

SYSTEMIC ADMINISTRATION OF PUTRESCINE

INDUCES GABA-LIKE BEHAVIORS IN RATS

by

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TABLE OF CONTENTS

	<u>Page</u>
I. General Introduction	1
A. Overview	1
B. Gamma-Aminobutyric acid	6
1. Historical Background	6
2. GABA neurochemistry	6
3. GABA as a neurotransmitter	11
4. Existence of GABA in the basal ganglia	13
5. GABA and dopamine neurochemical interaction	16
6. GABAergic behavior: Introduction	21
7. GABAergic behavior: anticonvulsant activity	21
8. GABAergic behavior: dopamine relationships	23
9. GABAergic behavior: rotational behavior	25
10. GABAergic behavior: dopamine stereotypy	26
11. GABAergic behavior: opiate-related effects	29
12. GABAergic behavior: systemic treatment with GABA agents	32
13. GABAergic behavior: anxiolytic effects and benzodiazepines	35
14. GABAergic behavior: summary	38
C. Putrescine (1,4-Diaminobutane) and the Polyamines	38
1. Background	38
2. Nature of polyamines	39
3. Polyamine neurochemistry	40
4. Metabolism of exogenous putrescine	49
5. Putrescine and polyamine-related behavior	53
D. Thesis Purpose, Rationale, and Hypotheses	62
1. Introduction	62
2. Pilot study data	63
3. Statement of purpose	67
4. Experimental rationale	67
5. Synopsis of experimental procedures	68

TABLE OF CONTENTS (continued)

	<u>Page</u>
6. Behavioral evaluation: subjects	69
7. Behavioral evaluation: time sampling procedures	69
8. Behavioral evaluation: other experimental procedures	73
9. Behavioral evaluation: statistical analysis of data	79
10. Behavioral evaluation: experimental hypotheses	81
11. Thesis format	82
II. Experiment 1	83
A. Introduction	83
B. Materials and Methods	87
1. Subjects	87
2. Apparatus	87
3. Drugs and Dosages	88
4. Procedures	88
C. Results	94
1. Formal Observations	94
a. Time sampling	94
b. Initial startle	108
c. Startle response magnitude	110
d. Open field performance	110
e. Grid coordination	114
f. Shock response thresholds	116
g. Body temperature	119
h. Food and water ingestion	121
i. Linearity of dose-response curves	123
2. Informal Observations	125
D. Discussion	127
1. Effects on Motor Behavior	128
a. Review of motor behavior effects	128-130
b. Motor behavior and polyamine literature	130

TABLE OF CONTENTS (continued)

	<u>Page</u>
c. Motor behavior and GABA literature	131
d. Motor behavior and other CNS substances	134
e. Motor behavior: summary	140
2. Effects on Sensory Reactivity	141
a. Initial startle intensity	141
b. Startle response magnitude	142
c. Shock response thresholds	142
d. Informal observations	142
e. Sensory reactivity: polyamine and GABA literature	143
f. Sensory reactivity and other CNS substances	144
g. Sensory reactivity: summary	147
3. Body Temperature and Other Behaviors	148
a. Body temperature	149
b. Food and water ingestion	149
c. Informal observations	149
d. Body temperature and CNS substances	149
e. Food and water ingestion and CNS substances	152
f. Other behaviors and the autonomic nervous system	157
g. Body temperature and other behaviors: summary	158
4. General Conclusions	159
III. Experiment 2	162
A. Introduction	162
B. Materials and Methods	165
1. Subjects	165
2. Apparatus	165
3. Drugs and dosages	165
4. Procedures	165

TABLE OF CONTENTS (continued)

	<u>Page</u>
C. Results	169
1. Formal Observations	169
a. Time sampling	169
b. Initial startle response intensity	193
c. Startle response magnitude	196
d. Open field performance	197
e. Grid coordination	201
f. Shock response thresholds	203
g. Body temperature	203
h. Food and water ingestion	206
i. Linearity of dose-response curves	208
2. Informal Observations	210
D. Discussion	212
1. Effects on Motor Behavior	213
a. Motor behavior: polyamine and GABA literature	213
b. Motor behavior and other CNS substances	216
c. Comparison of Experiment 1 and 2 results	220
d. Motor behavior: summary	224
2. Effects on Sensory Reactivity	226
a. Sensory reactivity: polyamine and GABA literature	227
b. Sensory reactivity and other CNS substances	228
c. Comparison of Experiment 1 and 2 results	229
d. Sensory reactivity: summary	230
3. Body Temperature and Other Behaviors	232
a. Body temperature and CNS substances	232
b. Food and water ingestion and CNS substances	232

TABLE OF CONTENTS (continued)

	<u>Page</u>
c. Other behaviors and the autonomic nervous system	233
d. Comparison of Experiment 1 and 2 results	234
f. Body temperature and other behaviors: summary	235
4. General Conclusions	236
IV. Experiment 3	241
A. Introduction	241
B. Materials and Methods	245
1. Subjects	245
2. Apparatus	245
3. Drugs and Dosages	245
4. Procedures	246
C. Results	250
1. Formal Observations	250
a. General locomotor activity	250
b. Specific activity	254
2. Informal Observations	264
D. Discussion	268
1. Locomotor Activity	268
a. General activity	268
b. Specific activity	269
2. Specific Activity: stereotypy and other behaviors	271
a. Apomorphine and behavior	272
b. Muscimol and behavior	273
c. GABAergic/dopaminergic interactions and behavior	276
d. Putrescine, muscimol effects and GABA/DA behavior	278
e. Informal observations and CNS substances	280

TABLE OF CONTENTS (continued)

