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EFFECT OF AGE ON DOPAMINE RECEPTOR FUNCTION AND THE ACTION OF NIALAMIDE IN THE NUCLEUS ACCUMBENS OF RATS

The Ohio State University

Ph.D. 1985

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EFFECT OF AGE ON DOPAMINE RECEPTOR FUNCTION AND THE ACTION
OF NIALAMIDE IN THE NUCLEUS ACCUMBENS OF RATS

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Kelley Martin Cousin, B.S. Pharmacy

*****

The Ohio State University

1985

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Division of Pharmacology
To my grandparents,
Bruce and Emily Martin, and Harry and Marjorie Doerr,
from whom I learned so much.
ACKNOWLEDGEMENTS

I would like to thank:

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INTRODUCTION

THE NUCLEUS ACCUMBENS

Neuroanatomy and Neurochemistry

The nucleus accumbens is a basal forebrain structure, lying ventral to the anterior horn of the lateral ventricle and perforated laterally by the anterior commissure (Figure 1). Medially, the nucleus accumbens appears to "lean into" the septal nucleus, and has sometimes been referred to as the nucleus accumbens septi. The nucleus accumbens is separated from the adjacent septal area by the ascending and descending fibers of the septum, which are easily seen in anterior coronal brain slices (Domesick, 1981). Over its entire longitudinal extent, the nucleus accumbens is bordered dorsally by the ventral striatum, and ventrally by the olfactory tubercle, and is continuous with the olfactory area via perforating cell bridges, referred to as "olfactory radiations". The lateral border of the nucleus accumbens is undefined and highly species variable (Chronister and DeFrance, 1981).
Figure 1.

Coronal section of the rat brain showing the location of the nucleus accumbens.

CC: Corpus callosum; CPU: Nucleus caudatus-putamen; V: ventricle; HPC: hippocampus; MPA: Medial parolfactorial area; CLA: Claustrum; ACB: Nucleus accumbens septi; CA: Anterior commissure; DBB: Diagonal Band of Broca; TOO: Olfactory tubercle; MFB: Median forebrain bundle; TP: Tuberculopiriform tract; PIR: Piriform cortex; TOL: Lateral olfactory tract; FR: Rhinal fissure. Kindly supplied by Pellegrino and Cushman.
On the basis of its location, neurogenesis, cytoarchitecture, histochemistry and connections, the nucleus accumbens has often been considered an extension of the ventral striatum. Most attempts to distinguish the nucleus accumbens from surrounding structures and define its borders anatomically focus on its connections to other brain areas. These connections have been described using autoradiography to map the anterograde transport of 3H-amino acids, anterograde and retrograde transport of horseradish peroxidase (Domesick, 1981; Fonnum and Walaas, 1981; Walaas and Fonnum, 1979) and electrophysiological techniques (Mogenson and Yim, 1981).

**Afferent Fibers**

The nucleus accumbens receives a prominent projection, traversing in the medial forebrain bundle, and referred to as the mesolimbic projection, from cell group A 10, the retrorubral nucleus, and various aggregates of ventral tegmental cells which form a continuum between A 10 and the retrorubral nucleus (Domesick, 1981) (Figure 2). Based on electrophysiological studies, this projection is believed to be primarily dopaminergic (Mogenson and Yim, 1981). In addition, two major limbic projections innervate the nucleus accumbens: 1) the fibers from the subiculum of the hippocampus which selectively innervate the medial portion of the nucleus accumbens and travel in the precommissural
Figure 2. Major Dopamine Pathways in the Brain (Lader, 1980).
fornix; and 2) a projection from the basal amygdaloid nucleus to the lateral accumbens. In addition, at least one part of the frontal neocortex also projects to the lateral nucleus accumbens (Domesick, 1981). Based on electrophysiology (Mogenson and Yim, 1981) and biochemical studies of the high affinity uptake of glutamate (Fonnum and Walaas, 1981; Walaas, 1981), both the limbic and cortical projections are thought to be excitatory and glutaminergic.

The nucleus accumbens is also projected upon by medial thalamic groups such as the nuclei paratenialis, paraventricularis and reuniens (Domesick, 1981); serotonergic fibers from the dorsal raphe nucleus via the dorsal raphe forebrain tract; and noradrenergic fibers from the locus coeruleus via the medial forebrain bundle (Fonnum and Walaas, 1981).

**Efferent Fibers**

The nucleus accumbens has two major efferent projections: 1) to the rostral part of the substantia innominata and the rostroventral part of the globus pallidus, collectively referred to as the ventral pallidum and 2) to the rostromedial substantia nigra (compacta and reticulata) and rostral ventral tegmental area. Electrocoagulation of the nucleus accumbens is associated with a decrease in glutamate decarboxylase in these structures, indicating that the
innervation from the nucleus accumbens is GABAergic (Fonnum and Walaas, 1981). Additionally, accumbal efferents connect with the limbic circuitry, i.e., the septum, preoptic area, and hypothalamus. Still longer fibers project to the mesencephalic tegmentum, central gray area and medial raphe nucleus (Domesick, 1981). With the exception of the efferents to the ventral pallidum, the descending projections from the nucleus accumbens generally course in the medial forebrain bundle.

The efferent association with limbic structures and the large volume of afferents from the hippocampus and amygdala help to differentiate the nucleus accumbens from the striatum anatomically and suggest a preferential association with the limbic system.

**Intrinsic Neurons**

In addition to the neurotransmitters whose presence in the nucleus accumbens can be attributed to afferent innervation, the nucleus accumbens also contains intrinsic neurons as suggested by the disappearance of marker enzymes following the local application of kainic acid. These include cholinergic (choline acetyltransferase and acetylcholinesterase) and gabaergic (glutamate decarboxylase) neurons in the medial accumbens; and glutaminergic neurons (loss of high affinity glutamate uptake sites) (Walaas and
Fonnum, 1979). The presence of various peptides in the nucleus accumbens has been suggested by radioimmunoassay, among them, substance-P, thyrotropin releasing hormone, somatostatin, enkephalin, vasoactive intestinal polypeptide and neurotensin (Johansson and Hokfelt, 1981)

Functional Significance

The presence of dopamine nerve terminals (Johansson and Hokfelt, 1981) and the high concentration of dopamine in the nucleus accumbens (second only to the caudate nucleus) and the preferential association of the nucleus accumbens with the limbic system suggested that this nucleus might be the site of action of neuroleptic drugs (dopamine receptor antagonists) in reversing the symptoms of psychosis. Indeed, within the past 10-15 years, a large volume of research has focused on the involvement of the nucleus accumbens in the etiology and treatment of schizophrenia. It has been postulated that excess dopamine within the mesolimbic-nucleus accumbens complex may play a major role in causing psychotic behavior. In support of this hypothesis, it has been reported that dopamine levels in the nucleus accumbens of schizophrenic patients determined on autopsy are approximately 150% of normal (Bird et al., 1977). In addition, increases in the high affinity binding of the neuroleptics haloperidol or spiroperidol in the nucleus accumbens from postmortem brains of schizophrenics
have also been described (Matthysse, 1981) suggesting an increase in dopamine receptors in this region.

The nucleus accumbens has also been implicated in the pathophysiology of Parkinson's disease, since reduced levels of dopamine and homovanillic acid are found not only in the striatum, but also throughout the nucleus accumbens and mesolimbic nuclei. Since deficits have been reported in postmortem determinations of choline acetyltransferase (a marker for cholinergic neurons) in the caudate, putamen and nucleus accumbens of Huntington's choreic patients (Palmer and Chronister, 1981), the nucleus accumbens may play a role in the symptomatology of this disease.

Because of its efferent projection to the globus pallidus (a component of the extrapyramidal system which channels motor output from the basal ganglia) and large afferent input from the amygdala and hippocampus (limbic areas of the brain believed to be concerned with the motivational and emotional aspects of behavior), the nucleus accumbens has been suggested to act as a limbic-motor interface. Evidence of the participation of the nucleus accumbens in motor function has been demonstrated by the local application of dopamine to this area, mimicking the release of dopamine from the dopaminergic projection originating in the ventral tegmental area. This local application of dopamine results in enhanced horizontal and/or exploratory activity in rats.
A similar increase in locomotor activity occurs after the injection of picrotoxin, a GABA receptor antagonist, into the ventral globus pallidus, an effect that can be inhibited by prior administration of GABA. This observation suggests that the GABAergic projection from the nucleus accumbens to the ventral globus pallidus may be important in the locomotor activity response of rats to dopamine injected into the nucleus accumbens (Mogenson and Nielson, 1983; Stevens, 1979; Mogenson and Yim, 1981).

An increase in locomotor activity in rats can also be induced by the administration of low doses of amphetamine (.5-1.0 mg/kg, i.p.), a dopamine agonist which acts by releasing newly synthesized dopamine from presynaptic nerve terminals (Van Rossum et al., 1977). The increase in locomotor activity seen at low doses of amphetamine is thought to be due to the release of dopamine within the nucleus accumbens, since 6-hydroxydopamine (6-OHDA) lesions of this area, which destroy dopaminergic nerve terminals, selectively block the locomotor activity response to low doses of amphetamine, but do not block the increase in stereotypy (sniffing and gnawing) produced by the administration of high doses of amphetamine (Iversen and Koob, 1977).
The nucleus accumbens has also been suggested to play a role in the circling response produced by the administration of dopamine agonists to rats with unilateral 6-OHDA lesions of the nigrostriatal dopamine tract. In evidence, amphetamine induced circling is blocked by destruction of the mesolimbic dopamine projection, or by injections of a dopamine antagonist directly into the nucleus accumbens (Kelly and Moore, 1977).

**Aging and the Nucleus Accumbens**

Little information is available concerning age-related changes in the mesolimbic dopaminergic system innervating the nucleus accumbens. McGeer and McGeer (1978) detected a sharp decline with age in tyrosine hydroxylase activity (the rate limiting enzyme in dopamine synthesis) in the nucleus accumbens of humans, with the greatest drop occurring before age 20 and then slowly progressing until age 80. Total tyrosine hydroxylase activity at age 80 is only 30-50% of that at age 5. This decrease in tyrosine hydroxylase could result in a decrease in dopamine synthesis (and dopamine levels) in the nucleus accumbens of humans. However, studies of the nucleus accumbens of rats have shown that the dopamine concentration was not less in the nucleus accumbens of old (24 month) compared to young (6 month) rats (Demarest et al., 1980).
An age related decline in the number of dopamine receptors has been described in the nucleus accumbens. For example, a decline in the density of 3H-spiperone (a dopamine receptor antagonist) binding sites has been reported from birth to approximately 95 years of age in humans (Severson et al., 1982). In addition, using 3H-spiroperidol, DeBlasi and Mennini (1981), showed a 36% decrease in dopamine receptor binding in limbic areas (anterior limbic cortex, nucleus accumbens and olfactory tuberculum) of rats 21-23 months compared to 3 months of age. In agreement with these decreases in dopamine receptor binding and indicative of a decrease in dopamine receptor function is the reduction in dopamine stimulated adenylate cyclase activity observed in aged (27 month) rats (Govani et al., 1977).

THE STRIATUM

Neuroanatomy

The striatum refers to the caudate and putamen, which along with the globus pallidus and amygdaloid nuclear complex comprise the basal ganglia (Carpenter, 1976). The striatum is projected upon by dopaminergic fibers (the nigrostriatal pathway) originating in the zona compacta of the substantia nigra, and differs from the nucleus accumbens
in that the majority of its afferent input derives from all areas of the cerebral cortex rather than primarily from limbic areas. Both the nucleus accumbens and striatum receive extensive thalamic projections (Domesick, 1981), and are thought to influence motor function via their GABAergic projections to the globus pallidus, albeit to different divisions (Scheel-Kruger et al., 1981).

Role in Motor Function

Sensorimotor Coordination

The importance of the striatum in maintaining normal motor function is most easily understood if one observes the disruptions in motor activity that occur when dopamine neurons in this area are lesioned. Thus, patients with Parkinson's disease, characterized by a loss of dopamine cells in the substantia nigra and a loss of dopamine nerve terminals in the striatum (as well as the nucleus accumbens) are plagued by akinesia, defects of synergistic (the combined action of muscles) and associative movements and profound abnormalities in postural tone (Marsden, 1980). For this reason, normal dopamine function in the striatum is hypothesized to be important in motor control in humans. In rats, lesions which produce a depletion of dopamine from the nigrostriatal fiber tracts produce marked impairments in the integration of sensory information with motor performance
(sensorimotor function). Thus, bilateral electrolytic lesions of the lateral hypothalamus, which damage the medial forebrain bundle (Levitt and Teitelbaum, 1975; Marshall et al., 1976) or intraventricular 6-OHDA injections (Marshall et al., 1976) have been shown to produce a substantial depletion of striatal dopamine and a state of somnolence (sleep like), akinesia (lack of spontaneous motion) and catalepsy (statue-like immobility) in rats. The inability to swim spontaneously after these lesions has also been reported (Levitt and Teitelbaum, 1975). It is interesting that these animals show improved motor function immediately following exposure to stimuli such as an ice bath or tail pinch or introduction to a rat colony (Marshall et al., 1976). It has been suggested that the response to "activating" stimuli of lesioned rats resembles the paradoxical kinesia sometimes observed in Parkinson's patients at times of severe stress.

Bjorklund and Stenevi (1979) have demonstrated that dopamine cells from the embryonic substantia nigra that have been transplanted into a pre-prepared lateral cortical cavity in adult rats previously lesioned in the nigrostriatal pathway with 6-OHDA, send neurites to the ventrolateral caudate-putamen and ameliorate akinesia, and improve limb use; i.e., catalepsy, forelimb placement, forelimb support, grid climbing, and limb withdrawal to pinch in rats.
**Stereotypy**

The peripheral administration of high doses of amphetamine (2-10 mg/kg, i.p.) produces a range of stereotypic (repetitive) behaviors in rats, including biting, sniffing, gnawing and licking, which can be blocked by the prior administration of low doses of neuroleptics (Van Rossum et al., 1977). The observation that intrastriatal injections of dopamine (Cools and Van Rossum, 1970) can produce stereotypic behavior suggests that the effect of amphetamine is mediated by the release of dopamine in the striatum. Bilateral 6-OHDA lesions of the ventral caudate (Iversen and Koob, 1977) block the amphetamine induced stereotypy, providing further evidence of striatal involvement in this behavior. It is interesting that in humans, the chronic use of amphetamine is associated with similar repetitive movements, most often pacing and oral contortions.

**Circling**

Rats unilaterally lesioned in the nigrostriatal tract characteristically display a circling response to dopamine agonists (Ungerstedt, 1971). This response is thought to result from a lesion-induced imbalance in the dopamine system in the striatum. Firstly, the striata on the
lesioned side is deficient in dopamine compared to the unlesioned side. Secondly, the lesion produces a proliferation of postsynaptic dopamine receptors on the lesioned side, resulting in a "supersensitive" response to direct acting dopaminergic agonists. Amphetamine, which acts by releasing dopamine from presynaptic terminals on the intact side, causes the animals to turn toward the lesioned side (ipsilateral circling). In contrast, apomorphine, a direct receptor agonist, has a greater effect on the supersensitive dopamine receptors on the lesioned side, causing the animals to rotate contralaterally.

**Age Related Changes in the Nigrostriatal Dopamine Tract**

**Biochemistry**

The age related changes that occur in the biochemistry of the dopamine neurons of the nigrostriatal tract have been extensively studied. Tyrosine hydroxylase (rate limiting) and dopa decarboxylase, two enzymes localized in dopamine neurons and involved in the biosynthesis of dopamine, are reduced in rat and human neostriatum (McGeer and McGeer, 1978). This reduction is characterized by a gradual decline in enzyme activity from 10 to 30 months in rats, while in humans the decline is characterized by a rapid drop from age 5 to 20, and a more gradual drop from age 20 to 80. In
agreement with these observations, a reduced synthesis of 3H-catecholamines, 3H-norepinephrine and 3H-dopamine, from L-3H-tyrosine and L-3H-dopa has been described in mice 28-30 months old (Finch, 1978). Similarly, reduced levels of dopamine have been detected in the striata of 25-28 month old mice (Finch, 1978); and rats greater than 24 months of age show a 25-50% decrease in striatal dopamine (Demarest et al., 1980; Joseph et al., 1978). Carlsson and Winblad (1976) also reported a significant reduction in dopamine levels in the striata of humans greater than 72.5 years of age amounting to a loss in dopamine of approximately 1% per year. In addition, a 30% decrease in dopamine uptake into striatal synaptosomes from senescent mice has also been reported (Reisine et al., 1980).

