Pharmacological, Behavioral and Neurochemical Assessment of the Selectivity of the Destruction of Central Serotonergic and Catecholaminergic Mechanisms by 6-Hydroxydopamine and 5,6-Dihydroxytryptamine in the Mouse

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PHARMACOLOGICAL, BEHAVIORAL AND NEUROCHEMICAL ASSESSMENT OF THE SELECTIVITY OF THE DESTRUCTION OF CENTRAL SEROTONERGIC AND CATECHOLAMINERGIC MECHANISMS BY 6-HYDROXYDOPAMINE AND 5,6-DIHYDROXYTRYPTAMINE IN THE MOUSE.

by

Frank J. Cann

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DEDICATION

To those who love: since you bring great joy.
To those who dream: since you set the course of life.
VITA

The author, Frank James Cann, is the son of Frank Cann and Mary (Chernetski) Cann. He was born July 6, 1944, in Newark, New Jersey.

His elementary education was obtained at Saint Casimir's School of Newark, New Jersey and secondary education at East Side High School, Newark, New Jersey, where he graduated in 1961.

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INTRODUCTION

ADVANTAGES OF NEUROTOXIN INDUCED LESIONS

The physical lesioning techniques used to alter the structure of the brain such as radio frequency generated heat, cutting, freezing and suctioning were rather unselective in their effects. These aforementioned techniques destroyed all neuronal cell bodies, dendritic arborizations, synaptic terminals and axonal projections which were physically located at the site of the lesion. Thus, when the physical techniques were utilized, any post-lesion changes in behavior could not be ascribed to the destruction of a single brain structure or a single neuronal type as characterized by its transmitter.

Recently, a method of lesioning the brain by means of neurotoxins came into vogue as neurotoxins with allegedly very specific destructive effects were developed. For example, a neurotoxin useful for the study of dopaminergic function should only destroy dopaminergic cells. It was expected that the use of such a neurotoxin would result in readily interpretable experimental findings and that the functional significance of the destroyed cells could be unequivocally deduced (Ungerstedt, 1973).
6-Hydroxydopamine (6-OHDA) and 5,6-dihydroxytryptamine (5,6-OHT) were two of the earliest agents claimed to have specific neurotoxic actions on catecholamine (Ungerstedt, 1971b) and indoleamine (Baumgarten et al., 1971) neurons, respectively. The research reported in these pages examines the specificity of action and certain pertinent problems concerning these two neurotoxic agents.

METHODS OF ADMINISTRATION OF NEUROTOXIC AGENTS

Neurotoxic agents may be administered peripherally or into the brain and its ventricles, and their desired effects may be upon either the peripheral nervous system, the central nervous system (CNS) or both. In the case of 6-hydroxydopamine (6-OHDA), peripheral administration results only in the destruction of the peripheral sympathetic nerves since the compound does not pass the blood brain barrier (Stone et al., 1962; Laverty et al., 1965; Clark et al., 1971). However, this peripheral selectivity is dependent upon the complete development of the blood brain barrier since brain norepinephrine (NE) can be depleted by the systemic administration of 6-OHDA to neonatal rats (Clark et al., 1972). Uretsky and Iversen (1969) demonstrated the central action of 6-OHDA after its intraventricular administration to adult rats.
In contrast, another neurotoxic agent, 6-hydroxydopa (6-OHDOPA) is able to penetrate the blood brain barrier and can induce destructive influences in the periphery as well as the CNS following its systemic administration (Sachs and Jonsson, 1972; Jacobowitz and Kostrzewa, 1971). However, selective lesions of the CNS, without involvement of the periphery, were achieved by direct application of 6-OHDOPA to the CNS (Kmieciak-Kolada, et al., 1974).

Pharmacological manipulations can also alter the actions of a neurotoxic agent. Jonsson and Sachs (1973) demonstrated that monamine oxidase inhibition enhances the neurotoxic action of 6-OHDOPA. Similarly, Breese and Traylor (1970) demonstrated that monamine oxidase inhibition markedly enhanced the dopamine depleting properties of intracisternally administered 6-OHDA. In contrast, the administration of desmethylimipramine almost completely prevented the degeneration of noradrenergic terminals by 6-OHDOPA (Jonsson and Sachs, 1973).

The route of administration can drastically affect the selectivity of 5,6-dihydroxytryptamine (5,6-OHT); systemic administration can result in a sympathectomy similar to that induced by 6-OHDA Baumgarten et al., 1972d). However, central administration results in specific lesions of indolaminergic neurons in preference to catecholaminergic neurons (Baumgarten et al., 1971).
In summary, 6-OHDA, as a neurotoxin, has been applied centrally either intraventricularly (Barnes et al., 1973a, b), intracisternally (Breese et al., 1970) or intracerebrally (Ungerstedt, 1973). The resultant destructive effects were upon the catecholaminergic terminals, axons and soma. 5,6-OHT, on the other hand, has been applied primarily via the intraventricular route (Baumgarten et al., 1971, 1972c; Barnes et al., 1973c; Da Prada et al., 1972; Costa et al., 1972).

METHODS OF ASSESSING THE SELECTIVITY OF NEUROTOXIN INDUCED LESIONS IN THE CNS

It is important to emphasize the distinction between the terms "specific" and "selective" since they are frequently used to describe neurotoxin actions. A neurotoxin is said to be specific if it possesses a single neurotoxic action; that is, at all effective doses the neurotoxin in question would be toxic to only one type of cell. In contrast a neurotoxin is said to be selective when it exerts a toxic effect upon one type of neuron at one dose, but at another dose the neurotoxin will also exhibit toxic effects upon several other neurons; this toxicity on several types of neurons may exhibit a dose response relationship.
NEUROCHEMICAL APPROACHES

In order to assess the specificity of neurotoxin action one would have to investigate the effects of the neurotoxin on many neurotransmitter systems. For example, with the neurochemical approach one may assess the destruction induced by a neurotoxin by measuring the amount of the neurotransmitter that would normally be present in the affected cells or nerve terminals. Other neurochemical approaches involve the measuring of alterations in the activity of biosynthetic enzymes or in the catabolism of exogenously administered neurotransmitter, or in membrane transport mechanisms.

In the case of the cholinergic system one may determine as neurochemical parameters the levels of ACh, the activity of acetylcholinesterase (AChE), uptake of choline, or the activity of choline acetyl transferase (ChAc). Decreases in any of the parameters mentioned above may be indicative of destructive influences on central cholinergic neurons.

Similarly, if the serotonergic system was destroyed one might expect to find decreases in the brain content of 5-HT, uptake of 5-HT or in the specific activity of tryptophan hydroxylase. Obviously the functional biochemical
characteristics of the specific neurotransmitter system involved would define the neurochemical approach to be taken.

The following findings with 6-OHDA should give insight as to the types of neurochemical changes that could be expected with a specific neurotoxin for catecholaminergic neurons. Sufficient neurochemical evidence exists in the case of 6-OHDA to indicate that destruction of central catecholaminergic systems occurred in rats after the central administration of the compound. For example, it was shown that the administration of 6-OHDA lowers the brain content of NE (Bloom et al., 1969; Uretsky and Iversen, 1969; Breese and Traylor, 1970; Scotti et al., 1971) and dopamine (Bloom et al., 1969; Breese and Traylor, 1970; Barnes et al., 1973b). As the major mechanism for the termination of central and peripheral catecholamine neurotransmission is thought to be a rapid presynaptic uptake of released catecholamines (Glowinski and Iversen, 1966), it may be expected that the destruction of catecholamine nerve terminals should impair this uptake. In fact, the uptake of NE was markedly diminished in rat brain following 6-OHDA treatment (Uretsky and Iversen, 1969; Uretsky et al., 1971). Similar effects were observed in the periphery (Jonsson and Sachs, 1970; Thonen, 1971).

Intraventricular administration of 6-OHDA to rats
significantly reduced the uptake of tritiated NE in hypothalamus and striatum but not in the pons-medulla region (Uretsky et al., 1971). These regional differences in uptake of tritiated norepinephrine after 6-OHDA are postulated to indicate a greater susceptibility to destruction of noradrenergic terminals than of noradrenergic cell bodies by the neurotoxin (Uretsky et al., 1971).

Additionally, as a result of neurotoxin action there may be changes in the activity of biosynthetic enzymes. In fact, following the 6-OHDA treatment the activity of tyrosine hydroxylase was markedly diminished (Breese and Traylor, 1970).

The catabolism of neurotransmitter may also be affected by a neurotoxin, as following 6-OHDA pretreatment, increased amounts of normetanephrine were observed after exogenously administered H\textsuperscript{3}-norepinephrine (Uretsky and Iversen, 1971; Breese and Traylor, 1970). Moreover, 6-OHDA treatment increased the formation of non-deaminated, ortho-methylated metabolites by catechol o-methyl-transferase (COMT) (Breese and Traylor, 1970). The increase in the amount of o-methylated metabolites mimicked a loss of MAO activity. However, 6-OHDA did not alter MAO activity (Breese and Traylor, 1970). Thus the increase in COMT metabolites most probably resulted from the loss of the presynaptic uptake of NE which reduced the availability of
NE for deamination by MAO (Breese and Traylor, 1970).

 Neurotoxins may also alter neurotransmitter turnover. 6-OHDA treatment decreased the turnover of NE (Bloom et al., 1969; Uretsky et al., 1971).

 Analogous findings with regard to the changes in the serotonergic system were observed following 5,6-OHT treatment. Long lasting depletions of serotonin have been reported following intraventricular injections of 5,6-OHT (Baumgarten et al., 1971; Baumgarten et al., 1972b; Bjorklund et al., 1974; Barnes et al., 1973c; Longo et al., 1974). The uptake of 5-HT was reduced by 5,6-OHT pretreatment (Daly et al., 1973). Furthermore, the activity of the biosynthetic enzyme tryptophan hydroxylase was also diminished following 5,6-OHT (Victor et al., 1973; Lovenberg and Victor, 1974). Thus, a convincing case may be made with a neurochemical approach for specific destructive effects of 6-OHDA and 5,6-OHT on the noradrenergic and serotonergic systems respectively.

 However, in order to classify a neurotoxin as having a specific action it must be demonstrated that the neurotoxin only affects a single neurotransmitter system. In the case of 6-OHDA, it was apparent that the compound affected both noradrenergic as well as dopaminergic nerve terminals (Breese and Traylor, 1970; Bell et al., 1970; Uretsky and Iversen, 1970). In addition, 6-OHDA may affect
rat brain 5-HT content (Bloom et al., 1969). On the other hand 6-OHDA had no effect on the brain content of GABA or several other amino acids postulated to have neurotransmitter roles (Uretsky and Iversen, 1970; Jacks et al., 1972). 6-OHDA was reported to decrease striatal acetylcholinesterase levels (Kim, 1973) and either to increase (Kim, 1973) or to have no effect (Consolo et al., 1974; Jacks et al., 1972) on striatal ACh. Thus, 6-OHDA may be able to affect several neurotransmitter systems. Similarly to 6-OHDA, the neurotoxin 5,6-OHT was found to have a selective rather than a specific action on brain neurotransmitter systems. Brain NE and DA contents were lowered by sufficiently high doses of 5,6-OHT (Bjorklund et al., 1974; Baumgarten et al., 1972b).

Thus, it is probably more correct to speak of neurotoxin selectivity rather than specificity. That is, until proven otherwise, one should assume that a neurotoxin does not affect a single neurotransmitter system (i.e. is not specific); however, the neurotoxin may be more effective with respect to one neurotransmitter system as compared to another (i.e. it may be selective). The degree of neurotoxin selectivity would be dependent primarily upon the degree of separation that would exist between the effect on one system versus another. These preceding statements are in essence a restatement of a basic pharmacological con-
cept; that drugs affect many biological systems and generally are selective rather than specific in their actions.

**HISTOLOGICAL APPROACHES**

Histological and histochemical examination of the brain and specific pathways, the neurotransmitter composition of which is known, is an extremely valuable tool in determining the extent of neurotoxin induced neuronal damage. A histochemical fluorescence technique is available to localize the catecholamines and indoleamines within the brain (Dahlstrom and Fuxe, 1964). This technique has been methodically applied to illustrate the cellular sequelae of the central administration of either 6-OHDA or 5,6-OHT.

The histological changes induced by 6-OHDA were similar in the periphery and in the CNS. In the periphery, 6-OHDA produces visible evidence of adrenergic nerve terminal degeneration (Tranzer and Thoenen, 1968). Centrally administered 6-OHDA also produces visual signs of neurotoxicity to NE and DA terminals and DA cell bodies (Ungerstedt, 1971b; Bloom et al., 1969). Similarly, microscopic and histochemical studies of neurons after 5,6-OHT have yielded visual evidence of destruction of noradrenergic terminals in the periphery (Baumgarten et al., 1972d) while in the CNS both 5-HT terminals (Baumgarten and Lachenmayer,
1972; Baumgarten et al., 1972c) and axons (Baumgarten, 1972a) show visible evidence of destruction after 5,6-OHT. Histological and histochemical analysis allows for a simultaneous evaluation of neurotoxin induced destruction of the target as well as non-target structures. Such an examination would be difficult if not impossible with the neurochemical approach. However, the approaches are complementary and taken together provide the strongest evidence for specificity and/or selectivity of the neurotoxin action.

**BEHAVIORAL APPROACHES**

Present day neuroscience demands the determination of the relevance of experimentally induced neurochemical and histological changes, particularly by means of investigations of behavioral correlates of the induced neurochemical or histological changes. It became apparent from such investigations that many behaviors have a multitransmitter basis. Thus, the behavioral effect of a neurotoxin cannot be readily ascribed to its action on a specific neurotransmitter system, till its action on other neurotransmitter systems can be excluded.

The biogenic amines have been implicated as modulators of many behaviors, and the behavioral correlates of
the action of the neurotoxins 6-OHDA and 5,6-OHT have been extensively investigated. In most cases the effects of these neurotoxins have supported the concept of a modulatory role of the biogenic amines in several behaviors; examples of the behavioral sequelae of the actions of 6-OHDA and 5,6-OHT are adduced below. It will become apparent on the basis of these examples that the question of selectivity of action of either 6-OHDA or 5,6-OHT has not been thoroughly investigated by means of the behavioral approach; in most cases, there are no data sufficient to correlate a specific neurochemical effect with the behavioral action.

One pertinent example concerns the avoidance response. The catecholamines have been implicated in avoidance responding for many years as the neuroleptics were found to be capable of inhibiting this response (Hanson et al., 1966). A cholinergic component was also implicated since anticholinergic agents reversed the block of avoidance induced by dopamine blocking agents (Hanson and Stone, 1964; Fibiger and Phillips, 1974). In addition, a modulatory role for serotonin has been suggested from genetic studies (Oliverio et al., 1974). Yet, 6-OHDA studies suggest that NE may not be needed for avoidance. Indeed, stereotaxic injections of 6-OHDA into the tegmental area containing the NE ascending fiber tracts did not impair the acquisition of
avoidance (Cooper et al., 1974). However, stereotaxic 6-OHDA injections which involved the ascending dopaminergic fiber bundles, or terminals in the caudate-putamen, resulted in a marked inhibition of acquisition (Cooper et al., 1974). Similar results were observed by Fibiger et al. (1974) who reported an impairment in the acquisition of one-way active avoidance in rats pretreated with 6-OHDA.

A similar situation occurs with regard to the "reward system". While these data may indicate that 6-OHDA exerts a specific dopaminergic rather than a noradrenergic effect on avoidance, the literature dealing with this matter is controversial, and the question is not settled. Previous work by Stein et al. (1968) employing the intracranial self-stimulation technique resulted in a noradrenergic theory of reward; that is, noradrenaline was proposed to be the neurotransmitter required for self stimulation. Recently, 6-OHDA has been used to support this concept (Stein, 1971). After 6-OHDA both the brain norepinephrine content and the self-stimulation rate were decreased (Stein, 1971). However, other investigators rejected a modulatory role of norepinephrine in self stimulation; since after 6-OHDA, they were able to initiate normal rates of self stimulation in spite of significant reductions in brain NE (Antelman et al., 1972). Furthermore, a facilitatory modulatory role for DA was proposed, when the intraventricular administra-
tion of haloperidol, a dopamine receptor blocker, significantly suppressed the rate of self stimulation (Lippa et al., 1972). Moreover, the identification of other modulatory neurotransmitters complicates the analysis of this behavior. For example, an inhibitory modulatory role for the serotonergic system has also been suggested to be an important regulator of intracranial self stimulation, i.e. reward behavior (Poschel and Ninteman, 1971).

The biogenic amines have also been implicated in nocioception. A serotonergic mechanism has been implicated in morphine analgesia (Tenen, 1968). It was found, using the flinch-jump method of Evans (1961) that the tryptophan hydroxylase inhibitor, parachlorophenylalanine (PCPA; Koe and Weissman, 1966) inhibited the analgesic effect of morphine. These data were supported by the fact that PCPA or a lesion of the median forebrain bundle which depleted brain 5-HT, increased the animal sensitivity to pain, without also increasing the animals' generalized responsiveness to a puff of air (Harvey and Yunger, 1973). However, these later investigators could not alter morphine analgesia by either method and concluded that morphine analgesia is not modulated by 5-HT (Harvey et al., 1974). In contrast, lesions of the midbrain raphe nuclei did not affect pain sensitivity but abolished the analgesic effect of morphine (Pepeu et al., 1974a). The lesions of the midbrain raphe
in addition to depleting brain 5-HT also decreased ACh content in the telendiencephalic portion of rat brain. The picture is further complicated by the fact that PCPA also depletes brain norepinephrine content (Miller et al., 1970), and MFB lesions deplete dopamine as well as NE content (Harvey et al., 1974). Thus, we are still at a controversial stage in the understanding of the role of neurotransmitters in pain sensitivity and drug induced analgesia. Selective neurotoxins may aid our understanding in this area.

Still another example illustrating the difficulty in relating a behavior to a specific neurotransmitter concerns aggression. The serotonergic system has been implicated in aggressive behavior but not without confounding influences from other neurotransmitter systems. Muricidal rats appear to have an alteration of serotonergic activity (Di Chiara et al., 1971) but an interaction with ACh can not be excluded in this behavior because of its induction by chronic dosing of pilocarpine (Haubrich and Reid, 1972; Vogel and Leaf, 1972). Similarly, in the case of isolation induced aggression in the mouse, it has been suggested that the decreased turnover in serotonergic as well as catecholaminergic neurons may be necessary (Valzelli, 1974). Thus, assessment of neurotoxin selectivity with this procedure would not be meaningful without a neurochemical analysis of
all the neurotransmitter systems involved.

The sexual behavior of male rats was found to be modulated by several biogenic amines. The sexual behavior of male rats was inhibited by activity of the serotonergic system (Tagliamonte et al., 1969). However, as PCPA has no effect on the sexual activity of cats, the general applicability of a serotonergic concept has been questioned (Zitrin et al., 1970). Furthermore, the sexual behavior of the rat may also be modulated by a dopaminergic facilitatory mechanism (Gessa and Tagliamonte, 1974). The use of the neurotoxin 5,6-OHT supports the serotonergic involvement in sexual behavior since after its administration a hypersexual response is induced (Da Prada et al., 1972). On the other hand, the failure to decrease the facilitatory effect of 5,6-OHT by means of 6-OHDA suggests that some additional clarification regarding the postulated facilitatory role of dopamine is indicated (Da Prada et al., 1972).

Locomotor activity has also been suggested to have a multineurotransmitter basis. There is much evidence to suggest that the central catecholaminergic system provides a facilitatory modulation of locomotion. Evidence for a relationship between central catecholamines and the locomotion was provided by the early work with reserpine. Reserpine treatment depressed locomotion and decreased the brain content of norepinephrine of rats (Holzbauer and
Vogt, 1956). However, reserpine also depleted serotonin and the remarkable behavioral depression which ensued was ascribed to a central release of serotonin (Brodie et al., 1959). The demonstration that a good correlation existed between reserpine induced locomotor depression and catecholamine depletion tended to refute the serotonergic hypothesis (Carlsson, 1961; Matsuoka et al., 1964). Additional support for a direct involvement of catecholamines was obtained by the discovery that L-DOPA effectively reversed the reserpine depression while 5-HTP did not (Blaschko and Chrusciel, 1960; Carlsson et al., 1957).

The role of the catecholamines in locomotor behavior became more firmly established with the discovery of alpha-methylparatyrosine (α-mpt), a compound which inhibits tyrosine hydroxylase, and selectively depletes brain catecholamines without altering brain 5-HT levels (Weissman, 1965). The locomotor depression induced by alpha-methylparatyrosine was consistent with the locomotor stimulation induced by amphetamine, primarily a releaser of catecholamines (Moore et al., 1970). Furthermore, the stimulant actions of amphetamine were reported to be blocked by alpha-methylparatyrosine (Weissman, 1966; Rech, 1970); this fact further supported the role of catecholamines in locomotor behavior. L-DOPA, which does not require the action of tyrosine hydroxylase to form catecholamines, was able to
reverse the α-mpt induced depression of locomotion (Moore and Rech, 1967). Altogether, these data suggest a strong facilitatory effect of catecholamines on the locomotor behavior of the rodent.

The results obtained with a potent dopamine-β-hydroxylase inhibitor, FLA-63, which blocked the synthesis of norepinephrine from dopamine suggested an involvement of NE in locomotion, as FLA-63 produced a reduction in locomotor activity at doses which depressed the brain content of NE (Svensson and Waldeck, 1969). Furthermore, the locomotor stimulant effect of L-DOPA was antagonized by FLA-63 (Stromberg and Svensson, 1971). Finally, the FLA-63 induced depression of locomotor activity was reversed by a monamine oxidase inhibitor (Svensson, 1970).

Studies with the centrally acting α-receptor agonist, clonidine and the α-receptor antagonist, phenoxybenzamine also implicated norepinephrine, a long recognized α-receptor agonist, as a facilitatory modulator of locomotion. Spinal cats were stimulated into active coordinated locomotor movements by clonidine (Forssberg and Grillner, 1973). A central blocking activity was demonstrated for phenoxybenzamine in spinal cats (Anden et al., 1967). Finally, clonidine enhances the locomotor stimulation induced by apomorphine following reserpine (Anden and Strombom, 1974). This stimulation of locomotion by clonidine was
antagonized by phenoxybenzamine (Anden and Strombom, 1974).

In addition, the cholinergic system may also contribute an inhibitory modulation of locomotion. The cholinergic modulation may be indirect through an influence on the catecholaminergic system. Indeed, the locomotor stimulation induced by muscarinic blocking drugs is dependent upon an intact catecholaminergic system (Thornburg and Moore, 1973a, b).