	<u>Page</u>
3. General Conclusions	282
V. General Discussion	288
A. Recapitulation	288
1. Systemic putrescine effects	288
2. Putrescine and GABA, dopamine behavior . .	291
B. Interpretations and Significance	293
C. General Findings	296
VI. Literature Cited	298
VII. Appendices	320
A. Pilot Study Data: temporal comparisons . . .	320
B. Experiment 1: additional behavioral data . .	321
C. Experiment 2: additional behavioral data . .	326
D. Glossary of Pharmacologic Agents	327
E. Tables for the General Discussion	329
1. Inhibitory and excitatory effects of systemic putrescine administration	329
2. Systemic putrescine effects and relation- ships to CNS substances	330
3. Experiment 3 effects and relationships to CNS function	331
VIII. Vitae	332
ABSTRACT	

GENERAL INTRODUCTION

Overview

Putrescine (1,4-diaminobutane), a simple diamine present in most vertebrate cells, has numerous functional capacities related to cellular metabolism and proliferation. Putrescine has been shown to be important in regard to general cellular replication, RNA synthesis, cyclic-AMP (c-AMP) synthesis, modification of the metabolic ornithine cycle, growth regulation of both normal and neoplastic tissues, and formation of the polyamines, spermidine and spermine (Konishi, Nakajima and Sano, 1976; McCann, Hornsperger and Seiler, 1979; Seiler and Al-Therib, 1974; Seiler and Eichtenopf, 1975; Shaw, 1979, and Shaw, 1972). In addition, putrescine has a role as a minor precursor of the major inhibitory neurotransmitter in the human brain, gamma-aminobutyric acid, or GABA (Seiler and Al-Therib, 1974; Seiler and Eichtenopf, 1975). In central nervous system (CNS) cells, putrescine catabolism to GABA is via a pathway that is independent of the major GABA synthetic pathway. The major pathway utilizes glutamic acid as the primary precursor of GABA, and glutamic acid decarboxylase (GAD) as the major synthetic enzyme.

Although putrescine is a simple diamine, it is classified as a polyamine together with spermidine and spermine (Figure 1). Putrescine is the primary precursor of both spermidine and spermine in a reversible synthetic pathway in which putrescine can be reformed and reutilized following spermidine degradative processes (Antrup and Seiler, 1980). Since the discovery of polyamines in the 1950's, much of the biochemical research involving them has been conducted using

cell cultures, derived from chicken, rodent, rabbit, primate, and human preparations and has included tissues such as liver, intestine, retina, muscle, skeleton, and neural cells, including glia and neuroblasts. In recent years, the number of in vivo biochemical as well as behavioral studies have steadily increased, using techniques such as intracerebral (IC), intraventricular (IVT), intravenous (IV), and intraperitoneal (i.p.) injection to administer the polyamines.

From a biochemical standpoint, the physiological significance of putrescine in neural tissue has been well established. In 1974, Seiler and Al-Therib demonstrated that putrescine catabolism occurs in the mammalian brain following IVT injection of radiolabeled ^{14}C -putrescine in mice and rats. Seiler and Eichertopf (1975) reported that GABA is an important intermediate of mammalian putrescine catabolism. Konishi, Nakajima, and Sano (1977) suggested that the metabolic pool of GABA in rat brain which is formed via the putrescine-GABA shunt, which is metabolically distinct from the glutamic acid-GABA pool. Thus, at present, the relevance of putrescine-derived GABA in neurotransmitter function is not clear.

Regulatory interrelationships between putrescine, spermidine and spermine, and GABA formation in rats have been characterized by Seiler (1979), and Antrup and Seiler (1980). Although it had previously been a point of contention, polyamine passage from the peripheral blood system into the brain after systemic administration has now been demonstrated with radiolabeled putrescine studies. Antrup and Seiler (1980) reported CNS conversion of ^{14}C -putrescine to GABA following IV and i.p. injection, and with similar i.p. results have been reported by

Caron, Cote and Kremzner (1980).

From a behavioral perspective, there is a paucity of comprehensive behavioral investigation concerning the systemic and central administration of the polyamines in vivo. Much of the behavioral information reported in the literature to date has been anecdotal in nature, devoid of any formal assessment and accompanying statistical analysis. Shaw (1972) reported that rats and mice receiving i.p. injections of spermidine and spermine became sedated, hypothermic and hypomotile; IV injection resulted in clonic convulsion, respiratory arrest and death. These observations were confirmed by Anderson, Crossland and Shaw (1975) with IVT studies in mice and rabbits. In these studies large IVT injections of putrescine produced convulsions or paralysis in mice. Anorexia, adipsia, and tachypnoea have also been reported following IVT polyamine administration. In 1980, Nistico, Ientile, Rotiroti and Di Giorgio reported that IVT administration of putrescine in chicks produced initial sedation followed by symptomology which included increased reactivity to stimulation, increased motor activity, vocalization, head jerking and rotation, circling and escape responses, myoclonic limb movement, and hypothermia accompanied by prolonged sedation. Thus, while the nature of pharmacologic action of the polyamines is not well understood, there appears to be evidence that CNS and systemic administration of these compounds do affect neurophysiological integrity and function in vivo.

In 1979, McCann (unpublished, personal communication, 1980) found unexpected increases of endogenous GABA levels following incubation of rat gliomal and hepatomal cell cultures with radiolabeled

putrescine. McCann demonstrated that 50% of the radiolabeled putrescine introduced into the cultures was converted to radiolabeled GABA. This conversion rate differed significantly from estimated in vivo conversion rates: it is estimated that endogenous putrescine contributes less than 10% to the total GABA pool in the human brain (McCann, personal communication).

