Aristotelian logic. Since the initial pharmacological data on facilitation of recovery,

presented in both this paper and the work of Dominguez and Longo (1969, 1970), indicate that the facilitating drugs do not act immediately, some series of processes must be set in motion which are not reversible by an acute administration of DA agonists. It is possible, however, that chronic treatment of recovered rats with pimozide might induce a return of irritability, or even a chronic blockade in irritable animals, retarding recovery.

Second, the ability of theophylline to induce irritability must be explained. It could possibly be acting through the same neural mechanisms responsible for septal irritability. On the other hand, the underlying mechanisms could be quite distinct. Since it was found that theophylline could induce irritability in normal animals, the problem might simply be one of drug sensitivity. It is known that rats with septal lesions are more sensitive to the depressant effects of barbiturates (Harvey et al., 1964). Such phenomena are usually rationalized as the result of supersensitivity of partially denervated tissue. The septal area has afferent and efferent connections with many areas of the brain (see introduction). It is therefore possible that the greater sensitivity of septal rats to the excitatory action of theophylline might be due to supersensitivity of certain neural tissues. It is also possible that septal lesions might disrupt tonic influences to critical brain areas making them more

sensitive to almost any pharmacological action.

CHAPTER 6

EFFECT OF CHRONIC PRESURGICAL ADMINISTRATION OF VARIOUS CATECHOLAMINE STIMULATING AND BLOCKING AGENTS ON SEPTAL LESION-INDUCED IRRITABILITY

In order to gain further insight into the neural mechanisms involved in the development and attenuation of septal lesion-induced irritability, a series of experiments on chronic presurgical treatment with pharmacological agents was undertaken. A growing body of experimental evidence suggests that pre-lesion manipulations can affect the manifestations of brain damage syndromes. For example, the LH lesion-induced aphagia and adipsia syndrome has been modified in a number of ways by such manipulations. Maintaining animals at reduced body weight prior to LH damage has been found to shorten the recovery period (Balagura & Harrell, 1974; Powley & Keesey, 1970). It has also been found that pharmacological pretreatment has measurable effects. The blocker of the catecholamine rate-limiting enzyme tyrosine hydroxylase, αMPT when administered for three days prior to LH surgery leads to facilitated recovery (Glick et al., 1972; Hynes et al., 1975). The DA blocker haloperidol and morphine have been found to have effects on the LH syndrome similar to that shown by αMPT (Hynes et al., 1975).

Only one chronic drug pretreatment study has been performed using the septal model. The serotonin depletor

pCPA was administered for five days prior to septal surgery (Harrell & Balagura, 1975). The authors indicate that the septal irritability was "mitigated" by high dosages (300 mg/kg/day) of pCPA. Essentially, the syndrome was still present, but certain components attenuated much faster in the drug group than in the saline control. Overall irritability scores of the pCPA animals were lower on each day rated, but the preoperative baseline level of emotional behavior was reached by all animals on the eighth day.

Since the previous data of this study is supportive of an underlying catecholaminergic substrate to the septal syndrome, it was decided to evaluate drugs affecting catecholamine synthesis and action. It was hypothesized that chronic presurgical blockade or interference with DA action would result in a decrease in the syndrome resulting from septal lesions. Animals receiving saline, or drugs not interfering with catecholamine synthesis would show a standard syndrome.

Method

Procedure

For each of seven days, individually housed Long-Evans hooded rats were injected intraperitoneally with one of the following drugs: apomorphine (20 mg/kg/day, n = 8); haloperidol (4 mg/kg/day; n = 8); ∝MPT (100 mg/kg/day, n = 8); morphine sulfate (60 mg/kg/day, in two divided dosages, n = 6); and, saline (1 cc/day, n = 10).

Surgery

On the seventh day, approximately two hours after the last injection, the rats were subjected to bilateral RF lesions of the septal area (see Chapter 2). Animals were rated for irritability every 24 hours for ten days after surgery.

Histology

Tissue was prepared for histology in the usual fashion (see Chapter 2).

Results

Projection microscope examination revealed large bilateral lesions of both the medial and lateral areas of the septum and adjacent structures. Lesion size and position did not differ significantly between groups. Composite histological reconstructions are presented in Figure 16.

Median irritability scores for animals receiving chronic ∝MPT, apomorphine, morphine and saline are presented in Figure 17. ∝MPT, apomorphine and saline rats all exhibited classic septal irritability on the first postoperative day. All three groups exhibited the characteristic gradual decline, reaching preoperative baseline by approximatedly the seventh postoperative day. There were no statistically significant differences in the overall intensity of the syndrome manifested by the rats in these groups, or in the rates of recovery over days.

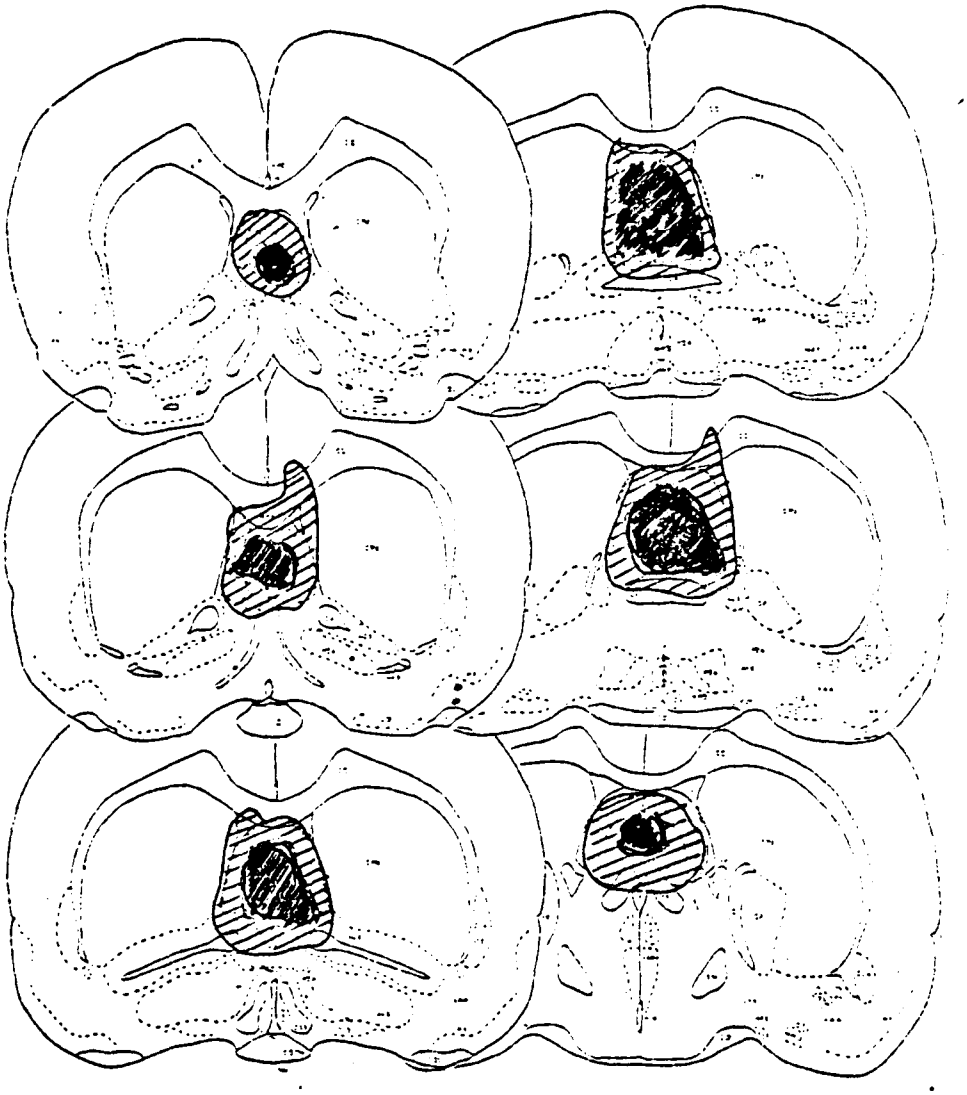


Figure 16: Coronal Reconstructions of Histology of Large Radio Frequency Lesions of the Septal Nuclei. The area of minimal damage is in black; the largest lesion is cross-hatched. Most lesions totally destroyed the septal area from the corpus callosum dorsally, to the anterior commissure ventrally, and from the wall of each caudate nucleus laterally. In 30% of the brains, there was some dilation of the third ventricle.