The exact nature of the modulatory role of serotonin in locomotor behavior is still controversial. For example, a facilitatory modulation of locomotion by serotonin has been postulated to be independent of the catecholamines (Modigh, 1974). In contrast, a serotonergic inhibition of catecholamine induced stimulation of locomotion has also been postulated (Mabry and Campbell, 1973). Further research will be needed to clarify the role of serotonin in this behavior.

6-Hydroxydopamine treatment has been shown to induce a transient decrease in locomotor activity (Breese and Traylor, 1970; Scotti de Carolis et al., 1971). Similarly, 5,6-ОHT has also been shown to depress locomotor activity of rodents (Barnes et al., 1973c; Longo et al., 1974). Thus, the neurotoxins' effects on locomotor behavior support the concept of a modulatory role of the bioamines in this behavior.
In conclusion, it is apparent that 6-OHDA and 5,6-OHT have dramatic influences on behavior. Moreover, because of the multineurotransmitter basis of the behaviors studied, the behavioral effects of 6-OHDA or 5,6-OHT treatments cannot stand alone as an argument for neurotoxin specificity or selectivity.

**PHARMACOLOGICAL APPROACHES**

The proof of neurotoxin selectivity by means of the pharmacological approach primarily depends upon the demonstration of selective changes in the responsiveness of the organism to drugs i.e. changes in the drug-receptor interaction. It may be expected that the pharmacological changes induced by a neurotoxin induced lesion of the brain should resemble those induced by physical induced lesions. These changes frequently take the form of a supersensitivity, although a subsensitivity may also develop. Thus, one may use the presence of either supersensitivity or subsensitivity to a specific agonist as a marker of the damage to a biological system which is normally affected by that agonist. This present investigation in part, explored the extent that supersensitivity or subsensitivity would reveal the nature of the neurotoxicity induced by 6-OHDA or 5,6-OHT.
At the time of this investigation very little was known about the nature of supersensitivity in the nervous system. Most of our knowledge about supersensitivity was derived from investigations of the denervation phenomena at the periphery. Therefore, supersensitivity phenomena at the peripheral level will be described to characterize their nature and to provide the implications for the studies of the selectivity of neurotoxin action in the CNS.

THE TERMINOLOGY OF SUPERSENSITIVITY PHENOMENA

Lesions of the nervous system can induce changes in the innervated structure's responsiveness to agonists. The change usually takes the form of an enhanced response. The term "denervation supersensitivity" was used to describe this phenomenon. Cannon and Rosenbluth (1949) indicated that the phenomenon was recognized as early as 1880 by Claude Bernard.

The phenomenon of denervation supersensitivity is demonstrable when a denervated tissue becomes more responsive than normal to its physiological neurotransmitter; supersensitivity should also result in an increased response of the tissue to agonist agents which mimic the action of the neurotransmitter.

Interestingly, it has recently been demonstrated that
denervation per se was not a prerequisite for the development of supersensitivity. Basically, it was discovered that a reduction in either the prejunctional content of the physiological neurotransmitter or in the rate of the prejunctional neuronal impulses was sufficient to induce supersensitivity. This supersensitivity which did not require denervation was termed "disuse supersensitivity".

As a result of numerous investigations, several types of supersensitivity have been described. Terms such as "prejunctional", "postjunctional", "decentralization", "deviation", "denervation", "disuse", "presynaptic", "postsynaptic", "cocainelike" and "nondeviation" have been used to describe these varied forms of supersensitivity. The aforementioned terms resulted primarily from investigations of supersensitivity in the periphery. It is probable that the list may become even more extensive as more knowledge about the nature of supersensitivity in the central nervous system is obtained.

**SUPERSENSITIVITY OF NICOTINIC RECEPTORS**

Supersensitivity is a complex subject as there is no universal mechanism to account for the development of supersensitivity even within one class of receptor. For example, various structures which contain nicotinic choli-
nergic receptors display different responses towards denervation.

Striated muscle contains a nicotinic cholinergic receptor and exhibits supersensitivity to acetylcholine following denervation. The supersensitivity of denervated striated muscle is not specific to acetylcholine. The denervated skeletal muscle also exhibits supersensitive responses to caffeine, potassium and epinephrine as well as acetylcholine (Cannon and Rosenbluth, 1949). In contrast a different response is observed following denervation of a sympathetic ganglion which also contains a nicotinic cholinergic receptor. Preganglionectomy of the sympathetic ganglion innervating the cat nictitating membrane apparently results in a subsensitivity of the nicotinic cholinergic receptor within that ganglion but strangely a supersensitivity of the muscarinic receptor (Dun et al., 1976) as well as the nictitating membrane (Sharpless, 1975).

**SUPERSENSITIVITY OF ADRENERGICALLY INNERVATED SMOOTH MUSCLE**

The phenomenon of supersensitivity of smooth muscle and its catecholamine receptors appears to be even more complex than that of the nicotine receptors in striated muscle and the ganglion. Many of the terms used to describe supersensitivity phenomena are a direct result of
research on supersensitivity in smooth muscle. In contrast to the somatic nervous system, the denervation supersensitivity which occurs in adrenergically innervated smooth muscle of the autonomic nervous system may be prejuncational as well as postjuncational (Trandelenburg, 1966). The terms "prejuncational and postjuncational supersensitivity" refer to a mechanism involving the prejuncational neuronal and postjuncional smooth muscle side of the neuromyial junction, respectively. The presence of both types of supersensitivity in smooth muscle occurs only in the case of a postganglionectomy, while in the case of a preganglionectomy only the postjuncional type occurs (Trandelenburg, 1966).

**PREJUNCTIONAL TYPE**

The prejuncional type of supersensitivity occurs soon after denervation and appears to be rather specific to norepinephrine. Moreover, as a rapid reuptake mechanism exists in the adrenergic nerve terminals and functions as the major mechanism for the termination of action of synaptically released norepinephrine (Glowinski and Iversen, 1966); any impairment of this reuptake mechanism would allow the synaptically released or exogenous NE to interact with the postsynaptic receptor for a prolonged period of
time. The net result of impaired reuptake would be to enhance the agonist action of a fixed amount of NE and to reduce the effective agonist concentration of exogenous NE. Thus, the loss of a presynaptic uptake process would result in a supersensitive response. Thus, the mechanism of prejunctional supersensitivity is thought to be due to the loss of the reuptake mechanism for liberated catecholamines by the degenerating nerve terminal. The term "cocaine-like supersensitivity" has been used as a synonym for the prejunctional type of supersensitivity as cocaine will block the uptake of NE by the catecholaminergic nerve terminals (Trandelenburg, 1966).

**POSTJUNCTIONAL TYPE**

The postjunctional type of supersensitivity in adrenergically innervated smooth muscle develops slowly and is not as specific as the prejunctional type. The smooth muscle develops a supersensitive response to acetylcholine, potassium ion and to norepinephrine.

Another term applied to the postjunctional type of supersensitivity is "decentralization type". It is in reference to decentralization that the term "disuse supersensitivity" developed. The concept of disuse was employed in pharmacological investigations in which reserpine was
utilized to deplete the neuron of norepinephrine and to induce a state of diminished rather than totally absent neuronal function. It was apparent that the type of supersensitivity which developed after reserpine, disuse, decentralization, or other procedures which diminished the flow of neuronal impulses to the adrenoceptive neuroeffect- or, mimicked that of the postjunctional type as it exhibited a long latency and a lack of specificity (Westfall, 1970).

**DENERVATION SUBSENSITIVITY**

Besides the development of supersensitivity, a subsensitivity to pharmacological agents may also develop. Subsensitivity may develop as a change in the agonist receptor site following extended periods of activation. For example, the number of beta adrenergic receptors in frog erythrocytes was markedly reduced following a 24 hour exposure to isoproterenol or norepinephrine (Mukherjee et al., 1975). Moreover, denervation subsensitivity to indirect acting agonists may occur as the latter depend for their action upon both an intact nerve terminal and normal supplies of releasable neurotransmitter (Flechenstein and Burn, 1953).
MECHANISMS OF SUPERSENSITIVITY DEVELOPMENT IN THE PERIPHERY

Varied mechanisms have been suggested for the development of supersensitivity in the periphery. One mechanism suggested for the induction of supersensitivity in striated muscle was the loss of a trophic factor from the nerve. This concept received less emphasis in recent times since it was demonstrated that exercising a denervated muscle with either electrical stimulation (Lomo and Rosenthal, 1972) or via acetylcholine (Drachman and Witzke, 1972) was sufficient to prevent the development of supersensitivity. Any trophic influence therefore, must reside within the muscle itself and may not be a result of a trophic factor released from the nerve terminal. The mechanism of this supersensitivity appears to be a proliferation of cholinceptive sites outside of the junctional area (Axelsson and Thesleff, 1959).

In sympathetically innervated smooth muscle the mechanism for the development of supersensitivity of the prejunctional type has been shown to be due to the loss of the catecholamine reuptake mechanism of the nerve terminal (Trandelenburg, 1966). In contrast, evidence is still being accumulated to characterize the mechanism for the development of supersensitivity of the postjunctional type. Fleming et al. (1975) demonstrated a partial depolariza-
tion, on the order of 10mv., in smooth muscle that exhibited supersensitivity of the postjunctional type. Furthermore, since no changes were observed in the junctional potentials, it was concluded that there were no changes in the postjunctional receptors (Fleming et al., 1975). Moreover, acute depolarization of this tissue results in an acutely developing supersensitivity similar to that observed after decentralization (Fleming et al., 1975). Thus, depolarization may play an important role in the postjunctional supersensitivity of sympathetically innervated smooth muscle. In addition, the postsynaptic type of supersensitive response of smooth muscle to potassium, acetylcholine and calcium are dependent upon the presence of calcium ion in the external tissue fluid (Carrier, 1975). In contrast, the supersensitive response of the same tissue to catecholamines was independent of external calcium concentration (Carrier, 1975). Morphologically, an increase in the number of nexial contacts was observed after postganglionic denervation of the vas deferens (Westfall et al., 1975). It was also suggested that an increase in ATP parallels or precedes the development of postsynaptic supersensitivity in denervated tissues (Westfall et al., 1975).

A novel mechanism was recently suggested to explain the hydrocortisone induced supersensitivity of the nicti-
tating membrane to catecholamines. Trandelenburg and Graefe (1975) demonstrated that inhibition of catechol-o-methyl transferase (COMT) induces supersensitivity of the cat nictitating membrane in a highly specific manner. The effect is limited to the response to NE, the physiological neurotransmitter, and only after the reuptake mechanism of the presynaptic terminal has been impaired either by denervation or cocaine. The hydrocortisone induced supersensitivity possessed characteristics similar to that induced by a COMT inhibitor. It was suggested that, hydrocortisone induced supersensitivity by blocking the high affinity uptake of NE into the high affinity methylation compartment, within which released catecholamines are actively being methylated by COMT (Trandelenburg and Graefe, 1975).

There are several conceivable mechanisms for the development of supersensitivity in a cholinergic junction. Examples of these mechanisms are: a loss in junctional cholinesterase content or activity, an increase in the amount of acetylcholine released per impulse and an increase in the responsiveness of the receptor to activation by ACh. It is interesting that the apparent mechanism for the development of supersensitivity in denervated striated muscle is the proliferation of extrajunctional cholinergic receptors (Axelsson and Thesleff, 1959).
SUPERSENSITIVITY OF CENTRAL DOPAMINE RECEPTORS

In contrast to the peripheral somatic and autonomic nervous systems, relatively few studies were reported which suggested the development of supersensitivity within the brain; most studies were primarily concerned with the dopaminergic system, and employed prolonged reductions of brain dopamine content to induce the supersensitivity. This approach was analogous to disuse supersensitivity in the periphery. Stolk and Rech (1967) utilized chronic reserpine treatment to reduce brain dopamine content; the treatment led to the development of a supersensitive locomotor response to d-amphetamine, a dopamine releasing agent. Similarly, a prolonged dopamine depletion was induced by the dietary administration of α-methylparatyrosine, and it resulted in a supersensitive locomotor response to ephedrine (Dominic and Moore, 1969). Similarly, the prolonged reductions in rat brain dopamine induced by 6-OHDA also resulted in a supersensitive response to the dopaminimetics, apomorphine and L-DOPA (Ungerstedt, 1971a; Uretsky and Schoenfeld, 1971).

More recently, other laboratories have verified the induction of dopaminergic receptor supersensitivity induced either by α-mpt, reserpine or 6-OHDA treatment (Friedman et al., 1976; Nahorski, 1975; Tarsy and Baldessarini, 1974;
Furthermore, persistent or acute blockade of dopaminergic receptors with neuroleptic agents also induced dopaminergic receptor supersensitivity (Christensen et al., 1976; Jackson et al., 1975; Von Voigtlander et al., 1975; Yarbrough, 1975). Agonist activation by L-DOPA of dopamine receptors during the period of 6-mpt treatment prevented the development of supersensitivity by 6-mpt (Gudelsky et al., 1975).

Supersensitivity of "dopaminergic autoreceptors" is another related phenomenon. Dopamine receptors have been postulated to exist on the dopaminergic neuron; hence, they were called autoreceptors (Bunney and Aghajanian, 1975). The dopaminergic autoreceptors have been postulated to be responsible for the suppression of firing by dopaminergic agents of dopaminergic cells in the substantia nigra (Bunney and Aghajanian, 1975); the inhibition of dopamine turnover by apomorphine was also thought to be the result of the activation of dopaminergic autoreceptors (Roth et al., 1975). Chronic haloperidol treatment reduced the threshold dose of apomorphine required to reduce the turnover of dopamine; this effect may be an example of supersensitivity of the dopaminoneceptive autoreceptors (Gianutsos et al., 1975). Moreover, the presence of presynaptic receptors may modulate the degree of supersensitivity evidenced by postsynaptic dopamine receptors. The evidence
for the modulation of postsynaptic receptor supersensitivity by presynaptic dopamine receptors was provided by the enhancement of reserpine-elicited dopaminergic supersensitivity by repeated administration of apomorphine or \( \alpha \)-methyl-p-tyrosine during the reserpinization (Friedman et al., 1976).

### SUBSENSITIVITY OF CENTRAL DOPAMINE RECEPTORS

Subsensitivity of dopaminergic receptors has also recently been suggested to occur. Apomorphine administration induced a hypothermic response in rodents; this response was characterized by acute desensitization, as a single dose of apomorphine prevented subsequent doses of apomorphine from inducing hypothermia (Costentin et al., 1975b). The response was specific for dopamine receptors since the hypothermic responses to clonidine, oxotremorine and promethazine were not altered by prior treatment with apomorphine (Costentin et al., 1975b). Chronic haloperidol induced supersensitivity to the apomorphine induced hypothermia (Costentin et al., 1975a). In contrast, 6-OHDA treatment induced a subsensitivity to the hypothermic response to apomorphine (Costentin et al., 1975a).
OVERUSE SUPERSENSITIVITY OF CENTRAL DOPAMINE RECEPTORS

In addition to the concept of disuse supersensitivity, recent experimental findings have suggested the development of overuse supersensitivity in central dopaminergic systems (Klawans and Margolin, 1975). The administration of amphetamine on a chronic basis and in high doses produced an enhanced response to amphetamine and apomorphine (Klawans and Margolin, 1975). It was suggested that L-DOPA induced dyskinesias, amphetamine choreatic movements and amphetamine psychosis may be a result of supersensitivity of dopaminergic receptors induced by their chronic excessive stimulation. The exact mechanism and implications of these findings remain obscure at present. Moreover, certain other amphetamine related phenomena must be excluded before overuse supersensitivity can be considered as a separate phenomenon. For example, amphetamine also produces supersensitivity of adrenergically innervated tissue in the periphery; this has been classified as the presynaptic type of supersensitivity, being due to amphetamine's blockade of uptake of NE by the adrenergic neuron (De Moraes and Carvalho, 1971). It is doubtful that blockade of the presynaptic NE uptake mechanism by amphetamine could account for the development, after chronic amphetamine treatment, of a supersensitive response to apomor-
phine, since apomorphine does not depend upon presynaptic stores of catecholamines for its action (Ernst, 1967, 1969). There also exists the problem of state dependency and drug conditioning phenomena which may mimic the development of supersensitivity (Tilson and Rech, 1973). Furthermore, chronic amphetamine treatment may induce dopaminergic receptor supersensitivity via a chronic catecholamine depletion (Lewander, 1974; Tonge, 1974). Thus, the mechanism of amphetamine induced supersensitivity of central dopamine receptors remains to be placed in proper perspective with the other known effects of chronic amphetamine administration.

In contrast, chronic amphetamine treatment may induce in the periphery a subsensitivity of noradrenoceptors which is evidenced as a decreased elevation of cyclic AMP by noradrenaline. The subsensitivity appeared to be specific since the cyclic AMP elevations induced by either dopamine, 5-HT or adenosine remained unaffected by the chronic amphetamine treatment (Martres et al., 1975). Chronic amphetamine treatment has not yet been observed to induce receptor subsensitivity in the CNS.

SUPERSSENSITIVITY OF CENTRAL NE RECEPTORS

In addition to the studies on dopamine receptor
supersensitivity some investigations have been concerned with demonstrating the supersensitivity of central noradrenergic receptors. These studies employed the technique of depletion of brain NE content to induce a disuse type of supersensitivity. For example, a supersensitive hypothermic response to clonidine was observed in rats three months after 6-OHDA induced lesions of the ascending NE containing pathways (Zis and Fibiger, 1975).

In another study, treatment with reserpine was reported, after 24 hours, to induce a supersensitive response of brain stem neurons to microiontophoretically applied norepinephrine (Boakes et al., 1971). Although an effect similar to that of reserpine was expected neither the dopamine-β-hydroxylase inhibitor, FLA-63, nor the tyrosine hydroxylase inhibitor, α-mpt, were able to induce changes in brain stem neuronal firing rates (Boakes et al., 1971).

Furthermore, a presynaptic as well as postsynaptic norepinephrine receptor supersensitivity has been suggested to occur in rat cerebral cortical slices following intraventricular 6-OHDA treatment (Kalisker et al., 1973; Huang et al., 1973). An acute short lasting subsensitivity and longer lasting supersensitivity to NE has been described following reserpine (Palmer et al., 1975). A beta-adrenergic receptor has been described in the rat pineal gland which regulates the activity of N-acetyltransferase and
therefore the degree of melatonin synthesis (Brownstein et al., 1973). Supersensitivity of the pineal β-adrenergic receptors has been demonstrated following denervation via a superior cervical ganglionectomy (Deguchi and Axelrod, 1972), disuse via constant light, reserpinization or 6-OHDA treatment (Deguchi and Axelrod, 1973; Weiss and Strada, 1972). Thus, supersensitivity of central noradrenergic catecholaminergic receptors has been demonstrated in a number of ways which primarily rely upon a diminution in the amount of stimulation of the catecholaminergic receptors.

**SUPERSENSITIVITY OF CENTRAL ACh RECEPTORS**

In contrast to the numerous investigations of supersensitivity in the peripheral somatic and autonomic nervous systems, there are relatively few examples of supersensitivity of the cholinceptive neurons of the central nervous system. Chronic scopolamine administration induced a supersensitive hypothermic response to pilocarpine (Friedman et al., 1969). More directly, Bird and Aghajanian (1975) demonstrated a supersensitive response of cholinceptive hippocampal pyramidal cells to acetylcholine following the destruction of a cholinergic input from the medial septal nucleus. Clinically, it has been suggested that the par-
kinsonian patient exhibits a supersensitivity to cholinergic agents; however, this supersensitivity has been suggested to be due secondarily to the loss of dopaminergic input to the striatum (Weintraub and Van Woert, 1975).

Evidence for a subsensitivity of central cholinergic receptors has also been accumulated. Tolerance develops to the disruptive effects of diisopropylfluorophosphate (DFP) on fixed ratio (FR) responding; a hyposensitivity of cholinergic mechanisms has been postulated to account for the development of this tolerance (Russell et al., 1975). Moreover, chronic dopaminergic blockade with haloperidol decreased the locomotor depressant action of pilocarpine. This has been suggested to be an example of central cholinergic receptor subsensitivity (Gianutsos and Lal, 1976).

**SUPERSENSITIVITY OF CENTRAL SEROTONIN RECEPTORS**

The advent of investigations into supersensitivity of central serotonin receptors began with the use of 5,6-OHT (Barnes et al., 1973c; Longo et al., 1974), (cf. Discussion).

**DEMONSTRATING POSTSYNAPTIC SUPERSENSITIVITY OF CENTRAL DA AND NE RECEPTORS WITH THE MICROIONTOPHORETIC TECHNIQUE**
Although there exists much indirect evidence suggesting postsynaptic receptor supersensitivity in the central nervous system, it has not been unequivocally demonstrated by a direct technique. The direct method for the demonstration of supersensitivity to a neurotransmitter in the central nervous system involves the use of the microiontophoresic application of that neurotransmitter to its receptive cells. The major difficulty with the iontophoresic technique in the central nervous system is that one can never be certain whether an observed response is the direct result of an interaction of the iontophoresed neurotransmitter with the postsynaptic receptor or whether it is the indirect result of an interaction of the iontophoresed neurotransmitter with receptors on the presynaptic boutons which impinge upon that cell. This difficulty is primarily a result of the presence of a large number of synaptic contacts which impinge upon cells in the mammalian central nervous system. Thus, it would be difficult to distinguish presynaptic from postsynaptic forms of supersensitivity in the CNS with this technique.

These practical difficulties with the microiontophoresic technique were observed recently in the course of the attempts to demonstrate postsynaptic receptor supersensitivity of central dopaminoceptive cells. The microiontophoresic application of dopamine to cells in the stria-
tum, following dopamine depleting lesions, resulted in the
description of both a subsensitivity as well as a supersen-
sitivity to dopamine. Spehlmann and Stahl (1974) reported
a subsensitivity of dopaminoceptive cells in the corpus
striatum following tegmental lesions in the cat. They
found, in cats with tegmental lesions, fewer spontaneously
firing cells which could be inhibited by dopamine. Fur­
thermore, in lesioned cats, the dopamine ejection current
required to inhibit striatal cells was significantly great­
er than in non-lesioned cats. The subsensitivity to dopa­
mine was specific since after the tegmental lesion there
were no significant changes in the striatal cells' re­
sponses to either GABA or L-glutamate. In contradiction to
the findings of Spehlmann and Stahl (1974), Yarbrough and
Phillis (1975) reported a supersensitive response of dopa­
minoceptive cells following unilateral lesions of the sub­
stantia nigra in rats. The supersensitive response was
demonstrated as an increased proportion of cells which were
inhibited by dopamine following the lesion. In addition,
Yarbrough and Phillis (1975) reported that following the
substantia nigra lesion there was a significant decrease in
the threshold current required for dopamine to inhibit the
firing of glutamate stimulated caudatal cells. Similar
supersensitivity of dopaminoceptive cells to dopamine has
been reported by Feltz and De Champlain (1972). A super-
sensitive response of dopaminoceptive cells was also observed following chronic haloperidol administration (Yarbrough, 1975).