McCann's (unpublished) in vitro findings prompted consideration of possible in vivo modification of GABA metabolism by exogenous GABA administration. It was hypothesized that in vivo conversion of putrescine to GABA might be increased in the presence of elevated putrescine concentrations. If endogenous GABA function could be modified by systemic putrescine administration, quantifiable behavioral changes should result. Thus, a series of pilot studies were undertaken to evaluate the merit of this preliminary hypothesis, which resulted in positive findings. It was found that oral and intraperitoneal administration of large amounts of putrescine did cause discernable effects in intact rats when compared to saline control animals.

Many of the obtained effects in both the oral and i.p. putrescine pilot studies were consistent with descriptions of polyamine-induced and GABA-like behaviors that have been reported in the literature. Thus, on the basis of these encouraging preliminary findings experimental procedures were designed that formally evaluated the behavioral modifications induced by putrescine administration and their relationship to endogenous GABA function. The resultant procedural designs are presented in four separate experiments which constitute this master's thesis.

Gamma-amino Butyric Acid

Historical background. In 1950, Eugene Roberts and Jorge Awapara independently discovered a unique brain metabolite that existed in high concentrations only in brain and spinal cord tissues. Utilizing pioneering paper chromatography techniques, these researchers are credited with discovering gamma-amino butyric acid in mammalian systems (Awapara, Laridua, Fuerst and Seale, 1950; Roberts and Frankel, 1950). Prior to their important findings, GABA was recognized as a product of microbial and plant metabolism (McGeer, Eccles and McGeer, 1978).

During the 1950's and 1960's intensive research was undertaken to establish the nature and function of brain neurotransmitters, including GABA. Crayfish and lobster neuronal and synaptic preparations were utilized by investigators to discern the apparent inhibitory nature of GABA. Elaborate biochemical evidence supporting the candidacy of GABA as a putative inhibitory neurotransmitter followed in the 1970's. Roberts and colleagues were able to purify the enzyme necessary for GABA synthesis, glutamic acid decarboxylase (GAD), and developed antibodies against it which could localize Purkinje cell terminals via neurohistochemical methods. Thus, by the mid-1970's, GABA was regarded as the major inhibitory neurotransmitter in mammalian and the human CNS. However, a detailed understanding of GABA metabolism and receptor function in the brain is still the subject of intensive investigation.

GABA neurochemistry. Neurophysiological studies have separated amino acids into two classes of neurotransmitter-like chemicals:

those which are excitatory, and those which are inhibitory. GABA is the most abundant neurotransmitter in the brain and is assumed to play a very important role in inhibitory neurotransmission since its brain content is 200- to 1000-fold greater than that of other transmitter substances, including dopamine (DA), norepinephrine (NE), acetylcholine (Ach), and 5-hydroxytryptamine, or serotonin (5-HT). The CNS-specific localization of GABA is generally accepted due to its low concentrations in peripheral tissues and the finding that as many as 25%-45% of all nerve endings in the brain may contain GABA (McGeer, Eccles and McGeer, 1978).

The main precursor of GABA synthesis, glutamic acid (or L-glutamate), is abundant in CNS tissues. Glutamic acid may be formed from glutamine or α -ketoglutarate, with brain glucose probably being the principal in vivo source of these metabolites. The major synthesizing pathway of GABA is known as the 'GABA shunt', which is a closed loop system that conserves endogenous GABA pools in the brain. There are three critical steps in the GABA shunt, depicted in Figure 2. The first step is the transamination of α -ketoglutarate, a Krebs cycle intermediate, into the primary GABA precursor, glutamic acid (Glu). Glu is then decarboxylated to form GABA with the synthesizing enzyme glutamic acid decarboxylase (GAD; EC 4.1.1.15). The final shunt step involves GABA transamination to succinic semialdehyde by the enzyme GABA- α -oxoglutarate transaminase (GABA-t) (EC 26.1.19). This reaction is rate-limiting for GABA synthesis and will only occur if α -ketoglutarate is the acceptor of the amine group. This last step accomplishes the resupply of glutamic acid via a cycle that allows the catabolism a