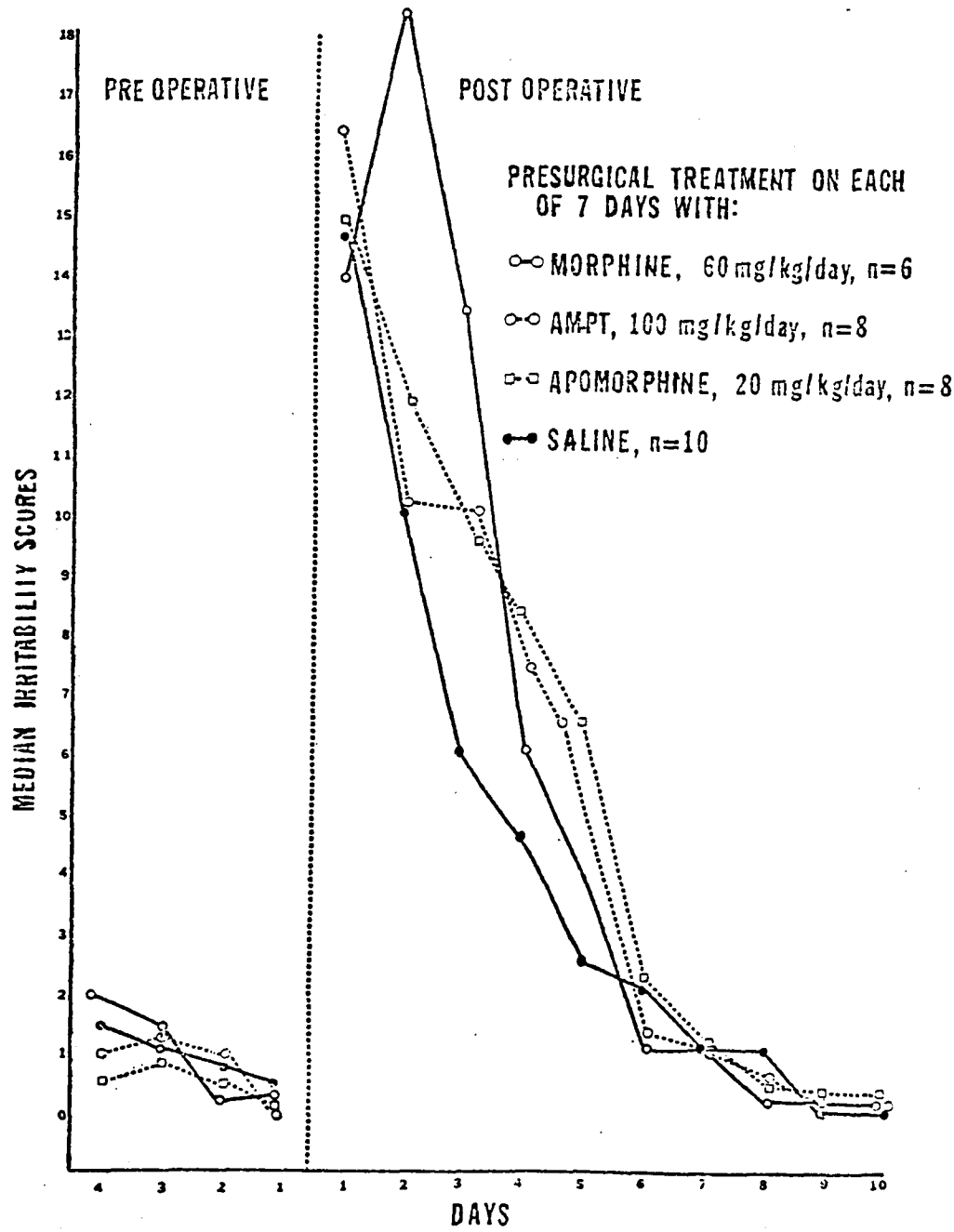


Figure 17: Pre- and Postoperative Irritability Ratings for Rats Receiving Intraperitoneal Injections of Morphine, ∝MPT, Apomorphine or Saline on each of Seven Days before Septal Lesioning.

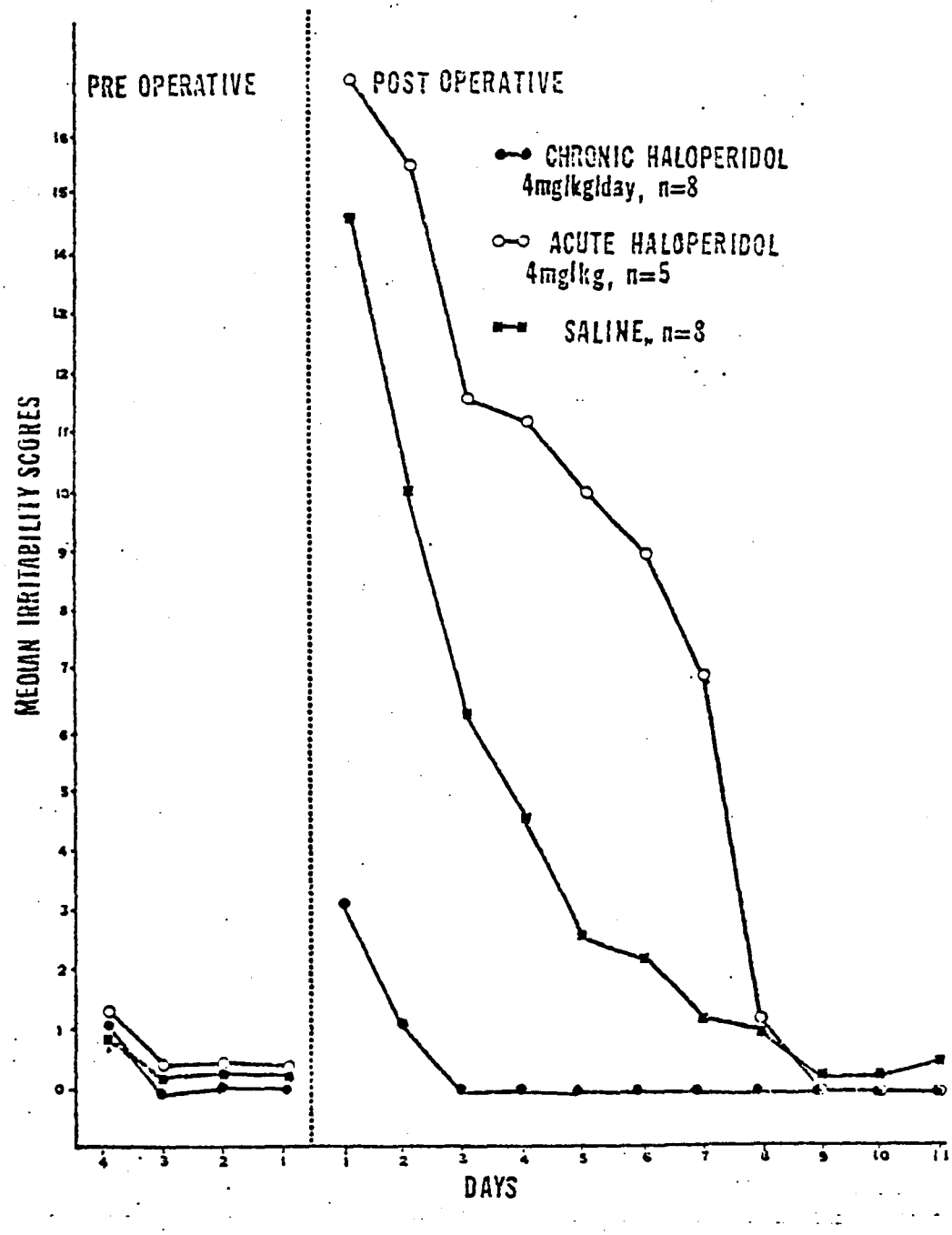


Figure 18: Pre- and Postoperative Irritability Ratings for Rats Receiving Intraperitoneal Injections of Haloperidol or Saline on each of Seven Days before Septal Lesioning, or a Single Injection of Haloperidol on the Day of Lesioning.

When chronic morphine animals were compared to controls, an exacerbation of intensity was evident for the first four postoperative days. However, the overall rate of recovery did not differ from that shown by control rats.

In contrast to these data is the truely remarkable result found in animals receiving chronic treatment with haloperidol (Figure 18). These rats exhibited extremely mild irritability on the first postoperative day. By the second postoperative day the rats were at preoperative levels of emotional behavior.

Since the haloperidol effect was so dramatic, it was necessary to ascertain if it was due to a chronic treatment effect, or due merely to the presence of the drug at the time of surgery. Therefore, an additional group of five rats were treated with haloperidol only on the day of septal surgery. These animals exhibited a classic septal syndrome with an even greater intensity and duration than that seen in control animals.

Discussion

The present findings indicate that it is possible to greatly attenuate the irritability that follows lesions of the septal area by the appropriate presurgical treatment. Thus, pretreatment with haloperidol seven days prior to surgery prevented the irritability characteristic of septally lesioned animals, while pretreatment with αMPT, apomorphine, or morphine did not affect the time course or

intensity of irritability. Harrell and Balagura (1975) have reported that septal irritability can be mitigated by six days of presurgical treatment with pCPA but not by chronic insulin pretreatment. There are, therefore, two pharmacological agents, haloperidol and pCPA, which are capable of modifying the appearance of the septal irritability syndrome. Haloperidol, however, seems to be the more effective agent. As the data reported in this paper indicate, rats pretreated with haloperidol exhibit preoperative levels of emotional behavior by the second postoperative day; the pCPA rats (Harrell & Balagura, 1975), although always exhibiting less irritability than controls, still showed irritability on the seventh postoperative day. Such differences, of course, might be attributed to procedural differences in the studies. It is also difficult to discuss the relative potency of drugs, although the dosages of both pCPA and haloperidol used in the respective studies are high. Such questions can only be answered by plotting dose response curves and standardizing procedures.

The major concern of this work is that of underlying mechanisms of action. Researchers studying the LH syndrome have suggested that the basis of recovery (Berger et al., 1971, 1973; Glick et al., 1972) is the development of supersensitivity of post-synaptic membranes (Sharpless, 1964).