However, Jonec and Finch (1975) saw a selective reduction in the Km of the high affinity uptake of 3H-dopamine into synaptosomes from 28 month old mice, but no change in maximum uptake, suggesting that the reduction in uptake may not have been due to a loss of dopamine neurons.

All of the above changes in dopamine biochemistry suggest a decline in the integrity of the dopamine neurons projecting from the substantia nigra to the striatum. In support of this hypothesis, the number of dopamine neurons in human substantia has been found to be inversely correlated with increasing age (McGeer and McGeer, 1978).
Dopamine Receptors

The number of dopamine receptors in the striatum has also been shown to undergo an age related decline, as evidenced by a reduction in the number of 3H-ligand binding sites. In humans, the number of 3H-spiperone or 3H-ADTN binding sites decreases significantly in the caudate nucleus but not the putamen. This loss in dopamine receptors amounts to a 7.5% receptor loss per decade (Severson et al., 1982). Wong and coworkers (1984) recently reported an age related decline in the number of 11C-labeled 3-N-methylspiperone binding sites in human caudate from 19-73 years. Most investigators have consistently shown a 50% decline in the number of 3H-spiroperidol binding sites (Misra et al., 1980; Memo et al., 1980; DeBlasi and Mennini, 1982; Joseph et al., 1981) and a 40% decline in the number of haloperidol binding sites (Govani et al., 1978) in the striata of rats over 24 months of age. Since in the striatum butyrophenones are thought to bind predominantly to the D2 receptor, it has been suggested that D2 receptors in the striatum decline with age.

Whether the decrease in dopamine receptors with age is specific for one or another receptor subtype (D1 or D2) is open to interpretation since there is some ambiguity regarding the selectivity of the ligands used for binding in these studies. However, dopamine stimulated adenylate
cyclase activity has been shown to be markedly reduced in the striata of rats over 24 months of age (Govani et al., 1977; Schmidt and Thornberry, 1978; Puri and Volicer, 1977) suggesting that there is an age related decline in the number of D1 dopamine receptors.

**Age Related Changes in Motor Function**

**Sensorimotor Coordination**

Motor performance has been shown to undergo an age related decline in rats, mice and humans. This decline in performance affects tasks requiring the coordinated control of motor function, rather than simple reflexive tasks and is more pronounced the more complex the task. For example, Wallace and coworkers (1980), found no change in simple reflexive tasks such as placing, hopping, negative geotaxis and surface and mid-air righting in Fischer-344 rats 6-24 months of age. In contrast, beginning at about 12 months, aging rats were less efficient in remaining suspended from a horizontal wire, were less coordinated in their descent of a wire mesh pole, were less successful in traversing an elevated platform and required a lower maximum speed on the rotorod than younger rats. Thus, those responses requiring a greater degree of motor coordination; balance, grasping, and climbing declined in an age related fashion. Similar observations have been made by other investigators (Janicke
The resemblance of these age related deficits in motor function to those produced by lesions which destroy the dopaminergic nigrostriatal tract (see above) suggests that these deficits might be related to a decline in the functioning of this pathway. In support of this hypothesis, several of the observed sensorimotor deficits in aged rats show partial or total reversal following implants of embryonic substantia nigra dopamine cells into the caudate and putamen (Gage et al., 1983). Prior to implants 21-23 month old rats showed impairments in: 1) the ability to maintain balance on a bridge of round or square cross section, 2) the length of time a rat could remain suspended from a taut wire, and 3) the ability to descend a vertical pole with wire mesh in a coordinated manner when compared to 2-3 month rats. Three months after transplant surgery, 24-26 month rats showed significant improvement in balance and limb coordination on the round and square bridge but no improvement in suspension from a wire (a measure of strength). These experiments suggest that intracerebral implants of dopaminergic neurons might be able to restore motor capabilities whose loss is related to dopaminergic neuronal systems. In fact, preliminary clinical trials have utilized this technique to treat patients with cases of reticulate Parkinson's disease, considered by some to be an
example of premature aging.

In addition to the reinnervation studies by Gage and co-workers, Joseph and coworkers (1983) determined that dopamine receptor upregulation via chronic haloperidol administration (alzet minipumps, 1.86 mg/day for 14 days) resulted in a better performance of motor tasks and a higher maximal binding of $3^H$-spiroperidol in the striata of rats of all ages. Furthermore, old (25 month) rats receiving chronic haloperidol performed better than or equal to young (6-8 month) untreated rats on all measures of motor function (rod walking, wire hanging, inclined screen, plank walking). These studies suggest that dopamine receptor proliferation may improve motor function in aged rats and indirectly, that the age related decrease in dopamine receptors in the brain may contribute to the appearance of deficits in motor behavior.

An age related decline in swimming ability has also been described in aged (24-27 month) rats (Marshall and Berrios, 1979; Gage et al., 1984). In general, this deficit is characterized by a decrease in the ability of the rats to keep their head out of water and a decline in the vigor with which the animal is able to move its limbs and resembles the swimming deficit which occurs when the nigrostriatal tract is disrupted after lesions of the lateral hypothalamus. Attempts have been made to correct these age related
impairments in swimming behavior. In a study by Marshall and Berrios (1979), the impairment in success and vigor seen in old rats was reversed if the animals were pretreated with apomorphine (a dopamine receptor agonist) or L-dopa (a biosynthetic precursor of dopamine). Although these studies do not conclusively relate deficits in swimming ability to declines in nigrostriatal function, they do suggest that age related alterations in brain dopamine systems may play a role in the decline with age of the performance of tasks requiring extensive sensorimotor integration.

Numerous investigators have also measured "spontaneous" activity in aging rats, however, the results are equivocal. This is due in part to the many methods available for measuring spontaneous activity. For example, Janicke and coworkers (1983) and Janicke and Wrobel (1984) saw no difference in spontaneous activity in 4 versus 32 month rats when activity was recorded by a motilimeter, which measures all activity greater than chewing. In contrast, Gage and coworkers (1983, 1984) reported reduced whole body locomotion in 24 to 25 month old rats verses 5 month old rats in the automated open field apparatus. Locomotor activity in this apparatus is recorded by 2 perpendicular photocell beams. Thus, it detects only gross ambulatory movements. The reduced whole body locomotion of old rats was not improved by grafts of embryonic substantia nigra (Gage et al., 1983) suggesting that the reduced activity
may be dependent on other neuronal systems.

**Stereotypy**

Smith and coworkers (1978) demonstrated an enhanced stereotypic response (sniffing, gnawing, licking, pawing) to apomorphine and amphetamine in 20 versus 2 month old rats. They attributed the enhanced response in old rats to a supersensitivity of the postsynaptic dopamine receptors or an age related decrease in drug metabolism. Subsequently, Campbell and coworkers (1984) were able to correlate enhanced sniffing and mouth movement in 24 versus 2 month old rats to apomorphine with the level of apomorphine in the brain. They suggested that the higher brain levels of apomorphine in old rats might be due to a decrease in the integrity of the blood brain barrier or an decreased elimination of the drug in these animals.

In contrast to the findings of Smith and Campbell, Joseph and coworkers (1983) described an age related decrease in the sniffing and licking response to apomorphine of Fischer-344 rats, 6-8, 12-18, and 25 months of age; and no age related decrease in grooming. The decreases in sniffing and licking in aged rats could be a result of the loss in dopamine receptors from the striatum. However, the response of old rats to dopamine receptor upregulation (via chronic haloperidol administration) was variable, with only the
Circling

Age related alterations in the rotational response of rats to dopamine receptor agonists after unilateral lesions of the nigrostriatal tract have also been described. After lesions of the left substantia nigra with 6-OHDA, old (24-26 month) rats responded to right intrastriatal injections of dopamine with a reduced rate of circling compared to young (6-8 month) rats (Cubells and Joseph, 1981). In this model dopamine is acting on intact dopamine receptors on the unlesioned side, suggesting that the receptor number or sensitivity may be reduced in old rats. Joseph and coworkers (1981) have been able to show a high positive correlation between rotation and $3^H$-spiroperidol specific binding, but not between rotation and adenylate cyclase stimulation. Since adenylate cyclase stimulation is thought to be a result of D1 receptor stimulation, the results of this experiment suggest that the decreased response of old rats to dopamine may reflect the loss of D2 receptors in the striatum.

Unilateral electrolytic lesions of the left substantia nigra in 6 and 25-29 month rats are accompanied by a decreased circling response to amphetamine (5 mg/kg i.p.) in the older rats (Joseph et al., 1978). Since the activity
of amphetamine is dependent on the release of dopamine from presynaptic neurons on the intact side, and since dopamine levels have been shown to be reduced in old rats, the reduced response to amphetamine may be due in part to a decreased release of dopamine in old rats. Alternatively, the reduced response could also be attributed to a reduced number of dopamine receptors in the striatum. Interestingly, no age related reduction in the circling response has been observed after the administration of apomorphine (5 mg/kg, i.p.). In contrast to amphetamine, apomorphine acts directly on the dopamine receptors, and has a greater effect in the lesioned striatum because of the development of supersensitivity of the dopamine receptors (upregulation of dopamine receptors). While old rats appear to have fewer dopamine receptors in the striatum, they show a similar development of receptor supersensitivity caused by denervation (Joseph et al., 1981). Thus the effect of apomorphine on the denervated striatum appears to be the same regardless of age.

It has recently been demonstrated that dietary restriction can delay the age associated loss of dopamine receptors as determined by 3H-spiperone binding in the striatum (Roth et al., 1984). A similar dietary restriction has been shown to increase the rotational response of old (24 mo) rats to intrastriatal injections of
amphetamine or dopamine (Joseph et al., 1983), bringing the level of rotation up to that of 6 month old rats. This apparent correlation between receptor binding and circling suggests that the reduced circling response to these drugs is due to a decreased number of dopamine receptors in old rats. In these studies, dietarily restricted rats were fed or fasted on alternate days from weaning and Ad Libitum fed rats had free access to food. The mechanism for the effectiveness of dietary restriction in preventing receptor loss is unidentified, but appears to affect other biochemical parameters of aging similarly.

Therefore, it appears that dopamine receptor down regulation in the striatum of aged animals may contribute to some of the decrements in rotation and stereotypy observed, as well as the decline in sensorimotor coordination. However, based on the improvement in sensorimotor function following intrastriatal nigral implants, and the improvement in swimming performance following administration of dopamine agonists, the age related decline in dopaminergic presynaptic function may also play a role in the decline in motor function with age.
Monoamine oxidase (MAO, monoamine O2: oxidoreductase (deaminating) EC 1.4.3.4) catalyzes the oxidation of primary and some secondary amines. For example, dopamine, tyramine, 3-methoxytyramine, tryptamine, 5-hydroxytryptamine, phenylethylamine, normetanephrine, metanephrine, epinephrine, norepinephrine, 1,4-methylhistamine and kynuramine, are all substrates for oxidative deamination via monoamine oxidase (Kapeller-Adler, 1970). In general, substrate oxidation by MAO proceeds by the overall reaction:

$$R-CH_2-NH_2 + O_2 + H_2O \rightarrow R-CHO + NH_3 + H_2O_2$$

A substrate must have 2 hydrogens on the \( \alpha \) carbon. One hydrogen appears to be necessary for attachment of the substrate to the enzyme; the second hydrogen is necessary for oxidation of the substrate (Pletscher, 1966).

In rats and man, oxidative deamination by MAO is the main degradative pathway for catecholamines (Pletscher, 1966). For example, MAO oxidizes dopamine to the corresponding aldehyde, dihydroxyphenylacetaldehyde, which then undergoes
further oxidation to dihydroxyphenylacetic acid (DOPAC) and methylation to homovanillic acid (HVA) as illustrated in Figure 3. In agreement, it has been shown that the inhibition of MAO reduces the levels of these dopamine metabolites in the brain and urine, and increases the concentration of intra- and extraneuronal dopamine in the brain. However, accumulation of endogenous amines is only evident after 85% inhibition of the enzyme (Pletscher, 1966).

MAO is found in varying concentrations in nearly all mammalian tissues. In humans, enzyme activity is highest in the liver, followed by the brain and heart (Pletscher, 1966). In rats, activity is highest in the heart followed by the liver and brain (Prange et al., 1967). MAO is located within the presynaptic neuron, where it is concerned with the oxidative deamination of the catecholamine neurotransmitters dopamine, norepinephrine and epinephrine and the indolealkyl amine neurotransmitter serotonin, subsequent to reuptake of these neurotransmitters from the synapse (Cooper et al., 1978). Using a dopamine uptake inhibitor to inhibit the deamination of dopamine by monoamine oxidase localized in the presynaptic nerve terminals, Azzaro and Schoepp (1982) have been able to demonstrate the significant metabolism of dopamine by postsynaptic monoamine oxidase. In both locations, the enzyme is associated with the outer mitochondrial membrane.
Metabolism of dopamine by monoamine oxidase. (Lader, 1980)

Figure 3.
Enzyme Biochemistry and Substrate Oxidation

MAO is a flavoenzyme with a molecular weight of 150,000 daltons. In its active form it contains 8 sulfhydryl (SH) groups (necessary for activity, but probably not located at the catalytic site) (Hellerman and Erwin, 1968) and 1 mole of flavin adenine dinucleotide (FAD) per mole of enzyme. FAD combines (via conjugase) with a SH group on a cysteine residue of the MAO "apoenzyme" to form the catalytically active monoamine oxidase enzyme (Sourkes, 1983; Maxwell and White, 1978), Figure 4. Both substrates and inhibitors combine in a 1:1 ratio with the enzyme (Chuang et al., 1974).

During substrate oxidation FAD is reduced (*N, Figure 4, Lehninger, 1975) by hydrogen atoms from the substrate amine, resulting in the conversion of the amine to an imino group (Figure 5 A). MAO-FADH2 is then reoxidized to the active enzyme (Figure 5 B) and the imino molecule undergoes hydrolysis to an aldehyde (Figure 5 C).

Isoenzymes

Two forms of MAO, MAO A and MAO B, were identified by Johnston in 1968. The A or B designation refers to the
MAO-serine-glycine-glycine-cysteine-tyrosine (apoenzyme)

\[
\begin{align*}
\text{FAD (oxidized form)} \\
\text{FAD (reduced form)}
\end{align*}
\]

\[\begin{align*}
\text{R} &= \text{ribityl moiety} \\
\star &= \text{site of reduction}
\end{align*}\]

APO MAO-FAD complex: formation of the catalytically active enzyme. Borrowed in part from Mutschler and Mohrke.

Figure 4.
A) \[ R-CH_2-NH_2 + MAO-FAD \rightarrow RCH=NH + MAO-FADH_2 \]

B) \[ MAO-FADH_2 + O_2 \rightarrow MAO-FAD + H_2O_2 \]

hydrolysis

C) \[ RCH=NH + H_2O \rightarrow RCHO + NH_3 \]

Sequential steps in substrate oxidation catalyzed by MAO. Taken in part from Pletscher et al., 1966.

Figure 5.
preference of some substrates and inhibitors for one or the other enzyme forms. For example, clorgyline is a selective inhibitor of MAO A, and norepinephrine and serotonin are preferred substrates for MAO A (Johnston, 1968). Deprenyl selectively inhibits MAO B (Knoll and Magyar, 1972), which preferentially deaminates benzylamine and B-phenylethylamine. However, not all substrates and inhibitors are selective (Table 1). At high concentrations, 'selective' substrates are no longer 'selective'; i.e., they are metabolized by either form of the enzyme (Youdim and Finberg, 1983). Likewise, inhibitors are only selective at low (nM-uM) concentrations (Tipton et al., 1983).

MAO A and B appear to be uniformly distributed in rat and human brain (Neff and Yang, 1974). In rats, the MAO A:MAO B ratio is 85:15; in humans that ratio is 25:75 (Oreland et al., 1983). MAO A is located primarily intraneuronally (Youdim and Finberg, 1983), while most MAO B is located extraneuronally in association with glial cells (Benedetti and Keane, 1980).