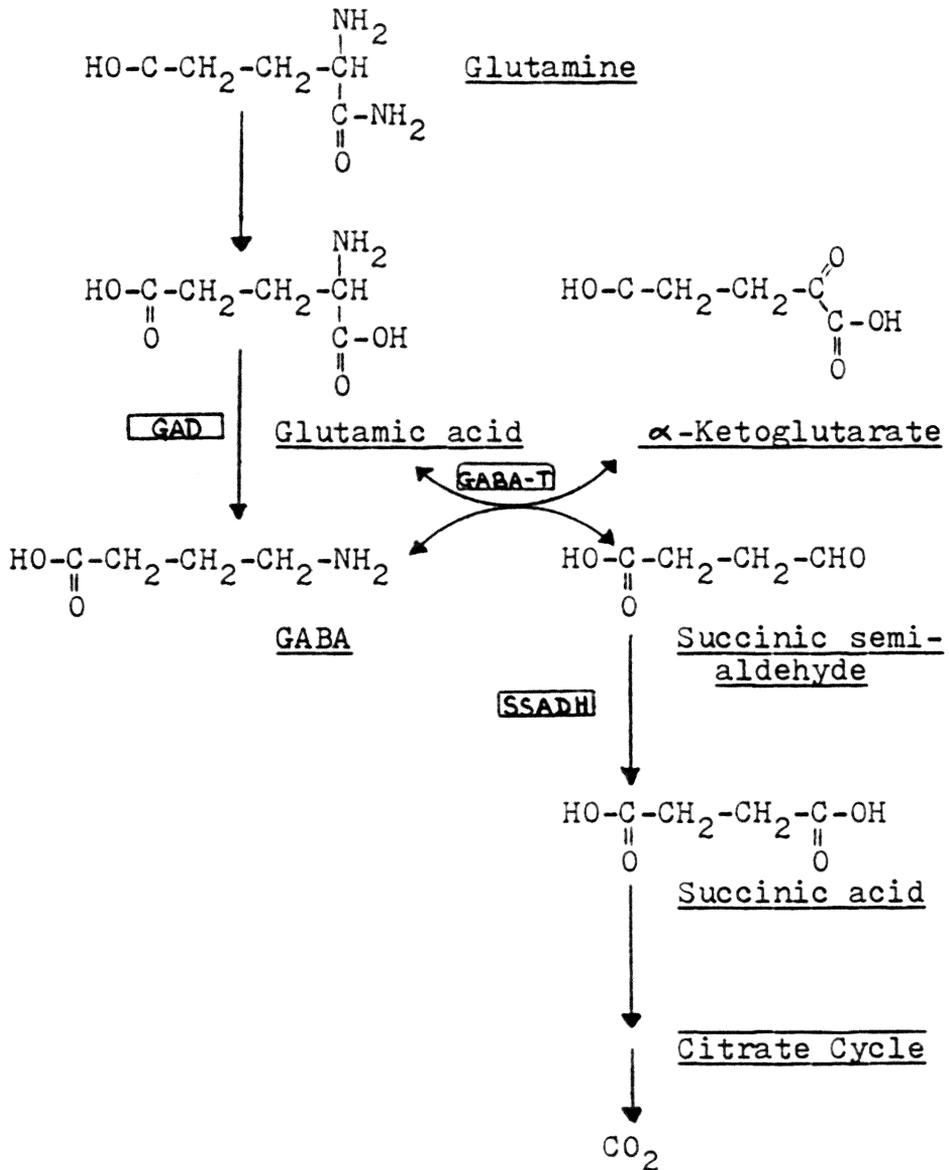


Figure 2. Scheme of primary gamma-aminobutyric acid (GABA) synthesis; pathway depicted represents the glutamic acid-GABA shunt, which uses the following enzymes: glutamic acid decarboxylase (GAD), GABA- α -oxoglutarate transaminase (GABA-T), and succinic acid dehydrogenase (SSADH) (adapted from McGeer, Eccles and McGeer, 1978).

molecule of GABA only if a molecule of its precursor is formed to take its place. Thus, a steady-state pool of GABA may be maintained in brain structures at all times. Lastly, succinic semialdehyde is oxidized to succinic acid by the enzyme succinic semialdehyde dehydrogenase (SSADH) (EC 1.2.1.24). Succinic acid can then reenter the citrate cycle of the normal metabolic Krebs cycle (Lehninger, 1978).

An alternate pathway for GABA synthesis from putrescine has been found in mouse brain (Seiler and Knodgen, 1971), fish brain (Seiler, Al-Therib and Kataok, 1973), rat brain (Seiler and Al-Therib, 1974; Seiler and Eichertopf, 1975), mouse neuroblastoma cells (Kremzner, Hiller and Simon, 1975), and chick retina (De Mello, Bachrach and Nirenberg, 1976). In this alternate pathway, the enzyme ornithine decarboxylase (ODC; EC 4.1.1.17) is the rate-limiting enzyme for the formation of putrescine and the polyamines, spermine and spermidine, from ornithine. Putrescine is converted to GABA via a direct pathway that uses the enzyme, diamine oxidase, which does not include glutamic acid as an intermediate (Figure 3). A more extensive explanation of putrescine catabolism to GABA is given in a subsequent selection of the general introduction dealing with putrescine neurochemistry. Recently Konishi, Nakajima and Sano (1977) and Tsuji and Nakajima (1978) have reported that a metabolic pool of GABA exists in the rat brain that is a result of putrescine catabolism, and that is different from the normal Glu-GABA pool.

Considerable evidence exists which implies that endogenous GABA concentrations have a discrete distribution within the mammalian CNS. Brain GABA levels have been reported to be on the order of μ moles/