Presurgical administration of αMPT, haloperidol or morphine to LH animals increased survival rates. All pharmacological agents (except possibly morphine) which facilitate recovery in either the LH or septal syndromes when the animals are presurgically treated, decrease action in some neurotransmitter system. αMPT acts by inhibiting the enzyme tyrosine hydroxylase (Moore, Wright & Bert, 1967), thereby depleting levels of both DA and NA. Tryptophan hydroxylase inhibition by pCPA (Jequier, Lovenberg & Sjoerdsma, 1967) depletes serotonin (Koe & Weissman, 1966). Haloperidol's major action is thought to be blockage of post-synaptic DA receptors (Gianutsos, Hynes & Lal, 1975). Morphine has most recently been described as acting directly on morphine receptors within a specific enkephalin system (Snyder, 1977). It has also been reported that morphine reduces activity in DA systems (Puri & Lal, 1973). This reduction in activity in DA systems caused by morphine is then postulated to result in the production of supersensitivity. If post-lesion recovery is due to supersensitivity, then the drug pretreatments give the experimental animals a head start on those processes necessary for recovery.

The data from the experiment reported here are not so simply interpreted. Both haloperidol and αMPT functionally reduce the action of DA at post-synaptic receptor sites. However, their mechanisms of action are different. Haloperidol blocks the post-synaptic receptor; αMPT depletes

DA which cannot then act on the receptor. Haloperidol, morphine and αMPT all facilitate recovery in the LH model. Only haloperidol facilitates recovery in the septal model. Therefore, it appears that only direct receptor blockade is an effective means of presurgical treatment for septal irritability. Haloperidol, morphine and αMPT differ in that DA synthesis is increased in the nigrostriatal system by both haloperidol and morphine but not by αMPT (Gunne, Jonsson & Fuxe, 1969; Janssen, 1967). This might help explain the difference between αMPT and haloperidol, but not between morphine and haloperidol. However, it can be concluded that buildup of supersensitivity is not a likely mechanism for the recovery seen in the animals pre-treated with haloperidol. It would seem that either chronic DA receptor blockade, or increased DA turnover secondary to this blockade are critical to the observed phenomenon. The action of chronic pCPA treatment on dopaminergic systems has not yet been described. More complete pharmacological understanding of the presurgical effects of drugs depends on further understanding of the action of both morphine and pCPA on catecholaminergic, and specifically dopaminergic, neurotransmitter systems.

Since chronic DA blockage results in attenuation of the development of the septal syndrome, it might be hypothesized that chronic stimulation with apomorphine should lead to the development of a more intense irritabil-

ity syndrome. The data do not support such an inference strongly; although the median irritability scores for apomorphine animals are higher than that of controls, there was no significant statistical difference. Additionally, the active half-life of apomorphine is relatively short compared to the sustained tonic effect initiated by a high dose of haloperidol. Therefore, administration of apomorphine in this experiment might be better termed repeated-pulse stimulation of the DA system, rather than chronic presurgical treatment.

CHAPTER 7

GENERAL DISCUSSION

The results of the present series of experiments may be summarized briefly.

Neurotoxic Lesions

Damage to CA systems of the septal area by direct micro-injection of 6-OHDA induces an irritability syndrome in form and intensity like that seen with large RF lesions of the septal nuclei.

Pretreatment of rats with DMI prior to intraseptal micro-injections did not prevent the appearance of this irritability syndrome, implying that damage to DA systems figures importantly in the induction of the syndrome.

Pharmacological Facilitation of Recovery

RF septal lesion-induced irritability can be attenuated by administration of DA agonists.

NA facilitating drugs transiently increase the intensity of septal irritability, but do not affect the duration of the syndrome.

Pharmacological Reintroduction of the Syndrome

Acute DA blockage in recovered septal rats does not reinstate the syndrome.

Recovered septal animals are more sensitive to the excitatory action of the CA facilitator theophylline.

Presurgical Drug Treatment

Chronic presurgical treatment with the DA blocking agent haloperidol prevents the appearance of irritability

after septal lesioning.

Taken together, these findings implicate dopaminergic mechanisms in the induction of, and recovery from, the irritability syndrome resulting from large lesions of the septal area of the rat. Although other authors have suggested that serotonergic mechanisms are causal in the septal syndrome (Dominguez & Longo, 1970; Fried, 1973; Lints & Harvey, 1969), it is suggested that the experiments reported in this paper, along with the auxiliary evidence of other investigators (Bernard et al., 1975; Gage & Olton, 1976) make a dopaminergic hypothesis more tenable in terms of our current concepts of neurochemical organization. Bernard and his associates (Bernard et al., 1975) found that septal lesions which induced irritability also lead to significant decreases in the CA, DA and NA content in various areas of the brain. In fact, the most recent work of this group (Bernard, White, Yutzey & Jones, 1977) indicates that recovered septal rats show higher levels of limbic DA than septal rats exhibiting the irritability syndrome. Also, Gage and Olton (1976) have reported facilitation of recovery in septal rats after the administration of L-DOPA. Further support for a critical role for CAs in the septal syndrome comes from the induction of irritability by intraventricular injections of high doses of 6-OHDA (Coscina et al., 1973; Nakamura & Thoenen, 1972).

Other evidence for a dopaminergic hypothesis is provided by excluding the critical action of other putative

neurotransmitter systems. A number of pharmacological agents only have a transient effect on the time course of recovery shown by septally lesioned rats. Among these agents are glycine, nalorphine (Stern & Catovic, 1975), physostigmine (Stark & Henderson, 1972), serotonin and methysergide (Dalhouse, 1974). This work does not exclude the possibility that the syndrome is due to some complex interaction among known putative neurotransmitter systems or, entirely unknown systems.

The possible complexities involved in a pharmacological understanding of recovery in the septal model can be high-lighted by considering the action of DA agonists on serotonin. Peripheral administration of L-DOPA not only leads to an increase in turnover of DA, but also leads to significant decreases in the whole brain content of serotonin (DaPrada, Caruba, Saner, O'Brien & Pletscher, 1973). It has also been reported that administration of apomorphine, in dosages found to be effective in facilitation of recovery in this study, can lead to depletion of serotonin in pargyline treated rats (Grabowska, 1975). These results are important, in this context, because pCPA can lead to attenuation of septal irritability (Dominguez & Longo, 1970) just as DA agonists can. Whole brain serotonin is depleted by pCPA, but pCPA also reduces NA levels (Welch & Welch, 1967). The story comes full circle since Antelman and Cagguila (1977) have presented evidence to suggest that NA inhibits DA functioning.

These results are not surprising since there is evidence that some neurotransmitter systems are "wired" in series (Cools & Van Rossum, 1976). Therefore, interference with the action of any given system should affect the others in some way.

These results do not necessarily detract from a dopaminergic hypothesis for septal irritability. The action of DA agonists give a relatively unequivocal picture of facilitation of recovery, while the failure of the serotonin blocker, methysergide, to attenuate irritability does not support the hypothesis of a serotonergic mechanism in the septal syndrome. The DA hypothesis is further supported by the ability of 6-OHDA to induce irritability in various paradigms.

One issue raised in this work concerns the site of action of the pharmacological agents which facilitate recovery. This paper is written with an explicit assumption of central action, but the possibility of peripheral mediation remains. Several authors have investigated the possibility of peripheral mediation through hormonal systems. Seggie and Brown (1973) observed that septal rats had larger adrenal glands than normal animals. They hypothesized that interference with adrenal action would attenuate the syndrome. Neither adrenalectomy, nor administration of dexamethasome, a blocker of ACTH, affected the intensity of irritability or the rate of recovery. Also, castration in adult rats did not have a marked effect on

irritability. These types of experiments do not exclude peripheral influences on sensory input, but examination of such processes would be exceedingly difficult to carry out.

Another way of approaching the problem of peripheral versus central action would be the injection of active DA agonists which are incapable of crossing the blood-brain-barrier. Interpretation of such data in the septal model would be rendered problematic by the likely alterations in blood-brain-barrier function by the large septal lesions. An alternate means of approaching the problem would be central administration of the drug in question. This technique is complicated by the necessity of knowing what anatomical site in which to inject the agent. If the site is unknown, intraventricular administration may be attempted. This method is also problematic since sufficient absorption will only occur if the critical site is near the ventricles.

There is inferential evidence to support the contention that there is central action of the drugs used in the studies reported here. L-DOPA, apomorphine, amphetamine, piribedil (Fuxe et al., 1975; Kehr, Carlsson & Lindqvist, 1975), theophylline (Fuxe & Ungerstedt, 1974), haloperidol and pimozide (Sedvall et al., 1975) all have known and well documented actions on CNS tissues when administered peripherally. Each drug easily crosses the blood-brain-barrier, and studies using these agents administered peripherally have been used to gain insight into the underlying neurochemical pathology of diseases such as Parkinsonism, chorea

and schizophrenia, as well as, insight into basic understanding of transmitter action in a number of behavioral paradigms.

Although the methodologies used in this research do not allow a detailed understanding of the underlying mechanisms of recovery in this syndrome, the results do allow the exclusion of a number of currently suggested theories for recovery after CNS damage.