MAO A and MAO B can be separated by gel electrophoresis (Youdim and Sandler, 1967) or immunoprecipitation (McCauley and Racker, 1973). It has been suggested that the MAO A or B character may reflect the amount of phospholipid that is associated with the particular MAO form (Houslay and Tipton, 1973; Kinemuchi et al., 1983).
Table 1. Some substrates and inhibitors of MAO.

<table>
<thead>
<tr>
<th></th>
<th>MAO A</th>
<th>MAO B</th>
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<tbody>
<tr>
<td><strong>Selective Substrates</strong></td>
<td>Serotonin</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td><strong>Selective Inhibitors</strong></td>
<td>Clorgyline</td>
<td></td>
</tr>
<tr>
<td><strong>Nonselective Substrates</strong></td>
<td>Dopamine</td>
<td>Tyramine</td>
</tr>
<tr>
<td><strong>Nonselective Inhibitors</strong></td>
<td>*Pargyline (Eutonyl)</td>
<td>**Tranylcypromine (Parnate)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Iproniazid</td>
</tr>
</tbody>
</table>

* used clinically for hypertension  
** used clinically for depression

Taken in part from Neff and Yang, 1974.
Enzyme Inhibitors

MAO is susceptible to reversible and irreversible enzyme inhibition. Table 2 lists some of the common inhibitors, their structures and classes. Reversible inhibition of the enzyme is competitive (Tipton et al., 1983), i.e., dependent on substrate and inhibitor concentrations, and is relatively short-lived. Any compound which binds to the enzyme at the active site but does not undergo oxidation can be a reversible inhibitor. Most of these inhibitors have one α hydrogen for binding but lack the second α hydrogen necessary for oxidation. Competitive reversible inhibitors include amphetamine, and harmaline. Tranylcypromine appears to have properties of both reversible and irreversible inhibitors (Planz et al., 1972A).

Irreversible inhibitors are covalently bound to the enzyme (Planz et al., 1972A, Hellerman and Erwin, 1968) and include the hydrazine derivatives, and the acetylenic (propargylamine) inhibitors. Inhibition by irreversible inhibitors is characterized by organ specific inhibition of long duration. What this means is that the length of inhibition is not characteristic of the specific inhibitor, but rather, depends on the rate of denovo enzyme synthesis in a particular tissue (Planz et al., 1972A and B). Half times for enzyme recovery in the brain and liver are 10
Table 2. Common Inhibitors of Monoamine Oxidase.

**IRREVERSIBLE**

**Hydrazine derivatives**

<table>
<thead>
<tr>
<th>Hydrazides: Nialamide</th>
<th><img src="image" alt="Nialamide Structure" /></th>
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<tbody>
<tr>
<td>Iproniazid</td>
<td><img src="image" alt="Iproniazid Structure" /></td>
</tr>
</tbody>
</table>

**Hydrazines: Phenelzine**

| Pheniprazine          | ![Pheniprazine Structure](image) |

**Acetylenic Inhibitors (Propargylamines)**

| Pargyline             | ![Pargyline Structure](image) |
| Clorgyline            | ![Clorgyline Structure](image) |
| Deprenyl              | ![Deprenyl Structure](image) |

**Cyclopropylamines**

| Tranylcypromine       | ![Tranylcypromine Structure](image) |

**REVERSIBLE**

**α-methyland substituted amines**

| Amphetamine           | ![Amphetamine Structure](image) |

**β-Carbolines**

| Harmaline             | ![Harmaline Structure](image) |
and 3-4 days respectively, indicating a greater rate of enzyme synthesis in the liver compared to the brain.

Since they in effect destroy the enzyme, irreversible inhibitors are sometimes referred to as suicide inhibitors. The combination of these inhibitors with the enzyme is initially competitive with substrate (Figure 6 A) and can be blocked by high concentrations of substrate or reversible inhibitors (Benedetti et al., 1983). Following the initial competitive component and regeneration of MAO-FAD (Figure 6 B) the inhibitor becomes irreversibly bound to the enzyme (Figure 6 C) as evidenced by the persistence of the enzyme-inhibitor complex in the presence of dialysis or high concentrations of substrate (Hellerman and Erwin, 1968; Patek and Hellerman, 1974). The irreversible binding of the hydrazine inhibitor phenylhydrazine to the enzyme is illustrated in Figure 7. Acetylenic inhibitors do not appear to bind to the same place on the FAD molecule, since the presence of pargyline does not interfere with the binding of hydrazines (Smith et al., 1965; Sourkes, 1983).

Both the hydrazine and acetylenic inhibitors are potent inhibitors of the enzyme in vitro (Maxwell et al., 1961; Zeller et al., 1955; Weikel and Salmon, 1962; Pletscher, 1966), and in vivo (Weikel and Salmon, 1962; Planz, 1972; Gorkin and Romanova, 1968).
**Irreversible inhibition of monoamine oxidase by phenylhydrazine. Taken in part from Sourkes, 1983.**

*Figure 6.*
Irreversible (covalent) combination of phenylhydrazine with the FAD component of MAO (MAO-FADH-Phe). Borrowed in part from Sourkes, and Mutschler and Mohrke.

Figure 7.
Metabolism, Distribution and Excretion of
Irreversible Inhibitors

In animals, tissue concentrations of the hydrazine derivatives are maximal about one hour after peripheral injection (Pletscher, 1966) and hydrazine derivatives unbound to the enzyme are excreted within 24 hours. The hydrazides nialamide and iproniazid are metabolized to the active inhibitor (hydrazine) form following cleavage of the amide bond (see Table 2). The active metabolite of iproniazid is isopropylhydrazine (Koechlin and Iliev, 1959); and of nialamide is probably B-[(N-benzylcarbamyl)-ethyl]-hydrazine (Pinson et al., 1962). The proposed metabolic scheme for iproniazid is shown in Figure 8.

The acetylenic group of the acetylenic inhibitors is essential for MAO inhibitory activity. Unlike the hydrazides, these inhibitors do not require metabolic activation to form the active inhibitor.

Therapeutics and Toxicity

MAO inhibitors have been used therapeutically. The first potent inhibitor of MAO activity used clinically was iproniazid. It was introduced in 1957 for the treatment of
Proposed metabolic scheme for iproniazid. Borrowed from Koechlin and Iliev.

Figure 8.
mental depression and angina pectoris. Currently, MAO inhibitors are used for atypical depression and hypertension. Although the mechanism of the therapeutic effectiveness of MAO inhibitors in these disorders is at best speculative, it is thought to be related to the MAO inhibitory activity since (Pletscher et al., 1966; Squires, 1978; Murphy, 1978):

1) structurally similar hydrazides (i.e. isoniazid) with no MAO inhibitory activity are not effective in the treatment of depression or hypertension,

2) structurally dissimilar inhibitors (hydrazines and acetylenic inhibitors) produce similar therapeutic effects, and

3) there is a "reasonable correlation" between MAO inhibitory activity and the therapeutic effects in humans.

The MAO inhibitors are thought to produce their therapeutic effects by influencing catecholamine levels. For example, it has been suggested that depression is related to a decline in serotonin and norepinephrine levels in the brain (the biogenic amine hypothesis) and MAO inhibitors have been shown to increase the concentrations of these transmitters at the synapse (Youdim and Finberg, 1983).

Hypertension is thought to be due to excess noradrenergic neurotransmission in the periphery which results in the
constriction of blood vessels. Many hypothesis of altered noradrenergic neurotransmission have been proposed to be responsible for the antihypertensive effects of MAO inhibitors (Squires, 1978), for example:

1) formation of false transmitter substances such as octopamine in the presence of MAO inhibitors,

2) feedback inhibition of norepinephrine synthesis and release due to an increased concentration of norepinephrine in the synaptic cleft,

3) desensitization of noradrenergic receptors following the accumulation of norepinephrine in the synapse, and

4) increased noradrenergic transmission in the nucleus tractus solitarii in the medulla.

It is quite possible that each of these effects contributes to the overall effectiveness of MAO inhibitors in reducing hypertension.

The use of MAO inhibitors is also associated with severe and sometimes fatal side effects, which limit their clinical usefulness. One classic side effect associated with the nonselective MAO inhibitors is the "tyramine" or "cheese" effect (Kupfer and Detre, 1978). Nonselective inhibitors of MAO inhibit the metabolism of tyramine and thereby enhance the release of norepinephrine by tyramine, an effect which can produce a severe hypertensive crisis. The potential for this reaction is high since tyramine is a component of many
foods (Kupfer and Detre, 1978). In addition, hydrazine derivatives produce severe hepatotoxicity and visual disturbances which are unrelated to monoamine oxidase inhibition.

Selective MAO A or MAO B inhibitors could have certain advantages if used clinically. For example, MAO A inhibitors selectively increase the concentrations of norepinephrine and serotonin both peripherally and in the brain, while having a smaller effect on dopamine, a mixed enzyme substrate. Thus, the antihypertensive and antidepressant effects might be selectively enhanced with the use of MAO A selective inhibitors. Use of a selective MAO A inhibitor might also reduce the chance of a hypertensive crisis resulting from tyramine intake, since tyramine is a nonselective substrate, equally well deaminated by MAO A or MAO B.

Currently, the MAO B selective inhibitor deprenyl is used in Europe in conjunction with L-dopa in the treatment of Parkinson's disease (Birkmayer et al., 1983). It is thought that inhibition of MAO B prevents the metabolism of dopamine in the brain of Parkinsonian patients and enhances the concentration of dopamine resulting from L-dopa administration. The "tyramine" effect might also be reduced with a selective MAO B inhibitor since MAO A is still available to deaminate tyramine in these patients.
Age related changes in monoamine oxidase activity

Changes in the activity of monoamine oxidase have been associated with aging in several species. For example, in humans, MAO activity increases progressively in brain, platelet and plasma, beginning at about 35 years of age (Robinson et al., 1972). While MAO activity in rat heart shows a progressive increase with both weight and age from about 2 weeks of age (Prange et al., 1967; Horita, 1967; Novick, 1961), liver and whole brain MAO levels vary little with age (Horita, 1967). However, MAO activity has been shown to increase with age in specific brain areas. Bhaskaran and Radha (1983) were able to show a significant increase in MAO activity in the cerebral cortex, medulla, hypothalamus, striatum and midbrain of 24 month old rats, compared to 3 and 6 month animals. Studies by Benedetti and Keane (1980) attribute the increase in MAO activity in brain tissue to an increase in MAO B (determined by b-phenylethylamine deamination) in the extrasynaptosomal mitochondrial fraction of all brain areas except the brain stem. This increase in MAO-B activity is most likely associated with the proliferation in glial cells shown to occur with age in the brain (Knoll et al., 1983). In contrast to the increase in MAO B activity, no age related increase in the activity of intrasynaptosomally located MAO
A in rat brain was detected using serotonin as a substrate. It is hypothesized that the increase in MAO B activity in rat brain may contribute to the reduced levels of dopamine in the striatum of senescent rats.
STATEMENT OF THE PROBLEM

Objective

The overall objective of this dissertation is to determine whether dopamine receptor function declines with age in the nucleus accumbens of rats. A number of laboratories have demonstrated a decline in coordinated motor activity in rodents and humans with increasing age. In rats, these motor deficits have been linked to an age related "down regulation" in the density of dopamine receptors in the striatum, or a decline in integrity of the dopaminergic neurons of the nigrostriatal tract.

The nucleus accumbens is an area of the brain closely related to but distinct from the striatum and is involved in the initiation and regulation of motor activity. The dopaminergic projection from the ventral tegmental area to the nucleus accumbens is important in this function. Thus, it is possible that altered dopaminergic transmission in the nucleus accumbens contributes to some of the motor deficits exhibited by aged rats. In fact, several studies suggest that an age related decrease in the number of dopamine receptors also occurs in the
nucleus accumbens. We have examined dopamine receptor function in the nucleus accumbens of aging rats using a behavioral model of dopamine receptor stimulation in which the locomotor activity response of rats to intraaccumbens injections of dopamine agonists is measured.

In these experiments, we, like others, used nialamide to inhibit dopamine metabolism and increase the intensity and duration of the locomotor activity response to intraaccumbens injections of dopamine. When compared to the response of younger rats, older rats had a much reduced response to dopamine. We found that this reduced response of older rats is specific for the nialamide pretreatment, and not due to a decline in dopamine receptor function. Thus, a second major objective of this dissertation is to determine why nialamide pretreatment produces a decreased response to dopamine in older rats.

Significance

The decline in motor function that occurs in humans is one of the most visible effects of aging. Parkinson's disease, characterized by a severe loss of voluntary motor activity, is considered by some to be an example of premature aging since its victims present some of the same lesions in dopaminergic neurons that occur in the brains of elderly patients. Studies which examine the age
related decline in motor performance in rats have begun to increase our understanding of the anatomical substrates underlying similar deficits in humans.

Nialamide is used routinely to inhibit monoamine oxidase activity in studies on the effects of dopamine in the striatum and the nucleus accumbens of aged and non aged rats. Changes in dopamine stimulated behaviors have recently been identified in old rats pretreated with nialamide. Thus, it is important to identify any alterations in effectiveness of nialamide with age.
CHAPTER I

LOCOMOTOR ACTIVITY RESPONSE OF NIALAMIDE PRETREATED OLD RATS TO INTRAACCUMBENS DOPAMINE

1.1. INTRODUCTION

Impairments in motor function have been demonstrated in aged rats that appear to be related to changes in dopaminergic activity in the brain. Thus, aged rats display deficits in sensorimotor coordination (Wallace et al., 1980) and swimming performance (Marshall and Berrios, 1979) that can be partially reversed by intrastriatal nigral grafts (Gage et al., 1983) or the administration of dopaminergic agonists (Marshall and Berrios, 1979) respectively. In addition, aged rats show a reduced rotational response to amphetamine or intrastriatally administered dopamine (Cubells and Joseph, 1981; Joseph et al., 1981).

The motor deficits observed in aged rats occur concomitantly with age related alterations in the biochemistry of the dopaminergic neurons of the
nigrostriatal tract. Declines have been reported in striatal tyrosine hydroxylase activity (McGeer and McGeer, 1978), striatal dopamine synthesis (Samorajski, 1975), striatal dopamine uptake (Jonec and Finch, 1975), and striatal dopamine levels (Joseph et al., 1978; Finch, 1978). In addition, decreases in striatal dopamine receptor binding (Joseph et al., 1978; Memo et al., 1980; De Blasi and Mennini, 1982) and dopamine stimulated adenylate cyclase (Govani et al., 1977; Schmidt and Thornberry, 1978) have been described.

The nucleus accumbens, a forebrain component of the mesolimbic system, is thought to be involved in the initiation and regulation of spontaneous locomotor activity (Mogenson et al., 1980). It has also been implicated in the pathophysiology of schizophrenia (Stevens, 1979; Mackay et al., 1980), Parkinson's disease (Price, 1978) and Huntington's chorea (Bots and Bruyn, 1981), and in the antipsychotic action of neuroleptics (Bartholini, 1977; Costa, 1977). It has previously been demonstrated that this nucleus receives a prominent dopaminergic projection from the ventral tegmental area (Lindvall and Bjorklund, 1978) and that bilateral intraaccumbens injections of dopamine produce a marked increase in locomotor activity (Price et al., 1978; Costall and Naylor, 1975; Jackson et al., 1975; Pijnenburg et al., 1975; Pijnenburg et al., 1976; Makajuola et al., 1980; Jones et al., 1981).
which can be blocked by neuroleptic drugs (Pijnenburg et al., 1975; Jackson et al., 1975; Pijnenburg et al., 1976; Costall et al., 1979; Makanjoula et al., 1980).

Because of its involvement in normal motor activity, the nucleus accumbens may also play a role in the decline in motor function observed in aged rats. For example, the rotational response of rats has been shown to require the nucleus accumbens (Kelly and Moore, 1977) and as mentioned above the intensity of this response shows an age related decline. The nucleus accumbens may also contribute to the reduced locomotor activity displayed by aged rats (Gage et al., 1983), and the age related decline in swimming performance (Marshall and Berrios, 1979).

The present experiments were designed to examine dopamine receptor function in the nucleus accumbens of young (6 mo), mature (15 mo), and old (26 mo) rats by measuring the locomotor response of these animals to bilateral intraaccumbens injections of dopamine after pretreatment with nialamide. This procedure has been shown to produce an intense and long lasting stimulation of locomotor activity (Jackson et al., 1975; Costall et al., 1979; Makanjoula et al., 1980). To further characterize dopaminergic function in the nucleus accumbens, the locomotor activity response of young, mature and old rats to
dopamine after pretreatment with the alternative monoamine oxidase inhibitors pargyline, iproniazid, and clorgyline and to ergometrine alone (a dopamine agonist), or to dopamine or amphetamine alone was determined.