In the case of norepinephrine, microiontophoretic studies have suggested that after 6-OHDA a supersensitivity develops in cerebellar Purkinje cells (Hoffer et al., 1971) and hippocampal pyramidal cells (Segal and Bloom, 1974) to iontophoretically applied NE. Disconcerting however, were the findings of a supersensitive response of brain stem neurons to iontophoretic application of NE after reserpine, but not after FLA-63 or \( \alpha \)-mpt (Boakes et al., 1971).

MECHANISMS OF SUPERSENSITIVITY PHENOMENA IN THE CNS

In the central nervous system the mechanism of the supersensitivity phenomenon has only recently been a subject of investigation. It was expected that denervation of dopaminergic pathways could result in an increased activation of dopamine stimulated adenylate cyclase activity by exogenously administered dopaminetics. The increased activation of adenylate cyclase might be a mechanism of supersensitivity. Dopamine stimulated adenylate cyclase was postulated to be involved in central dopaminergic neurotransmission (Kebabian and Greengard, 1971). In fact, the activation of adenylate cyclase by dopamine was increased
following radio frequency or 6-OHDA induced lesions of the substantia nigra of rats (Mishra et al., 1974). However, others could not confirm, after intrastriatal 6-OHDA, any alteration in the DA stimulated activity of mouse striatal adenylate cyclase (Von Voigtlander et al., 1973). Most recently, an endogenous protein activator which regulates adenylate cyclase and phosphodiesterase activities was increased after chronic treatment with either α-mpt, reserpine or haloperidol (Gnegy et al., 1976); this activator might be involved in the development of dopamine receptor supersensitivity. Thus, it remains a problem for further research to determine the role of DA stimulated adenylate cyclase in dopamine receptor supersensitivity.

There are several other mechanisms which speculatively may be involved in the development of central dopamine receptor supersensitivity. Hormonal mechanisms may alter receptor responsiveness as was demonstrated for thyroxine and hypothalamic factors such as thyrotropin releasing factor (TRF) and melanocyte inhibiting factor (MIF) for responses to L-DOPA (Engström et al., 1974; Plotnikoff et al., 1971; Huidobro-Toro et al., 1975). Since an inhibition of protein synthesis blocks the effects of L-DOPA, it may be speculated that an increase in protein synthesis may induce a supersensitive response to L-DOPA (Tang et al., 1974). Decreases in either the amount or activity of
the recently discovered heat stable activator of phospho-
diesterase may also induce supersensitivity (Strada et al.,
1974). Moreover, the finding of prolonged activation of
dopaminergic receptors by cholera toxin (Miller and Kelly,
1975) and the increased sensitivity of mice to apomorphine
and clonidine after repeated electroconvulsive shock
(Modigh, 1975) suggest the possibility of other mechanisms
which control central dopamine receptor sensitivity. In
addition, other mechanisms, such as an increase in the
amount of neurotransmitter released (Sharpless, 1975) or
the taking over of function by another neurotransmitter
system (Longo, 1975) were also suggested but not yet ex-
plored.

In the central nervous system, the mechanism of
changes in receptor sensitivity was most extensively in-
vestigated in the pineal gland. A beta-adrenergic receptor
is thought to regulate the activity of N-acetyltransferase
(NAT) in the pineal gland (Brownstein et al., 1973). In-
formation about environmental lighting is conveyed to the
pineal via postganglionic sympathetic fibers which have
their cell bodies located in the superior cervical ganglion
(Kappers, 1960). Denervation via a bilateral superior cerva-
vical ganglionectomy resulted in a superinduction of NAT
activity by isoproterenol (Deguchi and Axelrod, 1972). The
supersensitivity was characterized as the postsynaptic type
which occurred as soon as 24 hours following denervation (Deguchi and Axelrod, 1972). Denervation of the pineal did not result in a supersensitive response to dibutyryl cyclic AMP. Thus, the mechanism of supersensitivity appeared to be located before the cAMP, "second messenger" step (Deguchi and Axelrod, 1973). Furthermore, a catecholamine induced increase in cAMP could not account for the supersensitivity since a supersensitive response of adenyl cyclase to catecholamines did not occur until four weeks after denervation (Deguchi and Axelrod, 1973). Thus, one may speculate that some other as yet undefined aspect of the NE-receptor interaction in the pineal could account for the supersensitivity observed after denervation. Possible, some involvement of both RNA and protein synthesis may be involved as in the case of the changes in the diurnal responsiveness of NAT to induction by catecholamines (Romero and Axelrod, 1975).

In other experiments the mechanism of supersensitivity to NE appeared to be more clearly related to an enhanced response of adenyl cyclase to norepinephrine and the subsequent elevation of cyclic AMP levels. For example, two independent groups have documented an enhanced accumulation of cyclic 3'-5' AMP in rat cerebral cortical slices following intraventricular 6-OHDA treatment (Kalisker et al., 1973; Huang et al., 1973). In fact, both an early
developing prejunctional as well as a later developing postjuncional type of supersensitivity were suggested in this preparation. Thus, the development of supersensitivity of rat cerebral cortical slices to NE partly resembles that observed in sympathetically innervated peripheral tissues after a postganglionectomy (Trandelenburg, 1966).

In contrast to the catecholaminergic neurotransmitter system, our understanding of the mechanism of supersensitivity of central cholinceptive receptors is minimal. At least in one case the central supersensitivity to cholinonimetics was shown to arise from a presynaptic loss of acetylcholinesterase (Bird and Aghajanian, 1975). Furthermore, we have practically no knowledge concerning the mechanisms of supersensitivity in central serotonin receptors.

THE APPROACH TAKEN TO ASSESS THE SELECTIVITY OF THE NEUROTOXICITY OF 6-OHDA AND 5,6-OHT

It can be expected on the basis of the preceding discussion that a selective neurotoxin may induce, at a particular dose, the depletion of a single neurotransmitter. Therefore, to neurochemically assess the selectivity of 6-OHDA and 5,6-OHT the brain content of four neurotransmitters
was measured. The brain contents of norepinephrine, dopamine, serotonin and acetylcholine were measured after each neurotoxin treatment in order to: a) verify the destruction of a particular neurotransmitter system as evidenced by a decline in the levels of DA and/or NE in the case of 6-OHDA or 5-HT in the case of 5,6-OHT; b) to assess the selectivity of destruction by investigating alterations in the brain content of several other neurotransmitters such as ACh and 5-HT in the case of 6-OHDA and DA, NE and ACh in the case of 5,6-OHT.

Since neuronal destruction is considered to result in changes in postsynaptic receptor sensitivity the use of postsynaptic agonists acting on the dopaminergic, serotonergic or cholinergic receptor sites would help to elucidate the selectivity of neuronal destruction induced by either 6-OHDA or 5,6-OHT.

The postsynaptic state of dopaminergic receptors was assessed by the use of apomorphine, a postulated direct DA receptor agonist (Ernst, 1969). Since apomorphine is not taken up by dopaminergic nerve terminals it would not be useful to demonstrate supersensitivity of the prejunctional type. However, apomorphine would be useful in demonstrating DA receptor supersensitivity of the postsynaptic type. Thus, any 6-OHDA or 5,6-OHT induced increase in the behavioral response to apomorphine was considered to be evidence
for the postsynaptic type of DA receptor supersensitivity.

The behavioral response to L-DOPA was used to test for the presence of the presynaptic type of DA receptor supersensitivity as L-DOPA's action depends upon the formation of dopamine (Levitt et al., 1965) which may be taken up by dopaminergic nerve terminals. Thus, supersensitivity to L-DOPA but not to apomorphine would be indicative of a presynaptic type of DA receptor supersensitivity.

Furthermore, as neuronal destruction may result in the development of subsensitivity, this was also evaluated in the case of the dopaminergic neurons with the use of methamphetamine. Since methamphetamine's action depends upon a presynaptic store of catecholamines (Moore et al., 1970), any decrease in the action of methamphetamine after neurotoxin treatment would be indicative of a presynaptic type of subsensitivity of dopamine receptors.

To test for supersensitivity of serotonergic receptors the administration of L-5-HTP, which induced head twitches and tremors in mice (Corne, 1963) was utilized. It has been considered that the response to L-5-HTP is a result of 5-HT formed in the brain stem following its decarboxylation (Corne, 1963). Moreover, the pharmacologic action of L-5-HTP does not depend upon the rate limiting tryptophan hydroxylase step of 5-HT synthesis (Grahame-Smith, 1971). Thus, L-5-HTP could activate the postsynap-
tic 5-HT receptors in the absence of serotonergic nerve terminals. However, any observed supersensitive response to L-5-HTP may also be of the presynaptic type; as in the absence of nerve terminals there would be impaired reuptake of the 5-HT formed from 5-HTP. In contrast, L-tryptophan would be a poor choice as an agonist to test for 5-HT receptor supersensitivity since it depends upon the presence of brain tryptophan hydroxylase which is located in the serotonergic neurons, and which may be depleted by neurotoxin treatments.

In addition, the status of central muscarinic cholinoceptive receptors was assessed with tremorine, a compound the action of which is believed to result from a direct interaction with that receptor (Everett and Blockus, 1956; Cox and Potkonjak, 1969b). Thus, any increase in the tremorigenic response to tremorine may reflect a supersensitivity of central muscarinic cholinoceptive receptors.

Behavioral analysis consisted of an examination of locomotor activity following administration of the neurotoxins since the catecholaminergic system appears to have a facilitatory role in this behavior. Destruction of the catecholaminergic system should be reflected as a depression of motor activity. Thus, changes in spontaneous locomotor activity may be a supportive argument but may not be the only argument (cf. above) indicative of a selective neurotoxin action on the catecholaminergic system.
Indeed, if either 6-OHDA or 5,6-OHT changed the brain content of more than one neurotransmitter, or changed the sensitivity of more than one postsynaptic receptor, then 6-OHDA or 5,6-OHT may be classified as non-specific neurotoxins and possibly as non-selective neurotoxins. All results must also be interpreted to account for possible direct interactions or interrelationships between several neurotransmitter systems. Such interactions may mimic a non-specific action and must be excluded as the causal factors of multineurotransmitter changes that can be observed following neurotoxin treatment.

Regeneration of axons and axon terminals was reported following 6-OHDA or 5,6-OHT treatments (cf. below). In the case of 6-OHDA, De Champlain (1971) reported a regrowth of adrenergic fibers in the rat after systemic administration of 6-OHDA. The regeneration of monoaminergic neurons after 6-OHDA was also observed in the central nervous system (Nygren et al., 1971). Similarly, regeneration and sprouting was observed following 5,6-OHT treatment (Bjorklund et al., 1973; Nobin et al., 1973). In the case of 5,6-OHT treatment it was suggested that a functional serotonergic reinervation takes place (Fuxe et al., 1973) long after the 5,6-OHT treatment. Because of the regeneration phenomena, the aforementioned pharmacological, neurochemical and behavioral tests were investigated at various times
after the administration of either 6-OHDA or 5,6-OHT in order to determine the permanence of any observed alterations in receptor sensitivity.

The following pages report the findings of this study as they relate to the selectivity of action of the neurotoxins 6-OHDA and 5,6-OHT after intracerebral administration to mice. The neurochemical, behavioral and pharmacological approaches eluded to above were employed in this study to assess the selectivity of the aforementioned neurotoxins.
METHODS

ANIMALS AND INTRACEREBRAL INJECTION TECHNIQUE

Male mice of the ICR strain (Indiana Farms) ranging in weight from 20 to 25 grams were used. Seven mice were housed per cage with food and water ad lib. The mice were maintained on a light-dark cycle of 14 hours of light (0600-2000 hrs.) and 10 hours of darkness (2000-0600 hrs.) throughout the study.

Either 5,6-dihydroxytryptamine (5,6-OHT), 6-hydroxydopamine (6-OHDA) or the vehicle were injected intracerebrally as described by Haley and McCormick (1957). The drugs were always injected in a volume of ten microliters using 2.5 mm long 27 gauge stainless steel needle attached to a 50 \( \mu l \) Hamilton microsyringe; the tip of the needle was ground to a slight (0.25 mm) bevel. 5,6-OHT and 6-OHDA were freshly prepared prior to injection in a 1:1000 aqueous ascorbic acid solution. 5,6-OHT or 6-OHDA were administered at the doses of 43 and 70 \( \mu g/\text{gm} \) of the base, respectively. These doses were found to produce gross behavioral changes in pilot experiments. They were also reported to be without extensive lethality and were used successfully in rats.
India ink, 10 microliters, was used to determine the distribution of the drug solution following the intracerebral injection (Haley and McCormick, 1957). India ink was found throughout the ventricular system and the surface of the cortical areas. It was assumed that the drug injections had a similar distribution pattern. It was further assumed that the uptake mechanism of the catecholamine containing nerve terminals and the distribution of the injected drugs were the critical factors with regard to their destruction.

The site of the intracerebral injection was estimated in mice sacrificed for neurochemical evaluation. The site of injection was found to be $1.7 \pm 0.06$ mm (S. E.) lateral to the sagittal suture and $0.35 \pm 0.08$ mm (S. E.) posterior to an imaginary perpendicular to the sagittal suture; the perpendicular intersected the sagittal suture at the point where the left and right coronal sutures intersected the sagittal suture (100 animals). On average, the tip of the injection cannula was located in the right lateral ventricle. When the ventricle was missed the other structures receiving the injection were primarily the lateral septum and the putamen.
LOCOMOTOR ACTIVITY

Leigh Valley photoactometers were used to quantify the locomotor activity of mice. Groups of six mice were placed in photoactometers and their activity was recorded every fifteen minutes for three hours.

In order to evaluate the effect of 6-OHDA and 5,6-OHT on locomotor activity following their intracerebral injection, two types of experiments were performed. One measurement of activity following the treatment with either 6-OHDA, 5,6-OHT or vehicle involved the repeated assessment of locomotion on alternate days for a total of 20 days after intracerebral injection. Another approach, designed to negate the conditioning effect of repeated measures of motor activity, involved the single assessment of motor activity in different groups of naive animals 1, 4, 10 and 20 days after intracerebral injection.

LOCOMOTOR RESPONSES TO METHAMPHETAMINE AND L-DOPA

The locomotor response to methamphetamine, 2.5 mg/kg, i.p., was recorded in a similar fashion (cf. above) utilizing repeated measures of locomotor activity in the same
group as well as single measures in different groups of mice. The locomotor activity was recorded every 15 minutes for 90 minutes prior to and 90 minutes subsequent to the administration of methamphetamine.

The locomotor response of 5,6-OHT, 6-OHDA and vehicle treated mice to L-DOPA (100 mg/kg, i.p.) were recorded in a similar fashion; a 90 minute pre L-DOPA measurement was combined with a 90 minute post L-DOPA measurement. The tests were carried out on the same groups of animals 1, 4, 10, and 20 days after intracerebral injection.

**EVERETT TEST**

While a monoamine oxidase inhibitor, pargyline, was utilized by Everett; it was not utilized in this present investigation. Evaluation of the behavior response of 5,6-OHT, 6-OHDA and vehicle treated mice to L-DOPA, 100 mg/kg, i.p., was carried out by means of the test introduced by Everett (1962, 1967). Everett's scoring system was employed. Mice were placed in groups of four per cage after L-DOPA administration, 100 mg/kg, i.p., and observed every ten minutes for one hour. The score of 1+, 2+ and 3+ were assigned to each animal. The evaluation was based on the presence of piloerection, exophthalmos, salivation, Straub tail grades, reactivity to external stimulation (character-
ized by jumping, squeaking and kicking), and of aggressive and stereotypic behaviors. For each group of mice the percentage of animals exhibiting a maximal score of 1+, 2+ and 3+ was calculated.

All mice were scored after 100 mg/kg, i.p. of L-DOPA. The 1+ response consisted of none or slight salivation, a slight or no increase in locomotor activity, and Straub tail grade +1 or +2. The mouse was classified as having Straub tail grade +1 when it held its tail parallel to the horizontal surface with the tip pointing caudally. The mouse was classified as having Straub tail grade +2 when it held its tail erect and perpendicular to the horizontal surface. The vehicle treated mice typically would show the 1+ response.

The 2+ response to L-DOPA consisted of distinct salivation, a definite increase in motor activity, Straub tail grade +3, and increased reactivity to external stimuli characterized by darting or aggressive posturing. The mouse was classified as having Straub tail grade +3 when it held its tail arched over its back and with the tip pointing rostrally. The 2+ response to DOPA was observed only occasionally in vehicle treated mice, primarily at the early time after intracerebral injection, eg. 4 hours.

The 3+ response to L-DOPA, 100 mg/kg, i.p., consisted of marked salivation, a marked increase in locomotion
characterized by episodic or continuous running or darting, Straub tail grade +3, spontaneous jumping, stereotypic behavior, aggressive posturing and marked hyperirritability expressed by squeaking, jumping, kicking, darting, running and aggressive attack in response to external stimuli. The external stimuli consisted of lifting the cage top and lightly touching the side of each mouse with the side of a glass stirring rod. Mice which did not exhibit hyperirritability would tolerate the external stimuli, as following the stimulus, the mouse would walk away and resettle in a different corner of the cage. Vehicle treated mice never exhibited 3+ L-DOPA response.

BEHAVIORAL RESPONSE TO APOMORPHINE

The response of 5,6-OHT, 6-OHDA and vehicle treated mice to apomorphine, 40 mg/kg, i.p., was evaluated by observing spontaneous jumping, stereotypic climbing behavior, rigid gait, mouthing of sawdust and licking of the cage; in addition, some mice reacted to external stimuli by jumping, kicking and running. In an all or none fashion, the percentage of mice exhibiting hyperreactivity (the running, kicking and jumping response to external stimuli) and/or spontaneous jumping behavior were recorded and classified as having a supersensitive response to apomorphine. In
contrast to the supersensitive response of neurotoxin pretreated mice, the vehicle treated animals exhibited only stereotypic climbing behavior, licking of the cage and mouthing of sawdust along with slightly increased motor activity. Observation continued for one hour after apomorphine; after this time the mice appeared normal.

QUANTIFICATION OF TREMORS

Tremors elicited by tremorine (10 mg/kg, i.p.) or L-5-HTP (200 mg/kg) were quantified 4 days, 10 days and 20 days following intracerebral injection of 6-OHDA, 5,6-OHT or ascorbic acid vehicle. A single mouse was placed in a small plastic cage, suspended from a Grass FT-10 force transducer. The tremors were recorded on one channel of a Beckman Dynograph and integrated on another channel. The average number of integrator resets per minute during four respective five minute periods was calculated for each animal 5, 15, 30 and 60 minutes following the administration of either tremorine or L-5-HTP. The neurotoxin induced changes in the tremorigenic effect of tremorine were estimated by comparing the mean integrated tremor responses for each of the three groups.

Besides inducing tremor, L-5-HTP administration (200 mg/kg, i.p.) elicited additional symptoms which included
head-shaking, abnormal gait and body position (spread eagle posture) and retrograde movements; marked flushing of ears and paws could be readily observed; finally, when 5-HTP was given to 5,6-OHT pretreated mice, death occasionally resulted. The fraction of animals in which the presence or absence of these symptoms could be observed was evaluated in 5,6-OHT and vehicle pretreated animals following 5-HTP challenge both in the presence or absence of a decarboxylase inhibitor, RO 4-4602/1.

**NEUROCHEMICAL METHODS**

For the measurement of the bioamines the mice were sacrificed by decapitation, always between 7:00 and 11:00 A.M. to minimize diurnal influences. Brain levels of dopamine (DA), norepinephrine (NE), serotonin (5-HT) and acetylcholine (ACh) were measured in the portion of the brain composed of the cerebral hemispheres, basal ganglia, thalamus, hypothalamus, corpora quadrigemina and midbrain; the olfactory bulbs, cerebellum, pons and medulla were discarded (Figure 1). The sample was rinsed, blotted, weighed and placed in a liquid nitrogen within one minute of decapitation.

A modification of the ion exchange-column chromatographic method of Atack and Magnusson (1970) was used for
FIGURE 1. A sagittal view of the mouse brain. The dashed lines indicate the planes in which cuts were made to isolate the brain part used for neurochemical analysis. The portion of tissue between the dashed lines was used for the analysis.
the separation of NE, DA and 5-HT. Essentially, the spectrophotofluorometric methods of Bertler et al. (1958a, b), Carlsson and Waldeck (1958) and Maickel et al. (1967) were employed for the determination of NE, DA and 5-HT, respectively as described in detail below. Four brains were pooled for each determination of NE, DA and 5-HT.

After extraction with formic acid and electrophoretic separation, ACh was determined by the choline kinase method of Reid et al. (1971) as described in detail below. ACh determinations were carried out on single brains.

**EXTRACTION OF THE CATECHOLAMINES AND SEROTONIN**

The tissue was homogenized in 7.8 ml of 0.4 N perchloric acid (PCA) and 0.2 ml of 2% ascorbic acid; an additional 3 ml of 0.4 perchloric acid (PCA) was used to rinse the homogenizing pestle into the centrifuge tube. The homogenate was kept on ice and centrifuged at 3,000 RPM for 15 minutes. The supernatant was decanted and filtered through Whatman glass fiber filter paper. The filtrate volume was recorded and each sample was adjusted to pH 6.5. The neutralized extract was placed in -4 °C cold for 10 minutes. Following cooling the samples were centrifuged for 10 minutes at 3,000 RPM. The resultant supernatant was decanted onto the previously prepared columns utilizing the
procedure of Bertler et al. (1958).