Most neurological or behavioral processes invoked as explanations for recovery from brain damage have time courses that are usually measured in weeks (Goldberger, 1974). A number of these theories and proposed mechanisms have been discussed in the introduction to this work. Among these are: neuronal reorganization (Kennard, 1938), vicarious functioning (Lawrence & Kuypers, 1968), denervation supersensitivity (Trendelenburg, 1963; Ungerstedt, 1971b), axonal sprouting and regrowth (Schneider & Jhaveri, 1974) and behavioral substitution (Gazzaniga, 1974). However, drug-induced recovery described in this work is measured merely in hours. In fact, the reduction of emotional behavior seen with high doses of the DA agonist apomorphine reaches preoperative baseline within two hours of administration. It is difficult for any of the previously mentioned theories to account for such a fast drug-induced response.

Von Monakow (cited in Luria et al., 1969) suggested that many of the effects of CNS insult were not due to the destruction of nervous tissue itself. He distinguished a special form of transient disturbance of functions,

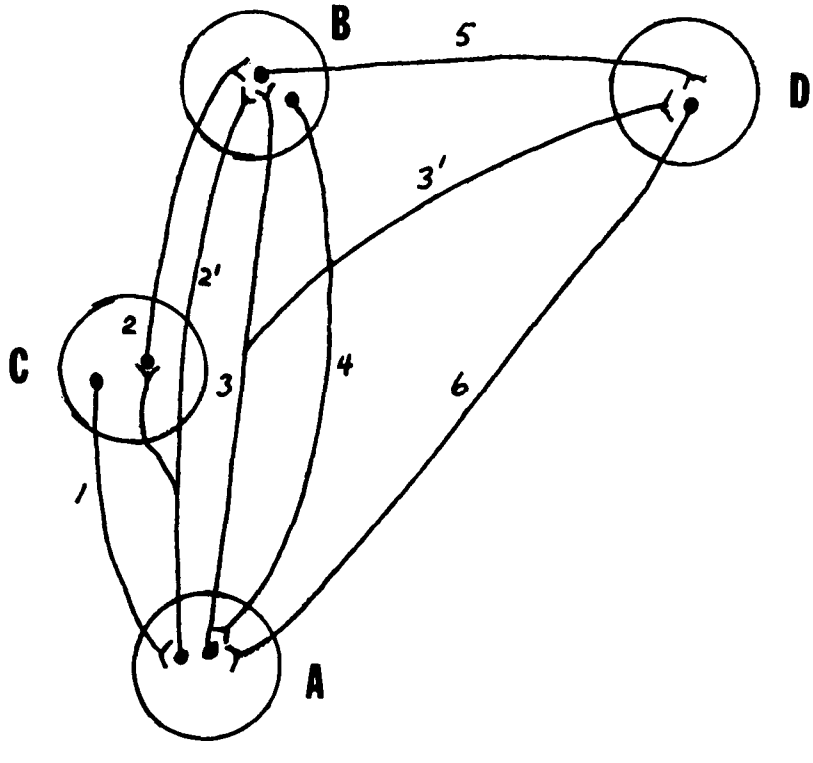
diaschisis, as the result of local brain lesions. Every localized brain lesion was considered an extremely strong stimulus, and Von Monakow conceived of this stimulus as a shock or trauma to the CNS. The shock could spread through the CNS to both adjacent and distal areas, and cause a lack of excitability in neural elements. Only the passage of time would allow for the the dissipation of the diaschitic shock. Von Monakow was unable to propose the specific mechanisms involved, but, he believed that local changes in blood circulation and cerebral spinal fluid flow, edema, release of toxic substances, and termination of tonic influences between connected brain areas might be responsible.

During and after the second world war, A. R. Luria and his associates (Luria et al., 1969) began to treat soldiers with penetrating wounds with cholinergic facilitating drugs. They found that in many cases of paresis or sensory loss, pharmacological treatment alone could induce permanent return of motor ability or sensation. In some patients, only a single administration of drug was needed to abolish a deficit which had been present for many months or even years.

Luria postulated that acetylcholinesterase was overactive in areas termed diaschitic. The anticholinesterase administration allowed a build up of acetylcholine. This potentiated cholinergic action and restored disturbed

synaptic activity. Luria et al. states that diaschisis is actually a synaptic conductance block which can be broken by appropriate pharmacological manipulation.

Although both Von Monakow's and Luria's hypotheses are not easily testable, the concept of diaschisis seems supportable by the present work. In the septal model there are several ways that diaschitic processes can be conceived. For instance, after damage to the septal area, certain non-destroyed neuronal systems may be enzymatically blocked and rendered physiologically inactive. Injections of the appropriate precursors and agonists may be sufficient to restore enzymatic activity and normal function. Thus, a model of recovery of function can be proposed as follows:



The model holds that, following lesion or trauma in brain locus C, the remaining anatomically intact portions of the neuronal system between A and B are physiologically blocked in the sense that chemical processes necessary for synaptic transmission have been rendered inactive. The model further holds that many of these blocked processes can be reinstated by the administration of appropriate precursors or agonists, thus restoring function to neuronal system A-B-D and the behavioral functions it subserves. Specifically, the initial dysfunction might be due to the cessation of input from axons which passed through lesioned area C (pathways 2 and 2'), or transient cessation of activity in axons and collaterals passing near C (pathways 3 and 3'). A slightly different alternative model would postulate that the observed behavioral abnormalities are due to cessation of normal neuronal feedback to some area (e.g., A) from either the lesioned area (pathway 1), or an area intimately influenced by the lesioned area (pathway 4 or 6). This lack of usual tonic input may interfere with an area's functional integrity by blocking or inhibiting biochemical or enzymatic processes. Again, the administration of appropriate precursors or agonists can reinstate blocked processes and restore the system to normal function.

This model is admittedly speculative. However, it can be tied to a known anatomical system. The ascending dopaminergic systems of the limbic region in general

(Lindvall & Björklund, 1974) and the septal area, in particular (Lindvall, 1975), have been described using the sensitive glyoxilic acid fluorescence method. The hypothetical loci proposed in the above model can, therefore, be associated with specific nuclei. Site A would be the DA source cells in the mesencephalon (nuclei A8, 9 and 10), site B the DA-rich olfactory tubercle and site D the amygdala. The explanatory power of this model becomes clear when one combines the anatomy with the data presented here and the recent report of Bernard's group (Bernard et al., 1977). Lesions of the septal nuclei do disrupt DA systems and pharmacological stimulation of DA systems does facilitate recovery. Additionally, recovery from septal irritability is associated with a return of rostral limbic system (olfactory tubercle and amygdala) DA levels to near normal.

Of course, other possible systems may be involved. These include the hippocampal-hypothalamic system via the fornix, and the amygdaloid-hypothalamic system via the stria terminalis. Still, the DA system and its anatomical components appear to be of critical importance.

Hypotheses concerning mechanisms in this system are testable by secondary lesion techniques, and assays of neurotransmitters and enzyme levels. It has now been shown that pharmacological and electrical stimulation of CNS areas can induce enzyme production across synapses (Theonen, 1975). Such trans-synaptic enzyme induction might very well be the mechanism whereby dopamine agonists exert their remarkable action in the septal syndrome, since these enzyme effects

take place within the same time frame as the drug-induced recovery. It should also be reiterated that somatosensory stimulation has been shown to increase dopamine turnover in the rat (Antelman et al., 1975). This may be the normal physiological substrate of recovery in septal rats.

Future experimental work in this area must be multi-disciplinary. Only by combining assays of both neurotransmitter levels and enzymes with lesioning and monitoring of recovery rates will the underlying mechanisms of recovery be understandable. The 6-OHDA intraseptal microinjection technique may be a better means of inducing irritability in this model system since it offers the possibility of inducing a dramatic behavioral effect with a specific biochemical lesion in a given locus. However, it will be necessary to validate the specificity of the damage before such a step is taken. Such a process demands the use of electronmicroscopy, fluorescence histochemistry and regional radio-enzymatic assays.

Relatively rapid attenuation by pharmacological means of some of the effects of brain damage, therefore, is a distinct possibility. This suggestion is supported not only by the data presented in this report, but also by the work of Luria et al. (1969) and O'Laughlin and Feldman (1976). Clinical applications may be an exciting and provocative possible outcome of these experiments.

BIBLIOGRAPHY

Adametz, J.H. Rate of recovery of functioning in cats with rostral reticular lesions. Journal of Neurosurgery, 1959, 16, 85-98.

Akert, K., Orth, O.S., Harlow, H.F. & Schiltz, K.A. Learned behavior of rhesus monkeys following neonatal bilateral prefrontal lobotomy. Science, 1960, 132, 1944-1945.

Andén, N.E., Corrodi, H., Fuxe, K. & Ungerstedt, U. Importance of nervous impulse flow for the neuroleptic induced increase in amine turnover in central dopamine neurons. European Journal of Pharmacology, 1971, 15, 193-199.

Antelman, S.M. & Caggiula, A.R. Norepinephrine-dopamine interactions and behavior. Science, 1977, 195, 646-653.

Antelman, S.M., Szechtma, H., Chin, P. & Fisher, A.E. Tail pinch-induced eating, gnawing and licking behavior in rats: Dependence on the nigrostriatal dopamine system. Brain Research, 1975, 99, 319-337.

Aring, C.D. & Fulton, J.F. Relation of the cerebrum to the cerebellum. Archives of Neurology and Psychiatry, 1936, 35, 439-466.