I.2. METHODS

A. Animals

Male COBS® CDF® F344/Crl rats, 6, 15, and 26 months of age were used in these studies and housed in AALAC approved cages. Food (Purina Rat Chow) and water were available ad libitum. The animal quarters were maintained on a 6 AM:6 PM light:dark cycle. Animals were permitted to acclimate to our laboratory environment for at least one week prior to experimentation.

B. Surgical Procedure

Rats were anesthetized with a halothane/oxygen mixture and secured in a stereotaxic frame (David Knopf Inst., CA.). Holes were then drilled on each side of the skull at A+9.8, L+1.6, DeGroot coordinates (Pellegrino and Cushman, 1967). Injections were made using a 10 μl Hamilton syringe (Hamilton Company, Reno, Nevada) which was attached by a clamp to the electrode carrier of the stereotaxic apparatus.
The needle tip was inserted through the holes to a depth of \( V - .15 \), and the solution was injected at a rate of \( 0.5 \mu \) 1/min. The needle was left in place for an additional minute to allow the solution to diffuse away from the needle tip and then withdrawn from the brain. Following bilateral injections, the skin incision was closed with a wound clip and covered with lidocaine to prevent any pain which might interfere with locomotion. Anesthesia was then discontinued and the rats were placed in individual activity cages for monitoring locomotor activity. Each animal was injected only once.

C. Locomotor Activity

Rats were placed in activity cages (Opto-Varimex-Minor, Columbus Inst., Cols., OH.) and allowed to acclimate for 30 min. They were then removed from the cages and anesthetized with the halothane/oxygen mixture prior to receiving bilateral intraaccumbens drug injections. Following drug administration, rats were returned to their respective cages and locomotor activity recorded as a function of time. The activity cages were designed to measure ambulatory movements, but not total horizontal or total vertical movement. The cages contained 12x12 infrared beams passing at a height of 5 cm from the bottom of the cage through a ventilated plexiglass box measuring 42 cm square and 20 cm high. Locomotor activity (i.e. ambulatory movement) was
recorded as the number of times 2 consecutive beams, 3.5 cm apart, were interrupted during a 60 minute period. The data were recorded by a digital counter (Columbus Inst., Columbus, Ohio). Animals were observed visually for convulsions, tremors or other non-ambulatory behavior. Observations were made in an isolated environmental room maintained at 21±1°C under diffuse light, beginning at approximately 1100 on day 1 and ending at 0200 on day 2. Pretreatment, if given, was administered at 0930. Whenever possible, the locomotor responses of rats of all 3 ages were determined simultaneously.

D. Histology

Injection coordinates for intraaccumbens injections were verified in each rat following activity experiments. Rats were sacrificed and the brain excised and placed in 4.0 % formaldehyde for 1-2 days. Frozen brain sections (40 μ thick) were sliced sequentially in a rostral to caudal direction using a Cryo-Cut Microtome (American Optical Corp., Buffalo, N.Y.) until injection tracts were visible. The needle tracts (site of injection) were compared to the location of the nucleus accumbens (using the stereotaxic atlas of Pellegrino and Cushman, DeGroot coordinates) for correct injection placement in each animal (Plate I).
E. **Drugs**

The following drugs were obtained from Sigma Chemical Co., St. Louis, Mo.: nialamide, dopamine HCl, ergometrine HBr, amphetamine S04, iproniazid PO4 and pargyline HCl. Clorgyline was a gift from May and Baker Co., England. Dopamine and ergometrine were dissolved in nitrogen bubbled distilled water containing 0.1% sodium metabisulfite, and adjusted to pH 5.5 - 6.0 with 0.5 N NaOH. These solutions were injected into the nucleus accumbens in a volume of 0.5μl. Control animals were injected with an equal volume of vehicle. Nialamide was dissolved in a minimum of 1.0 N HCl before the addition of an appropriate volume of double distilled water. Pargyline, iproniazid and clorgyline were dissolved in 0.9% NaCl. Intraperitoneal injections were administered in a volume of 1 ml/kg.

F. **Statistics**

Data is expressed as the mean and standard error of the mean (S.E.M.). Significant differences in locomotor activity and monoamine oxidase activity were evaluated using the two-tailed Student's t-test (Sokal and Rohlf, 1969) with a level of P<0.05 being considered significant. An analysis of variance with Student Neuman-Keuls test for multiple comparisons was performed when determining significant differences in activity among young, mature and old rats.
I.3. RESULTS

A. Effect of dopamine on the locomotor activity of young, mature and old rats pretreated with nialamide

Rats were pretreated with nialamide (100 mg/kg, i.p.) 1.5 hours prior to bilateral intraaccumbens injections of vehicle or various doses of dopamine. Figure 9 shows the mean locomotor activity of groups of nialamide pretreated young, mature, and old rats to intraaccumbens injections of vehicle. The locomotor activity of all 3 groups was much lower than that obtained after the intraaccumbens injection of dopamine (note the 10 fold greater scale on the y axis in Figures 10-13, the response to dopamine, compared to Figure 9, the response to vehicle) with the activity of old and young rats being significantly lower than that of mature rats at several of the time intervals. In order to determine the locomotor activity response to the intraaccumbens administration of dopamine, the mean activity value for each age group, recorded after the intraaccumbens administration of vehicle, was subtracted from that obtained after the intraaccumbens administration of dopamine.
The bilateral administration of dopamine into the nucleus accumbens of young and mature rats pretreated with nialamide produced an intense and long lasting stimulation of locomotor activity (Figures 10-13). This response to dopamine was dose dependent and, at all doses tested, was many times greater than the response to intraaccumbens injections of the vehicle. The locomotor activity response of old rats to dopamine at doses of 2.5-20 μg was significantly lower than that of young and mature rats. At a 40 μg dose, the response of old rats was significantly lower than that of young rats only.

B. Effect of dopamine on the locomotor activity of mature and old rats that were not pretreated with a monoamine oxidase inhibitor

In order to determine the response to dopamine in the absence of a monoamine oxidase inhibitor, mature and old rats were given bilateral intraaccumbens injections of dopamine (20 or 40 μg). As reported previously (Pijnenburg et al., 1976) the hypermotility response to dopamine in the absence of a monoamine oxidase inhibitor was brief, lasting approximately one hour. Both old and mature rats exhibited a hypermotility response to dopamine and there was a trend toward a greater response in the old rats (Table 3).
C. Effect of amphetamine on the locomotor activity response of young and old rats that were not pretreated with a monoamine oxidase inhibitor

Rats received intraaccumbens injections of amphetamine (20 μg bilaterally), an indirect dopamine agonist, and the locomotor activity response was recorded for 1 hour (Table 4). The response of 29 month old (old) rats to amphetamine was significantly greater than that of 9 month old (young) rats.

D. Effect of ergometrine on the locomotor activity of mature and old rats which were not pretreated with a monoamine oxidase inhibitor

To further characterize the response of mature and old rats to the intraaccumbens administration of direct acting dopamine receptor agonists, ergometrine (1 μg) was administered bilaterally into the nucleus accumbens of non-pretreated mature and old rats. In contrast to dopamine, and as reported by others (Pijnenburg et al., 1973) intraaccumbens administration of ergometrine produced a long lasting stimulation of locomotor activity of moderate intensity (Figure 14). The response of both mature and old rats was nearly identical.
E. Effect of dopamine on the locomotor activity of mature and old rats pretreated with pargyline

Mature and old rats were pretreated with pargyline (75 mg/kg, i.p.), a monoamine oxidase inhibitor, 1.5 hours prior to the bilateral intraaccumbens injection of vehicle or dopamine (20 μg), and the locomotor activity was recorded. The locomotor activity of groups of mature and old rats after the administration of vehicle is shown in Figure 15, with old rats showing significantly greater activity at 2, 3, and 4 hours after vehicle injection. This baseline activity was subtracted from the activity recorded after dopamine administration in order to obtain the hypermotility response to dopamine. Figure 16 shows that the intraaccumbens administration of dopamine produced a marked and prolonged hypermotility response in both mature and old rats pretreated with pargyline. In contrast to the effects after nialamide pretreatment, the peak response to dopamine was not significantly different between the two age groups, and the time course of the response for these groups was nearly identical.

F. Effect of iproniazid on the locomotor activity response of rats to intraaccumbens injections of dopamine
It was of interest to determine whether the reduced response to dopamine of nialamide pretreated old rats was a characteristic of pretreatment with all hydrazide type monoamine oxidase inhibitors. As can be seen in Figure 17, old rats pretreated with iproniazid did not respond less to dopamine compared to young and mature rats.

G. Response of mature and old rats pretreated with clorgyline to intraaccumbens dopamine

In order to determine if there was an age related difference in the response to intraaccumbens injections of dopamine after pretreatment with a selective monoamine oxidase inhibitor, rats were pretreated with clorgyline, a inhibitor selective for monoamine oxidase A. There was no age related difference in the locomotor activity response of mature and old rats to dopamine after pretreatment with clorgyline. The response of both age groups was nearly identical in both intensity and duration of response (Figure 18).

I.4. DISCUSSION

Previous studies have reported deficits in the motor function of aged rats that have been associated with an
impairment in dopaminergic neurotransmission in the corpus
striatum (Gage et al., 1983; Joseph et al., 1981;
Cubells and Joseph, 1981). However, other brain regions
containing dopamine nerve terminals may be involved in
producing these deficits. The nucleus accumbens, located in
the ventral forebrain, is known to be involved in the
initiation and maintenance of locomotor activity and
dopaminergic neurotransmission at this site plays an
important role in the regulation of this behavior (Mogenson
et al., 1980). Thus, abnormal dopaminergic
neurotransmission in the nucleus accumbens may be involved
in the motor deficits of aged rats. The present study was
designed to determine whether dopamine receptor function in
the nucleus accumbens is altered in old rats compared to
young and mature rats. Dopamine receptor function was
measured using a standard procedure in which the locomotor
activity response to dopamine was determined after its
injection directly into the nucleus accumbens of rats
pretreated with a monoamine oxidase inhibitor. This
procedure has been shown to produce an intense and long
lasting stimulation of locomotor activity (Jackson et al.
., 1975; Costall et al., 1979; Makanjoula et al.,
1980).

In our initial studies, dopamine was injected into the
nucleus accumbens of old, mature, and young rats after
pretreatment with nialamide. Compared to the response of
the rats from the other age groups, the response of old rats to dopamine was markedly reduced. Thus, dopamine did not significantly stimulate the locomotor activity of old rats at doses of 2.5 and 5.0 µg, while it produced a marked stimulation of locomotor activity in young and mature rats at these doses. At higher doses (20 and 40 µg), dopamine did stimulate the locomotor activity of aged rats but the response was reduced and of relatively short duration. Although these results are consistent with a decrease in dopamine receptor function in aged rats, the locomotor activity response of old rats to a dopamine agonist (ergometrine), dopamine alone, or dopamine after pargyline pretreatment do not support this conclusion.

In order to determine whether the decreased response of old rats to dopamine is related to the pretreatment with a monoamine oxidase inhibitor, we examined the response to dopamine of old and mature rats that were not pretreated with a monoamine oxidase inhibitor. Under these conditions, dopamine produced a brief stimulation of locomotor activity, lasting approximately one hour. This decrease in the duration of the response compared to that of rats pretreated with a monoamine oxidase inhibitor is consistent with the concept that dopamine, after intraaccumbens injection, is rapidly metabolized by monoamine oxidase. In contrast to the effects of dopamine in nialamide pretreated rats, it was found that the hypermotility response to dopamine of old
rats was not smaller than that of mature rats. In fact, there was a trend toward a greater response in the old rats compared to the mature rats. It has been reported that after injection of dopamine into rat striatum, the dopamine diffused to a greater extent in young compared to old animals (Bondareff et al., 1971). Since the response of old animals is not decreased in the absence of nialamide (Table 3), it is unlikely that the attenuated response of old animals in the presence of nialamide is related to diffusion differences. Our results suggest that the reduction in the response to dopamine in old rats pretreated with nialamide is related to the presence of the monoamine oxidase inhibitor.

This hypothesis is supported by our observation that ergometrine, a dopamine agonist that is not metabolized by monoamine oxidase, produced a similar intensity and duration of locomotor activity stimulation in old and mature rats. In addition, although the response of old rats to dopamine was reduced after nialamide pretreatment, it was not significantly decreased if the animals were treated with another monoamine oxidase inhibitors pargyline, iproniazid or clorgyline. This latter observation suggests that the reduced response of the old rats to dopamine is specific for nialamide and is not a general effect common to all monoamine oxidase inhibitors.
One explanation for the reduced response of nialamide pretreated old rats to dopamine would be that nialamide is unable to inhibit monoamine oxidase in the nucleus accumbens of old rats. Consequently, dopamine, after intraaccumbens injection, would be rapidly metabolized and would not accumulate in the vicinity of postsynaptic dopaminergic receptors. Studies on the metabolism of dopamine in vivo are needed to assess this possibility.

In summary, these studies show that after nialamide pretreatment, the response to the intraaccumbens administration of dopamine of old rats is markedly reduced compared to that of young and mature rats. The decreased response of old rats does not appear to be due to a decrease in dopamine receptor activity in the nucleus accumbens or to a physical impairment in old rats which prevents them from responding to dopamine with high rates of locomotor activity. Instead, the decreased response appears to be related to a selective effect of nialamide in old rats that does not occur in either young or mature rats. The nature of this unique action of nialamide in old rats is at present unclear, but it results in an attenuated response to the intraaccumbens injection of dopamine.
Plate I.

Photomicrograph showing bilateral needle tracts terminating in the nucleus accumbens.

Injection tracts were visible from A+9.0 to A+9.8 and were located at the site of or slightly medial to the anterior commissure and within the nucleus accumbens. This histological section is representative of sections from rats responding to bilateral injections of dopamine with high rates of locomotor activity.
Baseline locomotor activity of nialamide pretreated young, mature, and old rats injected with vehicle.

Rats were injected with nialamide (100 mg/kg i.p.) 1.5 hours prior to bilateral intraaccumbens injections of vehicle (0.5 µl). Motor activity was recorded at one hour intervals for 15 hours after vehicle injection. Each point represents the mean one hour activity ± S.E.M. of 7 rats. Analysis of variance: F(2,18) = 4.54, P < .05, 3 hr; F(2,18) = 9, P < .005, 4 hr; F(2,18) = 10.75, P < .001, 5 hr; F(2,18) = 6.35, P < .01, 6 hr; F(2,18) = 3.63, P < .05, 7 hr; F(2,18) = 5.62, P < .025, 8 hr; F(2,18) = 6.25, P < .01, 9 hr; F(2,18) = 20.85, P < .001, 10 hr; F(2,18) = 5.91, P < .025, 11 hr; F(2,18) = 12.15, P < .001, 12 hr; F(2,18) = 6.36, P < .01, 13 hr; F(2,18) = 4.69, P < .025, 14 hr. Student Newman-Keul's test: *P < .05 when compared to mature rats.
Figure 9.

LOCOMOTOR ACTIVITY/HOUR

△ YOUNG
□ MATURE
○ OLD

HOUR AFTER INJECTION
Figures 10-13.

Locomotor activity response of young, mature and old rats, pretreated with nialamide, to intraaccumbens injections of dopamine (DA).