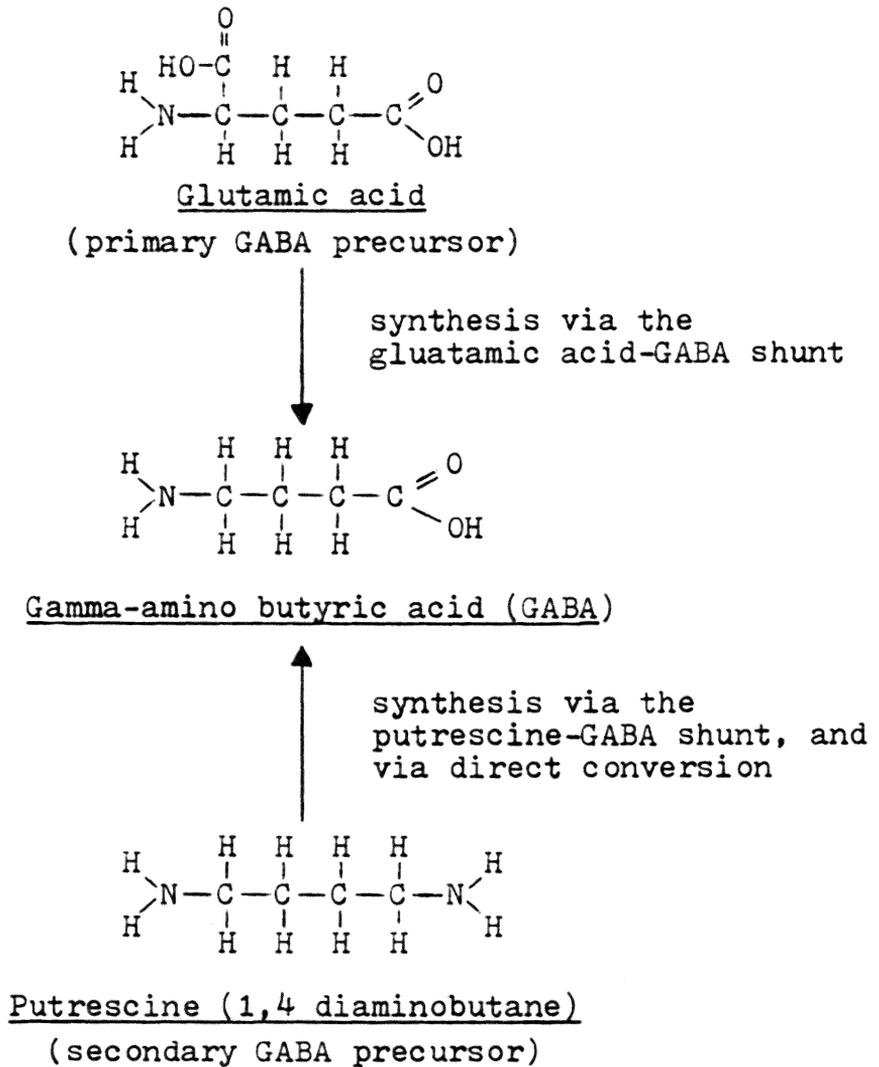


Figure 3. Chemical structures of gamma-amino butyric acid (GABA), and its primary and secondary precursors; glutamic acid (primary) and putrescine (secondary).

g-brain tissue (Fahn and Cote, 1968; Perry, 1971). The proposed neural sites and pathways which involve GABA have been identified by numerous techniques, including assays employing radiolabeled precursor, neuro-histochemical labeling procedures using enzymatic antibodies, enzyme (GAD) assays, iontophoretic GABA applications, and neurophysiological studies of isolated neurons. Specific cell types in the hippocampus, retina, olfactory bulb, cerebral cortex and spinal cord have been identified as GABAergic. Cerebellar cell types proposed to have GABAergic activity include Purkinje, golgi, basket and stellate cells. Central nervous system pathways which are thought to contain GABAergic mechanisms important to neurotransmission include neostriatal, striato-nigral, nigro-striatal, and pallido-nigral projections. Although the literature contains extensive biochemical and electrophysiological evidence that support the existence of GABAergic pathways in the CNS, definitive characterization of the mammalian GABA neural network is far from complete. Definitive studies are hampered by the complexity of higher order nervous systems, inherent problems encountered with in vivo research, and the existence of GABAergic interactions with other neurotransmitter systems in the brain and CNS.

GABA as a neurotransmitter. During the 1960's, much of the initial research exploring the pharmacology and neurophysiology of GABAergic neuron's utilized crustacean synaptic preparations. In 1958, Kuffler and Edwards produced evidence that GABA duplicated the action of a physiological transmitter substance in a crayfish spinal cord synapse, providing the first strong evidence that GABA may be an inhibitory neurotransmitter. Kravitz (1963) demonstrated that inhibitory

lobster neurons contained much higher concentrations of GABA than did excitatory neurons, while other researchers showed that GABA was released from these neurons during electrical stimulation (Otsuka, Iversen, Hall and Kravitz, 1966).

In 1966, Krnjevic and Schwartz demonstrated with microiontophoretic techniques that GABA mimicked the activity of inhibitory neurons of the mammalian cerebral cortex, increasing neuronal conductance to chloride and hyperpolarizing the membrane potential. Ito and Yoshida (1964) reported that Purkinje axons exerted inhibitory action on cerebellar nuclei. Obata and associates (Obata, Ito, Ochi and Sato, 1967) showed that iontophoretic application of GABA elicited IPSP-like (inhibitory postsynaptic potential) changes in Purkinje cells, mimicking the action of inhibitory transmitter release from the axon terminal. These investigators also found that GABA was released into the fourth ventricle of the brain following electrical stimulation of Purkinje cells. In 1970, Fonnum (Fonnum, Storm-Mathisen and Walberg, 1970) reported that the transection of Purkinje cell axons resulted in the disappearance of the enzyme, GAD, from axon terminals. In 1981, Matus, Pehling and Wilkinson reported the first direct evidence for the association of GABA transmitter receptors with postsynaptic sites which correlated with GABAergic inhibitory action. Matus, and associates were able to demonstrate, binding of ^3H -GABA and ^3H -musimol to subcellular fractions derived from crude rat brain homogenates. According to their data, synaptic plasma membranes contain two receptor-like GABA binding sites, one of which (a high affinity site) is located on postsynaptic densities.

Thus, strong biochemical and physiological data exists in the literature which supports the role of GABA as the major inhibitory neurotransmitter in both vertebrate and invertebrate systems. In fact, the concentration of GABA in the CNS is the highest concentration of any known neurotransmitter in vertebrate or invertebrate organisms. The establishment of the presence of all critical synthetic and catabolic enzymes necessary for GABA metabolism in inhibitory CNS axons and cell bodies is evidence of GABA's neurotransmitter function.

Existence of GABA in the basal ganglia. It has been demonstrated that very high concentrations of GABA and its synthesizing enzyme, GAD, are found in basal ganglia regions, including the globus pallidus, substantia nigra (SN) and nucleus accumbens (Anden, Anden and Wachtel, 1979; Balcom, Lenox and Meyerhoff, 1975). Research has suggested that GABA may have a fundamental role in the function of striatal structures (Costa, Cheney, Mao, and Moroni, 1978), and that GABAergic striato-nigral neurons may be important in influencing extrapyramidal side effects due to antipsychotics (Moroni, Peralta and Costa, 1979). Our knowledge of putative neurotransmitters in the CNS represents an integration of data obtained by electrophysiological, biochemical and histochemical methodology. This section is devoted to a review of evidence which supports the existence of GABA in basal ganglia and striatal regions of the brain.