**PREPARATION OF RESIN FOR THE ELUTION OF CATECHOLAMINES AND SEROTONIN**

The BioRad AG50W -4X, 200-400 mesh H⁺ form (Strong Cation Exchange) resin was batch processed in the following way prior to the preparation of the columns. 100 Gms. of resin was stirred with 100 ml of 2 N NaOH for 20 minutes. Triple distilled water was used in 100 ml quantities to wash away the NaOH until the final pH was less than 9.5. Subsequently, 100 ml of 2 N HCl was stirred with the resin for 20 minutes. This was followed by triple distilled water washes until the pH was greater than 5. The final washing was with 100 ml of redistilled ethanol; the mixture was stirred for 20 minutes, followed by triple distilled water washes until the odor of ethanol was no longer apparent. The resin was packed into columns with triple distilled water. A volume of 20 ml of 0.1 M phosphate buffer at pH 6.5 containing 0.1% EDTA was passed through each column prior to use. The elution procedure described below was followed.
ELUTION OF THE CATECHOLAMINES AND SEROTONIN

The amines were eluted according to the method of Atack and Magnusson (1970).

The sample was passed through the BioRad AG50W resin (4X, 200-400 mesh) at 1 drop per 10 seconds followed by 40 ml of triple glass distilled water at 1 drop every 5 seconds. Eleven ml of 1 N HCl was added and allowed to flow at 1 drop per 10 seconds. The first 3 ml of this HCl eluate were discarded and the subsequent 8 ml were collected and contained NE.

Following the elution of NE 13 ml of 1 N (50% ethanolic) HCl was allowed to pass through the column at a flow rate of 1 drop per 7 seconds. The first ml of the ethanolic HCl eluate was discarded. The next 5 ml were collected and contained DA; the next 7 ml were also collected and contained 5-HT.
ASSAY OF NOREPINEPHRINE

The trihydroxyindole technique of Bertler et al. (1958) was used. The following protocol was used.

<table>
<thead>
<tr>
<th></th>
<th>STANDARD</th>
<th>REAGENT BLANK</th>
<th>TISSUE SAMPLE</th>
<th>INTERNAL STANDARD</th>
<th>TISSUE BLANK</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled H₂O</td>
<td>3.80</td>
<td>3.90</td>
<td>2.90</td>
<td>2.80</td>
<td>2.90</td>
</tr>
<tr>
<td>0.1 M PO₄ buffer pH 6.5</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>sample eluate at pH 6.5</td>
<td>----</td>
<td>----</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>NE standard (1μg/m)</td>
<td>0.10</td>
<td>----</td>
<td>----</td>
<td>0.10</td>
<td>----</td>
</tr>
<tr>
<td>0.5% ZnSO₄</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
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</tr>
<tr>
<td>0.25% K₃Fe(CN)₆</td>
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<td>0.05</td>
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</tr>
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5 MINUTE WAIT

<table>
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<th>INTERNAL STANDARD</th>
<th>TISSUE BLANK</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% ascorbic acid, 5N NaOH</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
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<tr>
<td>5N NaOH</td>
<td>----</td>
<td>----</td>
<td>----</td>
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10 MINUTE WAIT

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<th>INTERNAL STANDARD</th>
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</tr>
</thead>
<tbody>
<tr>
<td>2% ascorbic acid</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>0.05</td>
</tr>
</tbody>
</table>
samples were placed in an Aminco Bowman spectrophotofluorometer and read with wave lengths for excitation of 400 m\(\mu\) and for emission of 505 m\(\mu\).

**ASSAY OF DOPAMINE**

A modification of the dihydroxyindole technique of Carlsson and Waldeck (1958) as modified by Glisson et al. (1972) was used. The following protocol was used for the development of the fluorophore.

<table>
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<tr>
<th></th>
<th>STANDARD</th>
<th>REAGENT</th>
<th>TISSUE</th>
<th>INTERNAL</th>
<th>TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Ethanolic HCl lN pH 5.4</td>
<td>2.00</td>
<td>2.00</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>distilled H(_2)O</td>
<td>1.05</td>
<td>1.30</td>
<td>1.30</td>
<td>1.05</td>
<td>1.30</td>
</tr>
<tr>
<td>citrate PO(_4) buffer pH 5.4</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>DA standard (1 (\mu)gm/ml)</td>
<td>0.25</td>
<td>----</td>
<td>----</td>
<td>0.25</td>
<td>----</td>
</tr>
<tr>
<td>sample (un-neutralized)</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>1.65</td>
</tr>
<tr>
<td>sample-pH 5.4</td>
<td>----</td>
<td>----</td>
<td>2.00</td>
<td>2.00</td>
<td>----</td>
</tr>
<tr>
<td>NaSO(_3)-NaOH</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>0.50</td>
</tr>
<tr>
<td>0.02 N Iodine</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
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**5 MINUTE WAIT**

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<th>Na(_2)SO(_3)-NaOH</th>
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**5 MINUTE WAIT**

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<tr>
<th></th>
<th>5N Acetic Acid</th>
<th>1.60</th>
<th>1.60</th>
<th>1.60</th>
<th>1.60</th>
<th>1.60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5N K(_2)CO(_3)</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Samples were heated at 100°C for fifteen minutes, cooled and the fluorescence read in an Aminco Bowman spectrophotofluorometer. The fluorescence wave lengths used were 330 m\(\mu\) for excitation and 380 m\(\mu\) for emission.

**ASSAY OF SEROTONIN**

The o-phthalaldehyde (OPT) technique of Maickel et al. (1968) was used. Briefly, 0.5 ml of the 5-HT eluate was mixed with 1 ml of 4 mg% OPT in 10 N HCl. The resultant mixture was heated at 100°C for ten minutes, cooled to room temperature and read in the Aminco Bowman spectrophotofluorometer with the wave lengths of excitation at 360 m\(\mu\) and emission at 470 m\(\mu\). The slit arrangement used was: excitation slit 2, cuvette slit 5, emission cuvette slit 2, emission slit 5, phototube slit 1.

**EXTRACTION AND ASSAY OF ACETYLCHOLINE**

The technique of Haubrich and Reid (1973) was utilized. The ACh and choline were extracted from brain tissue with a 15% solution of 1N formic acid in acetone (5 ml per gram of tissue). A second extraction was carried out with a 10% solution of 1N formic acid in acetone (1 ml per gram of tissue). The two extract supernants were com-
bined with an equal volume of water saturated ether and shaken to extract the acetone into the ether. The organic layer was then aspirated and discarded. The solvents remaining in the aqueous phase were blown off with a stream of air. After the extract was centrifuged to remove particulate matter the resulting supernatant was lyophylized.

The lyophylized extract was then dissolved in 20 ml of distilled water. 15 ml of the clear redissolved extract was spotted in three equal aliquots on Whatman 3 MM chromatographic paper allowing for drying between each spotting. The paper was also spotted with marker spots of choline and acetylcholine as well as internal standards. The paper was placed in a flat plate electrophoresis unit with the origin toward the positive electrode. The paper then was moistened with a brush so that the electrophoresis pyridine - formic acid buffer approached each side of the origin simultaneously. The electrophoresis unit was set to run at 800 volts for 2-3 hours.

Following electrophoresis the paper was air dried. The marker spots were identified by exposure to iodine vapor. After marking the location of these spots with a pencil the iodine was removed from the paper by exposure to steam. A 20x25 mm template was used to mark off the sample spots. The paper was cut and rolled into a tight cylinder with forceps. One ml of distilled water was added to a
culture tube containing the paper to elute the choline and acetylcholine by agitating with a vortex mixer for one minute and allowing it to stand for fifteen minutes.

Acetylcholine was hydrolysed as a 0.5 ml aliquot of the electrophoresis paper eluate was mixed with 10 μl of concentrated ammonium hydroxide. The resultant mixture was heated for twenty minutes on a boiling water bath. The samples were then dried in a vacuum at 50-60°C.

Following drying, 0.1 ml of a choline kinase and ATP solution containing ATP- λ-32 P (yielding approximately 1x10⁵ - 3x10⁵ CPM per 0.1 ml) was added to the sample tubes. The tubes were agitated to dissolve the choline. The samples were then incubated at 37°C for 2 to 4 hours. Ten μl containing 100 μg of phosphorylcholine chloride was added to each sample as a carrier. 1.5 ml of pH 10-Tris-MgSO₄ buffer was also added to each sample. The contents of the sample tubes were added to a column containing Dowex I anion exchange resin (chloride form, 8% cross linked, dry mesh 200-400). An additional 4.5 ml of pH 10-Tris-MgSO₄ buffer was also added to the column as a wash. The total effluent and eluate were collected and counted in a liquid scintillation counter. The brain content of ACh was calculated using the internal and external standards processed in parallel with the samples.
L-DOPA was dissolved with warming in sufficient 0.1 N HCl and distilled water to produce a 10 mg/ml solution at pH 3.5; RO 4-4602/1 and L-5-HTP were dissolved in water with warming. Tremorine HCl and methamphetamine HCl were dissolved in 0.9% saline. Apomorphine HCl was dissolved in 1:1,000 aqueous ascorbic acid. All drugs were administered intraperitoneally. Methamphetamine HCl, L-DOPA, L-5-HTP and DL-alpha-methyl tyrosine methyl ester HCl were purchased from the Sigma Chemical Company. RO 4-4602/1 and tremorine were graciously supplied by Roche, Inc., Nutley, New Jersey and Abbott Labs, Abbott Park, Illinois respectively. Apomorphine HCl was purchased from Mørck, Inc. Commercially available USP grade ascorbic acid was employed. Both 6-hydroxydopamine HBr and 5,6-dihydroxytryptamine creatinine sulfate dihydrate were purchased from the Regis Chemical Company, Morton Grove, Illinois.
Dr. T. Hoffman of the biostatistical department of Lederle Laboratory in Pearl River, New York served as the statistical consultant. The following statistical procedures were utilized. The "SAS" statistical analysis system developed by Anthony James Barr and James Howard Goodnight of the Department of Statistics, North Carolina State University, Raleigh, North Carolina was utilized on an IBM 360 computer. The neurochemical data were analysed by analysis of variance and multiple comparisons were made by means of the Newman-Keuls multiple range test. Repeated measures of motor activity were analysed by a least squares regression and analysis of variance. The locomotor activity data derived from the single test procedure were also analysed by analysis of variance and the mean activity of the groups were compared with the Newman-Keuls multiple range test. Significance of the occurrence of supersensitivity was assessed in both the Everett test and the apomorphine challenge test by means of the Chi-square statistic. The quantitative integrated readings of tremorigenic activity following either oxotremorine or L-5-HTP were analysed by means of the Mann Whitney U-test as well as the Kruskal-Wallis test.
RESULTS

NEUROCHEMICAL FINDINGS

The values for the various amines were determined in the contiguous brain part which included the cerebral hemispheres, basal ganglia, thalamus, hypothalamus, corpora quadrigemina and midbrain, hereafter referred to as "brain"; the brain parts which were not analyzed were the olfactory bulbs, cerebellum and pons-medulla (Figure 1).

SEROTONIN

Mouse brain levels of serotonin were altered by the intracerebral administration of either 6-OHDA or 5,6-OHT. The brain levels of 5-HT in vehicle treated mice were not found to differ significantly on any of the days tested. When compared to the vehicle treated animals, both 6-OHDA and 5,6-OHT significantly decreased the brain levels of serotonin; these decreased serotonin levels were observed on each of the days tested. Four, 10 and 20 days after the intracerebral injection of 70 µgm of 6-OHDA, mouse brain levels of 5-HT were decreased by 22% (p<0.05), 39% (p<0.01) and 38% (p<0.01), respectively. On the 4th,
<table>
<thead>
<tr>
<th></th>
<th>4 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5-HT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC</td>
<td>0.49 ± 0.03 (19)</td>
<td>0.59 ± 0.03 (16)</td>
<td>0.55 ± 0.02 (16)</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>0.38 ± 0.03 (7)</td>
<td>0.36 ± 0.05 (8)</td>
<td>0.34 ± 0.01 (8)</td>
</tr>
<tr>
<td>5,6-OHT</td>
<td>0.23 ± 0.06 (12)</td>
<td>0.29 ± 0.02 (8)</td>
<td>0.33 ± 0.04 (8)</td>
</tr>
<tr>
<td><strong>DA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC</td>
<td>1.22 ± 0.18 (25)</td>
<td>1.24 ± 0.09 (15)</td>
<td>1.04 ± 0.08 (15)</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>0.46 ± 0.10 (11)</td>
<td>0.37 ± 0.06 (8)</td>
<td>0.59 ± 0.06 (6)</td>
</tr>
<tr>
<td>5,6-OHT</td>
<td>0.55 ± 0.10 (16)</td>
<td>1.24 ± 0.14 (7)</td>
<td>0.89 ± 0.05 (8)</td>
</tr>
<tr>
<td><strong>NE</strong></td>
<td></td>
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<tr>
<td>ASC</td>
<td>0.56 ± 0.01 (23)</td>
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<td>0.52 ± 0.05 (20)</td>
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<td>6-OHDA</td>
<td>0.05 ± 0.01 (8)</td>
<td>0.20 ± 0.01 (8)</td>
<td>0.14 ± 0.01 (13)</td>
</tr>
<tr>
<td>5,6-OHT</td>
<td>0.17 ± 0.03 (15)</td>
<td>0.46 ± 0.03 (8)</td>
<td>0.62 ± 0.12 (8)</td>
</tr>
</tbody>
</table>
LEGEND FOR TABLE 1.

Mouse brain levels of 5-HT, DA, and NE in $\mu$gm/gm wet weight on various days after an intracerebral injection with either 6-OHDA, 5,6-OHT or ascorbic acid vehicle. Four brains were pooled for each determination. The number of determinations are indicated within the parenthesis. Significance of differences were determined by the Newman-Keuls multiple range test.
10th and 20th days after the intracerebral injection of 43 μg of 5,6-OHT, the mouse brain levels of 5-HT were found to be decreased by 53% (p<0.01), 51% (p<0.01) and 40% (p<0.01), respectively. There were no significant differences in the degree of serotonin depletion on the 4th, 10th or 20th days after 6-OHDA. Furthermore, there were no significant differences in the degree of serotonin depletion on the 4th, 10th and 20th days after 5,6-OHT (Table 1). Thus, both 6-OHDA and 5,6-OHT treatments resulted in prolonged decreases in mouse brain serotonin content.

DOPAMINE

Mouse brain levels of DA were differentially altered by the intracerebral injection of either 6-OHDA or 5,6-OHT. The levels of DA in vehicle treated mice were not found to differ significantly from each other on any of the days tested. Four, 10 and 20 days after the intracerebral injection of 70 μg of 6-OHDA, the mouse brain levels of DA were 62%, 70% and 43% below the levels observed in the vehicle treated mice, respectively. These reductions in brain DA levels by 6-OHDA were at the p<0.01 level of statistical significance. Furthermore, the decreased brain DA levels which were observed in the 6-OHDA treated animals
on the 4th, 10th and 20th post-treatment days did not differ significantly from each other. Thus, the intracerebral injection of 6-OHDA induced a prolonged and sustained depletion of DA in the mouse brain.

The depletion of brain DA by the 5,6-OHT treatment was not as long lasting as that induced by the 6-OHDA treatment (Table 1). Only on the 4th day after the intracerebral injection of 43 μgm of 5,6-OHT, was the mouse brain level of DA significantly below that of the vehicle treated mice (p < 0.01). Furthermore, on the 10th and 20th days after 5,6-OHT treatment, the level of DA was significantly greater than that observed on the 4th day after 5,6-OHT treatment (p < 0.05).

**norepinephrine**

The level of NE in vehicle treated mice was not found to differ significantly on any of the days tested. Mouse brain levels of NE were differentially altered after the intracerebral injection of either 6-OHDA or 5,6-OHT. In those mice which had received the 6-OHDA treatment, the levels of NE in brain were significantly below those of mice which had received isovolumetric, intracerebral injections of the ascorbic acid vehicle. The 6-OHDA treatment resulted in reductions of the NE content in brain by
91%, 63% and 73% respectively, on the 4th, 10th and 20th days after the injection (all the values were significantly below the control values at the level of \( p < 0.01 \)). 6-OHDA depleted the brain level of NE to a greater extent on the 4th as compared to the 10th post-treatment day \( (p < 0.05) \). Thus, 6-OHDA when administered intracerebrally to mice induces a prolonged depletion of brain NE content.

Similar to the 5,6-OHT effect on the brain DA levels and as compared to the effect of 6-OHDA, the depletion of brain NE by the 5,6-OHT treatment was not as long lasting as that induced by the 6-OHDA treatment (Table 1). The mouse brain level of NE was significantly below that of vehicle treated mice only on the 4th day after the intracerebral injection of 5,6-OHT. Furthermore, the levels of NE in the 5,6-OHT treated mice were significantly greater on the 10th and 20th days after treatment than those observed in mice which were tested 4 days after 5,6-OHT treatment. In addition, the levels of NE in the 5,6-OHT treated mice were also significantly greater than those observed in the 6-OHDA treated mice on both the 10th and 20th post-treatment days. Therefore, 5,6-OHT treatment depleted the brain content of NE in a transient manner with the levels returning to normal by the 10th day after treatment. This transient effect of 5,6-OHT treatment on brain NE content is in direct contrast to the prolonged (up to
20 days) depletion of brain NE which was induced by the 6-OHDA treatment.

**ACETHYCHOLINE**

The intracerebral injection of 70 μg of 6-OHDA or 43 μg of 5,6-OHT did not alter the mouse brain levels of ACh as compared to the vehicle treated mice (Table 2). The ACh data obtained following 6-OHDA treatment with the electrophoretic technique of Reid et al. (1971), confirm those previously reported by Barnes et al. (1973a, b) obtained with a bioassay method of ACh analysis.

**AMINES AFTER L-DOPA**

The i.p. administration of L-DOPA (100 mg/kg) lowered the brain 5-HT levels (p < 0.05) of mice which had received, 4 days earlier, an intracerebral injection of either ascorbic acid vehicle or 5,6-OHT (Table 3). L-DOPA did not appear to affect the brain levels of 5-HT in mice which had been pretreated with 6-OHDA. The 5-HT depleting action of L-DOPA has been previously reported (Bartholini, Da Prada and Pletscher, 1968; Butcher and Engel, 1969; Everett and Borcherding, 1970).

The mouse brain levels of DA were significantly
### TABLE 2

**BRAIN LEVELS (IN NANOM/GM) OF ACHE FOLLOWING**

**6-OHDA AND 5,6-OHT TREATMENT**

<table>
<thead>
<tr>
<th></th>
<th>4 DAYS</th>
<th>4 DAYS + L-DOPA</th>
<th>4 DAYS + 5-HTP</th>
<th>20 DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.5 + 0.6 (22)</td>
<td>15.3 + 1.3 (16)</td>
<td>22.2 + 2.1 (17)</td>
<td>15.6 + 1.1 (19)</td>
<td></td>
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<tr>
<td><strong>6-OHDA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.0 + 0.9 (11)</td>
<td>14.5 + 1.2 (12)</td>
<td>21.6 + 0.9 (11)</td>
<td>16.5 + 2.1 (12)</td>
<td></td>
</tr>
<tr>
<td><strong>5,6-OHT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.4 + 1.4 (19)</td>
<td>15.6 + 1.3 (16)</td>
<td>16.3 + 2.5 (15)</td>
<td>15.8 + 0.9 (8)</td>
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</table>
LEGEND FOR TABLE 2.

Mouse brain content of ACh in nM/Gm fresh weight 4 and 20 days after the intracerebral injection of either 6-OHDA (70 μgm/10 μl), 5,6-OHT (43 μgm/10 μl) or 10 μl of 0.1% ascorbic acid vehicle. Single mouse brains were used for each determination. The number of determinations are indicated in the parenthesis. Neither 6-OHDA nor 5,6-OHT affected the levels of ACh 4 or 20 days after i.c. injection. L-DOPA 100 mg/kg, i.p., 15 minutes prior to sacrifice did not affect the brain levels of ACh, in either 6-OHDA, 5,6-OHT or vehicle treated mice. L-5-HTP 200 mg/kg, i.p. 15 minutes prior to sacrifice increased the brain levels of ACh in vehicle treated (ASC) and 6-OHDA treated mice but not in 5,6-OHT treated mice (p < 0.025).
<table>
<thead>
<tr>
<th></th>
<th>4 DAYS</th>
<th>4 DAYS + L-DOPA</th>
<th>4 DAYS + L-5-HTP</th>
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<tbody>
<tr>
<td><strong>5-HT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC</td>
<td>0.49 ± 0.03 (19)</td>
<td>0.27 ± 0.1 (19)</td>
<td>0.93 ± 0.14 (16)</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>0.38 ± 0.03 (7)</td>
<td>0.41 ± 0.13 (8)</td>
<td>0.76 ± 0.19 (8)</td>
</tr>
<tr>
<td>5,6-OHT</td>
<td>0.23 ± 0.06 (12)</td>
<td>0.10 ± 0.01 (12)</td>
<td>0.70 ± 0.17 (8)</td>
</tr>
<tr>
<td><strong>DA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC</td>
<td>1.22 ± 0.18 (25)</td>
<td>1.87 ± 0.22 (24)</td>
<td>1.23 ± 0.10 (14)</td>
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<tr>
<td>6-OHDA</td>
<td>0.46 ± 0.10 (11)</td>
<td>0.74 ± 0.09 (7)</td>
<td>0.68 ± 0.06 (8)</td>
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<td>5,6-OHT</td>
<td>0.55 ± 0.10 (16)</td>
<td>1.60 ± 0.24 (16)</td>
<td>1.31 ± 0.08 (8)</td>
</tr>
<tr>
<td><strong>NE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASC</td>
<td>0.56 ± 0.01 (23)</td>
<td>0.56 ± 0.06 (24)</td>
<td>0.69 ± 0.08 (18)</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>0.05 ± 0.01 (8)</td>
<td>0.41 ± 0.06 (8)</td>
<td>0.31 ± 0.03 (12)</td>
</tr>
<tr>
<td>5,6-OHT</td>
<td>0.17 ± 0.03 (15)</td>
<td>0.26 ± 0.03 (16)</td>
<td>0.37 ± 0.04 (7)</td>
</tr>
</tbody>
</table>
**LEGEND FOR TABLE 3.**

Mouse brain levels of NE, DA and 5-HT in µgm/Gm. wet weight four days after intracerebral injection with either 6-OHDA, 5,6-OHT or ascorbic acid vehicle. Brain bioamine levels are also listed for animals which had received either L-DOPA, 100 mg/kg, i.p. or L-5-HTP, 200 mg/kg, i.p. fifteen minutes prior to sacrifice. Four brains were pooled for each determination. The number of determinations are indicated within the parenthesis. Significance of differences were determined by the Newman-Keuls multiple range test.
elevated \((p < 0.01)\) 15 minutes after L-DOPA administration (100 mg/kg, i.p.) in mice which had received an intracerebral injection, 4 days previously, of vehicle or 5,6-OHT. Although the increase in brain dopamine after L-DOPA in 6-OHDA treated mice was not statistically significant in this study, we have previously reported (Barnes et al., 1973b) that L-DOPA can elevate the depleted brain levels of DA in 6-OHDA treated mice. The evidence indicates that L-DOPA treatment can increase the brain levels of DA in 6-OHDA and 5,6-OHT treated mice, but the levels of DA did not exceed those of vehicle treated mice (Table 3) (Barnes et al., 1973b,c). Thus, the depletion of DA 4 days after intracerebral injection of 5,6-OHT and of 6-OHDA 2 and 10 days after intracerebral injection can be antagonized and the DA somewhat elevated by L-DOPA administration. Without further studies it cannot yet be established whether the maximal increases in brain DA induced by L-DOPA administration to vehicle treated mice can, in fact, be achieved in mice pretreated with the neurotoxins 6-OHDA or 5,6-OHT; no other published study has, as of yet, addressed itself to this question.