Arnand, B.K. & Brobeck, J.R. Localization of a feeding center in the hypothalamus of the rat. Proceedings of the Society of Experimental Biology and Medicine, 1951, 77, 323-324.

Balagura, S. & Harrell, L.E. The lateral hypothalamic syndrome: Its modification by obesity and leanness. Physiology and Behavior, 1974, 13, 345-347.

Balagura, S., Harrell, L.E. & Ralph, T. The modification by glucodynamic hormones of the lateral hypothalamic recovery period. Science, 1973, 182, 59-60.

Berger, B., Wise, C. & Stein, L. Norepinephrine: Reversal of anorexia in rats with lateral hypothalamic damage. Science, 1971, 172, 281-284.

Berger, B., Wise, C. & Stein, L. Nerve growth factor: Enhanced recovery of feeding after hypothalamic damage. Science, 1973, 180, 506-509.

Bernard, B., Berchek, J. & Yutzey, D. Alterations in brain monoaminergic functioning associated with septal lesion induced hyperreactivity. Pharmacology, Biochemistry and Behavior, 1975, 3, 121-126.

Bernard, B., White, T., Yutzey, D. & Jones, L. Septal lesion hyperemotionality: Amines in search of a function. Federation Proceedings, 1977, 36, 951.

Bernstein, H. & Moyer, K. Aggressive behavior in the rat: Effects of isolation and olfactory bulb lesions. Brain Research, 1970, 20, 75-84.

Björklund, A. & Stenevi, U. Nerve growth factor: Stimulation of regenerative growth of central noradrenergic neurons. Science, 1972, 175, 1251-1253.

Bowen, F.P. Behavioral alterations in patients with basal ganglia lesions. Association for Research in Nervous and Mental Disease, 1975, 55, 169-180.

Brady, J. & Nauta, W. Affective changes following septal forebrain lesions in the albino rat. Journal of Comparative and Physiological Psychology, 1953, 46, 339-346.

Brady, J. & Nauta, W. Subcortical mechanisms in emotional behavior: Septal and habenular lesions. Journal of Comparative and Physiological Psychology, 1955, 48, 412-420.

Braun, J.J., Meyer, P.M. & Meyer, D.R. Sparing of a brightness habit in rats following visual decortication. Journal of Comparative and Physiological Psychology, 1966, 61, 79-82.

Breese, G. & Traylor, T.D. Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. British Journal of Pharmacology, 1971, 42, 88-99.

Brody, J. Behavioral effects of serotonin depletion and of p-chlorophenylalanine (a serotonin depletor) in rats. Psychopharmacologia, 1970, 17, 14-30.

Brownstein, M., Saavedra, J.M. & Palkovits, M. Norepinephrine and dopamine in the limbic system of the rat. Brain Research, 1974, 79, 431-436.

Butcher, R.W. & Sutherland, E.W. Adenosine 3'-5'-phosphate in biological materials. Journal of Biological Chemistry, 1962, 237, 1244-1250.

Cain, D. Olfactory bulbectomy: Neural structures involved in irritability and aggression in the male rat. Journal of Comparative and Physiological Psychology, 1974, 86, 213-220.

Caplan, M. An analysis of the effects of septal lesions on negatively reinforced behavior. Behavioral Biology, 1973, 9, 129-167.

Carpenter, M.B. & Correll, J.W. Spinal pathways mediating cerebellar dyskinesia in rhesus monkey. Journal of Neurophysiology, 1961, 24, 534-551.

Carpenter, M.B. & Hanna, G.R. Effects of thalamic lesions upon cerebellar dyskinesia in the rhesus monkey. Journal of Comparative Neurology, 1962, 119, 127-148.

Carrea, R.M.E. & Mettler, F.A. Function of the primate brachium conjunctivum and related structures. Journal of Comparative Neurology, 1955, 102, 151-322.

Cole, D.D., Sullins, W.R. & Isaac, W. Pharmacological modification of the effect of spaced occipital ablations. Psychopharmacologia, 1967, 11, 311-316.

Cools, A.R. & Van Rossum, J.M. Excitation-mediating and inhibition-mediating dopamine-receptors: A new concept towards a better understanding of electro-physiological, biochemical, pharmacological, functional and clinical data. Psychopharmacologia, 1976, 45, 243-254.

Cooper, I.S. Dystonia: Surgical approaches to treatment and physiologic implications. In M.D. Yahr (Ed.), The basal ganglia. New York: Raven Press, 1975.

Corrodi, H., Fuxe, K. & Ungerstedt, U. Evidence for a new type of dopamine receptor stimulating agent. Journal of Pharmacy and Pharmacology, 1971, 23, 989-991.

Corrodi, H., Fuxe, K., Hökfeld, T., Lindbrink, P. & Ungerstedt, U. Effects of ergot drugs on central catecholamine neurons and evidence for stimulation of central dopamine neurons. Journal of Pharmacy and Pharmacology, 1973, 25, 409-411.

Coscina, D., Seggie, J., Godse, D. & Stancer, H. Induction of rage in rats by central injection of 6-hydroxy-dopamine. Pharmacology, Biochemistry and Behavior, 1973, 1, 1-6.

Costall, B. & Naylor, R.J. Actions of dopaminergic agonists on motor function. Advances in Neurology, 1975, 9, 285-297.

Cotzias, G.C., Van Woert, M.H. & Schiffer, L.M. Aromatic amino acids and modification of Parkinsonism. New England Journal of Medicine, 1967, 276, 374.

Coyle, J.T. & Snyder, S.H. Catecholamine uptake by synaptosomes in homogenates of rat brain: Stereospecificity in different areas. Journal of Pharmacology and Experimental Therapeutics, 1969, 170, 221-231.

Cytawa, J. & Kutulas, G. Influence of chlorpromazine on emotional hyperreactivity resulting from septal forebrain injury. Psychopharmacologia, 1972, 27, 389-392.

Cytawa, J. & Teitelbaum, P. Spreading depression and recovery of subcortical functions. Acta Biologica Experimentalis, 1967, 27, 343-353.

Dalhouse, A. The role of serotonin in the septal behavior syndrome of rats. (Doctoral dissertation, Ohio State University, 1974). Dissertation Abstracts International, 1975, 35, 4211B.

Da Prada, M., Caruba, M., Saner, A., O'Brien, R. & Pletscher, A. The action of L-DOPA on sexual behavior of male rats. Brain Research, 1973, 55, 383-389.

Dominguez, M. & Longo, V. Taming effect of para-chlorophenylalanine on septal rats. Physiology and Behavior, 1969, 4, 1031-1033.

Dominguez, M. & Longo, V. Effects of para-chlorophenylalanine and methyl-para-tyrosine and other indol and catecholamine depletors on the hyperirritability syndrome of septal rats. Physiology and Behavior, 1970, 5, 607-610.

Dudar, J. The effects of septal nuclei stimulation on the release of acetylcholine from the rabbit hippocampus. Brain Research, 1975, 83, 123-134.

Ernst, A.M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. Psychopharmacologia, 1967, 10, 316-323.

Escourolle, R. & Poirier, J. Manual of basic neuropathology. Philadelphia: W.B. Saunders Co., 1973.

Falck, B., Hillarp, N.A., Thieme, G. & Torp, A. Fluorescence of catecholamine and related compounds condensed with formaldehyde. Journal of Histochemistry and Cytochemistry, 1962, 10, 348-354.

Fox, C. Certain basal telencephalic centers in the cat. Journal of Comparative Neurology, 1940, 72, 1-62.

Franz, D.N. Central nervous system stimulants. In L. Goodman & A. Gilman (Eds.), Pharmacological basis of therapeutics (5th Ed.). New York: Macmillan, 1975, 359-366.

Fried, P. The septum and behavior: A review. Psychological Bulletin, 1972, 78, 292-310.

Fried, P. The septum and hyperirritability: A review. British Journal of Psychology, 1973, 64, 267-275.

Fulton, J.F. & Ingraham, E. Emotional disturbances following electrical lesions in the base of the brain. Journal of Physiology, 1929, 67, xxvii-xxviii.

Fuxe, K., Agnati, L., Corrodi, H., Everitt, B., Hökfelt, T., Löfström, A. & Ungerstedt, U. Action of dopamine receptor agonists in forebrain and hypothalamus: Rotational behavior, ovulation, and dopamine turnover. Advances in neurology, 1975, 9, 223-242.

Fuxe, K. & Ungerstedt, U. Action of caffeine and theophylline on super-sensitive dopamine receptors: Considerable enhancement of receptor response to treatment with DOPA and dopamine receptor agonists. Medical Biology, 1974, 52, 48-54.

Gage, F.H. & Olton, D.S. L-DOPA reduces hyperreactivity induced by septal lesions in rats. Behavioral Biology, 1976, 27, 213-218.

Gál, E.M. Metabolism of para-chlorophenylalanine and the molecular aspects of its action. In J. Barchas & E. Usdin (Eds.), Serotonin and behavior. New York: Academic Press, 1973, 9-17.

Garth, L. The problem of paraplegia in man. Bethesda, Md.: National Institute of Neurological Diseases and Blindness, 1972.

Gazzaniga, M.S. Determinants of cerebral recovery. In D.G. Stein, J.J. Rosen & N. Butters (Eds.), Plasticity and recovery of function in the central nervous system. New York: Academic Press, 1974.