Rats were pretreated with nialamide (100 mg/kg i.p.) 1.5 hours prior to bilateral intraaccumbens injections of dopamine: 2.5 µg (Figure 10), 5.0 µg (Figure 11), 20 µg (Figure 12), and 40 µg (Figure 13). Motor activity was recorded at one hour intervals for 15 hours after the injection of dopamine. Each point represents the mean one hour activity ± S.E.M. of 3-6 rats. Baseline activity (nialamide + vehicle, Figure 9) was always subtracted from activity recorded after the injection of dopamine to get the locomotor activity response/hour. Analysis of variance, DA 2.5 µg: F(2,8) = 12.39, P < .005, 2 hr; F(2,8) = 23.88, P < .001, 3 hr; F(2,8) = 15.39, P < .005, 4 hr; F(2,8) = 9.86, P < .01, 5 hr; F(2,8) = 5.35, P < .05, 6 hr. DA 5.0 µg: F(2,10) = 9.90, P < .005, 2 hr; F(2,10) = 27.48, P < .001, 3 hr; F(2,10) = 41.01, P < .001, 4 hr; F(2,10) = 40.94, P < .001, 5 hr; F(2,10) = 76.43, P < .001, 6 hr; F(2,10) = 36.25, P < .001, 7 hr; F(2,10) = 7.44, P < .025, 8 hr. DA 20 µg: F(2,8) = 12.09, P < .005, 3 hr; F(2,8) = 17.81, P < .005, 4 hr; F(2,8) = 5.68, P < .05, 5 hr; F(2,8) = 7.94, P < .025, 6 hr; F(2,8) = 8.26, P < .025, 7 hr. DA 40 µg: F(2,11) = 4.71, P < .05, 5 hr; F(2,11) = 5.14, P < .05, 6 hr; F(2,11) = 4.74, P < .05, 7 hr. Student Newman-Keul's test: *P < .05 when compared to young and mature rats; **P < .05 when compared to mature rats; ***P < .05 when compared to young rats.
Figure 10.
Figure 11.
Figure 12.
Figure 13.
TABLE 3

One hour locomotor activity response of mature and old rats to intraaccumbens injections of dopamine.

Dopamine at doses of 20μg/0.5μl or 40μg/0.5μl was injected bilaterally into the nucleus accumbens of groups of 4 rats. Activity was monitored for one hour following the central injections. Baseline activity (one hour activity of rats receiving intraaccumbens injections of vehicle, data not shown) was always subtracted from dopamine stimulated activity to get the locomotor activity response/hour.

<table>
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<th>TREATMENT</th>
<th>LOCOMOTOR ACTIVITY/HOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MATURE</td>
</tr>
<tr>
<td>dopamine 20μg</td>
<td>624 ± 173</td>
</tr>
<tr>
<td>dopamine 40μg</td>
<td>744 ± 80</td>
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</table>

*Activity is statistically different from that of mature rats, P < .05.
TABLE 4

One hour locomotor activity response of mature and old rats to intraaccumbens injections of amphetamine

Two groups of 4 rats, 9 and 29 months of age, received bilateral intraaccumbens injections of amphetamine (20μg). Each value represents the mean one hour activity response of each age group ± SEM.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>LOCOMOTOR ACTIVITY / HOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 mos.</td>
</tr>
<tr>
<td>Amphetamine 20μg</td>
<td>2353 ± 228</td>
</tr>
</tbody>
</table>

* Activity is statistically different from that of 9 month rats, P<.05.
Figure 14.

Effect of intraaccumbens injections of ergometrine on the locomotor activity response of mature and old rats.

Locomotor activity was recorded at one hour intervals for 7 hours after the injection of ergometrine (1 μg). Each point represents the mean activity ± S.E.M. of 4-6 rats.
Figure 14.
Figure 15.

Time course of the locomotor activity response of pargyline pretreated mature and old rats to intraaccumbens injections of vehicle.

Rats were pretreated with pargyline, 75 mg/kg i.p., 1.5 hours prior to bilateral intraaccumbens injections of vehicle (0.5 µl). Locomotor activity was recorded at one hour intervals for 15 hours after the injection of vehicle. Each value represents the mean activity of 3-4 rats ± S.E.M.. *Activity significantly different from that of mature rats, P<.05.
Figure 15.
Figure 16.

Time course of the locomotor activity response of pargyline pretreated mature and old rats to intraaccumbens injections of dopamine.

Rats were pretreated with pargyline (75 mg/kg, i.p.) 1.5 hours prior to bilateral intraaccumbens injections of dopamine (20 μg/0.5 μl). Locomotor activity was recorded at one hour intervals for 15 hours after the injection of dopamine. Baseline activity (pargyline + vehicle, Figure 15) was subtracted from activity recorded after the injection of dopamine to get the locomotor activity response/hour. Each value represents the mean activity of 3-4 rats ± S.E.M.
LOCOMOTOR ACTIVITY/HOUR (X1000)

□ MATURE
□ OLD

HOUR AFTER INJECTION

Figure 16.
Figure 17.

Effect of iproniazid pretreatment on the locomotor activity response of young, mature and old rats to intraaccumbens injections of dopamine.

Rats were pretreated with iproniazid (100 mg/kg, i.p.) 1.5 hours prior to intraaccumbens injections of dopamine (20μg, bilaterally). Locomotor activity was recorded at one hour intervals for 15 hours after the dopamine injection. Each point represents the mean 1 hour locomotor activity response of groups of 3-4 rats at one hour time intervals after the intraaccumbens injection of dopamine. There were no significant age related differences in the locomotor activity response to dopamine of iproniazid pretreated rats.
Figure 17.
Response of mature and old rats pretreated with clorgyline to intraaccumbens injections of dopamine.

Rats were pretreated with clorgyline (5 mg/kg, i.p.) 1.5 hours prior to bilateral intraaccumbens injections of dopamine (20 μg). The locomotor activity response was recorded at 1 hour intervals for 10 hours after the dopamine injection. Each point represents the mean 1 hour activity response of 3-4 rats ± SEM. Activity was not significantly different for the 2 age groups.
Figure 18.
CHAPTER II

EFFECT OF NIALAMIDE ON THE METABOLISM OF DOPAMINE INJECTED INTO THE NUCLEUS ACCUMBENS OF OLD RATS

II.1. INTRODUCTION

The injection of dopamine into the nucleus accumbens of rats after pretreatment with a monoamine oxidase inhibitor results in a strong and long lasting stimulation of locomotor activity (Costall and Naylor, 1975; Jackson et al., 1975; Pijnenburg et al., 1975; Makanjoula et al., 1980). We have recently reported (Cousin et al., 1985; Chapter I) that the locomotor activity response of old (26 mo) rats to intraaccumbens injections of dopamine is less than that of young (6 mo) or mature (15 mo) rats if the rats are pretreated with nialamide (a monoamine oxidase inhibitor). In these experiments, the locomotor activity response to the injection of dopamine (20 μg bilaterally) lasted at least 6 hours in young and mature rats, while the response of old rats lasted only 2 hours and was significantly less than that of young and mature animals from 3-6 hours after the injection of dopamine.
The mechanism of this attenuated response to dopamine of nialamide pretreated old rats is not clear. The reduced response does not appear to be due to a decline in dopamine receptor function, since old rats respond equally well to intraaccumbens injections of ergometrine or dopamine in the absence of pretreatment with a monoamine oxidase inhibitor (Cousin et al., 1985; Chapter I). Neither is this reduced response a characteristic of pretreatment with all monoamine oxidase inhibitors, since rats pretreated with pargyline, iproniazid or clorgyline show no age related difference in the locomotor activity response to intraaccumbens dopamine (Chapter I).

Monoamine oxidase inhibitors are frequently used in studies on the effect of dopamine on locomotor activity because they prevent the metabolism of dopamine, and thereby enhance the intensity and duration of the stimulatory effects of injected dopamine. It is possible that the response to dopamine is reduced in old animals because nialamide is not an effective inhibitor of dopamine metabolism (and monoamine oxidase) in these animals. Therefore, the present studies were designed to determine the effect of nialamide pretreatment on the concentration of dopamine and its metabolites after the direct injection of dopamine into the nucleus accumbens. In addition, the ability of nialamide to inhibit monoamine oxidase in the nucleus accumbens after intraperitoneal administration or
following in vitro preincubation with nucleus accumbens homogenates was determined.

II.2. METHODS

A. Animals

Male COBS® CDF® F344/Crl rats, 6, 15, and 26 months of age supplied by the National Institute on Aging were used in these studies and housed in AALAC approved cages. Food (Purina Rat Chow) and water were available ad libitum. The animal quarters were maintained on a 6 AM:6 PM light:dark cycle. Animals were permitted to acclimate to our laboratory environment for at least one week prior to experimentation.

B. Intraaccumbens Injections

Rats were anesthetized with a halothane/oxygen mixture and secured in a stereotaxic frame (David Knopf Inst., CA.). Holes were then drilled on each side of the skull at A+9.8, L±1.6, DeGroot coordinates (Pellegrino and Cushman, 1967). Injections were made using a 10 μl Hamilton syringe (Hamilton Company, Reno, Nevada) which was attached by a clamp to the electrode carrier of the stereotaxic apparatus. The needle tip was inserted through the holes to a depth of
and the solution was injected at a rate of 0.5 μl/min. The syringe was left in place for an additional minute to allow the solution to diffuse away from the needle tip. Following bilateral injections, the skin incision was closed with a wound clip and covered with lidocaine.

C. Spectrofluorometric Assay for Dopamine, DOPAC and HVA

The nucleus accumbens was dissected as described by Horn et al., (1974) and placed on dry ice until analyzed. Dopamine, DOPAC and HVA were extracted and separated using Sephadex G-10 columns according to the method of Earley and Leonard (1978). Briefly, the nucleus accumbens was weighed and homogenized in 1.0 ml of 0.4 N perchloric acid. Excess perchlorate was precipitated with 50 μl KOH/HCOOH buffer. Each sample was centrifuged at 4800 x g for 20 min at 4°C and the supernatant decanted onto the sephadex columns. Dopamine was eluted from the column with 0.01 N HCl and 0.005 M Na2HPO4; DOPAC and HVA were eluted with 0.005 M Na2HPO4. The concentrations of dopamine and metabolites in the eluate were determined spectrofluorometrically according to the method of Chang (1964), and expressed as ng/mg tissue.
D. Monoamine Oxidase Assay

Monoamine oxidase activity was determined in duplicate in nucleus accumbens homogenates (McCamen et al., 1965). The nucleus accumbens was dissected (Horne et al., 1974) and placed on dry ice. The nucleus was weighed and homogenized in 10 Vol of 0.001 M phosphate buffer, pH 7.0. 100 μl of buffer substrate containing 0.3 μl 3H-dopamine (30.4 Ci/mmol) and 2.5 mM dopamine in 0.1 M potassium phosphate, pH 6.75, was added to 20 μl of homogenate (2.0 mg of tissue), and incubated for 30 min at 38° C. In experiments where the enzyme was preincubated with nialamide in vitro, nialamide was added to the enzyme in a pH 7.0 phosphate buffer solution and incubated for 30 min at 38° C, prior to the addition of substrate. The reaction was stopped by the addition of 10 μl of 3 N HCl. 3H-metabolites were extracted into 500 μl of ethyl acetate. 250 μl of the ethyl acetate phase (containing 3H-metabolites) was added to 250 μl of fresh ethyl acetate and re-extracted using 120 μl of acidified buffer. 200 μl of this ethyl acetate phase was then transferred to a counting vial and the 3H-metabolite concentration determined by liquid scintillation counting. Blanks contained 100 μl of buffer substrate and 20 μl of the buffer used to homogenize the tissue. This assay was linear with time (Figure 19) and tissue (Figure 20) concentration.
E. Statistics

Data is expressed as the mean and standard error of the mean (S.E.M.). Significant differences between age groups were evaluated using analysis of variance with Student Neuman-Keuls test, with a level of P < 0.05 being considered significant.

F. Drugs

The following drugs were obtained from Sigma Chemical Co., St. Louis, Mo.: nialamide, pargyline HCl, and dopamine HCl. Dopamine was dissolved in nitrogen bubbled distilled water containing 0.1% sodium metabisulfite, and adjusted to pH 5.5 - 6.0 with 0.5 N NaOH. Drug solutions were injected into the nucleus accumbens in a volume of 0.5 μl. Control animals were injected with an equal volume of vehicle. Nialamide was dissolved in a minimum of 1.0 N HCl before the addition of an appropriate volume of double distilled water. Pargyline was dissolved in 0.9% NaCl. Intraperitoneal injections were administered in a volume of 1 ml/kg. 3H-dopamine was obtained from New England Nuclear.
A. Effect of nialamide on the concentration of dopamine in the nucleus accumbens after direct injection of dopamine

Dopamine levels were measured in the nucleus accumbens of nialamide pretreated rats, 0.5, 1.5, 3.5, and 6 hours after the direct injection of dopamine (20 μg)(Table 5). Figure 21 shows the dopamine concentration in the nucleus accumbens of nialamide pretreated young, mature or old rats after subtraction of control (nialamide + vehicle) values. At 0.5 hour after the dopamine injections, the dopamine concentration in the nucleus accumbens was almost identical in rats of all ages. Dopamine levels then declined in all rats over the 6 hour period; however, the concentration of dopamine in the nucleus accumbens of old rats declined more rapidly than that of young and mature rats, and was significantly lower than that of young rats at 3.5 hours and young and mature rats at 6 hours after the intraaccumbens injection of dopamine.

B. Effect of pargyline on dopamine levels in the nucleus accumbens of rats receiving bilateral intraaccumbens injections of dopamine

In order to determine whether the reduced concentration
of injected dopamine in the nucleus accumbens of old rats was unique to nialamide pretreatment, rats were pretreated with pargyline (75 mg/kg i.p., 1.5 hours) and dopamine levels in the nucleus accumbens were determined 3.5 hours after intraaccumbens injections of dopamine (20 µg)(Table 6). Under these conditions the dopamine concentration in the nucleus accumbens of old rats was not lower than that of young and mature rats (Figure 22).

C. Effect of nialamide or pargyline pretreatment on dopamine and dopamine metabolite levels in the nucleus accumbens

In order to determine if the lower levels of dopamine in the nucleus accumbens of old rats that received nialamide and intraaccumbens dopamine might be related to the inability of nialamide to inhibit the metabolism of dopamine, the concentrations of dopamine (DA), homovanillic acid (HVA), and dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens were determined .5, 1 and 2 hours after the injection of dopamine (Table 7). Figure 23 represents the dopamine concentration in the nucleus accumbens of saline or nialamide (100 mg/kg), or saline or pargyline (75 mg/kg) pretreated rats at 2 hours after the injection of dopamine. As expected, the dopamine levels of the saline pretreated animals of all ages were already markedly reduced as most of the injected dopamine had been metabolized. In
contrast, the dopamine concentration in the nucleus accumbens of pargyline pretreated rats of all ages was markedly elevated. Pretreatment with nialamide also produced a significant elevation in dopamine levels in the nucleus accumbens of mature and young rats. However, the dopamine level in the nucleus accumbens of old rats was significantly lower than that of young and mature rats.

Figure 24 shows the DOPAC levels in the nucleus accumbens of rats in the three different age groups. After saline pretreatment, DOPAC concentrations in rats of all ages were much higher than those in rats pretreated with a monoamine oxidase inhibitor. Pargyline effectively inhibited the metabolism of injected dopamine in rats of all ages, as reflected in the very low concentration of DOPAC in the nucleus accumbens. Similarly, DOPAC concentrations were also reduced in the nucleus accumbens of young and mature rats pretreated with nialamide. However, the DOPAC concentration in the nucleus accumbens of old rats pretreated with nialamide was significantly greater than that in young or mature rats.

Pargyline pretreatment reduced the HVA concentration in rats of all ages (Figure 25). Unexpectedly, the HVA concentration in the nucleus accumbens of young rats pretreated with pargyline was significantly greater than that of mature or old rats, suggesting a smaller inhibition
of monoamine oxidase. However, HVA was not detected in the nucleus accumbens of pargyline pretreated old rats. Pretreatment with nialamide reduced the HVA concentration in the nucleus accumbens of young and mature rats. However, the HVA concentration in the nucleus accumbens of old rats was significantly greater than that in young and mature rats.

D. Effect of pargyline on monoamine oxidase activity in the nucleus accumbens

Rats of the three different age groups were injected with pargyline (75 mg/kg i.p.) or saline 3 hours prior to sacrifice and the monoamine oxidase activity in the nucleus accumbens was determined in vitro (Table 8). There was no significant age difference in the monoamine oxidase activity in the nucleus accumbens of rats that were injected with saline, and pargyline completely inhibited enzyme activity in rats of all ages.