The mouse brain levels of NE were also elevated 15 minutes after L-DOPA administration (100 mg/kg, i.p.) in mice which had previously received an intracerebral injection, 4 days earlier, of 6-OHDA \((p < 0.05)\). After L-DOPA
administration the brain content of NE in 6-OHDA treated mice was not significantly different from that of vehicle treated mice, suggesting a restoration by L-DOPA of the brain NE content of 6-OHDA treated mice to normal or near normal levels. Although the 5,6-OHT treated animals also exhibited an increase in the brain content of NE after L-DOPA, that increase was not statistically significant. In fact, after L-DOPA the 5,6-OHT treated mice still had a brain level of NE which was significantly below that of vehicle treated mice (p < 0.05) (Table 3). Control mice which were treated with the ascorbic acid vehicle did not exhibit NE elevation after L-DOPA administration. Thus, the difference in the capacity to restore the brain content of NE of the 5,6-OHT and 6-OHDA treated mice in response to L-DOPA administration may, in part, explain the differences in behavioral response of these mice to L-DOPA; these results will be described in a subsequent section.

The i.p. administration of L-DOPA (100 mg/kg) did not affect the brain content of ACh mice which had, 4 days previously, received intracerebral injections of either 6-OHDA, 5,6-OHT or vehicle (Table 2).

**AMINES AFTER 5-HTP**

Fifteen minutes after the injection of L-5-HTP (200
mg/kg, i.p.), the brain content of 5-HT was increased in mice which received, 4 days previously, an intracerebral injection of either vehicle, 6-OHDA or 5,6-OHT (Table 3). The L-5-HTP treatment increased the 5-HT content of vehicle treated mice by 90% ($p < 0.01$), of 6-OHDA treated mice by 100% ($p < 0.05$) and of 5,6-OHT treated mice by 204% ($p < 0.01$). Thus one may conclude that the pretreatment with either ascorbic acid vehicle, 6-OHDA or 5,6-OHT, in the dose employed, did not change the ability of L-5-HTP to increase the brain content of 5-HT. After L-5-HTP the levels of 5-HT in 6-OHDA or 5,6-OHT treated mice were not significantly different; a fact which may account for a lack of difference between these groups in their behavioral response to L-5-HTP (cf. below).

L-5-HTP administration did not alter the levels of DA in mice which, 4 days earlier, received an intracerebral injection of vehicle or 6-OHDA. After L-5-HTP administration, the content of DA in brains of mice which received 6-OHDA was still below that of mice had received vehicle treatment ($p < 0.05$). An unusual result was that after L-5-HTP, the level of DA was increased in 5,6-OHT treated mice when compared to 5,6-OHT treated mice which were not given L-5-HTP. Everett (1974) had reported that 5-HTP does not markedly affect the levels of DA in mice which did not receive either 6-OHDA or 5,6-OHT.
Fifteen minutes after the administration of L-5-HTP (200 mg/kg, i.p.) the brain levels of NE were not significantly altered in vehicle or 5,6-OHT treated mice. Although the brain NE content increased after L-5-HTP in 6-OHDA treated mice ($p < 0.05$) the NE level was still markedly below that of mice which had received an intracerebral injection of the vehicle and, 4 days later, 5-HTP ($p < 0.01$) (Table 3). Thus, L-5-HTP administration (200 mg/kg, i.p.) could not restore the brain content of NE to normal levels in mice which had been depleted of NE by prior intracerebral treatment with either 6-OHDA or 5,6-OHT.

L-5-HTP (200 mg/kg, i.p.) increased the mouse brain content of ACh in animals which had received, 4 days previously, an intracerebral injection of vehicle or 6-OHDA (Table 2). After L-5-HTP, the content of ACh in mouse brain was increased by 53% ($p < 0.025$) in vehicle treated animals and by 66% ($p < 0.025$) in 6-OHDA treated animals. L-5-HTP was without effect on the brain content of ACh in mice which, 4 days previously, had received an intracerebral injection of 5,6-OHT (Table 2). These results suggest an interaction between the serotonergic and the cholinergic systems and require further study.
BEHAVIORAL FINDINGS

LOCOMOTOR ACTIVITY - SINGLE TEST PROCEDURE

The locomotor activity of mice was assessed at various times after 6-OHDA, 5,6-OHT or vehicle treatment. In the single test procedure naive mice were exposed only once to the locomotor activity chamber. The locomotor activity of mice in the single test procedure was markedly depressed on the 1st day following the intracerebral injection of either 6-OHDA or 5,6-OHT in doses of 70 or 43 μgm/10 μl, respectively (Figure 2). When compared on the 1st post-treatment day to the ascorbic acid treated groups, the 6-OHDA and 5,6-OHT treated groups exhibited a depression of spontaneous locomotor activity which was statistically significant to the \( p < 0.001 \) level. The activity of the 6-OHDA treated groups were not significantly different from that of the vehicle treated animals on the 4th, 10th and 20th post-treatment days. Thus, the locomotor activity of 6-OHDA treated mice recovered completely by the 4th day after treatment. However, the depression of locomotor activity lasted longer in 5,6-OHT treated animals. Although some recovery was evident as on day 4, the activity was significantly greater than on day 1 (\( p < 0.005 \)), the 5,6-OHT treated animals did not show any additional in-
RECOVERY OF LOCOMOTOR ACTIVITY AFTER 6-OHDA and 5,6-OHT
SINGLE TEST

DAY 1  DAY 4  DAY 10  DAY 20

COUNTS x 1000

MINUTES

Fig. 2
The cumulated locomotor activity of mice, recorded at fifteen minute intervals for three hours on various days following intracerebral injection of ascorbic acid vehicle (Asc), 6-OHDA or 5,6-OHT. Each point represents the mean locomotor activity of three groups of six mice; S.E.M. values are shown for the cumulative activity of 180 minutes. Different groups of mice were employed on each day ("single test"). The Newman-Keuls multiple range test as applied to a two factor ANOV was used to determine the significance of the differences from control values at three hours. On day 1 both 5,6-OHT and 6-OHDA treated groups were significantly less active than controls (***; p < 0.001). Only the 5,6-OHT treated mice were significantly less active than controls on days 4 and 10 (*: p < 0.05); there were no significant differences between the three groups on day 20.
REGRESSION OF LOCOMOTOR ACTIVITY AFTER VEHICLE, 6-OHDA, OR 5,6-OHT TREATMENT

Fig. 3
LEGEND FOR FIGURE 3.

The locomotor activity of mice was recorded for a total of 90 minutes on various days after intracerebral injection of either ascorbic acid vehicle, 70 μgm of 6-OHDA or 43 μgm of 5,6-OHT. The same mice were tested every other day beginning with the 1st day after intracerebral injection ("repeated" test). Each point represents the mean locomotor activity of 3 groups of 6 mice. A least squares fit of a quadratic (parabolic) curve represents the regression observed for the Asc and the 5,6-OHT groups (r-square = 0.9222 and 0.7978, respectively). A simple linear regression represents the 6-OHDA regression (r-square = 0.5013). The 6-OHDA treated groups were significantly below control (p < 0.05) on each of the 19 days. The 5,6-OHT groups were significantly less than controls (p < 0.05) up to the 5th day after intracerebral injection. The 5,6-OHT and 6-OHDA treated groups were not significantly different from each other.
crease in activity between the 4th and 10th day. The data indicate that in the case of the 5,6-OHT treated mice, a full recovery of the spontaneous locomotor activity did not occur until sometime after the 10th post-treatment day.

**LOCOMOTOR ACTIVITY - REPEATED TEST PROCEDURE**

The results obtained with the repeated locomotor activity test (cf. Methods) were similar to those obtained with the single test procedure. A marked depression of spontaneous locomotor activity was observed on the 1st day following the intracerebral injection of either 6-OHDA or 5,6-OHT (Figure 3). The vehicle treated animals showed the expected habituation to the locomotor chamber, their locomotor activity decreasing throughout the 19 days of the experiment. In contrast, the marked initial reduction in locomotor activity exhibited by the 5,6-OHT treated mice on the 1st day after 5,6-OHT was followed by a gradual increase in activity until the 11th post-treatment day; subsequently, the locomotor activity began to decrease progressively. This result can be construed as indicating a block of the habituation response by the 5,6-OHT pretreatment and/or as a recovery from the effect of 5,6-OHT treatment. Similarly, in the repeated test design, the 6-OHDA treated animals showed a depression of locomotor activity through
the 19 days (Figure 3). Since the activity levels decreased each day after 6-OHDA treatment it seems that 6-OHDA did not affect the habituation response (Figure 3). The spontaneous motor activity of the 6-OHDA treated groups were significantly below that of vehicle treated groups \((p < 0.05)\) on each test day. However, the 5,6-OHT treated groups were no longer significantly below vehicle treated animals on the 5th and subsequent days of testing.

Finally, in the repeated measure design, there were no significant differences observed in the spontaneous motor activity between the 6-OHDA or 5,6-OHT treated mice on any post-injection day.

**PHARMACOLOGICAL FINDINGS**

**LOCOMOTOR RESPONSE TO METHAMPHETAMINE - SINGLE TEST PROCEDURE**

The locomotor stimulation induced by 2.5 mg/kg of methamphetamine, i.p., was significantly less in animals that were pretreated with 6-OHDA as compared to vehicle treated controls (Figure 4). This marked reduction in activity was observed on the 1st \((p < 0.005)\) as well as the 4th, 10th \((p < 0.05)\) and 20th \((p < 0.005)\) days following the administration of 6-OHDA.
LOCOMOTOR RESPONSE OF 6-OHDA and 5,6-OHT TREATED ANIMALS TO METHAMPHETAMINE CHALLENGE

SINGLE TEST

DAY 1  DAY 4  DAY 10  DAY 20

MINUTES AFTER METHAMPHETAMINE

Fig. 4
LEGEND FOR FIGURE 4.

The cumulated locomotor activity of mice was recorded at 15 minute intervals for 90 minutes after methamphetamine (2.5 mg/kg) administration on several days after treatment with either ascorbic acid vehicle (Asc), 6-OHDA or 5,6-OHT. Different groups of mice were employed on each day ("single test"). Each point represents mean locomotor activity of 3 groups of 6 mice. S.E.M. values are shown for the cumulated locomotor activity at 90 minutes. The Newman-Keuls multiple range test applied to a 2 factor ANOV was used to determine the significance of the differences from control values at 90 minutes. The 5,6-OHT mice differed significantly from controls on day 1, day 4 and day 20. 6-OHDA treated animals differed significantly from controls on each day tested (* p < 0.05, ** p < 0.01, *** p < 0.005).
At certain times, the 5,6-OHT treated mice could be differentiated from the 6-OHDA treated animals with regard to their locomotor responses to methamphetamine. The 5,6-OHT treated mice were similar to the 6-OHDA treated mice in their locomotor response to methamphetamine only on the 1st day following the intracerebral injection of the neurotoxins; in both groups of animals locomotor stimulation induced by methamphetamine was markedly reduced (p < 0.005). However, on the subsequent testing days the 5,6-OHT treated mice exhibited locomotor stimulatory effects, in response to methamphetamine administration, which were greater than those of 6-OHDA treated animals. Additionally, on the 4th and 20th days after the intracerebral injection of 5,6-OHT, these animals exhibited an enhanced response to methamphetamine; i.e. this response was greater than that observed in vehicle treated mice (p < 0.05 and < 0.01, respectively). Those mice which had received intracerebral injections of the ascorbic acid vehicle exhibited stable locomotor stimulatory responses to methamphetamine on each of the days tested.

LOCOMOTOR RESPONSE TO METHAMPHETAMINE - REPEATED TEST PROCEDURE

Figure 5 depicts the daily locomotor stimulation
REGRESSION OF LOCOMOTOR RESPONSE TO METHAMPHETAMINE AFTER VEHICLE, 6-OHDA OR 5,6-OHT TREATMENT

Fig. 5
The cumulated locomotor activity of mice was recorded at 15 minute intervals for 90 minutes after methamphetamine (2.5 mg/kg) challenge on various days after either ascorbic acid vehicle Asc, 6-OHDA or 5,6-OHT treatment. The same mice were tested on alternate days ("repeated test"). Each point represents mean locomotor activity of 3 groups of 6 mice. A simple linear regression was fitted to the data of each group; r-square for the 5,6-OHT was 0.7804 (p < 0.05). Tolerance to methamphetamine did not occur on this dose regimen since the slopes of the regression line of both the vehicle treated and the 6-OHDA treated groups were not significantly different from zero (i.e. the time effect curve was parallel to the X axis). The 5,6-OHT treated mice differed significantly from controls on days 1 thru 7; the response of 6-OHDA treated mice was significantly less than that of the controls on each of the days tested (p < 0.05). The bar (I) represents ± the SEM of the activity.
induced by the i.p. administration of methamphetamine (2.5 mg/kg) administered every other day to the same groups of mice which were previously treated with either 6-OHDA, 5,6-OHT or vehicle. The figure depicts these results as linear regression curves fitted to the data by the least squares method. It is obvious that tolerance did not develop in the vehicle treated mice to the locomotor stimulatory effects of methamphetamine as administered on this alternate days schedule; the slope of the regression line was not significantly different from zero. Thus, the vehicle treated mice exhibited stable locomotor responses to methamphetamine in the repeated test procedure.

The 6-OHDA treated mice also exhibited stable locomotor responses to methamphetamine in the repeated test. The stability is evidenced by the slope of the regression line calculated for the 6-OHDA data, as the slope did not differ significantly from zero. As in the case of the single test procedure, the 6-OHDA treated mice exhibited a markedly diminished response to methamphetamine beginning on the first day after 6-OHDA, and continuing throughout the test period ($p < 0.05$).

Similarly, the 5,6-OHT treated mice also exhibited a marked reduction in their locomotor response to amphetamine on the 1st day after 5,6-OHT, substantiating the results observed with the single test procedure. However,
the 5,6-OHT treated mice exhibited in the repeated test procedure a response to methamphetamine which gradually approached that of the vehicle treated mice. Specifically, the 5,6-OHT treated mice exhibited on days 1, 3, 5 and 7 a locomotor response to methamphetamine which was significantly less than that of vehicle treated mice; subsequently, there was no significant difference between these two groups.

**LOCOMOTOR RESPONSE TO L-DOPA**

The locomotor response of vehicle treated mice to L-DOPA (100 mg/kg, i.p.) was unremarkable and constant on each of the days tested (Figure 6). However, 6-OHDA pre-treatment resulted in a marked increase in the locomotor response of mice to L-DOPA on the 1st, 4th, 10th and 20th days after intracerebral 6-OHDA treatment ($p < 0.001$); in fact, 100 mg/kg of L-DOPA induced in 6-OHDA treated mice wild running and jumping behavior.

In contrast to the 6-OHDA treated animals, the L-DOPA response of the 5,6-OHT treated mice resembled that of the vehicle treated mice. Except for an occasional mouse which exhibited some slight stimulation, the majority of 5,6-OHT treated animals did not exhibit responses which were different from vehicle treated mice. In fact, there were no
Fig. 6

LOCOMOTOR RESPONSE TO 100 Mg/Kg OF L-DOPA FOLLOWING 6-OHDA and 5,6-OHT
LEGEND FOR FIGURE 6.

The cumulative locomotor activity of mice was recorded at 15 minute intervals after L-DOPA administration (100 mg/kg, i.p.) on various days following intracerebral injections of either ascorbic acid vehicle (Asc), 70 μgm of 6-OHDA, or 43 μgm of 5,6-OHT. The points represent the mean locomotor activity of 3 groups of 6 mice each (n = 18) under each treatment condition. S.E.M. values are shown for the cumulative mean locomotor activity at 90 minutes after L-DOPA. The 6-OHDA and 5,6-OHT treated groups were tested for significant differences from the vehicle treated mice at 90 minutes after L-DOPA. Asterisks indicate significant differences with respect to vehicle treated mice: * p < 0.05; ** p < 0.001; significance of differences were determined with the Newman-Keuls multiple range test applied to a ANOV.
statistically significant differences between 5,6-OHT and vehicle treated mice, in their locomotor response to L-DOPA on any of the post-treatment days.

**EVERETT TEST**

Initial experiments (Barnes et al., 1973b) indicated that L-DOPA, 100 mg/kg, i.p. when administered 8 hours after pargyline, 40 mg/kg, i.p., produced few signs in vehicle treated mice (piloerection, exophthalmos, grade 1 Straub tail and a slight increase in reactivity). However, L-DOPA administration to 6-OHDA-treated mice induced behavior previously described by Everett and Weigand (1962); this behavior was characterized by hyperreactivity, squeaking, piloerection, exophthalmos, grade 3 Straub tail, spontaneous jumping and running; a few mice also exhibited aggression and/or stereotypic behavior. It is important to note that even when 6-OHDA treated animals exhibited a marked depression of spontaneous motor activity e.g. on the 1st and 2nd days after intracerebral injection of 6-OHDA, the administration of L-DOPA did not produce a return to normal behavior, but rather the hyperreactive, hyperirritable behavior described above (cf. Methods). It was also observed (Barnes et al., 1973b) that pargyline pretreatment was not prerequisite for the response; accordingly, subse-
TABLE 4
RESPONSE TO L-DOPA CHALLENGE IN 6-OHDA TREATED MICE

<table>
<thead>
<tr>
<th>TIME AFTER 6-OHDA TREATMENT</th>
<th>% OF POPULATION SHOWING THE RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GROUP</td>
</tr>
<tr>
<td>4 Hr</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
</tr>
<tr>
<td>8 Hr</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
</tr>
<tr>
<td>16 Hr</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
</tr>
<tr>
<td>24 Hr</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
</tr>
<tr>
<td>2 Days</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
</tr>
<tr>
<td>10 Days</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
</tr>
<tr>
<td>20 Days</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
</tr>
<tr>
<td>30 Days</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
</tr>
</tbody>
</table>
LEGEN FOR TABLE 4.

The average percentage of mice scoring +1, +2 or +3 in response to L-DOPA 100 mg/kg, i.p. at various times after 6-OHDA or vehicle treatment is tabulated. The hypersensitive response to L-DOPA was observed as soon as four hours after intracerebral injection and seemed to be fully developed by eight hours. The response was evident throughout the thirty day test period n= 16 per group at each time after intracerebral injection.
quent investigations were carried out without pargyline pretreatment.

A time course evaluation was carried out to determine at what point the exaggerated response to L-DOPA may be demonstrated after intracerebral 6-OHDA; the results are illustrated in Table 4. The potentiation of the L-DOPA response seemed evident as early as 4 hours following 6-OHDA treatment; it was obvious and marked 8 hours after 6-OHDA. The response persisted for at least 30 days following the intracerebral injection of 6-OHDA (Table 4).

A comparison was made between the L-DOPA responses of vehicle, 6-OHDA and 5,6-OHT treated mice. Mice typically exhibited a +1 response to L-DOPA whether tested 4, 10 or 20 days after the vehicle pretreatment; only infrequently did a vehicle treated mouse exhibit a response as high as +2 (Table 5). The exaggeration of the Everett test following 6-OHDA pretreatment described by Barnes et al. (1973b) was confirmed in the present experiments for the 4th, 10th and 20th days following the 6-OHDA treatment. At these times, from 92 to 100% of L-DOPA challenged 6-OHDA treated mice exhibited a 3+ response. This difference in response of 6-OHDA and vehicle treated mice was statistically significant (Table 5).

The response to L-DOPA of 6-OHDA pretreated animals differed from that recorded in the 5,6-OHT treated animals.
### TABLE 5

**RESPONSE TO L-DOPA IN 5,6-OHT OR 6-OHDA TREATED MICE**

<table>
<thead>
<tr>
<th>DAYS AFTER INTRACEREBRAL INJECTION</th>
<th>GROUP</th>
<th>PERCENT OF POPULATION SHOWING THE RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+1</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT b)</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>6-OHDA a)</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT b)</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>6-OHDA a)</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>Vehicle</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT b)</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>6-OHDA a)</td>
<td>0</td>
</tr>
</tbody>
</table>

The percentages (rounded off to the nearest whole number) represent the number of animals meeting the criteria described in the methods. N=24 for each group. The differences between test scores of 6-OHDA and 5,6-OHT treated mice and their controls were evaluated for statistical significance by the Chi-squared statistic. a) In the case of 6-OHDA treated mice the null hypothesis was rejected for the fourth ($p < 0.0005$, d.f. = 2, $x^2 = 48$), tenth ($p < 0.0005$, d.f. = 2, $x^2 = 23$), and twentieth ($p < 0.0005$, d.f. = 2, $x^2 = 23$) day after intracerebral injection. b) In the case of 5,6-OHT treated mice the null hypothesis was accepted for the fourth ($p < 0.2$, d.f. = 2, $x^2 = 4.4$), tenth ($p < 0.2$, d.f. = 2, $x^2 = 4.3$) and twentieth ($p < 0.2$, d.f. = $x^2 = 2.3$) day after intracerebral injection.
While there was a mild increase of the irritability following L-DOPA administration in 5,6-OHT treated animals, only on the 10th and 20th day following such treatment was a 3+ response noticed in a small, 17 to 25%, segment of the population; however, the differences between the L-DOPA responses of the 5,6-OHT and the vehicle treated animals were not statistically significant (Table 5). Thus, 6-OHDA treatment, but not vehicle or 5,6-OHT treatment induced a significant supersensitive response to L-DOPA. This suggests that a greater change in the catecholaminergic system was induced by 6-OHDA than by 5,6-OHT treatment.