Geschwind, N. Late changes in the nervous system: An overview. In D.G. Stein, J.J. Rosen & N. Butters (Eds.), Plasticity and recovery of function in the central nervous system. New York: Academic Press, 1974, 467-508.

Gianutsos, G., Hynes, M.D. & Lal, H. Enhancement of apomorphine induced inhibition of striatal dopamine turnover following chronic haloperidol. Biochemical Pharmacology, 1975, 24, 581-582.

Glass, J. & Thomas, G. Effects of cortical ablations upon recovery from the septal syndrome in hooded rats. Physiology and Behavior, 1970, 5, 879-882.

Gleese, P. & Cole, J. Recovery of skilled motor functions after small repeated lesions of motor cortex in macaque. Journal of Neurophysiology, 1950, 13, 137-148.

Glick, S.D. & Greenstein, S. Facilitation of recovery after lateral hypothalamic damage by prior ablation of frontal cortex. Nature, 1972, 239, 187-188.

Glick, S.D., Greenstein, S. & Zimmerberg, B. Facilitation of recovery by ∝methyl-p-tyrosine after lateral hypothalamic damage. Science, 1972, 177, 534-535.

Glowinski, J. & Axelrod, J. Effects of drugs on the release and metabolism of ^{3}H norepinephrine in the rat brain. Journal of Pharmacology and Experimental Therapeutics, 1965, 149, 43-49.

Glusman, M. The hypothalamic "savage" syndrome. Proceedings of the Association for Research in Nervous and Mental Disease, 1974, 53, 52-92.

Goldberger, M.E. Recovery of movement after central nervous system lesions in monkeys. In D.G. Stein, J.J. Rosen, & N. Butters (Eds.), Plasticity and recovery of function in the central nervous system. New York: Academic Press, 1974, 265-338.

Goldman, P. Grawford, H., Stokes, L., Galkin, T. & Rosvold, H. Sex dependent behavioral effects of cerebral cortical lesions in the developing rhesus monkey. Science, 1974, 186, 540-542.

Goldman, P. & Rosvold, H. Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. Experimental Neurology, 1970, 27, 291-304.

Goldstein, M. & Nakajima, K. The effect of disulfiram on catecholamine levels in the brain. Journal of Pharmacology and Experimental Therapeutics, 1967, 167, 96-102.

Gotsick, J. & Marshall, R. Time course of the septal rage syndrome. Physiology and Behavior, 1972, 9, 685-691.

Grabowska, M. Influence of apomorphine on brain serotonin turnover rate. Pharmacology, Biochemistry and Behavior, 1975, 3, 589-591.

Green, J.D. & Arduini, A.A. Hippocampal electrical activity in arousal. Journal of Neurophysiology, 1954, 17, 533-557.

Greengard, P. Water-soluble vitamins. In L. Goodman & A. Gilman (Eds.), Pharmacological basis of therapeutics (5th Ed.). New York: Macmillan, 1975, 1549-1563.

Growden, J.H., Chambers, W.W & Liu, C.N. An experimental study of cerebellar dyskinesia in the rhesus monkey. Brain, 1967, 90, 603-632.

Gunne, L.M., Jonsson, J. & Fuxe, K. Effect of morphine intoxication on brain catecholamine neurons. European Journal of Pharmacology, 1969, 5, 338-342.

Hammond, G. & Thomas, G. Failure to reactivate the septal syndrome in rats. Physiology and Behavior, 1971, 6, 598-601.

Harlow, H.F., Thompson, C., Blomquist, A. & Schiltz, K. Learning in rhesus monkeys after varying amounts of prefrontal lobe destruction during infancy and adolescence. Brain Research, 1970, 18, 343-353.

Harrell, L. & Balagura, S. Septal rage: Mitigation by pre-surgical treatment with p-chlorophenylalanine. Pharmacology, Biochemistry and Behavior, 1975, 3, 157-159.

Harris, J.E. & Baldessarini, R.J. Uptake of ^{3}H-catecholamines by homogenates of rat corpus striatum and cerebral cortex: Effects of amphetamine analogues. Neuropharmacology, 1973, 12, 669-679.

Harvey, J. Comparison between the effects of hypothalamic lesions on brain amine levels and drug action. Journal of Pharmacology and Experimental Therapeutics, 1965, 147, 244-251.

Harvey, S.C. Hypnotics and sedatives. In L. Goodman & A. Gilman (Eds.), The pharmacological basis of therapeutics (5th Ed.). New York: Macmillan, 1975, 102-123.

Harvey, J., Heller, A., Moore, R., Hunt, H. & Roth, L. Effects of central nervous system lesions on sleeping time in the rat. Journal of Pharmacology and Experimental Therapeutics, 1964, 144, 24-36.

Heller, A. & Moore, R. Effects of central nervous system lesions on brain monoamines in the rat. Journal of Pharmacology and Experimental Therapeutics, 1965, 150, 1-9.

Hicks, S.P. & D'Amato, C.J. Motor-sensory and visual behavior after hemispherectomy in newborn and mature rats. Experimental Neurology, 1970, 29, 416-438.

Horita, A. Beta-phenylisopropylhydrazine, a potent and long lasting monoamine oxidase inhibitor. Journal of Pharmacology and Experimental Therapeutics, 1958, 122, 176-181.

Hynes, M., Anderson, C., Gianutsos, G. & Lal, H. Effects of haloperidol, methyltyrosine and morphine on recovery from lesions of lateral hypothalamus. Pharmacology, Biochemistry and Behavior, 1975, 3, 755-759.

Isaac, W. Role of stimulation and time in the effects of spaced occipital ablations. Psychological Reports, 1964, 14, 151-154.

Iversen, S.D. & Creese, I. Behavioral correlates of dopaminergic supersensitivity. Advances in Neurology, 1975, 9, 81-92.

Jacks, B.R., De Champlain, J. & Cardeau, J.P. Effects of 6-hydroxydopamine on putative transmitter substances in the central nervous system. European Journal of Pharmacology, 1972, 18, 353-360.

Jackson, J.H. On some implications of dissolution of the nervous system. In J. Wilson (Ed.), Selected writings of J.H. Jackson (Vol.2). New York: Basic Books, 1958, 29-44./ Medical Press and Circular, 1882, ii, 411-432.

Jackson, J.H. Remarks on the diagnosis and treatment of diseases of the brain. British Medical Journal, 1888, 2, 59-63, 111-117.

Janssen, P.A. The pharmacology of haloperidol. Neuropsychiatry, 1967, 3, 510-518.

Jequier, E., Lovenberg, W. & Sjoerdsma, A. Tryptophan hydroxylase inhibition: The mechanisms by which p-chlorophenylalanine depletes brain serotonin. Molecular Pharmacology, 1967, 3, 274-278.

Jonason, K.R., Enloe, L, Contrucci, J. & Meyer, P.M. Effects of simultaneous and successive septal and amygdaloid lesions on social behavior of the rat. Journal of Comparative and Physiological Psychology, 1973, 83, 54-61.

Katzman, R. Cerebral edema (Neuropathology Lectures, 1975) Unpublished manuscript, Albert Einstein College of Medicine, 1975.

Katzman, R., Björklund, A., Owman, C.H., Stenevi, U. & West, K.A. Evidence of regenerative axon sprouting of central catecholamine neurons in rat mesencephalon following electrolytic lesions. Brain Research, 1971, 25, 579-596.

Kehr, W., Carlsson, A. & Lindqvist, M. Biochemical aspects of dopamine agonists. Advances in Neurology, 1975, 9, 185-195.

Kennard, M.A. Reorganization of motor function in the cerebral cortex of monkeys deprived of motor and premotor areas in infancy. Journal of Neurophysiology, 1938, 1, 477-496.

Kennard, M.A. Relation of age to motor impairment in man and subhuman primates. Archives of Neurology and Psychiatry, 1940, 44, 377-397.

Kennard, M. Cortical reorganization of motor function: Studies on series of monkeys of various ages from infancy to maturity. Archives of Neurology and Psychiatry, 1942, 47, 227-240.

King, F. Effects of septal and amygdaloid lesions on emotional behavior and conditional avoidance responses in the rat. Journal of Nervous and Mental Disease, 1958, 126, 57-63.

King, F. & Meyer, P. Effects of amygdaloid lesions upon septal hyperemotionality in the rat. Science, 1958, 128, 655-656.

Kleiner, F.B. & Meyer, P.M. Effects of simultaneous septal and amygdaloid lesions upon emotionality and retention of a black-white discrimination. Brain Research, 1967, 5, 459-468.

Koe, B.K. & Weissman, A. P-chlorophenylalanine: A specific depletor of brain serotonin. Journal of Pharmacology and Experimental Therapeutics, 1966, 154, 499-516.

Koelle, G.B. Anticholinesterase agents. In L.S. Goodman & A. Gilman (Eds.), The pharmacological basis of therapeutics (5th Ed.). New York: Macmillan, 1975, 445-466. (a)

Koelle, G.B. Parasympathomimetic agents. In L.S. Goodman & A. Gilman (Eds.), The pharmacological basis of therapeutics (5th Ed.). New York: Macmillan, 1975, 467-476. (b)

Koelle, G.B. & Friedenwald, J.A. A histochemical method for localizing cholinesterase activity. Proceedings of the Society of Experimental Biology, 1949, 70, 617-622.