Regional distribution studies of GABA in the mammalian CNS have shown that GABA is heterogeneously distributed throughout most brain regions (Fahn, 1976). Neurochemical investigations have demonstrated that the highest GABA concentrations are in the substantia nigra,

globus pallidus and hypothalamus; intermediate levels are found in the caudate nucleus, the putamen, and the dentate nucleus of the cerebellum; and low concentrations are observed in the cerebral and cerebellar cortices, hippocampus and spinal cord (Enna, 1979). The difference in GABA levels between the lowest and highest regions constitutes only a three- to four-fold ratio which is small compared to most transmitters (Enna, 1979). Biochemical markers for GABAergic nerve terminals, including GAD activity and high affinity GABA uptake, exhibit regional distributions similar to that observed for GABA content (Enna, Kuhar and Snyder, 1975). Measurement of GABA turnover rates by mass fragmentation of GABA and GAD have shown that GABA turnover is highest in the caudate nucleus and lowest in the SN, while the caudate nucleus has the lowest and SN the highest GABA content in these respective regions (Moroni, Peralta and Costa, 1979).

It appears that there are two types of GABA neurons in the striatum: 1) short axon neurons which are intrinsic to the striatum, and 2) long axon neurons which project from the striatum to other brain regions, including the SN (McGeer and McGeer, 1975). Long axon GABA neurons are postulated to form the descending striato-nigral GABAergic pathway which controls feedback regulation of DA nigro-striatal neurons (Ribak, Vaughn, Saito, Barber and Roberts, 1977).

A significant reduction of GABA and GAD content in the SN can be caused by electrolytic lesions of the globus pallidus, destruction of the striatum by suction, or hemitransection of the brain at the level of the subthalamus (Ribak, Vaughn, Saito, Barber and Roberts, 1977). Ribak et al. (1977) have demonstrated that GAD-containing neurons have

a functional linkage between the basal ganglia and SN, with SN nerve endings exhibiting GABA uptake in slice preparations, as well as containing GAD. These studies suggest that cell bodies containing GABA are located within the striatum and that they project to the SN.

Double labeling experiments utilizing i.v.t. injections of 6-hydroxydopamine (an agent which selectively destroys adrenergic nerve terminals) and ^3H -leucine yielded results which suggested that some descending GABAergic fibers are located in the globus pallidus and terminate on DA neurons in the SN (Hattori, Fibiger and McGeer, 1975). These experiments demonstrated that ^3H -leucine injected into the globus pallidus traveled by axoplasmic flow to SN nerve endings which made contact with dendrites of DA neurons which were degenerating due to 6-hydroxydopamine treatment (Hattori et al., 1975). Immunocytochemical labeling procedures using antibodies which attach themselves to GAD-containing terminals have shown that GAD-positive axon terminals in the SN: 1) originate from GABA neurons in the striatum and globus pallidus, and 2) exert postsynaptic inhibition on neurons in the SN (Ribak, Vaughn, Saito, Barber and Roberts, 1977).

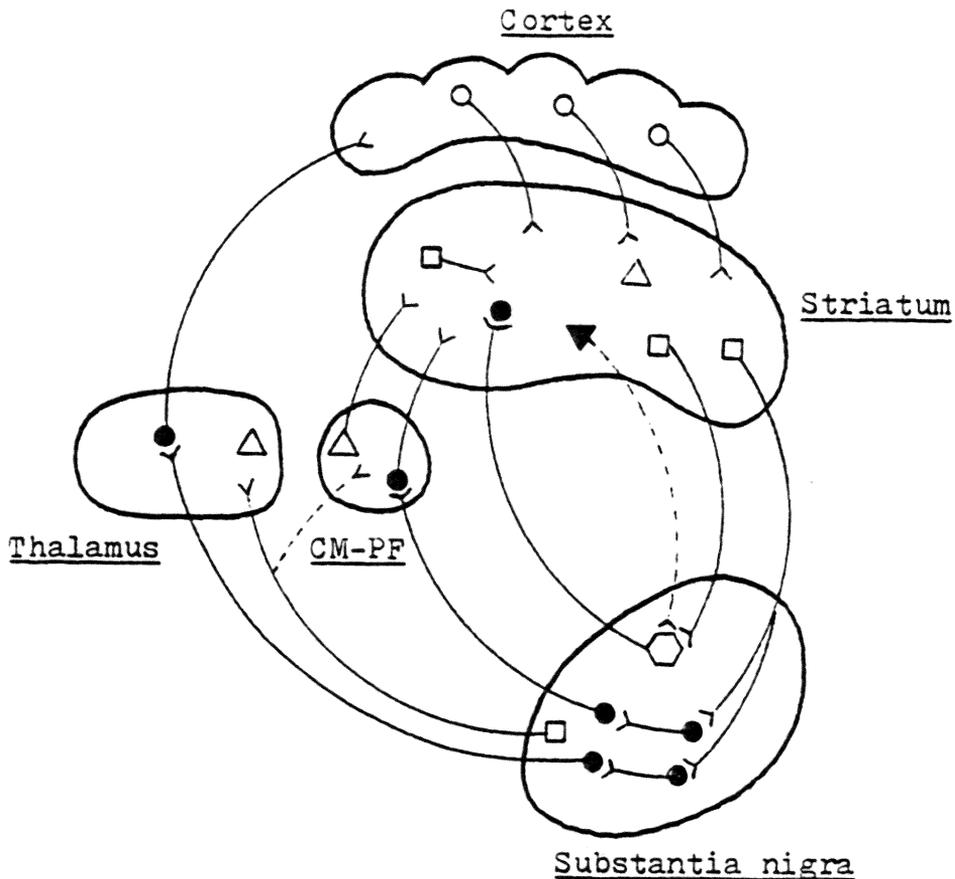
GABA appears to be the major transmitter substance in the striatonigral pathway (Dray, 1980), although striatal enkephalinergic (Hong, Yang, Fratta, and Costa, 1978) and substance P (Hong and Costa, 1978) neurons may also have a role in feedback loops between the caudate nucleus and SN. Some researchers have also proposed that some GABA-containing neurons may be intrinsic to the SN and may give rise to a nigral efferent pathway (Dray, 1980).