**EVERETT TEST AFTER DECARBOXYLASE INHIBITION**

RO 4-4602, an aromatic amino acid decarboxylase inhibitor (Pletscher and Bartholini, 1971) interfered with the response of the 6-OHDA treated mice to L-DOPA as evaluated by the Everett test (Table 6). The response was blocked by RO 4-4602 at the ED$_{50}$ dose of 70 (95% confidence limits 42-117) mg/kg; at this dose, 50% of animals showed only a 1+ or 2+ response to L-DOPA. A 25 mg/kg, i.p., dose of RO 4-4602, which presumably blocked only peripheral decarboxylase and not central decarboxylase, did not inhibit the response to L-DOPA of 6-OHDA treated mice.
TABLE 6. Seventy micrograms of 6-OHDA were given intracerebrally to all mice 45 days prior to L-DOPA. RO4-4602/1 was administered intraperitoneally 30 minutes prior to L-DOPA. L-DOPA (100 mg/kg) was administered intraperitoneally and observations were made 30 minutes later. The ED$_{50}$ and 95% confidence limits for blockade of the +3 response by RO4-4602 was 70 (42-117) mg/kg.

<table>
<thead>
<tr>
<th>DOSE OF RO4-4602 IN MG/KG</th>
<th>% OF ANIMALS SHOWING SCORES OF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+3</td>
</tr>
<tr>
<td>0</td>
<td>75%</td>
</tr>
<tr>
<td>25</td>
<td>100%</td>
</tr>
<tr>
<td>50</td>
<td>41%</td>
</tr>
<tr>
<td>100</td>
<td>55%</td>
</tr>
<tr>
<td>200</td>
<td>10%</td>
</tr>
<tr>
<td>400</td>
<td>16%</td>
</tr>
<tr>
<td>800</td>
<td>0%</td>
</tr>
</tbody>
</table>
RESPONSE TO APOMORPHINE

A supersensitive response to apomorphine consisting of spontaneous jumping behavior and/or hyperreactivity (cf. Methods) was induced in a large percentage of mice treated with 6-OHDA; this supersensitivity was observed on all 3 days of testing (Table 7). The observed differences between the number of 6-OHDA treated and vehicle treated mice exhibiting supersensitivity (hyperactivity and spontaneous jumping, cf. Methods) were statistically significant on the 4th ($p<0.01$), 10th ($p<0.0005$), and 20th days ($p<0.01$) following intracerebral injection (Chi squared statistic). The administration of apomorphine resulted, particularly in the case of 6-OHDA pretreated mice in spontaneous jumping and hyperactivity (the mice exhibited jumping and kicking on introduction of a foreign object). Other signs observed in mice after apomorphine included a rigid gait, mouthing of sawdust, and stereotypic climbing on the wire top of the cage (cf. Methods).

The 5,6-OHT pretreated animals also showed a supersensitive response to apomorphine. However, fewer 5,6-OHT than 6-OHDA treated mice exhibited spontaneous jumping behavior following apomorphine. The differences between the number of vehicle and treated mice exhibiting the supersensitivity was significant in the case of 5,6-OHT treated
### TABLE 7

**RESPONSE TO APOMORPHINE IN 5,6-OHT OR 6-OHDA TREATED MICE**

<table>
<thead>
<tr>
<th>DAYS AFTER INTRACEREBRAL INJECTION</th>
<th>GROUP</th>
<th>PERCENT OF GROUP EXHIBITING SUPERSENSITIVITY AS *:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SPONTANEOUS HYPER-JUMPING</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HYPER-REACTIONITY</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT a)</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>6-OHDA b)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT a)</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>6-OHDA b)</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>Vehicle</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT a)</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>6-OHDA b)</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
</tr>
</tbody>
</table>

\* The remaining mice did not exhibit a supersensitive response to apomorphine.
The table shows the proportion (in percent) of mice exhibiting either jumping and hyperreactivity, or only hyperreactivity at various days following the vehicle, 6-OHDA or 5,6-OHT treatment. Twelve mice were employed in each test.

The differences between the numbers of mice exhibiting supersensitivity (hyperreactivity alone or jumping as well as hyperreactivity) to apomorphine after 5,6-OHT or 6-OHDA and vehicle treatment were evaluated for statistical significance by the Chi-squared statistic. a) The null hypothesis that the differences following 5,6-OHT were due to chance was rejected for the 4th ($p \leq 0.025$, d.f. = 1, $x^2 = 6.2$) and 10th ($p \leq 0.001$, d.f. = 1, $x^2 = 11.0$) days but accepted for the 20th day ($p > 0.1$, d.f. = 1, $x^2 = 3.0$) after intracerebral injection. b) The null hypothesis that the observed differences following 6-OHDA were due to chance was rejected for the 4th ($p \leq 0.01$, d.f. = 1, $x^2 = 7.3$) 10th ($p \leq 0.0005$, d.f. = 1, $x^2 = 15.7$) and 20th days ($p \leq 0.01$, d.f. = 1, $x^2 = 7.3$).
animals on the 4th ($p < 0.025$) and 10th ($p < 0.001$) days but not on the 20th day. The percentages of responding animals were always greater in the case of 6-OHDA than in that of 5,6-OHT treated mice.

**RESPONSE TO TREMORINE**

A tremorigenic response was present after tremorine (10 mg/kg, i.p.) in vehicle treated mice (Table 8). The peak tremor response was observed at 15 minutes after injection. There were no significant differences among the mean tremor responses observed in the vehicle treated mice on any of the 3 days tested.

On the 4th day after intracerebral injection of 6-OHDA the tremorigenic response to tremorine was significantly increased. As in the case of the vehicle treated mice the peak tremor response in the 6-OHDA treated mice occurred 15 minutes after tremorine administration. However, this increased response to tremorine was not observed on the 10th and 20th days after 6-OHDA treatment (Table 8).

The peak tremor response was induced by tremorine 15 minutes after its administration to the 5,6-OHT treated mice as was the case with vehicle or 6-OHDA treated mice. Similar to the 6-OHDA treated mice, the 5,6-OHT treated mice exhibited an enhanced tremor response to tremorine on
TREMORIGENIC RESPONSE TO TREMORINE

<table>
<thead>
<tr>
<th>DAYS AFTER INTRACEREBRAL INJECTION</th>
<th>GROUP</th>
<th>AVERAGE NUMBER OF INTEGRATOR RESETS PER MINUTE ± S.E.</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Vehicle</td>
<td>8.64 ± 3.73 (9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>21.25 ± 8.90 (4) (a)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT</td>
<td>26.24 ± 8.93 (5) (b)</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>6.66 ± 1.12 (17)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>6.98 ± 4.58 (4)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT</td>
<td>9.89 ± 2.73 (13)</td>
<td>N.S.</td>
</tr>
<tr>
<td>20</td>
<td>Vehicle</td>
<td>7.82 ± 1.96 (17)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-OHDA</td>
<td>6.80 ± 1.49 (8)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>5,6-OHT</td>
<td>13.38 ± 3.09 (9) (a)</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

TABLE 8. The average number of integrator resets per minute was calculated for each group 15 minutes after tremorine (10 mg/kg, i.p.). Statistical significance was determined by the Mann Whitney U test. N.S. means not significant.
the 4th day after intracerebral injection (p < 0.025). However, unlike the 6-OHDA treated mice, the 5,6-OHT treated mice also exhibited an enhanced tremor response on the 20th day following intracerebral injection (p < 0.05). On the 10th day after injection, the 5,6-OHT also exhibited an apparent enhanced tremor response to tremorine, but this response was not great enough to be statistically significant. Thus, 6-OHDA and 5,6-OHT treated mice were similar in their response to tremorine only on the 4th day after intracerebral injection, whereas 5,6-OHT treatment appeared to induce a longer lasting enhancement of the tremor response to tremorine than did 6-OHDA treatment. The onset of tremors was not significantly changed in any of the groups on any of the days tested.

RESPONSE TO L-5-HTP

L-5-HTP, 200 mg/kg, i.p., induced a very mild tremorigenic effect in vehicle treated animals. The effect was relatively stable with no clear peak effect. In contrast, mice which received an intracerebral injection of 6-OHDA exhibited a greatly enhanced tremorigenic response to 5-HTP (Table 9) on the 4th (p < 0.05), 10th (p < 0.01) and 20th (p < 0.005) days after 6-OHDA treatment. Only on the 4th post-treatment day was the onset of tremors in 6-OHDA
<table>
<thead>
<tr>
<th>DAYS AFTER INTRACEREBRAL INJECTION</th>
<th>MINUTES AFTER L-5-HTP</th>
<th>GROUP</th>
<th>AVERAGE NUMBER OF INTEGRATOR RESETS PER MINUTE ± S. E.</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>30</td>
<td>Vehicle</td>
<td>12.2 ± 4.72 (10)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-OHDA</td>
<td>18.2 ± 2.79 (5)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Vehicle</td>
<td>3.38 ± 1.74 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5,6-OHT</td>
<td>11.36 ± 2.76 (5)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>Vehicle</td>
<td>4.97 ± 2.09 (6)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-OHDA</td>
<td>28.17 ± 7.45 (3)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Vehicle</td>
<td>2.92 ± 0.96 (8)</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5,6-OHT</td>
<td>39.64 ± 18.72 (4)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>Vehicle</td>
<td>4.91 ± 1.66 (9)</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-OHDA</td>
<td>18.53 ± 3.05 (3)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Vehicle</td>
<td>4.49 ± 1.39 (11)</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5,6-OHT</td>
<td>22.11 ± 6.39 (7)</td>
<td></td>
</tr>
</tbody>
</table>
Tremorogenic response to L-5-HTP (200 mg/kg, i.p.) was quantified for mice on various days after the intra-cerebral injection of vehicle, 6-OHDA or 5,6-OHT. The peak response in 6-OHDA treated mice occurred 30 minutes after 5-HTP, while that of 5,6-OHT treated mice occurred 5 minutes after 5-HTP. Significance of differences was determined by the Mann Whitney U test.
12 MINUTES AFTER 5-HTP

<table>
<thead>
<tr>
<th>ASCORBIC</th>
<th>6-OHDA</th>
<th>5,6-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>REC.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INTG.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7
LEGEND FOR FIGURE 7.

The upper tracing illustrates the unintegrated recording of tremor activity in treated mice fifteen minutes after L-5-HTP administration on the fourth day after intracerebral injection of vehicle, 6-OHDA or 5,6-OHT (REC). The lower tracings represent the integrated activity (INTG.) of the upper tracing which was used in quantifying the data. Significance of differences were based upon the mean number of integrator resets per minute determined for selected five minute periods. Ten seconds of recording are illustrated.
treated mice significantly more rapid than the onset in the vehicle treated mice (1.6 minutes versus 4.1 minutes, \( p < 0.05 \)). The peak tremor response in 6-OHDA treated mice occurred 30 minutes after L-5-HTP.

5,6-OHT treatment also markedly increased the response to L-5-HTP on the 4th \(( p < 0.05 \)), 10th \(( p < 0.025 \)) and 20th days \(( p < 0.025 \)). The onset of tremors was more rapid only on the 4th day after 5,6-OHT treatment \(( 2.1 \text{ minutes versus } 4.1 \text{ minutes for controls, } p < 0.05 \)). Thus, mice which were treated with either 6-OHDA or 5,6-OHT exhibited a marked potentiation of the tremorigenic effect of L-5-HTP on each of the days tested; in this case, the two neurotoxins were indistinguishable at the doses employed. Figure 7 illustrates the actual recordings of the tremor responses to 5-HTP obtained in a typical experiment.

RESPONSE OF 5,6-OHT TREATED MICE TO L-5-HTP AFTER DECARBOXYLASE INHIBITION

The decarboxylase inhibitor affected in a biphasic fashion the response to 5-HTP challenge of mice pretreated with 5,6-OHT. At the dose of 25 mg/kg, RO 4-4602 increased significantly \(( p < 0.05 \)) the incidence of intermittent tremor or (Table 10) and spread eagle response in the 5,6-OHT treated animals as compared to vehicle treated mice or mice treated with the vehicle and RO 4-4602; at the dose of
**SYMPTOMOLOGY IN 5,6-OHT TREATED MICE AFTER L-5HTP AND VARIOUS DOSES OF RO4-4602/1**

<table>
<thead>
<tr>
<th>DOSE OF RO4-4602 IN MG/KG</th>
<th>GROUP</th>
<th>FLUSHED EARS AND PAWS</th>
<th>HEAD SHAKING</th>
<th>INTERMITTANT SPREAD EAGLE</th>
<th>DEATH</th>
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**TABLE 10.** All mice received RO4-4602/1 30 minutes before L-5HTP. Observations were made for one hour following L-5HTP. RO4-4602/1 was administered i.p. All mice received 43 μgm/10 μl of 5,6-dihydroxytryptamine (5,6-OHT) or 10 μl of a 1:1000 aqueous ascorbic acid vehicle (ASC) 45 days prior to L-5HTP (200 mg/kg, i.p.).
800 mg/kg, RO 4-4602 decreased significantly (p < 0.005) the incidence of these responses as well as of the head shaking response. On the other hand, at both 25 and 800 mg/kg dose, RO 4-4602 blocked completely the appearance of the peripheral response to 5-HTP, the flush of ears and paws, in both vehicle and 5,6-OHT treated mice.
This study compares the effects of two neurotoxic compounds, 5,6-OHT and 6-OHDA, for their selectivity of action. Selectivity was assessed by measuring the brain contents of four neurotransmitters: DA, NE, 5-HT and ACh. Furthermore, the permanence of the neurotoxic sequelae were also evaluated in terms of pharmacological and behavioral responses of 5,6-OHT and 6-OHDA treated animals to challenges with appropriate drugs and precursors.

As a catecholamine congener, 6-OHDA might be expected to affect brain catecholamines to a greater extent than brain indolamines (Malmfors and Thoenen, 1971). In fact, it was observed that 6-OHDA was taken up more selectively by catecholaminergic rather than indolaminergic terminals of the rat brain (Heikkela and Cohen, 1973). Thus, it was expected that the uptake of 6-OHDA by catecholaminergic neurons would result in their selective destruction as described by Javoy et al. (1976). The marked declines in the brain contents of NE and DA after 6-OHDA indicated that a lesion of catecholamine neurons occurred (cf. Table 1). It was reported that very similar catecholamine depletion
occurred after 6-OHDA in the rat (Breese and Traylor, 1970).

Aromatic amino acid decarboxylase is an enzyme which can synthesize dopamine from L-DOPA (Levine and Sjoerdsma, 1964). Our data indicate that the activity of aromatic amino acid decarboxylase is not significantly reduced by 6-OHDA because even after 6-OHDA, the brain content of DA can still be elevated by L-DOPA (Table 3). Similarly, 6-OHDA treatment did not affect the decarboxylation of L-5-HTP to 5-HT (Table 3). Similar results were also reported in rats treated with 6-OHDA (Uretsky and Schoenfeld, 1971).

However, there occurred after 6-OHDA (70 μgm/10 ml) treatment a significant reduction in the brain content of 5-HT. The reduction in 5-HT content was still evident at 20 days post treatment. This indicates a non selective action for 6-OHDA. Thus, it appears that the present method cannot be utilized in mice to achieve a total selective lesion of central catecholaminergic neurons. The decrease in the brain 5-HT content has been previously reported by some (Bloom et al., 1969) and denied by other investigators (Uretsky and Iversen, 1970; Jacks et al., 1972). According to our data 6-OHDA was only relatively selective at the dose employed in the mouse as it induced a greater depletion of DA and NE than of 5-HT. It must be emphasized that most investigators measured 5-HT at 21 days or later, after 6-OHDA administration and may have missed earlier unselective ef-
fects of 6-OHDA on brain 5-HT levels (Bloom et al., 1969; Uretsky and Iversen, 1970).

Altogether, our data suggest that 6-OHDA is not taken up selectively by catecholaminergic neurons as indicated also by neurochemical and histochemical evidence (Fuxe and Ungerstedt, 1968; Fozard and Clarke, 1970; Iversen, 1970). At the dose of 6-OHDA employed, 6-OHDA appeared to be only relatively selective in its action in the mouse; alternatively, a functional change may have occurred in the serotonergic system following 6-OHDA treatment and this may have resulted in the decreased 5-HT levels.

**NEUROCHEMICAL FINDINGS WITH 5,6-OHT**

At the intracerebral dose employed, 43 μg/mL, 5,6-OHT induced marked (up to 53%) and prolonged diminution of mouse brain levels of 5-HT. This effect was comparable to that obtained by others (Bjorklund et al., 1974; Saner et al., 1974). The action was not entirely specific as NE and DA levels were also significantly diminished; however, the action of 5,6-OHT on catecholamines was less pronounced and brief compared to its action on 5-HT. Longo et al. (1974) who used doses essentially identical with ours reported effects of 5,6-OHT on DA content similar to those obtained by us; on the other hand, the changes in NE content which they
reported were variable. Changes in brain DA and/or NE content were also obtained by Bjorklund et al. (1974) with intraventricular administration and by Breese et al. (1974b) with intracisternal administration of 5,6-OHT. Also, Saner et al. (1974) who injected 5,6-OHT into median forebrain bundle noticed a considerable decrease in DA of the rat telencephalon; a similar lack of specificity of 5,6-OHT was reported by Baumgarten et al. (1972b, 1973).

It should be emphasized that the depletions of NE and DA which were observed in this study after 5,6-OHT were of a transient nature since the brain content of these catecholamines was not significantly different from that of the vehicle treated mice on the 10th and 20th days after 5,6-OHT treatment (Table 1). Thus, in our hands 5,6-OHT may have a relatively selective effect on the serotonergic system in the mouse, as the catecholamines recovered from their initial depletion while the 5-HT content remained depleted. Furthermore, 5,6-OHT treatment did not inhibit the capacity of the brain to synthesize catecholamines and serotonin from systemically administered L-DOPA and L-5-HTP, respectively (Table 3). Finally, 5,6-OHT treatment did not alter the resting level of ACh in mouse brain at any time tested.
INTERACTIONS BETWEEN THE DOPAMINERGIC, CHOLINERGIC AND SEROTONERGIC SYSTEMS.

The ability of anticholinergic agents to reverse the tremor and rigidity of Parkinsonian patients is well known. Also well recognized is the effectiveness of anticholinergic agents in the treatment for drug induced extrapyramidal reactions. However, the role of dopamine in these conditions was not emphasized until the pathological and neurochemical etiology of Parkinson's Disease was identified as a deficiency of striatal dopamine (Hornykiewicz, 1966, 1973). As a consequence of this discovery an interrelationship between striatal cholinergic and dopaminergic systems was envisioned and extensively studied. The resultant experimentation has generally supported such an interrelationship. The following discussion will first indicate those experiments which support the concept of a dopaminergic cholinergic interaction; second, it will indicate recent findings with 6-OHDA which are quite unexpected and which may require a slight revision of the concept.

Several investigations suggest that nigral dopaminergic neurons exert a tonic inhibitory effect on striatal cholinergic neurons. For example, Sethy and Van Woert (1974 a, b) reported that dopamine receptor stimulation with dopamimetics diminishes ACh release and increases striatal
ACh content. Conversely, they reported that dopamine receptor blockers increase ACh release and decrease striatal ACh content. Similar findings were reported by Consolo et al. (1974b). Furthermore, dopaminetics reduce ACh turnover in striatum (Trabucchi et al., 1975a). In contrast, DA receptor blockers increase striatal ACh turnover (Trabucchi et al., 1974). Nigral stimulation induces a release of DA in the striatum (Portig and Vogt, 1969) as well as inhibits unit activity in the caudate (Connor, 1970; Krnjevic, 1975). Moreover, transection of the nigrostriatal pathway diminishes DA release from the ventricular area of the caudate nucleus (Besson et al., 1974). Thus, if the nigrostriatal dopaminergic system exerts a physiological tonic inhibition of striatal cholinergic cells, it may be expected that diminution of the presynaptic supply of DA should result in enhanced cholinergic function in the striatum. Inhibition of dopamine synthesis by means of \( \alpha \)-mpt, or nerve terminal destruction by means of 6-OHDA or lesions of the nigrostriatal pathway were used to decrease the presynaptic DA content; the results of these studies are discussed below.

Surprisingly, the literature which describes the effects of \( \alpha \)-mpt, nigrostriatal pathway lesions, or 6-OHDA treatment on striatal cholinergic function remains controversial. For example, Beani and Bianchi (1973) reported the expected increase in striatal ACh after \( \alpha \)-mpt treatment.
However, two other groups found α-mpt to be without effect on striatal ACh levels (Jones et al., 1973; Guyenet et al., 1975a).

The effect of nigrostriatal pathway lesions on striatal function also is surprising. Such lesions did not alter the activity of choline acetyltransferase or acetylcholinesterase in the brains of either rats (McGeer et al., 1973) or baboons (Katoaka et al., 1974).

Furthermore, most investigations which employed 6-OHDA to diminish the presynaptic supply of DA, found 6-OHDA to be without effect on striatal cholinergic function. For instance, Consolo et al. (1974a) found that 6-OHDA did not affect either ACh levels or choline acetyltransferase activity of rat striatum. Similar results were obtained by Grewal et al. (1974). Furthermore, 6-OHDA treatment did not alter the choline acetyltransferase activity of either rat striatum (Kim, 1973), substantia nigra (McGeer et al., 1973) or mouse cortex (Kostrzewa, 1973). Kim's (1973) findings did not agree with the others since he reported after 6-OHDA a marked elevation of rat striatal ACh as well as a decrease in acetylcholinesterase activity in rat striatum; however, Kim's report (1973) was not confirmed by others.

It is also interesting to note that 6-OHDA treatment did not enhance the increase in striatal ACh utilization induced by dopamine receptor antagonists (Stadler et al.,
1973). Conversely, 6-OHDA treatment did not antagonize the diminution of ACh utilization induced by dopamine receptor agonists (Ladinsky et al., 1975). Lastly, and even more surprising is the fact that the inhibitory effect of nigral stimulation is not inhibited by 6-OHDA treatment (Krnjevic, 1974).

In summary, directly acting DA receptor agonists have consistent effects on striatal ACh; the effects of indirectly acting DA receptor agonists on striatal ACh remain controversial. Thus, it appears that not all problems have been resolved in establishing a concept of a physiological, dopaminergic, tonic inhibition of striatal cholinergic cells.