E. Effect of nialamide on monoamine oxidase activity in the nucleus accumbens

Table 9 represents the enzyme activity as well as the percent inhibition determined in the nucleus accumbens of groups of young, mature and old rats injected with nialamide (25 to 300 mg/kg, i.p.) or saline 3 hours prior to
sacrifice. As can be seen from the table, the enzyme activity in the nucleus accumbens of young, mature, and old rats after saline injection was similar. Nialamide produced a dose related inhibition of enzyme activity in young and mature rats, reaching 100% inhibition at 100 mg/kg, the dose used for the locomotor activity studies and dopamine and metabolite determinations. However, the enzyme activity in old rats was significantly greater than that of young and mature rats at all doses tested. In fact, when monoamine oxidase was completely inhibited in young and mature rats at the 100 mg/kg dose of nialamide, activity was only inhibited by 61% in old rats. Very high doses of nialamide (300 mg/kg, i.p.) produced a 93% inhibition of enzyme activity.

F. Monoamine oxidase inhibition following in vitro preincubation with nialamide

In order to determine whether the reduced inhibition of monoamine oxidase might be due to a reduced accessibility of nialamide to the nucleus accumbens after peripheral injection rather than a change in the enzyme that made it resistant to inhibition by nialamide, monoamine oxidase activity in the nucleus accumbens was determined after in vitro preincubation of homogenates with nialamide (Table 10). Under these conditions, nialamide dose dependently inhibited enzyme activity in rats of all ages, and there was no age difference in enzyme activity or percent inhibition.
of the enzyme.

II.4. DISCUSSION

Bilateral injections of dopamine into the nucleus accumbens of rats has been shown to produce a marked stimulation of locomotor activity. This response can be enhanced by pretreating the animals with a monoamine oxidase inhibitor to prevent the metabolism of dopamine. We studied the effects of intraaccumbens dopamine and the monoamine oxidase inhibitor nialamide, in order to determine whether changes occur in dopamine receptor function in the nucleus accumbens of aging rats (Cousin et al., 1985, Chapter I). In these studies we detected a reduced locomotor activity response to intraaccumbens injections of dopamine in old rats. To ascertain that the reduced response of old rats was not peculiar to pretreatment with nialamide, the response of mature and old rats to intraaccumbens injections of dopamine after pretreatment with pargyline (an alternative monoamine oxidase inhibitor) was also determined. Surprisingly, old and mature rats responded equally well to intraaccumbens injections of dopamine after pretreatment with pargyline. In the present studies, we have attempted to characterize the age related effect of nialamide in rats.
One explanation for the reduced locomotor activity response of old rats to intraaccumbens injections of dopamine after nialamide pretreatment might be that there is a reduced retention of injected dopamine in these animals. To test this possibility, we measured dopamine concentrations in the nucleus accumbens of rats of the different age groups that were pretreated with nialamide or pargyline (in doses used for behavioral experiments) at different times after the intraaccumbens injection of dopamine. We found that dopamine levels in the nucleus accumbens of nialamide pretreated old rats were significantly less at 3.5 and 6 hours than those in nialamide pretreated young and young and mature rats respectively. This decreased accumulation of dopamine in old rats after nialamide pretreatment corresponds to the reduced stimulation of locomotor activity observed in these animals from 3 to 6 hours after intraaccumbens dopamine injection (Cousine et al., 1985; Chapter I). In comparison and consistent with the results of the locomotor activity experiments, we saw no age related decrease in dopamine retention when rats were pretreated with pargyline. These results suggest that nialamide is not effectively inhibiting dopamine metabolism in the nucleus accumbens of old rats.

To determine the effect of nialamide on dopamine metabolism, we measured the levels of dopamine and the
dopamine metabolites, DOPAC and HVA, simultaneously in nuclei from young, mature and old rats pretreated with saline, nialamide, or pargyline two hours after bilateral injections of dopamine into the nucleus accumbens. In the absence of a monoamine oxidase inhibitor (saline pretreatment), the dopamine levels were not different from those of untreated rats (data not shown) and there was a marked accumulation of DOPAC and HVA in rats of all ages. Pargyline pretreatment reduced the DOPAC and HVA concentrations in young, mature and old rats, consistent with a marked inhibition of monoamine oxidase. In contrast to pargyline, the DOPAC and HVA concentrations in the nucleus accumbens of old rats pretreated with nialamide were significantly higher than those in young and mature rats.

These results suggest that nialamide did not totally inhibit monoamine oxidase activity in the nucleus accumbens of old rats. To test this possibility directly, we determined the degree of enzyme inhibition in homogenates prepared from nucleus accumbens tissue after the peripheral injection of saline, pargyline or nialamide. As expected, pargyline (75 mg/kg, i.p.) totally inhibited monoamine oxidase activity in the nucleus accumbens of young, mature and old rats. In contrast, although nialamide (100 mg/kg, i.p.) completely inhibited monoamine oxidase activity in the nucleus accumbens of young and mature rats, it only produced partial inhibition of this enzyme in old rats (61%
inhibition). Lower doses of nialamide (25 and 50 mg/kg) produced a significantly greater inhibition of the monoamine oxidase activity in young and mature rats compared to old rats. The reduced level of inhibition of monoamine oxidase activity after nialamide pretreatment in old animals could account for the lower concentration of dopamine and the increased concentration of HVA and DOPAC in the nucleus accumbens of these animals. It also can explain the attenuated locomotor activity response of old rats to dopamine after nialamide pretreatment.

The decreased effectiveness of injected nialamide in inhibiting monoamine oxidase activity in the nucleus accumbens of old rats could be due to a change in the enzyme, making it resistant to inhibition by this drug. To test this hypothesis, homogenates from the nucleus accumbens of young, mature and old rats were incubated with nialamide in vitro, prior to the determination of monoamine oxidase activity. Under these conditions, nialamide produced a dose dependent inhibition of enzyme activity and the degree of inhibition was similar for all 3 age groups. These results suggest that the reduced effectiveness of injected nialamide in inhibiting monoamine oxidase in the nucleus accumbens of old rats is not caused by a change in the enzyme. It is more likely related to some change in the distribution and/or metabolism of nialamide that occurs with age, resulting in a decreased amount of nialamide available
to inhibit monoamine oxidase in the nucleus accumbens of old rats.

In summary, the reduced response of old rats to intraaccumbens injections of dopamine after nialamide pretreatment appears to be due to a decreased retention of injected dopamine in the nucleus accumbens of these animals. This decreased retention of dopamine is apparently the result of a reduced inhibition of dopamine metabolism (and monoamine oxidase) in the nucleus accumbens of old rats after the peripheral administration of nialamide.
Figure 19.

Linearity of monoamine oxidase assay as a function of incubation time.

Nucleus accumbens homogenates (2 mg tissue) were incubated with 3H-dopamine substrate for increasing intervals of time, and the amount of 3H-product formed (in nmoles) was determined. Each point represents the mean of 2 determinations. Linear regression analysis correlation coefficient: $r^2 = 0.97$. 
Figure 20.

Linearity of monoamine oxidase assay as a function of tissue concentration.

Nucleus accumbens homogenates (1.5-4.0 mg tissue) were incubated for 30 min with 3H-dopamine substrate and the nmoles of 3H-product formed were measured. Each point represents the mean of 2 determinations. Linear regression analysis correlation coefficient: $r^2 = .91$. 

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TABLE 5

Dopamine concentrations in the nucleus accumbens of nialamide pretreated rats after intraaccumbens injections of dopamine.

Rats were pretreated with nialamide (100mg/kg, i.p.) or saline 1.5 hours prior to bilateral intraaccumbens injections of dopamine, 20µg. Rats were killed .5, 1.5, 3.5, or 6 hours after dopamine injections and the concentration of dopamine in the nucleus accumbens of each rat was determined spectrofluorometrically. Each value represents the mean dopamine concentration ± SEM in the nucleus accumbens of 4 young (Y), mature (M), or old (O) rats.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>HOUR AFTER INJECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>SALINE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>+</td>
<td>9.53 ± .68</td>
</tr>
<tr>
<td>VEHICLE</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>9.63 ± .56</td>
</tr>
<tr>
<td>NIALAMIDE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y</td>
</tr>
<tr>
<td>+</td>
<td>15.96 ± 1.15</td>
</tr>
<tr>
<td>VEHICLE</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>15.21 ± .78</td>
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<tr>
<td>NIALAMIDE</td>
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</tr>
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<td></td>
<td>Y</td>
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<tr>
<td>+</td>
<td>199.88 ± 18.5</td>
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<tr>
<td>DOPAMINE</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>183.75 ± 26.05</td>
</tr>
</tbody>
</table>

N.D. = not determined.
Figure 21.

Time course of the decline in dopamine levels in the nucleus accumbens of nialamide pretreated young, mature and old rats after direct injections of dopamine.

Rats were pretreated with nialamide (100 mg/kg, i.p.) 1.5 hours prior to bilateral intraaccumbens injections of dopamine, 20 μg/0.5 μl, or vehicle. Dopamine levels were determined spectrofluorometrically 0.5, 1.5, 3.5 and 6 hours after the injection of dopamine. Each point represents the mean dopamine concentration ± SEM in the nucleus accumbens of 4 rats receiving nialamide and dopamine injections, after subtraction of control levels (nialamide + vehicle). Statistics were performed on actual dopamine levels (nialamide + dopamine) after subtraction of control values (nialamide + vehicle). Dopamine concentrations (ng/mg tissue ± SEM) in the nucleus accumbens of young, mature and old rats respectively, receiving nialamide + vehicle injections (controls) were, 0.5 hr: 15.96 ± 1.15, 15.21 ± 1.95, 11.47 ± .83; 3.5 hr: 13.52 ± .83, 7.05 ± .63, 7.13 ± .47; 6 hr: 10.96 ± .52, 10.87 ± 1.04, 10.10 ± .25; and for mature and old rats respectively at 1.5 hr: 12.52 ± 1.24, 9.26 ± .53. * Significantly less than dopamine concentrations in the nucleus accumbens of old rats, P<0.05. ** Significantly less than dopamine concentrations in the nucleus accumbens of young and mature rats, P<.05.
Figure 21.
TABLE 6

Dopamine concentrations in the nucleus accumbens of pargyline pretreated rats 3.5 hours after intraaccumbens injections of dopamine

Rats were pretreated with pargyline (75 mg/kg, i.p.) or saline 1.5 hours prior to bilateral intraaccumbens injections of dopamine, 20 µg. 3.5 hours after the injections of dopamine, the rats were killed, and the dopamine concentration was determined in the nucleus accumbens of each rat. Each value represents the mean dopamine concentration ± SEM in the nucleus accumbens of 4 young (Y), mature (M) or old (O) rats.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>AGE</th>
<th>ng dopamine/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALINE</td>
<td>Y</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>6.43 ± .62</td>
</tr>
<tr>
<td>VEHICLE</td>
<td>O</td>
<td>6.44 ± .28</td>
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<tr>
<td>PARGYLINE</td>
<td>Y</td>
<td>10.12 ± .96</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>17.30 ± 1.25</td>
</tr>
<tr>
<td>VEHICLE</td>
<td>O</td>
<td>11.01 ± 1.50</td>
</tr>
<tr>
<td>PARGYLINE</td>
<td>Y</td>
<td>95.45 ± 10.18</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>130.00 ± 19.99</td>
</tr>
<tr>
<td>DOPAMINE</td>
<td>O</td>
<td>118.72 ± 8.53</td>
</tr>
</tbody>
</table>

N.D. = not determined.
Figure 22.

Dopamine levels in the nucleus accumbens of pargyline pretreated young, mature and old rats receiving intraaccumbens injections of dopamine.

Rats were pretreated with pargyline (75 mg/kg i.p) 1.5 hours prior to bilateral intraaccumbens injections of dopamine, 20 μg/0.5μl, or vehicle. Dopamine levels in the nucleus accumbens were determined spectrofluorometrically 3.5 hours after the injection of dopamine. Each bar represents the mean dopamine concentration ± SEM in the nucleus accumbens of young, mature and old rats receiving pargyline and dopamine injections, after subtraction of control dopamine levels (pargyline + vehicle). Statistics performed on the actual dopamine concentrations (pargyline + dopamine) after subtracting control levels (pargyline + vehicle), showed no significant age difference in dopamine concentrations. Dopamine concentrations (ng/mg tissue ± SEM) in the nucleus accumbens of young, mature and old rats respectively, receiving pargyline + vehicle injections (controls), were: 10.12 ± .96, 17.30 ± 1.25, 11.01 ± 1.50.
Figure 22.

DA CONCENTRATION (ng/mg tissue)

PARCYLINE + DA 20μg

YOUNG  MATURE  OLD
### TABLE 7

Effect of nialamide or pargyline pretreatment on the metabolite (DOPAC + HVA) to dopamine (DA) ratio.

Rats were pretreated with saline, nialamide (100 mg/kg, i.p.) or pargyline (75 mg/kg, i.p.) 1.5 hours prior to bilateral intraaccumbens injections of dopamine, 20 μg. Rats were killed .5, 1.0, and 2.0 hours after the dopamine injections and the concentration of dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and dopamine (DA) in each nucleus was determined spectrofluorometrically. Each value represents the mean ratio determination ± SEM in the nucleus accumbens of 4 rats.

<table>
<thead>
<tr>
<th>HOUR AFTER DOPAMINE INJECTION</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DOPAC + HVA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YOUNG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline + DA</td>
<td>.36 ± .10</td>
<td>1.94 ± .41</td>
<td>5.68 ± .78</td>
</tr>
<tr>
<td>Nialamide + DA</td>
<td>.02 ± .01</td>
<td>.03 ± .01</td>
<td>.06 ± .01</td>
</tr>
<tr>
<td>Pargyline + DA</td>
<td>N.D.</td>
<td>N.D.</td>
<td>.06 ± .01</td>
</tr>
<tr>
<td>MATURE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline + DA</td>
<td>.56 ± .25</td>
<td>2.03 ± .42</td>
<td>4.44 ± 1.05</td>
</tr>
<tr>
<td>Nialamide + DA</td>
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<td>.02 ± .003</td>
<td>.10 ± .01</td>
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<td>Pargyline + DA</td>
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<td>N.D.</td>
<td>.03 ± .01</td>
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<td>OLD</td>
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<tr>
<td>Saline + DA</td>
<td>.56 ± .18</td>
<td>1.85 ± .47</td>
<td>5.47 ± 1.55</td>
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<tr>
<td>Nialamide + DA</td>
<td>.16 ± .03*</td>
<td>.37 ± .12*</td>
<td>3.21 ± .82*</td>
</tr>
<tr>
<td>Pargyline + DA</td>
<td>N.D.</td>
<td>N.D.</td>
<td>.01 ± .002</td>
</tr>
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</table>

* Significantly different from young and mature rats receiving the same pretreatment, P<.05.
Effect of saline, nialamide or pargyline pretreatment on dopamine (DA) levels in the nucleus accumbens following bilateral intraaccumbens injections of dopamine.

Young, mature and old rats were pretreated with saline (1 ml/kg), nialamide (100 mg/kg i.p.), or pargyline (75 mg/kg i.p.) 1.5 hours prior to bilateral intraaccumbens injections of dopamine, 20 μg/0.5μl. Dopamine (DA) levels in the nucleus accumbens were determined spectrofluorometrically 2 hours following the dopamine injections. Each bar represents the mean concentration of DA ± SEM in the nucleus accumbens of groups of 4 rats. * Significantly different from dopamine levels in the nucleus accumbens of young and mature rats receiving the same treatment, P < 0.05.

Figure 23.
Figure 23.
Figure 24.

Effect of saline, nialamide or pargyline pretreatment on dihydroxyphenylacetic acid (DOPAC) levels in the nucleus accumbens following bilateral intraaccumbens injections of dopamine.

Young, mature and old rats were pretreated with saline (1 ml/kg), nialamide (100 mg/kg, i.p.) or pargyline (75 mg/kg, i.p.) 1.5 hours prior to bilateral intraaccumbens injections of dopamine, 20 μg/0.5 μl. DOPAC levels in the nucleus accumbens were determined spectrofluorometrically 2 hours following the dopamine injections. Each bar represents the mean concentration of DOPAC ± S.E.M. in the nucleus accumbens of groups of 4 rats. * Significantly different from DOPAC levels in the nucleus accumbens of young and mature rats receiving the same pretreatment, P < .05.
Figure 24.
Effect of saline, nialamide or pargyline pretreatment on homovanillic acid (HVA) levels in the nucleus accumbens following bilateral intraaccumbens injections of dopamine.