Evidence has been reported which argues for the existence of three

major populations of GABAergic neurons in the striatum, including 1) caudatal interneurons, 2) strio-pallidal (long axon) neurons, and 3) strio-nigral (long axon) neurons (Moroni, Peralta and Costa, 1979). With respect to GABAergic neurons, the strio-pallidal projection may be inhibited by direct DA innervation, while the strio-nigral pathway is indirectly inhibited, possibly by a cholinergic interneuron (Moroni et al., 1979). Finally, endogenous opiates are thought to inhibit GABA interneurons in the caudate nucleus (Moroni et al., 1979).

Thus, GABA appears to have an important role in the CNS, inhibiting excitatory neural activity and synaptic stimulation, especially in the substantia nigra and striatum. GABA is generally accepted as functioning by inhibiting postsynaptic activity, but data has been reported which suggests that GABA may also be involved in presynaptic inhibition (Haefly, Polc, Schaffner, Keller, Pieri and Mohler, 1979). GABA has been hypothesized to interact with other neurotransmitters besides dopamine, including acetylcholine, serotonin, and most recently, endogenous opiates (Cattabeni, Bugatti, Groppetti, Maggi, Parenti and Racagni, 1979; Moroni et al., 1979). In Figure 4, a schematic model of neurotransmitter interaction in the strio-nigral pathway is depicted, showing hypothesized GABAergic tracts in the following brain regions: striatum, cortex, SN, thalamus, and CM-PF complex (thalamic center median-parafasicular complex) (as adapted from Scheel-Kruger, Arnt, Braestrup, Christensen and Magelund, 1979).

GABA and dopamine neurochemical interaction. The two brain regions that contain highest concentrations of brain GABA, in the substantia nigra and nucleus accumbens, also contain high concentrations



Neurotransmitters:

- GABA (inhibitory)
- acetylcholine (excitatory)
- dopamine (inhibitory)
- glutamic acid (excitatory)
- △ endogenous opiate peptides; enkephalins/endorphins
- ▼ Substance P (excitatory)

Figure 4. Schematic model of neurotransmitter interaction and the striato-nigral pathway; depicts descending pathways from striatum to substantia nigra which regulate posture and behavioral stimulation; also, related pathways of the cortex, thalamus, and CM-PF (thalamic center median parafasicular complex), with dashed lines (---) showing hypothesized interactions (adapted from Scheel-Kruger, Arnt, Braestrup, Christensen and Magelund, 1979).

of dopamine (Anden, Anden and Wachtel, 1979). The existence of multiple DA-GABA interactions within the basal ganglia has been demonstrated.

Injection of GABA into the SN of rats results in an increase of DA in the forebrain region (Anden and Stock, 1973). In fact, electrophysiological data (Crossman, Walker and Woodruff, 1973; Precht and Yoshida, 1971) and pharmacological data (Anden and Stock, 1973; Gale and Guidotti, 1976; Guidotti, 1976) have supported the notion that descending GABAergic nerve fibers may regulate SN dopaminergic cells by exerting an inhibitory influence on their activity. However, recent research suggests that GABA-DA interactions are more complex than originally postulated in this striato-nigral feedback loop. Cattabeni et al. (1979) reported that GABA can modulate DA cells either directly or indirectly via interneurons. Cholinergic receptors appear to be located on descending striatal GABAergic fibers, while GABA interneurons in the striatum directly innervate DA terminals. Cheramy, Nieousllion and Glowinski (1978) showed that intranigral injection of GABA in cats caused a biphasic DA response: initial stimulation of DA release, and then inhibition.

Other research has shown that GABA-DA neurotransmission dynamics may be very complex and it may be difficult to predict a priori the result of an experimental manipulation. DiChara, Porceddu, Morelli, Mulas and Gessa (1979) have presented data which indicate that the strio-nigral pathway is not only a feedback pathway, but also an efferent route for striatal impulses. Thus striatal neurons GABA mediate the level of excitation of DA neurons in the nigra. These authors also

report that non-DA neurons in the nigra are sensitive to GABA and that these non-DA neurons may mediate striatal neurotransmission beyond the nigra (such as the GABAergic nigro-thalamic pathway).

Studies utilizing the GABA-receptor agonist, muscimol, have suggested that increases in GABAergic activity may have confounding interactions, making predictions about GABA experimental manipulations difficult (Walters, Lakoski, Eng and Waszczak, 1979). These authors state that the striato-nigral feedback loop may affect some DA neurons indirectly and that several GABA synapses may be involved in the loop. Thus GABA agonists might produce a complex "spectrum" of effects in this pathway making prediction of GABA-agonist modification of DA activity difficult. Waszczak, Eng and Walters (1980) reported that a population of neurons in the SN pars reticulata is affected to a larger degree by a GABA input than SN pars compacta DA neurons. Differential responses to systemic (i.v.) muscimol, picrotoxin, and iontophoresed GABA in these SN regions added impetus to the view that GABA may not exert an exclusive inhibitory influence upon the nigro-striatal DA system, and that the role of GABA in the SN is more complex than conceptualized by the classic feedback loop model (Waszczak, Eng and Walters, 1980).