Since the brain part that we analyzed contained the striatum, our data have relevance to the concept of a striatal dopaminergic cholinergic interaction. Basically, neither 6-OHDA nor 5,6-OHT changed endogenous ACh levels (Table 2). The data with 6-OHDA are in direct agreement with those of Jacks et al. (1972) and Consolo et al. (1974a). The data with 5,6-OHT are novel. Our data with 6-OHDA contributes to a growing body of evidence which suggests that 6-OHDA treatment is without effect on striatal ACh levels.

Furthermore, our own data and those of others (cf. above) suggest that excessive DA receptor stimulation rather than tonic DA receptor stimulation inhibits striatal ACh utilization. This latter statement may be supported by the
results of Agid et al. (1975) who demonstrated again that 6-OHDA alone did not reduce striatal ACh levels; however, 6-OHDA did inhibit the increase in striatal ACh levels induced by a high dose (10 mg/kg) of amphetamine.

In an attempt to put the 6-OHDA data in line with the hypothesis of a tonic inhibitory action of DA on striatal ACh cells the following hypotheses may be considered. It may be argued that we did not achieve sufficient depletion of DA to modify cholinergic function. However, Guynet et al. (1975a) achieved after 6-OHDA a DA level of only 5% of normal, without being able to detect alterations in ACh content. It may also be argued that the dopaminceptive cholinergic neurons may only be a small percentage of the total population of cholinergic neurons in striatum. Alternatively, it may also be argued that after 6-OHDA a quasi-normal state of cholinergic function could be maintained by supersensitive DA receptors. Obviously, further studies of the interaction of the dopaminergic system on the cholinergic system with respect to both levels and turnover values of acetylcholine, after presynaptic alterations of the nigrostriatal pathway (Costa and Neff, 1968), are needed for the resolution of this important point.

Also related to this matter of a dopaminergic cholinergic interaction are our results following L-DOPA treatment. Our results indicate that L-DOPA administration to
either vehicle, 6-OHDA or 5,6-OHT treated animals, and the resultant increase in the level of DA, did not affect brain ACh levels. Similarly, Sethy and Van Woert (1973) found no change in rat brain ACh levels with a single administration of L-DOPA but a small (8-13%) increase in ACh content was observed with chronic L-DOPA. The lack of effect of L-DOPA on neostriatal levels of ACh was also reported by others (Beani et al., 1966; Beani and Bianchi, 1973). It was recently reported that a decarboxylase inhibitor may be necessary to demonstrate acute effects of L-DOPA on the striatal cholinergic neurons (Sethy and Van Woert, 1974 a, b). This later result appears to be a sufficient explanation of the present results. In summary, since 6-OHDA did not reduce brain ACh content, we may conclude that 6-OHDA did not destroy central cholinergic neurons. The results of others (cf. above) also lead to a similar conclusion.

However, the results obtained with tremorine appear inconsistent with respect to this concept. An enhanced response to tremorine was observed on the fourth, but not on the tenth or twentieth days after 6-OHDA. The enhancement of the response to tremorine on the 4th day suggests a supersensitivity of cholinergic receptors. At least three other published hypotheses may be related to this result. The first is that 6-OHDA induced a diminution of DA and resulted in a disinhibited cholinergic state (see previous discussion).
The second is that striatal cells become more sensitive to ACh or to muscarinic agonists, following the loss of DA as suggested by Spehlmann (1975). The third is that the supersensitive response to tremorine was indirect and mediated by the 6-OHDA induced supersensitivity of the catecholaminergic system (Tables 4, 5, 7).

To expand upon and to explain the last hypothesis, several studies suggested that the cholinergic system may exert its effects by means of the catecholaminergic system. For instance, the antirecruitment effects of the anticholinesterase, physostigmine, requires a non-depleted pool of catecholamines (Van Meter and Karczmar, 1971). Furthermore, increases in ACh levels can induce an increase in DA and a decrease in NE levels (Glisson et al., 1972). This latter effect may occur in the case of both a normal or a particularly increased supply of catecholamines (Glisson et al., 1972, 1974). Moreover, oxotremorine, a muscarinic agonist, increases striatal DA content (Javoy et al., 1974 a, b). Finally, the locomotor stimulatory effects of anticholinergic compounds, even those which do not block DA uptake (Coyle et al., 1969), are dependent upon a presynaptic supply of dopamine (Thornburg and Moore, 1973 a, b).

While any one of these hypotheses may explain the enhanced response to tremorine on the 4th day after 6-OHDA, they do not explain the lack of an increased response to
tremorine on the 10th and 20th days after 6-OHDA. The latter result may be explained as a long term accommodation of cholinergic function which occurs in response to an acute 6-OHDA induced perturbation of cholinergic function (Agid et al., 1975).

In summary, since 6-OHDA treatment did not induce a decrease in brain ACh levels nor did it induce a long term supersensitivity to tremorine, it may be concluded that 6-OHDA did not induce a lesion of cholinergic neurons. Instead, the transitory increased response to tremorine on the 4th day may reflect a 6-OHDA induced alteration in neurotransmitter system interactions. Obviously, much additional research would be required to adequately resolve this complex issue.

A special consideration should be given to the novel results obtained with respect to an interaction between 5-HT and ACh. While the diminution of 5-HT levels by the 5,6-OHT treatment did not affect ACh levels, the latter were markedly increased following the 5-HTP challenge of the control and 6-OHDA treated mice. However, the 5-HTP challenge did not increase the levels of ACh in the 5,6-OHT pretreated mice. The results indicate that 5-HTP may increase the ACh levels only in the presence of relatively intact serotoninergic neurons as in the case of the ascorbic acid or the 6-OHDA treated animals. Somewhat similar conclusions were
reached by Pepeu et al. (1974a) who demonstrated an increase in ACh release after a 5-HTP administration to parachlorophenylalanine treated rats, but not in rats with lesions of the raphe nuclei.

A serotonergic-cholinergic interaction has also been suggested by the observation of parallel decreases in both ACh and 5-HT levels in animals with septal lesions (Heller et al., 1962). Recently, Barnes et al. (1974) have shown that central accumulation of ACh leads to marked increases of 5-HT in several brain parts. The data of Haubrich and Reid (1972) also support the concept of a serotonergic-cholinergic interaction as they reported an increase in 5-HT turnover following muscarinic agonists. Furthermore, the serotonomimetic agent, quipazine, raises striatal ACh levels; this effect is considered to be mediated through the serotonergic system (Euvrard et al., 1977). Harmaline, a monoamine oxidase inhibitor of indole derivation, increases striatal ACh levels. The effect is not mediated by an interaction with the dopaminergic or gabaminergic systems. It also appears that the effect of harmaline may not be mediated through the serotonergic system (Javoy et al., 1977). Thus, although evidence is accumulating for a serotonergic-cholinergic interaction there may be even other neurotransmitter cholinergic interactions in striatum which are still to be identified. Altogether, the data of others as well as
our own suggest that the serotonergic and cholinergic systems may affect each other's function.

Evidence exists which suggests that the dopaminergic and serotonergic systems also interact. L-DOPA has been shown to cause a reduction in brain 5-HT content (Everett et al., 1970; Bartholini et al., 1968; Butcher et al., 1969). A serotonin depleting effect of L-DOPA was also observed in this study (Table 3). Moreover, this effect of L-DOPA on brain 5-HT is attenuated by 6-OHDA treatment as shown by Algeri et al. (1974) and as observed in this study as well (Table 3). The L-DOPA induced inhibition of spontaneous motor activity and the hypothermia were attributed to the release of cerebral 5-HT stores (Maj et al., 1971, 1973, 1974). Furthermore, 6-OHDA and L-mpt (Jouvet et al., 1973) were shown to increase 5-HT turnover in brain.

Some data also indicate that L-DOPA-serotonin interaction exists in man as the lumbar cerebrospinal fluid levels of 5-HIAA were elevated following L-DOPA treatment (Chase, 1974). In fact, an inverse correlation exists between the degree of akinesia and rigidity in Parkinsonism and the degree of 5-HT depletion (Chase, 1974). However, the addition of PCPA to L-DOPA treatment of Parkinsonism did not affect the therapeutic efficacy (Chase, 1974). More recently, apomorphine was reported to increase the turnover of
brain 5-HT (Grabowska, 1974). To the contrary, another
dopaminergic agonist, ET 495, was without effect on the rat
brain levels of 5-HT or 5HIAA (Consolo et al., 1975). These
findings merely illustrate the complexity of interpretation
of neurotransmitter interactions and the need for additional
research to identify functional relationships between these
neurotransmitter systems. In summary our data support the
reported L-DOPA induced depletion of 5-HT and also confirms
the reported impairment of this effect by 6-OHDA treatment.

PHARMACOLOGICAL AND BEHAVIORAL RESULTS AND THEIR RELA-
TIONSHP TO THE 5-HT, ACh AND CATECHOLAMINE SYSTEMS

RELEVANCE OF THE EFFECTS OF 6-OHDA AND 5,6-OHT ON LOCO-
MOTOR ACTIVITY TO THE FUNCTIONAL STATE OF BIOAMINE SYSTEMS

While the opinion was not unanimous, 6-OHDA was re-
ported to cause behavioral and locomotor depression (Cooper
et al., 1973) as it did in this study. There were relative-
ly few studies of the locomotor effects of 5,6-OHT; the
results of Longo et al. (1974) and the present as well as
our earlier data (Barnes et al., 1973 a, c) indicate that
this compound markedly decreased motor activity, contrary to
the non-quantified data of Baumgarten et al. (1972b), Da
Prada (1972) and Longo et al. (1974). On the other hand,
disinhibitory or excitatory effects of 5,6-OHT were more in keeping with the opinion held by many since the early suggestion of Brodie and Shore (1957) that 5-HT was a behavioral suppressant; indeed, some of our own data (cf. below) may be consistent with this concept.

The 5,6-OHT treated animals while exhibiting initially a depression of locomotor activity, showed subsequently some recovery in the continuous test paradigm as their locomotor activity was no longer significantly depressed, compared to the controls, after the fifth post-treatment day (Figure 3). On the other hand, when the single test procedure was employed the motor activity of the 5,6-OHT treated group remained depressed through the tenth post-treatment day (Figure 2). The apparent earlier recovery of motor activity in 5,6-OHT treated mice in the repeated test may be explained as follows. The vehicle treated animals show habituation to the cage and exhibit, in repeated tests, a progressive reduction in locomotor activity; this habituation may not have occurred in the 5,6-OHT treated mice. Indeed, the locomotor activity of the vehicle treated mice progressively decreased and by the 5th day approached the level exhibited by the 5,6-OHT treated mice. The 5,6-OHT treated mice eventually did exhibit habituation but only after the 11th day; interestingly, exploratory activity returned to normal at this time in the single test procedure. Thus, the
data suggest that a return of the habituation phenomenon in mice may reflect in a repeated test design the recovery of exploratory motor activity.

In the 5,6-OHT treated mice the catecholamines were at normal levels on the 10th post-treatment day; it may be inferred therefore that the occurrence of the habituation phenomenon in 5,6-OHT treated mice may be a reflection of the recovery of the catecholamines. The lack of dependence of the habituation response upon brain catecholamine level was implied by the results in the repeated tests with 6-OHDA treated mice. The habituation response was observed as early as the 5th post-treatment day and continued thereafter inspite of the greater depletion of catecholamines in the 6-OHDA treated mice as opposed to the 5,6-OHT treated mice. Moreover, recovery of exploratory activity in 6-OHDA treated mice also occurred early (on the 4th post-treatment day), again inspite of low catecholamine levels.

Controversy surrounds the mechanism of the locomotor depression induced by 6-OHDA or 5,6-OHT. Some have suggested that the depression of locomotor activity by 6-OHDA was a non-specific toxic effect (Taylor et al., 1970; Evetts and Iversen, 1970; Scotti de Carolis et al., 1971). There is evidence for independence of locomotor depression from the catecholamine depleting action of 6-OHDA; for instance, even though pretreatment with desipramine prevented the depletion
of NE by 6-OHDA, it did not prevent the locomotor depression induced by 6-OHDA (Scotti de Carolis et al., 1971). However, the evidence presented was not completely convincing since the levels of NE were determined but not those of DA. Moreover, it has been previously reported that NE uptake blockers such as protriptyline can spare the noradrenergic terminals but not the dopaminergic terminals from destruction by 6-OHDA (Evetts and Iversen, 1970). Thus, the behavioral depressant effects in the absence of NE depletion may reflect normal NE levels and depleted DA levels; the latter may, per se, depress locomotor behavior. The role of catecholamine depletion in the depression of locomotion was also suggested by our own 5,6-OHT data, as 5,6-OHT induced early catecholamine depletion and depression of locomotion.

Not only does controversy exist concerning the cause of locomotor depression by 6-OHDA or 5,6-OHT but also concerning the nature of the recovery of exploratory locomotor behavior. Aside from the suggestion of recovery from nonspecific toxic effects it was suggested that supersensitiv-
recovery of spontaneous exploratory locomotion in rodents after either 6-OHDA or 5,6-OHT treatments may be due to the restoration of a functional catecholamine pool. The latter has been suggested as the mechanism for the behavioral recovery from 6-OHDA induced anorexia in rats (Zigmond and Stricker, 1973).

Our data are relevant to the nature of the depression as well as recovery of locomotor activity after 6-OHDA. The data indicate that L-DOPA stimulated the locomotor activity of 6-OHDA treated mice to a greater degree than that of the vehicle treated mice as early as one day after 6-OHDA (Figure 6); this was at the time when the spontaneous exploratory activity of 6-OHDA treated mice was markedly depressed. Similarly, on the 4th and 10th days after 5,6-OHT treatment, mice exhibited a supersensitivity to apomorphine; on these days they also exhibited a depression of spontaneous locomotor activity. Thus, while supersensitivity of DA receptors was present it could not account for the recovery of spontaneous locomotor activity which occurred several days later. As L-DOPA stimulated the locomotor activity of 6-OHDA treated mice to a greater extent than that of vehicle treated mice, it appeared that 6-OHDA did not have a non-specific toxic effect on the efferent pathways responsible for locomotor behavior. A pertinent suggestion was that 6-OHDA induced a sensory deficit (Ljungberg and
As there does not appear to be deficit in the efferent locomotor pathway (cf. above) and in view of the strong implication of the role of catecholamines in locomotor behavior from previous studies (cf. Introduction), a catecholamine hypothesis for the recovery of exploratory behavior is still tenable if viewed in the following way. The neurotoxins, 6-OHDA and 5,6-OHT, induce a loss of a functional pool of catecholamines. The loss of this functional pool may be responsible for the initial depression of locomotion observed after each neurotoxin. Furthermore, the recovery of the activity of this functional pool may parallel the recovery of exploratory behavior and habituation phenomenon in mice which received either neurotoxin. The effects of the functional pool may be amplified by the existence of a concomitant receptor supersensitivity. Thus, our data appear to be consistent with the concepts of a catecholamine involvement with locomotion and with that of a functional catecholamine pool. Further experiments are needed to support these postulated functional relationships involved in recovery of locomotion which occurs in spite of prolonged catecholamine depletion in 6-OHDA treated mice.

That the methamphetamine effect was reduced by 6-OHDA pretreatment was not surprising, as amphetamines act at least in part, via a presynaptic release of NE and DA (Moore
et al., 1970; Carlsson, 1970; Costa and Groppeti, 1970), and as the stores of NE and DA were diminished in the 6-OHDA treated mice. There was little or no recovery of this effect throughout the test period in both the repeated and single test procedure (Figures 4 and 5), in agreement with the prolonged effect of 6-OHDA on brain catecholamines (Table 1). Similar results were obtained by others (Fibiger et al., 1973; Creese and Iversen, 1974).

Soon after 5,6-OHT treatment, mice exhibited a reduced response to methamphetamine. This effect was observed in the case of the single test procedure on the 1st but not on the 4th post-treatment day, and through the 7th post-treatment day in the case of the repeated test. In the repeated test, the 5,6-OHT treated mice exhibited a trend of a gradually increasing response to methamphetamine after the initial inhibition observed on the 1st post-treatment day (Figure 3). The slow progressive trend may have reflected a gradual return of the catecholamines to normal from their depleted levels. Normal catecholamine levels were observed on the 10th post-treatment day. After the initial diminished response to methamphetamine on the first day, the 5,6-OHT treated mice subsequently exhibited in the single test procedure an increased locomotor response to methamphetamine (Figure 4). This increased response to methamphetamine in 5,6-OHT treated animals may have reflected a loss of the in-
hibitory serotonergic system which was postulated (Baldessarini et al., 1975; Creese and Iversen, 1974; Green and Harvey, 1974; Mabry and Campbell, 1973, 1974; Mogilnicka et al., 1977) to modulate catecholamine arousal in the rat; although the concept was questioned by Jacobs et al. (1975a) it was recently reconfirmed in rats by Breese et al. (1974a). The absence of an enhanced response to methamphetamine in 6-OHDA treated animals whose 5-HT is depleted may have been a result of the persistent marked depletion of NE and DA in these mice. Moreover, on the 20th day following 5,6-OHT treatment the catecholamines but not 5-HT levels were restored and there was an enhanced locomotor response to methamphetamine. Thus, the present data support the concept of a serotonergic inhibition of catecholamine mediated locomotion and also of the presynaptic, indirect mechanism for the action of methamphetamine.

PHARMACOLOGICAL ASSESSMENT OF CNS FUNCTION AFTER 6-OHDA OR 5,6-OHT TREATMENT

It was established that supersensitivity phenomena in the periphery are associated with either the denervation or disuse of synaptic junctions (cf. Introduction). 6-OHDA and 5,6-OHT were expected to chemically denervate and induce disuse of central catecholaminergic and serotonergic path-
ways, respectively. Thus, the development of supersensitive responses to central agonists was considered in this study to be indicative of the neuronal destruction induced by 6-OHDA and 5,6-OHT. It was expected that lesions of serotonergic nerve terminals would induce a supersensitive response to L-5-HTP, whereas lesions of dopaminergic nerve terminals would induce supersensitive responses to apomorphine and L-DOPA. Finally, lesions of cholinergic terminals were expected to induce a supersensitive response to the centrally acting, cholinergic agonist, tremorine.

Some properties of supersensitivity phenomena in the central nervous system may be expected to resemble these phenomena in the periphery. In the case of the loss of central presynaptic nerve terminals, which possess active high affinity neurotransmitter uptake mechanisms, it would be expected that a rather specific supersensitive response of the prejunctional type would develop. As in the periphery, the central prejunctional type of supersensitivity would be expected to develop soon after the loss of the neurotransmitter uptake process. Moreover, a prejunctional type of supersensitivity may be expected in the case of central catecholaminergic, serotonergic and cholinergic neurons all of which have well documented high affinity uptake mechanisms (Iversen and Neal, 1968; Yamamura and Snyder, 1972).
On the other hand, demonstration of the postsynaptic type of supersensitivity in the central nervous system poses several problems. One problem is whether postsynaptic supersensitivity of central receptors will exhibit the same characteristics of non-specificity and delayed development as exhibited by postjunctional supersensitivity in the periphery (cf. Introduction). Indeed, since central neurons are sensitive to several neurotransmitters (Yarbrough and Phillis, 1975) denervation or disuse of one neurotransmitter input to a neuron may result in the development of supersensitivity to all of its other neurotransmitter inputs. In contrast, it is conceivable that postsynaptic supersensitivity of central receptors can be very specific in nature. This question is difficult to answer because polysynaptic pathways are involved in the ultimate expression of behavioral responses. Therefore, it is difficult to demonstrate that supersensitivity of only one neuronal pool is responsible for the behavioral expression of supersensitivity. Indeed, neurotoxin-induced alteration of a single receptor of a synapse constituting a link in a synaptic chain, may initiate secondary changes in the other synaptic junctions of the chain which utilize different neurotransmitters. These secondary compensatory changes may mimic non-specific supersensitivity of the originally affected synaptic junction.
Other methodological difficulties occur when one considers the possibility of the existence of isoreceptors. For example, some investigators have postulated the existence of several different types of dopamine receptors. DA receptors have been characterized as being excitatory or inhibitory (Cools and Van Rossum, 1976) as well as presynaptic and postsynaptic (Bunney and Aghajanian, 1975). Thus, with the possibility of the existence of several receptor subtypes the interpretation of the behavioral expressions of neurotoxin-induced central receptor supersensitivity becomes even more complex. The following pages will describe the responses of mice to several pharmacological agonists after 6-OHDA and 5,6-OHT treatments and relate them to the development of changes in receptor sensitivity.

**CHOLINERGIC SYSTEM**

Originally, it was suggested that the action of tremorine was indirect, via a stimulated synthesis and release of ACh (Marchbanks, 1969). This hypothesis may need revision (Cox and Potkonjak, 1969b). Oxotremorine increased the brain levels of acetylcholine in the rat (Cox and Potkonjak, 1969a) and cat (Pepeu et al., 1968); this is consistent with decreased ACh turnover. In fact, oxotremorine decreased ACh turnover (Trabucchi et al., 1975b) and ACh re-
lease (Szerb and Somogyi, 1973). Furthermore, a causal role of increased ACh levels in tremor production following tremorine was questioned since anticholinesterase agents potentiated the increase in brain ACh but did not potentiate the tremor (Cox and Potkonjak, 1969b). Furthermore, drugs such as propranolol, reserpine, α-mpt and diethyldithiocarbamic acid were capable of inhibiting oxotremorine induced tremor while they did not affect the increase in ACh levels (Cox and Potkonjak, 1970). On the other hand, tremor induced by oxotremorine is readily blocked by atropine at doses which did not prevent the increase in whole brain ACh (Cox and Potkonjak, 1969b). These recent data suggest that tremorine after conversion to oxotremorine probably induces tremor through a direct action on central muscarinic receptors (Cox and Potkonjak, 1969a,b).

Tremorine also induces changes in the bioamines. Dopamine levels were reported to increase after tremorine in the rat, guinea pig and rabbit (Friedman et al., 1967). Furthermore, brain stem levels of norepinephrine decreased and those of serotonin increased (Friedman et al., 1963). Recently, the increase in 5-HT was correlated to the hypothermic action of tremorine (Cox and Potkonjak, 1967); the latter investigators could not find any changes in the brain DA content of tremorine treated rats. The decrease in NE was substantiated and found to be independent of tremorine's hypothermic effect (Cox and Potkonjak, 1967). A muscarinic
mechanism was suggested for the decrease in NE (Cox and Potkonjak, 1967). Some role of NE in tremor production by tremorine was implied since NE depleting agents and beta-adrenergic blocking agents inhibit tremorine tremor (Cox and Potkonjak, 1970; Leslie et al., 1972). However, the local anesthetic effects of beta-adrenergic blocking agents or a non-specific depression of motor function with NE depleting agents may be responsible for the antagonism of tremor (Cox and Potkonjak, 1970).