Young, mature and old rats were pretreated with saline, nialamide (100 mg/kg, i.p.) or pargyline (75 mg/kg, i.p.) 1.5 hours prior to bilateral intraaccumbens injections of dopamine, 20 μg. HVA levels in the nucleus accumbens were determined spectrofluorometrically 2 hours following the dopamine injections. Each bar represents the mean concentration of HVA ± S.E.M. in the nucleus accumbens of groups of 4 rats. * Significantly different from HVA concentrations in the nucleus accumbens of young and mature rats receiving the same pretreatment, P < .05. ** Significantly different from HVA concentrations in the nucleus accumbens of mature and old rats receiving the same pretreatment, P < .05.
Figure 25.
TABLE 8

Determination of monoamine oxidase activity in the nucleus accumbens of young, mature and old rats injected with pargyline.

Rats were injected with either saline or pargyline (75 mg/kg i.p.) 3 hours prior to sacrifice. The nucleus accumbens was dissected and the monoamine oxidase activity in each nucleus was determined in duplicate. Each value represents the mean enzyme activity in the nuclei of 4-6 rats ± SEM. Control values obtained for age groups were not statistically different.

<table>
<thead>
<tr>
<th>MONOAMINE OXIDASE ACTIVITY (nmoles/mg/hour) (MEAN ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALINE</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>YOUNG</td>
</tr>
<tr>
<td>MATURE</td>
</tr>
<tr>
<td>OLD</td>
</tr>
</tbody>
</table>

ND = not detectable; activity was not above blank values.
TABLE 9

Dose response relationship for nialamide induced inhibition of monoamine oxidase in the nucleus accumbens of rats.

Rats were pretreated with nialamide 3 hours prior to sacrifice. The nucleus accumbens was dissected and monoamine oxidase activity was determined in duplicate in each tissue. Each value represents the mean activity ± SEM in the nucleus accumbens of 3 rats.

<table>
<thead>
<tr>
<th>AGE</th>
<th>TREATMENT</th>
<th>MEAN ACTIVITY ± SEM nmoles/mg tissue/hr</th>
<th>%INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>YOUNG</td>
<td>Saline</td>
<td>5.79 ± .33</td>
<td>control</td>
</tr>
<tr>
<td>MATURe</td>
<td>Nialamide 25 mg</td>
<td>1.39 ± .16</td>
<td>76</td>
</tr>
<tr>
<td>OLD</td>
<td>Nialamide 50 mg</td>
<td>0.32 ± .19</td>
<td>94</td>
</tr>
<tr>
<td>YOUNG</td>
<td>Nialamide 100 mg</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>MATURe</td>
<td>Nialamide 200 mg</td>
<td>1.68 ± .46</td>
<td>76</td>
</tr>
<tr>
<td>OLD</td>
<td>Nialamide 300 mg</td>
<td>0.49 ± .11</td>
<td>93</td>
</tr>
</tbody>
</table>

* Significantly different from activity in the nucleus accumbens of young and mature rats receiving the same treatment, p <0.05.
TABLE 10

Percent inhibition of monoamine oxidase activity in the nucleus accumbens of young, mature and old rats following in vitro preincubation with nialamide.

Rats were killed and the nucleus accumbens was dissected and homogenized. 2 mg of tissue was preincubated for 30 min with various doses of nialamide prior to the addition of substrate. MAO activity was determined in duplicate in each nucleus. Each value represents the mean activity in the nucleus accumbens of groups of 3-4 rats ± SEM. Values between age groups were not statistically different.

<table>
<thead>
<tr>
<th>AGE</th>
<th>[NIALAMIDE]</th>
<th>MEAN ACTIVITY ± SEM (nmoles/mg tissue/hr)</th>
<th>%INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>YOUNG</td>
<td></td>
<td>5.95 ± .54</td>
<td>control</td>
</tr>
<tr>
<td>MATURE</td>
<td>no nialamide</td>
<td>5.49 ± .41</td>
<td>control</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>5.70 ± .43</td>
<td>control</td>
</tr>
<tr>
<td>YOUNG</td>
<td>7.8 X 10 -7</td>
<td>2.65 ± .65</td>
<td>55</td>
</tr>
<tr>
<td>MATURE</td>
<td></td>
<td>2.38 ± .19</td>
<td>57</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>2.45 ± .48</td>
<td>57</td>
</tr>
<tr>
<td>YOUNG</td>
<td>7.8 X 10 -6</td>
<td>.43 ± .27</td>
<td>93</td>
</tr>
<tr>
<td>MATURE</td>
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<td>.30 ± .06</td>
<td>95</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>.28 ± .08</td>
<td>95</td>
</tr>
<tr>
<td>YOUNG</td>
<td>7.8 X 10 -5</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>MATURE</td>
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<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>ND</td>
<td>100</td>
</tr>
</tbody>
</table>
CHAPTER III

CHARACTERIZATION OF THE REDUCED ABILITY OF NIALAMIDE TO INHIBIT MONOAMINE OXIDASE IN OLD RATS

III.1. INTRODUCTION

Nialamide, a monoamine oxidase inhibitor, is commonly used in studies on the locomotor activity response of rats to dopamine injected onto the nucleus accumbens because it prevents the metabolism of injected dopamine and thereby increases the duration and amount of locomotor activity stimulation. We have determined that the locomotor activity response of old rats to bilateral injections of dopamine into the nucleus accumbens after nialamide pretreatment is less than that of young and mature rats (Chapter I). The reduced response of old rats appears to be due to a decreased inhibition of monoamine oxidase in the nucleus accumbens of these animals (Chapter II). The present studies were designed to characterize the reduced effectiveness of nialamide in old rats.
III.2. METHODS

A. Animals

Male COBS® CDF® F344/Crl rats, 6, 15, and 26 months of age (referred to as young, mature and old rats respectively) supplied by the National Institute on Aging were used in these studies and housed in AALAC approved cages. Food (Purina Rat Chow) and water were available ad libitum. The animal quarters were maintained on a 6 AM:6 PM light:dark cycle. Animals were permitted to acclimate to our laboratory environment for at least one week prior to experimentation.

B. Monoamine Oxidase Assay

Monoamine oxidase activity was determined in duplicate in nucleus accumbens homogenates (McCamen et al., 1965). The nucleus accumbens was dissected (Horn et al., 1974) and placed on dry ice. The nucleus was weighed and homogenized in 10 Vol of 0.001 M phosphate buffer, pH 7.0. 100 μl of buffer substrate containing 0.3 μl 3H-dopamine (30.4 Ci/mmol) and 2.5 mM dopamine in 0.1 M potassium phosphate, pH 6.75, was added to 20 μl of homogenate (2.0 mg of tissue), and incubated for 30 min at 38° C. The reaction was stopped by the addition of 10 μl of 3 N HCl. 3H-metabolites were extracted into 500 μl of ethyl acetate.
250 μl of the ethyl acetate phase (containing 3H-metabolites) was added to 250 μl of fresh ethyl acetate and re-extracted using 120 μl of acidified buffer. 200 μl of this ethyl acetate phase was then transferred to a counting vial and the 3H-metabolite concentration determined by liquid scintillation counting. Blanks contained 100 μl of buffer substrate and 20 μl of the buffer used to homogenize the tissue. This assay was linear with time and tissue concentration in nucleus accumbens, heart and liver homogenates.

C. Injections

Intraperitoneal and subcutaneous injections were administered in a volume of 1 ml/kg. Intravenous injections were administered into the femoral vein of rats anesthetized with chloral hydrate (420 mg/kg, i.p.) in a volume of .25 ml/kg.

D. Statistics

Data is expressed as the mean and standard error of the mean (S.E.M.). Significant differences between age groups were evaluated using analysis of variance with Student Neuman-Keuls test, with a level of P < .05 being considered significant.
E. Drugs

The following drugs were obtained from Sigma Chemical Co., St. Louis, Mo.: nialamide, iproniazid phosphate, and dopamine HCl. 3H-dopamine (30.4 Ci/mmole) was obtained from New England Nuclear. Nialamide was dissolved in a minimum of 1.0 N HCl before the addition of an appropriate volume of double distilled water. Iproniazid phosphate was dissolved in 0.9% NaCl.

III.3. RESULTS

A. Dose response relationship for nialamide induced inhibition of monoamine oxidase in the heart

Since the inhibition of monoamine oxidase by nialamide administered intraperitoneally is reduced in the nucleus accumbens of old rats (Chapter II) it was of interest to determine whether this reduced response also occurred in peripheral tissues. Table 11 shows the monoamine oxidase activity in homogenates prepared from the hearts of groups of young, mature and old rats, 3 hours after intraperitoneal injections of nialamide (25-100 mg/kg) or saline. As reported by Horita (1968), monoamine oxidase activity in the hearts of control (saline) rats increased with age.
Nialamide produced a dose-related inhibition of monoamine oxidase in rats of all ages; however, monoamine oxidase activity in the hearts of old rats was significantly greater than that of young and mature rats at both the 25 and 50 mg/kg dose. Expressed as percent inhibition of monoamine oxidase activity in age matched controls, nialamide produced less enzyme inhibition in old rats than in young and mature rats at all doses tested. This effect was most pronounced at the 25 mg/kg dose. At this dose, nialamide produced no detectable inhibition of monoamine oxidase in old rats, while enzyme activity was inhibited 76% and 58% in young and mature rats respectively. In addition, enzyme inhibition was greatest in the young animals, less in the mature animals and smallest in the old animals, indicating that the reduced response to nialamide was age related.

B. Dose response for nialamide induced inhibition of monoamine oxidase in the liver

Groups of young, mature and old rats were injected with nialamide (25 and 100 mg/kg, i.p.) or saline and the activity of monoamine oxidase was determined in liver homogenates 3 hours later (Table 12). There was no age related change in the activity of monoamine oxidase in the liver of groups of rats injected with saline (controls), and enzyme activity after the injection of nialamide was not different between age groups at either dose.
C. Effect of iproniazid on monoamine oxidase activity in the nucleus accumbens

In order to determine whether the reduced ability of nialamide to inhibit monoamine oxidase activity in the nucleus accumbens of old rats was a property of all hydrazide type inhibitors, rats were injected with iproniazid (25 or 100 mg/kg, i.p.), or saline, 3 hours prior to the in vitro determination of monoamine oxidase activity in nucleus accumbens homogenates (Table 13). Like nialamide, iproniazid produced a marked inhibition of monoamine oxidase activity which was dose related. At high doses of iproniazid (100 mg/kg) there was no age difference in the inhibition of monoamine oxidase, i.e., the enzyme was inhibited 99% in rats of all ages. However, at the lower dose of iproniazid (25 mg/kg), monoamine oxidase activity was slightly greater in old rats compared to young and mature rats. Similarly, the percentage of enzyme inhibition was 80% in old rats, and 85% and 86% in mature and young rats respectively.

D. Effect of subcutaneous injections of nialamide on monoamine oxidase activity in the nucleus accumbens

In an attempt to avoid the passage of large amounts of
injected nialamide through the liver via the hepatic portal system and minimize the possible effects of liver metabolism, rats were injected subcutaneously with nialamide (100mg/kg) and monoamine oxidase activity in the nucleus accumbens was determined (Table 14). After the injection of nialamide, monoamine oxidase activity in the nucleus accumbens of old rats was significantly greater than that of young and mature rats. Expressed as percent inhibition of age matched controls, nialamide inhibited monoamine oxidase activity in old rats 38 %, compared to 79 % and 86 % in mature and young rats respectively.

E. Effect of intravenous injections of nialamide on monoamine oxidase activity in the nucleus accumbens and heart of rats

In order to determine whether a decreased absorption of nialamide after peripheral injection might contribute to the reduced ability of nialamide to inhibit monoamine oxidase in old rats, rats were injected with intravenously with nialamide (25 mg/kg) and the monoamine oxidase activity in homogenates prepared from the nucleus accumbens and heart determined 3 hours later (Table 15). As reported for intraperitoneal (Chapter II) and subcutaneous injections, monoamine oxidase activity in the nucleus accumbens of old (29 month) rats injected with nialamide was significantly greater than that of young (9 month) and mature (18 month)
rats. Expressed as percent inhibition of age matched control rats, enzyme inhibition was 15% in old rats, compared to 42% and 32% in young and mature rats.

Nialamide reduced the monoamine oxidase activity in the hearts from rats of all ages; however, the enzyme inhibition was less in old rats (7%) compared to young (33%) and mature (12%) rats. Actual enzyme activity values after the injection of nialamide were significantly different for all 3 age groups in the heart.

III.4. DISCUSSION

The decrease in the locomotor activity response of old rats to intraaccumbens injections of dopamine after nialamide pretreatment appears to be due to an age related decrease in the ability of nialamide to inhibit monoamine oxidase in these animals. In the present studies, we have looked at some of the characteristics of this age related reduction in the effectiveness of nialamide.

It was of interest to determine whether the reduced inhibition of monoamine oxidase by nialamide was specific to the nucleus accumbens (brain). Therefore, we determined the degree of enzyme inhibition in heart and liver tissues taken from young, mature and old rats after intraperitoneal
injections of nialamide. As reported by Horita (1968), monoamine oxidase activity increased with age in the hearts of control rats. In fact, enzyme activity in old rats was twice that of young rats. After nialamide injection (25-100 mg/kg, i.p.) there was a decrease in the percent inhibition of monoamine oxidase with increasing age, similar to that determined in the nucleus accumbens (Chapter II). These results suggest that the reduced inhibition of monoamine oxidase in the nucleus accumbens is not specifically related to a decreased ability of nialamide to penetrate the blood brain barrier in old rats.

As reported by Horita (1961) nialamide (i.p.) produced a greater inhibition of monoamine oxidase in the liver than in the heart or brain. In our studies, at the 25 mg/kg dose the liver enzyme was inhibited 86% in young rats, the heart enzyme was inhibited 76%, and the nucleus accumbens enzyme was inhibited 76%. The greater inhibition in the liver may be attributed to the direct passage of nialamide to the liver through the hepatic portal system after intraperitoneal injection. In contrast to the age related decrease in the inhibition of monoamine oxidase by nialamide that occurred in heart and nucleus accumbens tissue, we observed no age related difference in the ability of nialamide to inhibit monoamine oxidase activity in the liver. It is possible that a lower dose of nialamide is necessary to reveal an age related effect of nialamide in
the liver since the effect is notably greater the lower the
dose, especially in the heart. However, it should be noted
that in heart and nucleus accumbens tissue, doses which
produced greater than 90% inhibition of monoamine oxidase
in young and mature rats, produced significantly less
inhibition of this enzyme in old rats.

There was no age related difference in the response of
rats to enzyme inhibition produced by high doses of
iproniazid (100 mg/kg, i.p.), a hydrazide type monoamine
oxidase inhibitor structurally similar to nialamide. This
dose was the same as that used in behavioral studies
(Chapter I) in which the locomotor activity response of old
rats to dopamine after pretreatment with iproniazid was not
less than that of young and mature rats. However, at a
lower dose of iproniazid (25 mg/kg, i.p.), the activity of
monoamine oxidase in the nucleus accumbens of old rats was
significantly greater than that of young and mature rats,
although the difference in enzyme activity was much smaller
than that observed after nialamide injections.

It is possible that the liver is metabolizing nialamide
to an inactive form at a faster rate in old rats and this
effect might contribute to the decreased effectiveness of
nialamide in these animals. Therefore, it was of interest
to determine whether rats injected subcutaneously (to avoid
absorption directly into the hepatic portal system) with
nialamide would show the same age related difference in enzyme inhibition. The pattern of enzyme inhibition in the nucleus accumbens after subcutaneous injections of nialamide was very similar to that observed when nialamide was injected intraperitoneally, suggesting that the liver may not be of primary importance in the differential metabolism of nialamide with age, if such a difference does exist.

The absorption of compounds administered peripherally may be reduced with age, due to a reduction in peripheral blood flow that occurs with age. We injected nialamide directly into the femoral vein in an attempt to bypass any age related changes in absorption that might affect the amount of nialamide absorbed after subcutaneous or intraperitoneal injections. The differences in percent inhibition of monoamine oxidase in the nucleus accumbens after the i.v. injection of nialamide were very similar to those reported after subcutaneous or intraperitoneal injections. These results suggest that an age related decrease in the absorption of nialamide after peripheral administration may not play a large role in the decreased ability of nialamide to inhibit monoamine oxidase in aged rats.