Lashley, K.S. Factors limiting recovery after central nervous system lesions. Journal of Nervous and Mental Disease, 1938, 88, 733-755.

Lawrence, D.G. & Hopkins, D.A. Bilateral pyramidal lesions in infant rhesus monkeys. Brain Research, 1970, 24, 543-544.

Lawrence, D.G. & Kuypers, H. The functional orgainzation of the motor system in the monkey. II. The effects of lesions of the descending brain stem pathways. Brain, 1968, 91, 15-36.

Lenneberg, E.H. Biological foundations of language. New York: John Wiley & Sons, 1967.

Lewis, P. & Shute, C. The cholinergic limbic system: Projections to the hippocampal formation, medial cortex, nuclei of the ascending cholinergic system and the subfornical organ and supraoptic crest. Brain, 1967, 90, 521-541.

Lindvall, O. Mesolimbic dopaminergic afferents to the lateral septal nucleus of the rat. Brain Research, 1975, 87, 89-95.

Lindvall, O. & Björklund, A. The organization of the ascending catecholamine neuron systems in the rat brain. Acta Physiologica Scandinavica, 1974, Suppl. 412, 3-48.

Lints, C. & Harvey, J. Altered sensitivity to footshock and decreased brain content of serotonin following brain lesions in the rat. Journal of Comparative and Physiological Psychology, 1969, 67, 23-31.

Liu, C.N. & Chambers, W.W. Intraspinal sprouting of dorsal root axons. Archives of Neurology and Psychiatry, 1958, 79, 46-61.

Ljungberg, T. & Ungerstedt, U. Reinstatement of eating by dopamine agonists in aphagic dopamine denervated rats. Physiology and Behavior, 1976, 16, 277-283.

Loizzo, A. & Massotti, M. Taming effect of nonnarcotic analgesics on the septal syndrome in rats. Pharmacology, Biochemistry and Behavior, 1973, 1, 367-370.

Luria, A., Nayden, U., Tsvetkova, L. & Vinarskaya, E. Restoration of higher cortical function following local brain damage. In P. Winken & G. Bruyn (Eds.), Handbook of clinical neurology (Vol. 3). Amsterdam: North Holland Pub., 1969, 368-433.

Lynch, G., Deadwyler, S. & Cotman, C. Post lesion axonal growth produces permanent functional connections. Science, 1973, 180, 1364-1366.

Lynch, G., Mosko, S., Parks, T. & Cotman, C. Relocation and hyperdevelopment of the dentate gyrus commissural system after entorhinal lesion in immature rats. Brain Research, 1973, 50, 174-178.

Malick, J. A behavioral comparison of three lesion-induced models of aggression in the rat. Physiology and Behavior, 1970, 5, 679-681.

Maling, H.M. & Acheson, G.H. Righting and other postural activity in low decerebrate and in spinal cats after d-amphetamine. Journal of Neurophysiology, 1946, 9, 379-386.

Mark, V.H., Takada, I., Tsutumi, H., Takamatsu, H., Toth, E. & Mark, D.B. Effects of exogenous catecholamines in the amygdala of a "rage" cat. Applied Neurophysiology 1975, 36, 61-72.

Marotta, R., Potegal, M., Gardner, E. & Glusman, M. Abolition of the septal syndrome in the rat by L-DOPA. Paper presented at the meeting of the American Psychological Association, Chicago, 1975.

Matinian, L.A. & Andieasian, A.S. Enzyme therapy in organic lesions of the spinal cord. Los Angeles, California: Brain Information Service, 1976.

Max, D., Cohen, E. & Lieblich, J. Effects of capture procedures on emotionality scores in rats with septal lesions. Physiology and Behavior, 1974, 13, 617-620.

McDowell, F.H. & Sweet, R. Actions of dopaminergic agonists in Parkinsonism. Advances in Neurology, 1975, 9, 367-371.

Mellgren, S. & Srebro, B. Changes in acetylcholinesterase activity and distribution of degenerating fibers in the hippocampal region after septal lesion in the rat. Brain Research, 1973, 52, 19-36.

Merlo, A.B. & Izquierdo, I. Effects of inhibition of O-methyl transferase and of adrenergic blocking agents on conditioning and extinction in rats. Medicina et Pharmacologia Experimentalis, 1965, 13, 217-226.

Meyer, D.R., Isaac, W & Maher, B. The role of stimulation in spontaneous reorganization of visual habits. Journal of Comparative and Physiological Psychology, 1958, 51, 546-548.

Meyer, P.M., Horel, J.A. & Meyer, D.R. Effects of dl-amphetamine upon placing responses in neodecorticate cats. Journal of Comparative and Physiological Psychology, 1963, 56, 402-404.

Mishra, R., Gardner, E., Katzman, R. & Makman, M. Enhancement of dopamine-stimulated adenylate cyclase activity in rat caudate after lesions in substantia nigra: Evidence for denervation supersensitivity. Proceedings of the National Academy of Sciences of the United States of America, 1974, 71, 3883-3887.

Montgomery, A. & Christian, E. Norepinephrine concentrations in brain and heart of hyperreactive septally-lesioned rats. Pharmacology, Biochemistry and Behavior, 1973, 1, 491-492.

Moore, K.E. & Dominic, J.A. Tyrosine hydroxylase inhibitors. Federation Proceedings, 1971, 30, 859-870.

Moore, K.E., Wright, P.F. & Bert, K.K. Toxicological studies with alpha-methyl tyrosine on inhibition of tyrosine hydroxylase. Journal of Pharmacology and Experimental Therapeutics, 1967, 155, 505-515.

Nakamura, K. & Thoenen, H. Increased irritability: A permanent behavioral change induced in the rat by intraventricular administration of 6-hydroxydopamine. Psychopharmacologia, 1972, 24, 359-372.

Nauta, W. An experimental study of the fornix system in the rat. Journal of Comparative Neurology, 1956, 104, 247-272.

Nauta, W. Some neural pathways related to the limbic system. In E.R. Ramsey & D. Doherty (Eds.), Electrical studies on the unanesthetized brain. New York: Hoeber, 1960, 11-16.

Nielson, H., McIver, H. & Boswell, R. Effects of septal lesions on learning, emotionality, activity, and exploratory behavior in rats. Experimental Neurology, 1965, 11, 147-157.

Nurimoto, S., Ogawa, N. & Ueki, S. Hyperemotionality induced by lesions in the olfactory system of the rat. Japanese Journal of Pharmacology, 1974, 24, 175-184.

O'Laughlin, E. Personal communication, March 17, 1977.

O'Laughlin, E. & Feldman, S. Recovery from hypothalamic aphagia after a single intrahypothalamic injection of apomorohine. Neuroscience Abstracts, 1976, 2, 306.

Olton, D.S. & Gage, F.H. Role of the fornix in the septal syndrome. Physiology and Behavior, 1974, 13, 269-279.

Palkovitz, M., Brownstein, M., Saavedra, J. & Axelrod, J. Norepinephrine and dopamine content of hypothalamic nuclei of the rat. Brain Research, 1974, 77, 137-144.

Pellegrino, L.J. & Cushman, A.J. A stereotaxic atlas of the rat brain. New York: Appleton-Century-Crofts, 1967.

Pepeu, G., Mulas, A. & Mulas, M. Changes in acetylcholine content in the rat brain after lesions of the septum, fimbria, and hippocampus. Brain Research, 1973, 57, 153-164.

Petsche, H., Stumpf, C. & Gogolak, G. The significance of the rabbits septum as the relay station between the midbrain and hippocampus. I. The control of hippocampus arousal activity by the septum cells. Electro-

encephalography and Clinical Neurophysiology, 1962, 14, 202-211.

Pirch, J. & Norton, S. Beta-phenylisopropylhydrazine (JB-516) on septal hyperirritability and brain amine levels in the rat. Psychopharmacologia, 1965, 8, 181-190.

Poirier, L.J., Langelier, P., Roberge, A., Boucher, B. & Kitsikis, A. A non-specific histopathological change induced by the intracerebral injection of 6-hydroxy-dopamine. Journal of Neurological Sciences, 1972, 16, 401-416.

Poncy, M., Bernard, P. & Chernov, H. Biochemical and behavioral modifications in septal and hypothalamically-lesioned animals. Neuropharmacology, 1972, 11, 39-44.

Powell, E. Septal efferents revealed by axonal degeneration in the rat. Experimental Neurology, 1963, 8, 406-422.

Powley, T.L. & Keesey, R.E. Relationship of body weight to the lateral hypothalamic feeding syndrome. Journal of Comparative and Physiological Psychology, 1970, 70, 25-36.

Puri, S.K. & Lal, H. Effect of morphine, haloperidol, apomorphine and benztropine on dopamine turnover in rat corpus striatum: Evidence showing morphine induced reduction in central nervous system dopaminergic activity. Federation Proceedings, 1973, 32, 758.

Quenzer, L., Feldman, R. & Moore, J. Towards a mechanism of the anti-aggression effects of chlordiazepoxide in rats. Psychopharmacologia, 1974, 34, 81-93.

Raisman, G. The connections of the septum. Brain, 1966, 89, 317-349.