The locus of tremor production appears to be with the extrapyramidal motor system; the tremor may be initiated in the neostriatum. Tremorine and oxotremorine, its active metabolite, induce tremor in a cerebellectomized preparation (Everett, 1956) and in decerebrate animals (Everett et al., 1956); mesencephalic tegmental lesions, however, inhibit tremor induced by these drugs (Tasker and Kertesz, 1965). Moreover, stereotaxic implantation of tremorine into either globus pallidus, caudate nucleus and substantia nigra result in tremor production (Cox and Potkonjak, 1969a). Similarly intracaudate injections of another muscarinic cholinergic agent carbachol also induced tremor (Connor et al., 1966). Thus, an intact neostriatal and lower brain stem structures are necessary for the expression of tremorine tremor.

Since 6-OHDA decreased DA and NE, it may be expected that this depletion of catecholamines would inhibit the
action of tremorine. A similar inhibition of tremor might be expected early after 5,6-OHT treatment when the catecho-
lamines were depleted. These expectations were not realiz-
ed. On the 4th day following either 6-OHDA or 5,6-OHT treatment, the tremorine responses were greatly exaggerated (Table 8). It should be emphasized that these exaggerated responses were obtained in the absence of any significant change in ACh levels whether in the 6-OHDA treated or in 5,6-OHT treated animals. The exaggerated response to tremorine appeared to be longer lasting in the case of 5,6-OHT treated mice since it was still evident on the 20th post-
treatment day. The particular sensitivity of the 5,6-OHT treated animals to tremorine suggests that the phenomenon may depend in part on the lesion of the serotonin containing neurons, since on the 20th post-treatment day both the levels of NE and DA had returned to normal levels and since there was no evidence of supersensitivity of catecholamine receptors in these mice as evidenced by no enhanced response to apomorphine or L-DOPA. Tremorine increased central levels of ACh (Holmstedt and Lundgren, 1966); which in turn may induce increased 5-HT levels (Barnes et al., 1974) to which the 5,6-OHT treated animals were shown to be supersen-
sitive (cf. next section). Altogether, the finding of an exaggerated response to tremorine, in spite of lowered brain levels of NE and DA in both 5,6-OHT and 6-OHDA treated mice
on the 4th day (Table 8), was surprising in light of the expectations.

It may be suggested that 1) either the serotonergic system supersensitivity is involved in the elaboration of tremor following tremorine; indeed 5-HT but not NE or DA was markedly depleted 20 days following 5,6-OHT treatment and at that time the exaggerated response to tremorine was still evident; 2) a hitherto undetected change in central cholinergic function may have occurred following either 6-OHDA or 5,6-OHT treatment.

**SEROTONERGIC SYSTEM**

L-5-HTP was used to stimulate postsynaptic serotonceptive receptors and thereby assess their functional state. 5-HTP and high serum tryptophan levels increase the brain content of 5-HT (Bogdanski et al., 1958; Fernstrom and Wurtman, 1972); 5-HTP retained this ability after either 6-OHDA or 5,6-OHT treatment (Table 3). Unfortunately, 5-HTP is taken up by the neurons rather unspecifically and it may displace endogenous stores of NE (Dahlstrom and Fuxe, 1964; Corrodi et al., 1967). Tryptophan may be a more selective precursor of 5-HT than is 5-HTP; this is evidenced by the selective enhancement of the fluorescence of raphe terminals (Aghajanian and Asher, 1971) and by increased brain 5-HT
after tryptophan loading (Grahme-Smith, 1971). However, tryptophan may be inappropriate to test serotononoceptive receptor function since in the case of 5,6-OHT treatment a decrease in tryptophan hydroxylase has been observed and this may limit the amount of conversion of L-tryptophan to 5-HT (Victor et al., 1973; Lovenburg and Victor, 1974). Furthermore, it is not yet clear whether the observed increase in brain indole fluorescence following tryptophan is due to the accumulation of serotonin or, in addition, to other indolamines (Bjorklund et al., 1971). Anden (1968) suggested that 5-HTP stimulates serotonergic receptors as evidenced by an enhancement of the hindlimb extensor reflex. In addition, blockers of 5-HT uptake also enhance the hindlimb extensor reflex by enhancing the amount of serotonin at the synapse (Meek et al., 1970). The extensor reflex was utilized to demonstrate the destruction of serotonergic terminals by 5,6-OHT (Daly et al., 1973); a combination of drugs requiring the presynaptic integrity of 5-HT terminals could no longer exert their influence on the reflex after 5,6-OHT treatment.

The tremorogenic response to 5-HTP was greatly increased throughout the testing period of 20 days in the 5,6-OHT and 6-OHDA treated mice. The results of this investigation were the first description of central 5-HT receptor supersensitivity (Barnes et al., 1973 a, c). The phenomena
of central 5-HT receptor supersensitivity has been recently confirmed by others (Gerson and Baldessarini, 1975; Jacobs et al., 1975b; Klawans et al., 1975; Longo et al., 1974; Stewart et al., 1976). The mechanism of this supersensitivity may be analogous to that hypothesized for the increased sensitivity to L-DOPA of 6-OHDA treated animals (Barnes et al., 1973b); while the latter may be due to the impairment of NE and/or DA uptake by the catecholamine nerve terminals (Bell et al., 1970) or due to changes in postsynaptic NE and/or DA receptor sensitivities (Ungerstedt, 1971a), the increased sensitivity to 5-HTP in 5,6-OHDT treated animals may be due to a lesion of the serotonergic neurons (Baumgarten et al., 1973). Thus, a lesion of serotonergic terminals may result in an impairment of the 5-HT uptake by serotonergic neurons and induce a presynaptic type of supersensitivity (Daly et al., 1973; cf. Introduction). There may also be an increase in the sensitivity of the postsynaptic 5-HT receptor. In a similar vein, the supersensitivity to 5-HTP observed also in the case of 6-OHDA treated animals may be related to a lesion of 5-HT containing neurons which may be surmised on the basis of the long lasting depletion of 5-HT recorded in these animals (Table 1).

In fact, the question might be raised as to the significance of dopamine or norepinephrine depletion as possible mechanisms for the enhancement of the 5-HTP response since
these occurred in mice treated with either 6-OHDA or 5,6-OHT (Table 1). However, a serotonergic involvement can be suggested by examination of the tremor responses and neurochemical findings in mice on the 20th day after intracerebral injection of 5,6-OHT or 6-OHDA. In the case of 6-OHDA, 5-HT was still depleted as were the catecholamines. However, in the case of 5,6-OHT treated mice only the levels of 5-HT but not those of NE and DA were significantly depleted on the 20th day after intracerebral injection. In fact, in view of the results obtained with the DOPA test and apomorphine test on the 20th day after 5,6-OHT (cf. next section) it could be concluded that supersensitivity of catecholaminergic receptors was not present on that day. Therefore, the enhanced response of 5,6-OHT treated mice to 5-HTP was most probably related to a destruction of brain serotonergic systems, and this mechanism probably accounts for the similar findings in 6-OHDA treated mice.

It would be interesting to examine the possibility that 5-HTP induced tremors may be induced by activation of the inferior olive cells and the corresponding climbing fiber input into the cerebellum (Llinas and Volkind, 1973) similar to that induced by harmaline. Such a mechanism should help elucidate the 5-HT-DA mechanism involved in Parkinson's disease, especially since it has not been possible to induce a Parkinsonism tremor in primates unless both
striatal DA and 5-HT were lowered by a ventrotegmental lesion (Poirier and Sourkes, 1976); this has cast some doubt as to a purely dopaminergic etiology for the disease. Admittedly, it is a frequent finding that both DA and 5-HT are depleted in the Parkinsonism patient (Hornykiewicz, 1973 a,b). It has been suggested that tremor results when the dopaminergic system is impaired in animals with lesions in the rubro-olivo-cerebellar pathway (Larochelle et al., 1971). Obviously, additional research is needed to elucidate the physiological role of 5-HT and the other neurotransmitters in the extrapyramidal motor system.

CATECHOLAMINERGIC SYSTEM

The response of the 5,6-OHT treated animals to L-DOPA should be compared with those of 6-OHDA treated animals (Tables 5 and 7, Figure 6). Without DOPA treatment neither group displayed aggression or hyperirritability as described by Nakamura (1972) for 6-OHDA treated rats. In confirmation of the data of others (Ungerstedt, 1971a; Uretsky and Schoenfeld, 1971) the 6-OHDA treated animals exhibited in our hands an exaggerated response to L-DOPA, whether this response was evaluated by the Everett test (Everett, 1962, 1967) or in terms of changes in the locomotor activity after L-DOPA. In sharp contrast, the response to the L-DOPA
challenge of the 5,6-OHT treated mice was not statistically different from those of the controls whether in the case of the Everett test or the locomotor activity test; this suggested a lack of prejunctional type of dopamine receptor supersensitivity in 5,6-OHT treated mice. Longo et al. (1974) reported with the Everett test a transient increase in response to L-DOPA after 5,6-OHT. However, they did not evaluate their data statistically.

Some dopaminergic supersensitivity of the postjuncti- n-5,6-OHT as well as 6-OHDA treated mice, because both groups exhibited enhanced responses to apomorphine. An enhanced response to apomorphine is generally interpreted as the phenomenon of postsynaptic DA receptor supersensitivity (Ungerstedt, 1971a). The response to apomorphine of 5,6-OHT treated mice was less than that of 6-OHDA treated mice. The enhanced response to apomorphine was of a transient nature in 5,6-OHT treated mice while it persisted in the 6-OHDA treated mice. It should be emphasized that the response to apomorphine, besides its well recognized effect on the DA receptor (Ernst, 1969), may also depend in part on the state of the serotonergic neurons (Grabowska et al., 1974). Altogether, these data are consistent with the concept that the supersensitivity to L-DOPA and apomorphine is due to the nerve terminal lesion induced by 6-OHDA, as well as with the notion that the action of the
latter, just as that of 5,6-OHT is only relatively specific.

The interaction of central noradrenergic and dopaminergic receptors proposed by some investigators (Nyback et al., 1970; Consolo et al., 1975; Anden and Strombom, 1974) may have relevance to the lack of an enhanced response of 5,6-OHT treated mice to L-DOPA. For example, Stromberg and Svensson (1971) suggested that the L-DOPA induced hyperactivity in mice required both DA and NE receptor stimulation. The lack of a marked supersensitivity of 5,6-OHT treated mice to L-DOPA may suggest that the postulated noradrenergic-dopaminergic interaction was not altered in the same way by 5,6-OHT and 6-OHDA.

SEVERAL COMMENTS ON THE NATURE OF CNS SUPERSENSITIVITY

One might conclude that a decentralization type (cf. Introduction) of supersensitivity developed in the CNS following both 5,6-OHT and 6-OHDA. The decentralization concept was implied by the results concerned with the cholinergic system. 6-OHDA and 5,6-OHT did not reduce brain ACh levels, suggesting that neither agent destroyed cholinergic neurons. However, both agents induced a supersensitivity to the cholinomimetic, tremorine. Thus, one might conclude that the cells containing the catecholamine and serotonin
receptors became supersensitive to the muscarinic agent, and that the supersensitivity induced, was similar to the non-specific decentralization type of supersensitivity that occurs in the periphery. Furthermore, as 6-OHDA induced a supersensitivity to L-5-HTP and as 5,6-OHT induced a supersensitivity to apomorphine these findings also suggested that a non-specific decentralization type of postsynaptic supersensitivity developed. Moreover, a 6-OHDA induced decentralization type of supersensitivity of DA receptors was suggested by others (Costall and Naylor, 1976). However, the results of Costall and Naylor (1976) are difficult to interpret as they could not demonstrate a supersensitivity to dopamine in the same preparations that exhibited the "non-specific supersensitivity" to NE and 5-HT. This discrepancy casts doubt as to the utility of the method which they employed to demonstrate non-specific supersensitivity.

In contrast, careful review of our data suggests an alternative hypothesis to the concept of a decentralization type of CNS receptor supersensitivity. The data suggest the existence of the phenomena of specific postsynaptic receptor supersensitivity in the CNS. 6-OHDA induced a prolonged depletion of brain 5-HT; this suggests that 6-OHDA induced a lesion of 5-HT neurons. Thus the fact that 6-OHDA treatment induced a supersensitivity to L-5-HTP is consistent with the
concept of a specific 5-HT receptor supersensitivity that is secondary to a lesion of serotonergic neurons. Similarly, the fact that 5,6-OHT treatment induced a supersensitivity to apomorphine is consistent with the development of specific DA receptor supersensitivity that is secondary to a lesion of DA neurons; indeed, a 5,6-OHT induced lesion of dopaminergic neurons is suggested by the depletion of brain DA induced by this agent. Furthermore, the fact that after 5,6-OHT, both the depletion of brain DA and the supersensitivity to apomorphine were reversible suggested that these two phenomena were related. Furthermore, both the 5,6-OHT induced depletion of 5-HT as well as the supersensitivity to L-5-HTP were long lasting and these findings suggest that these phenomena were related. Lastly, both the 6-OHDA induced depletion of DA as well as the supersensitivity to apomorphine were long lasting and these findings suggest that both of these phenomena were related. All these data emphasize the important concept that supersensitivity phenomena are correlated closely in time with neuronal destruction (i.e. neurotransmitter depletion).

When receptors are in a state of decentralization supersensitivity, they simultaneously exhibit supersensitive responses to a variety of agonists (Sharpless, 1975). Thus, if 6-OHDA induced a state of decentralization supersensitivity of either DA or 5-HT receptors these receptors would
exhibit simultaneous supersensitive responses to apomorphine, L-5-HTP and to tremorine. However, the 6-OHDA treated mice exhibited supersensitive responses to dopaminimetics and L-5-HTP for up to 20 days but to tremorine only up to the 4th day after treatment. Thus, a primary characteristic of non-specific decentralization supersensitivity has not been fulfilled, as the mice were not simultaneously supersensitive to apomorphine and tremorine or L-5-HTP on all of the days tested. Altogether, it appears that the 6-OHDA induced short term alteration of the cholinergic system can not be correlated with a lesion of cholinergic neurons (i.e. no ACh depletion) nor with a long term induction of supersensitivity of DA and 5-HT receptors. Thus, the data suggest that the supersensitivity to tremorine may be related to other phenomena.

Altogether, the data support a concept of specific rather than non-specific postsynaptic receptor supersensitivity in the CNS. Thus, other mechanisms must be postulated to account for the enhanced response to tremorine. For example, if tremorine's action is dependent in part upon ACh release as suggested by Pepeu et al. (1974b), a 6-OHDA induced loss of striatal acetylcholinesterase activity may result in an apparent supersensitivity to tremorine. This latter concept as well as the alternative hypotheses of neurotoxin induced changes in cholinergic-dopaminergic or cholinergic-serotonergic neuronal interactions (cf. previous
Discussion) may be involved in the tremorine response. Lastly, the induction of secondary compensatory changes within synaptic chains may also be a more likely explanation than to hypothesize the non-specific decentralization type of supersensitivity as an explanation for the tremorine responses.

In summary, the data obtained suggest that a lesion of catecholamine neurons induced a supersensitivity to catecholaminergic agonists. Furthermore, a lesion of serotonergic neurons induced a supersensitive response to serotonergic agonists. Thus, a specific supersensitivity developed in the case of serotonergic and catecholaminergic neurons. The analogous case in the periphery is the prejunctional type of supersensitivity where the supersensitivity is specific in nature. In fact, the data suggest the development of both a prejunctional (L-DOPA) as well as a postjunctional (apomorphine) type of supersensitivity of dopaminergic receptors. In the case of the 5-HTP response a prejunctional as well as postjunctional supersensitivity of serotonergic receptors could also have developed. Therefore, the data suggest an important concept: the possibility that the postjunctional form of supersensitivity of central catecholamine and serotonergic receptors is specific in nature, as opposed to non-specific in nature as it exists in the periphery. In fact, recent microiontophoretic studies also support the
concept of specific postsynaptic receptor supersensitivity in the CNS, as 6-OHDA treatment enhanced the response of striatal cells to DA but not to 5-HT or NE (Yarbrough and Phillis, 1975). Admittedly, a great deal of additional research would be required to resolve this complex and important issue.

One may hypothesize that a specific postsynaptic supersensitive response may indicate that dopaminoceptive and serotonooceptive striatal cells receive only one physiological transmitter input, dopamine or serotonin, respectively. Thus, postsynaptic cells may develop a supersensitive response to their physiological transmitter because they lack receptors for other neurotransmitters. This speculation, however, is not tenable in light of the demonstration of the receptiveness to both 5-HT and DA by striatal cells (Yarbrough and Phillis, 1975).

Therefore, it is suggested that neurons of the CNS may develop a differential supersensitive response. Thus, when hypofunction occurs in only one neurotransmitter input, the postsynaptic neuron may respond by developing a postsynaptic supersensitive response to only that neurotransmitter which is deficient. Admittedly, such a corollary to Dale's principle may be very speculative and much additional experimentation would be required to substantiate such a phenomenon. However, the possibility of the presence of both a specific
postsynaptic as well as a specific prejunctional type of supersensitivity in the CNS represents a very elegant mechanism for maintenance of brain function within normal limits.

Presynaptic as well as postsynaptic mechanisms of supersensitivity, as well as the recovery of functional pools may all be important in maintaining or restoring brain function. One can see from the results presented that even after "behavioral recovery" an abnormal state may still be present; such a state can be reflected by pharmacological hypofunction (subsensitivity) or hyperfunction (supersensitivity). Thus, 6-OHDA decreased amphetamine's action as the latter depends on the presynaptic store of catecholamines. Similarly, 5,6-OHT induced a subsensitivity to the facilitation by chlorimipramine of the hindlimb extensor reflex which depends upon a presynaptic supply of 5-HT in the spinal cord (Daly et al., 1973). On the other hand, 6-OHDA and 5,6-OHT induce supersensitivity to apomorphine and L-5-HTP respectively. Thus, a pharmacological presynaptic subsensitivity may exist concomitantly with a postsynaptic pharmacologic supersensitivity while a minimal functional pool may be maintaining behavior within normal limits.

The finding of supersensitivity in the catecholaminergic systems is not a new concept, but the employment of neurotoxins to induce this supersensitivity is novel.
Previously, supersensitivity has been demonstrated in the central catecholaminergic systems following: chronic reserpine treatment (Stolk and Rech, 1967; Rech and Stolk, 1970), axotomy (Trandelenburg, 1966) or the inhibition of catecholamine synthesis (Dominic and Moore, 1969). Furthermore, recent research suggests that the sensitivity of catecholamine receptor may be altered by hormonal influences (Emlen et al., 1972; Engstrom et al., 1974; Huidobro-Toro et al., 1974).

The conclusion that the 5-HTP and L-DOPA supersensitivity in the 5,6-OHT and 6-OHDA treated mice, respectively, is dependent upon the ultimate synthesis of the normal neurotransmitters, is evidenced by the fact that at effective doses, the decarboxylase inhibitor, RO 4-4602, completely blocked the 5-HTP as well as the L-DOPA response. It appears that the catecholamines and 5-HT rather than their precursors, are responsible for the responses to the L-DOPA and 5-HTP challenge, respectively, and that when the formation of the bioamines is prevented by the decarboxylase inhibitor (Pletscher and Bartholini, 1971; Hyttel et al., 1972) the response to the precursor is blocked. Furthermore it is of interest that at low doses, RO 4-4602 potentiated the central while attenuating the peripheral actions of 5-HTP. This is consistent with the fact that at these low doses RO 4-4602 blocks decarboxylase peripherally but not
centrally (Pletscher and Bartholini, 1971), thus increasing the availability of 5-HTP to the CNS.

**SUMMARY**

The results of this study indicate no specificity and a relative rather then an absolute selectivity for both agents. In the case of 6-OHDA a specific lesion of the catecholamine system was expected and the following findings supported such a contention: a) NE and DA were depleted for a long time period; b) the responses to L-DOPA or apomorphine were exaggerated over the same time period whether evaluated in terms of the locomotor activity, the Everett test or finally by observation; c) the response to amphetamine was decreased throughout the study; d) the brain content of ACh was not affected. However, a case for non-specificity of the action of 6-OHDA may also be made: a) brain 5-HT was depleted throughout the study; b) the response to L-5-HTP was exaggerated throughout the study; c) transient changes in cholinergic function were observed as evidenced by an enhanced tremorogenic effect to tremorine on the fourth post-treatment day.

Similarly, in the case of 5,6-OHT a specific lesion of the indolamine system was expected. The following experimental results suggest that the expectation was realized:
a) long lasting depletion of brain 5-HT; b) long lasting exaggerated responses to L-5-HTP; c) a lack of effect on ACh content. However, a case for non-specificity of 5,6-OHT may also be made since transient decreases in brain NE and DA were observed up to the 10th post-treatment day and an enhanced response to apomorphine existed to the 10th post-treatment day. Finally, the tremor response to tremoreine was increased for some time implying either a serotonergic mechanism, a cholinergic-serotonergic interaction, or a biochemically undetected change in the function of the cholinergic system was induced by 5,6-OHT treatment.

The results of this study emphasize the multitransmitter phenomena involved in the actions of 6-OHDA and 5,6-OHT, as well as the concomitant complexities of their effects on drug responses and on behavioral paradigms. A relatively selective lesion of indolamine neurons may be an achievable goal with 5,6-OHT if sufficient time is allowed for the restoration of NE and DA levels following their initial depletion. However, without concomitant morphological or kinetic studies such a selectivity may only be suggested by the present data. Furthermore, it is interesting to note that although behavioral recovery may be manifested following these neurotoxic agents (i.e. normal locomotive behavior), there may remain a "pharmacodynamic alteration" (i.e. hypersensitivity or subsensitivity). Such lasting
pharmacological alterations may be exploited by the judicious use of agonist agents such as apomorphine, L-5-HTP and tremorine as neuropsychodiagnostic tools to help elucidate the nature of neuropathology whether in the case of emotive, cognitive or neurological involvements. Such an approach may be desirable to help identify the etiologies of dyfunctions which may result in common behavioral expressions such as the schizoid states, depressions and choreas. Obviously, different behavioral substrates may have different susceptibilities to both impairment and recovery and each needs to be evaluated individually in that regard.
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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Date  9/30/1977  Director's Signature