In conclusion, it appears that the heart but not the liver shares with the nucleus accumbens the age related resistance to the monoamine oxidase inhibitory effect of nialamide. Iproniazid, a hydrazide structurally similar to
nialamide, at low doses appears to be a less effective inhibitor of monoamine oxidase in old animals, since it did inhibit monoamine oxidase in old rats slightly less than in young and mature rats at a 25 mg/kg dose. However, at high doses of iproniazid (100 mg/kg, i.p.) there was no age difference in the activity of monoamine oxidase or in the locomotor activity response of rats to intraaccumbens injections of dopamine (Chapter I). In addition, experiments in which nialamide was injected subcutaneously or i.v., suggest that the liver is not important in a metabolic effect, if one exists, and the i.v. experiments further suggest that absorption may not be a major consideration. Thus, it appears that the reduced response to nialamide may be some effect of metabolism or distribution of nialamide (and possibly other hydrazide monoamine oxidase inhibitors) that is peculiar to aged rats.
TABLE 11

Dose response relationship for nialamide induced inhibition of monoamine oxidase in the heart.

Rats were injected with nialamide (25, 50, and 100 mg/kg, i.p.) or saline 3 hours prior to sacrifice. The heart was dissected and the monoamine oxidase activity in each nucleus was determined in duplicate. Each value represents the mean enzyme activity in the nuclei of groups of 3-10 rats ± SEM.

<table>
<thead>
<tr>
<th>AGE</th>
<th>TREATMENT</th>
<th>MEAN ACTIVITY ± SEM nmoles/mg tissue/hr</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>YOUNG</td>
<td>SALINE</td>
<td>10.93 ± .69</td>
<td>control</td>
</tr>
<tr>
<td>MATURE</td>
<td>SALINE</td>
<td>15.99 ± 1.04</td>
<td>control</td>
</tr>
<tr>
<td>OLD</td>
<td>SALINE</td>
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<td>control</td>
</tr>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>MATURE</td>
<td>NIALAMIDE 50</td>
<td>.91 ± .27</td>
<td>94</td>
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<tr>
<td>OLD</td>
<td>NIALAMIDE 50</td>
<td>7.51 ± 1.96*</td>
<td>64</td>
</tr>
<tr>
<td>YOUNG</td>
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<td>100</td>
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<tr>
<td>MATURE</td>
<td>NIALAMIDE 100</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>OLD</td>
<td>NIALAMIDE 100</td>
<td>.98 ± .50</td>
<td>95</td>
</tr>
</tbody>
</table>

ND = not detectable, activity was not above blank values. *Significantly different from enzyme activity in the hearts of young and mature rats injected with the same dose of nialamide, p<0.05.
TABLE 12

Dose response for nialamide induced inhibition of monoamine oxidase in the liver.

Rats were injected with nialamide (25 and 100 mg/kg, i.p.) or saline. Rats were killed 3 hours later and the liver was dissected. Monoamine oxidase activity was determined in duplicate in liver homogenate from each rat; each value represents the mean enzyme activity ± SEM in the nuclei of 3–4 rats.

<table>
<thead>
<tr>
<th>AGE</th>
<th>TREATMENT</th>
<th>MEAN ACTIVITY ± SEM nmoles/mg tissue/hr</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td>16.39 ± .20</td>
<td>control</td>
</tr>
<tr>
<td>MATURE</td>
<td>SALINE</td>
<td>18.58 ± .92</td>
<td>control</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>18.92 ± 1.24</td>
<td>control</td>
</tr>
<tr>
<td>YOUNG</td>
<td>NIALAMIDE 25</td>
<td>2.34 ± .60</td>
<td>86</td>
</tr>
<tr>
<td>MATURE</td>
<td></td>
<td>2.52 ± .39</td>
<td>86</td>
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<tr>
<td>OLD</td>
<td></td>
<td>2.86 ± .42</td>
<td>85</td>
</tr>
<tr>
<td>YOUNG</td>
<td>SALINE</td>
<td>11.25 ± 1.23</td>
<td>control</td>
</tr>
<tr>
<td>MATURE</td>
<td></td>
<td>13.29 ± .41</td>
<td>control</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>12.13 ± .85</td>
<td>control</td>
</tr>
<tr>
<td>YOUNG</td>
<td>NIALAMIDE 100</td>
<td>.28 ± .12</td>
<td>98</td>
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<tr>
<td>MATURE</td>
<td></td>
<td>.06 ± .03</td>
<td>100</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>.25 ± .03</td>
<td>98</td>
</tr>
</tbody>
</table>
TABLE 13

Effect of iproniazid on monoamine oxidase activity in the nucleus accumbens.

Rats were injected with iproniazid (100 mg/kg, i.p.) 3 hours prior to sacrifice. The nucleus accumbens was dissected and the monoamine oxidase activity in each nucleus was determined in duplicate. Each value represents the mean enzyme activity ± SEM in the nucleus accumbens of groups of 3-7 rats.

<table>
<thead>
<tr>
<th>AGE</th>
<th>TREATMENT</th>
<th>MEAN ACTIVITY ± SEM</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmoles/mg tissue/hr</td>
<td></td>
</tr>
<tr>
<td>YOUNG</td>
<td></td>
<td>5.66 ± .34</td>
<td>control</td>
</tr>
<tr>
<td>MATURE</td>
<td>SALINE</td>
<td>5.49 ± .39</td>
<td>control</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>5.94 ± .50</td>
<td>control</td>
</tr>
<tr>
<td>YOUNG</td>
<td></td>
<td>.77 ± .04</td>
<td>86</td>
</tr>
<tr>
<td>MATURE</td>
<td>IPRONIAZID 25</td>
<td>.83 ± .05</td>
<td>85</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>1.20 ± .07*</td>
<td>80</td>
</tr>
<tr>
<td>YOUNG</td>
<td></td>
<td>.07 ± .07</td>
<td>99</td>
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<tr>
<td>MATURE</td>
<td>IPRONIAZID 100</td>
<td>.06 ± .04</td>
<td>99</td>
</tr>
<tr>
<td>OLD</td>
<td></td>
<td>.04 ± .02</td>
<td>99</td>
</tr>
</tbody>
</table>

* Significantly different from enzyme activity in the nucleus accumbens of young and mature rats receiving the same dose of iproniazid, p<0.05.
TABLE 14

Effect of subcutaneous injections of nialamide on monoamine oxidase activity in the nucleus accumbens.

Rats were injected subcutaneously with nialamide (100 mg/kg) or saline 3 hours prior to sacrifice. The nucleus accumbens was dissected and the monoamine oxidase activity was determined in duplicate in each nucleus. Each value represents the mean activity ± SEM in the nuclei of groups of 3-4 rats.

<table>
<thead>
<tr>
<th>AGE</th>
<th>TREATMENT</th>
<th>MEAN ACTIVITY ± SEM</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmoles/mg tissue/hr</td>
<td></td>
</tr>
<tr>
<td>YOUNG</td>
<td>SALINE</td>
<td>5.77 ± .37</td>
<td>control</td>
</tr>
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<td>MATURE</td>
<td>SALINE</td>
<td>5.39 ± .42</td>
<td>control</td>
</tr>
<tr>
<td>OLD</td>
<td>SALINE</td>
<td>5.24 ± .43</td>
<td>control</td>
</tr>
<tr>
<td>YOUNG</td>
<td>NIALAMIDE 100</td>
<td>0.82 ± .27</td>
<td>86</td>
</tr>
<tr>
<td>MATURE</td>
<td>NIALAMIDE 100</td>
<td>1.11 ± .21</td>
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<tr>
<td>OLD</td>
<td>NIALAMIDE 100</td>
<td>3.23 ± .32*</td>
<td>38</td>
</tr>
</tbody>
</table>

* Significantly different from monoamine oxidase activity in the nucleus accumbens of young and mature rats, p < 0.05.
TABLE 15

Effect of intravenous injections of nialamide on monoamine oxidase activity in the nucleus accumbens and heart of rats.

Young (9 month), mature (18 month) and old (29 month) rats received intravenous injections of nialamide (25 mg/kg) into the femoral vein 3 hours prior to sacrifice. The heart and nucleus accumbens were dissected from each rat, and the monoamine oxidase activity was determined in duplicate in tissue homogenates. Each value represents the mean enzyme activity ± SEM in tissues from groups of 3-6 rats.

<table>
<thead>
<tr>
<th>AGE</th>
<th>TREATMENT</th>
<th>MEAN ACTIVITY + SEM</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>nmoles/mg tissue/hr</td>
<td></td>
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</tbody>
</table>

NUCLEUS ACCUMBENS

<table>
<thead>
<tr>
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<th>Control</th>
<th>nmoles/mg tissue/hr</th>
<th></th>
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<tbody>
<tr>
<td>Young</td>
<td>6.23 ± .27</td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td>6.00 ± .64</td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>6.63 ± .64</td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>3.59 ± .27</td>
<td>control</td>
<td>42</td>
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<tr>
<td>Mature</td>
<td>4.07 ± .41</td>
<td>control</td>
<td>32</td>
</tr>
<tr>
<td>Old</td>
<td>5.63 ± .23*</td>
<td>control</td>
<td>15</td>
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</table>

HEART

<table>
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<tr>
<th></th>
<th>Control</th>
<th>nmoles/mg tissue/hr</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>14.12 ± .93**</td>
<td>control</td>
<td></td>
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<tr>
<td>Mature</td>
<td>22.34 ± 2.89</td>
<td>control</td>
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<tr>
<td>Old</td>
<td>28.41 ± 2.01</td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>9.46 ± .93***</td>
<td>control</td>
<td>33</td>
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<tr>
<td>Mature</td>
<td>19.75 ± 1.25***</td>
<td>control</td>
<td>12</td>
</tr>
<tr>
<td>Old</td>
<td>26.54 ± 1.43***</td>
<td>control</td>
<td>7</td>
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</table>

Table continued on next page.
* Significantly different from monoamine oxidase activity in the nucleus accumbens of mature and young rats, p<0.05.
** Significantly different from monoamine oxidase activity in the hearts of mature and old rats, p<0.05. ***
Monoamine oxidase activity values for young, mature and old rats are significantly different from each other, p<0.05.
CHAPTER IV

SUMMARY

The dopaminergic neurons which innervate the striatum (the nigrostriatal tract) and the dopamine receptors within the striatum have been shown to undergo an age related decline. This decline is thought to contribute to some of the age related deficits in motor function, i.e., rotation, swimming, and sensorimotor coordination, observed in rats. It is possible that the nucleus accumbens, known to influence the initiation and regulation of spontaneous locomotor activity via the dopaminergic mesolimbic pathway, may play a role in some of the age related decrements in motor function previously attributed to the striatum. Our initial experiments were designed to measure the integrity of dopamine receptor function in the nucleus accumbens, using a behavioral model of dopamine receptor stimulation in which the locomotor activity response of young (6 month), mature (15 month) and old (26 month) rats to intraaccumbens injections of dopamine after pretreatment with a monoamine oxidase inhibitor was determined. Consistent with a decline in dopamine receptor function, old rats pretreated with nialamide responded with a lower intensity of activity to
intraaccumbens injections of dopamine than did young or mature rats. However, this decreased response of old rats did not occur if the rats were pretreated with the alternative monoamine oxidase inhibitors pargyline, iproniazid, or clorgyline or after the injection of dopamine or amphetamine alone. Thus, the reduced response of old rats to intraaccumbens injections of dopamine after nialamide pretreatment appears to be specific for nialamide pretreatment rather than indicative of a decline in dopamine receptor function.

Monoamine oxidase inhibitors are commonly used in studies on the effect of dopamine on locomotor activity, to prevent the metabolism of injected dopamine, thus prolonging the retention of dopamine within the nucleus accumbens and thereby enhancing the locomotor stimulatory effects of dopamine. One explanation for the reduced response of old nialamide pretreated rats to dopamine might be that nialamide is not an effective inhibitor of dopamine metabolism in these animals. To test this hypothesis, we measured the concentrations of dopamine and the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the nucleus accumbens after nialamide or pargyline pretreatment and direct injections of dopamine. As expected, pretreatment of rats with pargyline in doses used for locomotor activity studies produced a marked retention of injected dopamine in the nucleus
accumbens of rats of all ages and low levels of the dopamine metabolites, DOPAC and HVA. In contrast, and consistent with the low levels of motor stimulation in old rats, the concentration of dopamine in the nucleus accumbens after nialamide pretreatment was significantly lower and metabolite concentrations significantly higher in old rats than in young or mature rats. These results suggest that nialamide was not an effective inhibitor of dopamine metabolism (and monoamine oxidase) in old rats.

In agreement with this hypothesis, we found that nialamide (25-300 mg/kg, i.p.) produces a smaller inhibition of monoamine oxidase in old rats than in young and mature rats at all doses tested. In contrast, pargyline (75 mg/kg, i.p.) produces equivalent enzyme inhibition in rats of all ages. The reduced response of old rats to nialamide does not appear to be due to some change in the enzyme which makes it resistant to inhibition by nialamide, since in vitro preincubation of the enzyme with nialamide produced equivalent inhibition in rats of all ages.

The reduced ability of nialamide to inhibit monoamine oxidase in old rats after peripheral administration also occurs in the heart, but not in the liver at the doses tested. A decreased absorption of nialamide in old rats does not appear to contribute to the reduced effect, since the i.v. administration of nialamide also produced a smaller
inhibition of monoamine oxidase in both the heart and nucleus accumbens. Rather, the reduced effectiveness may be due to some age related change in distribution or metabolism of nialamide in old rats. It is possible that the liver may be involved in the metabolism of nialamide to an inactive form, an effect that might occur more rapidly in older animals. To test this hypothesis, we injected nialamide subcutaneously to avoid absorption directly into the hepatic portal system and then measured monoamine oxidase activity in the nucleus accumbens of rats of different ages. As with i.p. administration, nialamide was a less effective inhibitor of monoamine oxidase in the nucleus accumbens of old rats when administered subcutaneously. This result suggests that the liver does not play a large role in a metabolic effect if one occurs. Preliminary experiments with iproniazid suggest that this reduced effectiveness of nialamide may be a property of all hydrazides, although to a lesser extent, since a low dose of iproniazid produced a smaller inhibition of monoamine oxidase in the nucleus accumbens of old rats than in young or mature rats after i.p. administration.

In conclusion, we have identified a reduced locomotor activity response of old rats to intraaccumbens injections of dopamine which is specific for nialamide pretreatment. This reduced response appears to be due to a reduced ability of nialamide to inhibit dopamine metabolism and monoamine
oxidase activity in the nucleus accumbens of old rats. The mechanism of this reduced response of old rats to nialamide remains to be defined.
LIST OF REFERENCES


Kelly, P.H., K.E. Moore. Mesolimbic dopamine neurons:


and the activity in relation to psychiatric disorders. 
In: Modern Problems in Pharmacopsychiatry, Vol. 19, 
Monoamine Oxidase and its Selective Inhibitors, edited by 
246-254.

Oreland, L., H. Kinemuchi, B.Y. Yoo. The mechanism of 
action of the monoamine oxidase inhibitor pargyline. Life 

Palmer, G.C. and R.B. Chronister. The biochemical 
pharmacology of the nucleus accumbens. In: The 
Neurobiology of the Nucleus Accumbens, edited by R.B. 
Chronister and J.F. DeFrance. Haer Institute for 

Patek, D.R. and L. Hellerman. Mitochondrial monoamine 
oxidase: mechanism of inhibition by phenylhydrazine and by 

Pellegrino, J., and A.J. Cushman. A Stereotaxic Atlas of 

Pijnenburg, A.J.J., W.M.M. Honig, J.A.M. Van Der Heyden, 
and J.M. Van Rossum. Effects of chemical stimulation of 
the mesolimbic dopamine system upon locomotor activity. 

Effects of antagonists upon locomotor stimulation induced 
by injection of dopamine and noradrenaline into the 
nucleus accumbens of nialamide-pretreated rats. 

Inhibition of d-amphetamine-induced locomotor activity by 
injection of haloperidol into the nucleus accumbens of the 

Pijnenburg, A.J.J., and J.M. Van Rossum. Stimulation of 
locomotor activity following injection of dopamine into 
the nucleus accumbens. J. Pharm. Pharmacol. 25: 

Ergometrine induced locomotor activity following 
intracerebral injection into the nucleus accumbens. Brain 

Pinson, R., B.M. Bloom, C.J. Buck. Some central nervous 
system drugs designed from metabolic considerations. Ann. 