Raisman, G. Comparison of the mode of termination of the hippocampal and hypothalamic afferents to the septal nuclei as revealed by electron microscopy of degeneration. Experimental Brain Research, 1969, 7, 317-343. (a)

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

Raitt, J., Nelson, J. & Tye, A. Effect of chlorpromazine on septal hyperactivity in the rat. British Journal of Pharmacology, 1961, 17, 473-478.

Randrup, A. & Munkvad, I. Role of catecholamines in the amphetamine excitatory response. Nature, 1966, 211, 540.

Rasmussen, H. Cell communication, calcium ion, and cyclic adenosine monophosphate. Science, 1970, 170, 404-412.

Reeves, A. & Plum, F. Hyperphagia, rage, and dementia accompanying a ventromedial hypothalamic neoplasm. Archives of Neurology, 1969, 20, 616-624.

Reynolds, R. Equivalence of radio frequency and electrolytic lesions in producing septal rage. Psychonomic Science, 1965, 2, 35-36.

Rosen, J., Stein, D.G. & Butters, N. Recovery of function after serial ablation of prefrontal cortex in the rhesus monkey. Science, 1971, 173, 353-356.

Rosner, B. Recovery of function and localization of function in historical perspective. In D.G. Stein, J.J. Rosen & N. Butters (Eds.), Plasticity and recovery of function in the central nervous system. New York: Academic Press, 1974, 1-30.

Salama, A. & Goldberg, M. Norepinephrine turnover and brain monoamine levels in septal lesioned aggressive rats. Life Sciences, 1973, 12, 521-526.

Saavedra, J., Brownstein, M. & Axelrod, J. Specific and sensitive enzymatic isotopic microassay for serotonin in tissue. Journal of Pharmacology and Experimental Therapeutics, 1973, 186, 508-515.

Saavedra, J., Brownstein, M. & Palkovitz, M. Serotonin distribution in the limbic system of the rat. Brain Research, 1974, 79, 437-441.

Schalleck, W., Loehn, A. & Jew, N. Effects of chlordiazepoxide (Librium) and other psychotropic agents on the limbic system of the brain. Annals of the New York Academy of Science, 1962, 96, 303-314.

Schildkraut, J.J. Neuropharmacology of the affective disorders. Annual Review of Pharmacology, 1973, 13, 427-454.

Schneider, G.E. & Jhaveri, S.R. Neuroanatomical correlates of spared or altered function after brain lesions in the newborn hamster. In D.G. Stein, J.J. Rosen & N. Butters (Eds.), Plasticity and recovery of function in the central nervous system. New York: Academic Press, 1974, 65-110.

Schnuir, R. Localization of the septal rage syndrome in Long Evan's rats. Journal of Comparative and Physiological Psychology, 1972, 81, 291-296.

Schwartzbaum, J.S. & Gay, P.E. Interacting behavioral effects of septal and amygdaloid lesions in the rat. Journal of Comparative and Physiological Psychology, 1966, 61, 59-65.

Sedvall, G., Fyrö, B., Nybäck, H. & Wiesel, F. Actions of dopaminergic antagonists in the striatum. Advances in neurology, 1975, 9, 131-140.

Seggie, J. & Brown, G. Effects of dexamethasome on affective behavior and adrenal reactivity following septal lesions in the rat. Journal of Comparative and Physiological Psychology, 1973, 83, 60-65.

Sethy, V., Kuhar, M., Roth, R., Van Woert, M. & Aghajanian, G. Cholinergic neurons: Effect of acute septal lesion on acetylcholine and choline content of the rat hippocampus. Brain Research, 1973, 55, 481-484.

Sharpless, S.K. Reorganization of function in the nervous system: Use and disuse. Annual Review of Physiology, 1964, 26, 357-388.

Siegel, M. & Landis, S. Afferents to the septal area of the rat studied with the method of retrograde transport of horseradish peroxidase. Brain Research, 1975, 82, 263-268.

Siegel, S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill, 1956.

Singh, B. Comparison of hyperemotionality caused by lesions in the septal and ventromedial hypothalamic areas in the rat. Psychonomic Science, 1969, 16, 3-4.

Snyder, S.H. Opiate receptors and internal opiates. Scientific American, 1977, 236, 44-56.

Sorensen, J. & Harvey, J. Decreased brain acetylcholine after septal lesions in rats: Correlation with thirst. Physiology and Behavior, 1971, 6, 723-725.

Sourkes, T.L. Psychopharmacology. In R.W. Albers, G.J. Siegel, R. Katzman & B.W. Agranoff (Eds.), Basic neurochemistry. Boston: Little, Brown and Company, 1972, 581-606.

Srebro, B. & Mellgren, S. Changes in post-natal development of acetylcholinesterase in hippocampal region after early septal lesions in the rat. Brain Research, 1974, 79, 119-131.

Stark, P. & Henderson, J. Central cholinergic suppression of hyper-reactivity and aggression in septal-lesioned rats. Neuropharmacology, 1972, 11, 839-847.

Stein, D.G. Some variables influencing recovery of function after central nervous system lesions in the rat. In D.G. Stein, J.J. Rosen & N. Butters (Eds.), Plasticity and recovery of function in the central nervous system. New York: Academic Press, 1974, 373-428.

Stern, P. & Catovic, S. Brain glycine and aggressive behavior. Pharmacology, Biochemistry and Behavior, 1975, 3, 723-726.

Stricker, E.M. & Zigmond, M.J. Recovery of function after damage to central catecholamine containing neurons: A neurochemical model for the lateral hypothalamic syndrome. Progress in Psychobiology and Physiological Psychology, 1976, 6, 121-188.

Teitelbaum, B. Sex differences in delayed alternation performance following single or multiple stage frontal lesions in rats. Paper presented at the meeting of the American Psychological Association, Montreal, August, 1973.

Teitelbaum, P. & Epstein, A.N. The lateral hypothalamic syndrome: Recovery of feeding and drinking after lateral hypothalamic lesions. Psychological Review, 1962, 69, 79-90.

Teuber, H.L. Unity and diversity of frontal lobe functions. Acta Neurobiologica Experimentalis, 1972, 32, 615-656.

Teuber, H.L. Recovery of function after lesions of the central nervous system: History and prospects. Neuroscience Research Program, 1974, 12, 197-211.

Theonen, H. Trans-synaptic enzyme induction. Advances in Neurology, 1975, 9, 67-72.

Thorne, B.M., Aaron, M. & Latham, E.E. Olfactory system damage in rats and emotional, muricidal, and rat pup killing behavior. Physiological Psychology, 1974, 2, 157-163.

Trendelenburg, U. Supersensitivity and subsensitivity to sympathomimetic amines. Pharmacological Review, 1963, 15, 225-277.

Tucker, T.J. & Kling, A. Differential effects of early and late lesion of frontal granular cortex in the monkey. Brain Research, 1967, 5, 377-389.

Turner, B. Neural structures involved in the rage syndrome of the rat. Journal of Comparative and Physiological Psychology, 1970, 71, 103-113.

Ungerstedt, U. Histochemical studies on the effects of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In T. Malmfors & H. Thoenen (Eds.), 6-Hydroxydopamine and catecholamine neurons. Amsterdam: North-Holland Press, 1971. (a)

Ungerstedt, U. Presynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigra-striatal dopamine system in the rat brain. Acta Physiologica Scandinavica, 1971, Suppl. 82, 69-93. (b)

Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiologica Scandinavica, 1971, Suppl, 367, 1-48. (c)

Valenstein, E. & Nauta, W. A comparison of the distribution of the fornix system in the rat, guinea pig, cat, and monkey. Journal of Comparative Neurology, 1969, 113, 337-363.

Ward, A.A., Jr. & Kennard, M. Effect of cholinergic drugs on recovery of function following lesions of the central nervous system in monkeys. Yale Journal of Biology and Medicine, 1942, 15, 189-228.

Watson, C.W. & Kennard, M. The effect of anticonvulsant drugs on recovery of function following cerebral cortical lesions. Journal of Neurophysiology, 1945, 8, 221-231.

Welch, A. & Welch, B. Effect of p-chlorophenylalanine on brain noradrenaline in mice. Journal of Pharmacy and Pharmacology, 1967, 19, 632-634.

Wolf, A. A method for shortening the duration of lower motor neurone paralysis by cholinergic facilitation. Journal of Nervous and Mental Disease, 1940, 92, 614-622.

Wolf, G. Elementary histology for neuropsychologists. In R.D. Meyers (Ed.), Methods in psychobiology. New York: Academic Press, 1971.

Yutzey, D.A., Meyer, P.M. & Meyer, D.R. Emotionality changes following septal and neocortical ablations in rats. Journal of Comparative and Physiological Psychology, 1964, 58, 463-465.

Yutzey, D.A., Meyer, D.R. & Meyer, P.M. Effect of simultaneous septal and neo or cortical lesions upon emotionality in the rat. Brain Research, 1967, 5, 452-458.

Zeigler, H.P. & Karten, H.J. Central trigeminal structures and lateral hypothalamic syndrome in the rat. Science, 1974, 186, 636-638.

Zeman, W. & King, F. Tumors of the septum pellucidum and adjacent structures with abnormal affective behavior: An anterior midbrain structure syndrome. Journal of Nervous and Mental Disease, 1958, 127, 490-503.

Zis, A.P., Fibiger, H.C. & Phillips, A. Reversal by L-DOPA of impaired learning due to destruction of the dopaminergic nigro-neostriatal projection. Science, 1974, 185, 960-962.