In addition to the research demonstrating GABAergic mediation of DA activity, other data have shown that DA may influence GABAergic functions. Systemic administration of apomorphine, a known DA-agonist, increased the release of GABA in the SN and striatum (van der Heyden, Venema and Korf, 1980). Blockade of DA receptors with neuroleptics causes an increase of GABA turnover in several brain nuclei, including

the globus pallidus (Marco, 1978). In vitro studies have also shown that exogenously applied DA stimulates the release of ^3H -GABA in SN slice preparations (Reubi, 1977). In 1980, van der Heyden, Venema and Korf substantiated these findings with in vivo studies. These authors concluded that DA may exert an inhibitory action on the endogenous release of GABA in the SN.

In 1980, Ferkany, Strong and Enna, using GABA-transaminase inhibitors to prevent the catabolism of GABA, chronically elevated in vivo GABA levels in mice to evaluate the effect on DA function. Using DA and GABA receptor binding techniques, these investigators discovered that chronically elevated GABA content leads to a significant increase in the number of DA receptors and a significant decrease in GABA receptors in the corpus striatum of the basal ganglia. These data suggest that elevated GABA levels caused a DA receptor supersensitivity reaction due to a GABA-induced decrease in the firing of DA neurons (Ferkany, Strong and Enna, 1980).

Thus, the functional link between GABA and DA has been extensively investigated in basal ganglia structures, including the SN, the striatum, nucleus accumbens, and globus pallidus. While it appears that GABAergic neurons do exert influence on DA function, recent evidence has questioned the validity of the classic striato-nigral feedback loop model. In addition to direct inhibitory influence of GABA on DA neurons within this pathway, it may be that multiple GABAergic synapses, GABA nigral interneurons, GABA autoreceptors, or other complex interactions exist making specification of GABA-DA transmitter dynamics difficult.

GABAergic behavior: introduction. In recent years, a considerable amount of research has been conducted on the behavioral effects of enhanced GABAergic function. Modification of endogenous GABAergic activity is primarily accomplished by one of the following methods: intracranial injection of GABA itself, systemic and intraventricular (i.v.t.) administration of GABA agonists (such as muscimol), and the inhibition of GABA metabolism with the systemic administration of GABA-transaminase inhibitors (such as gamma-vinyl GABA). The following sections will review recent literature concerned with behavioral changes observed following experimentally induced increases in GABA content, or the facilitation of GABAergic CNS function by administration of GABA-agonists. The behavioral research will be presented in five sections: 1) anticonvulsant action of GABA, 2) GABA-mediated behaviors related to DA (rotational behavior, stereotypic behavior, and activity), 3) analgesic properties of GABA, 4) GABA-mediation of opiate function, 5) anxiolytic properties of GABA, and 6) behavioral effects of systemic treatment with GABA agents.

GABAergic behavior: anticonvulsant activity. Convulsant drugs acting on GABA-mediated inhibition are broken down into three categories, including 1) those which impair GABA synthesis; 2) drugs blocking synaptic release of GABA; and 3) drugs which impair the neuronal inhibitory action of GABA (Meldrum, 1979). Isoniazid is an inhibitor of GAD, the enzyme involved in GABA synthesis, and seizures induced by isoniazid treatment are thought to be due to reductions in brain GABA content (Meldrum, 1979).

The selective antagonism of isoniazid-induced seizures compared

to strychnine-induced convulsions is accepted as an index of GABA-mimetic activity (Meldrum, 1979). In 1976, Naik, Guidotti and Costa found that muscimol selectively antagonized isoniazid-induced seizures in rats. Further evidence of muscimol's anticonvulsant activity in rodents has been reported (Anlezark, Collins and Meldrum, 1978).

In 1979, Mathews and McCafferty studied the effect of the GABA-receptor agonist, muscimol, on seizures caused by agents which impair GABA-mediated neurotransmission in rats. They found that IV administration of muscimol delayed the onset of isoniazid- and picrotoxin-induced convulsions, but had no effect on strychnine-induced convulsions (which are mediated by glycine instead of GABA). Tonic forelimb extension due to bicuculline (a GABA-antagonist) treatment and metrazol-induced convulsions were also attenuated by muscimol. In sum, this research demonstrated that systemically administered muscimol resulted in anticonvulsant activity against a number of chemical convulsants known to impair CNS-GABA function by GABA receptor blockage, or depletion of GABA content.

In 1980, Frey and Loscher reported that increasing GABA levels in mice by i.p. and oral administration of cetyl-GABA resulted in significant anticonvulsant activity. This anticonvulsant activity was reflected in the increases of thresholds for convulsions induced by electroshock and pentetrazole injection. Cetyl-GABA is a synthetic transport form of GABA which can be converted to GABA by metabolic processes in the CNS.

Wood, Russell, and Kurylo (1980) experimentally increased GABA levels in mice and demonstrated the anticonvulsant effect of this

increase. They elevated GABA with three techniques: 1) with agents which inhibit GABA-T but do not affect GAD (gabaculine); 2) those which inhibit GABA-T with a slight elevation of GAD (amino-oxyacetic acid, or AOAA); and 3) those that strongly inhibit both types of enzymes (hydrazine). They found that these treatments caused significant, time-dependent increases in GABA content of both whole brain and synaptosomal preparations from brain tissue. In addition, a strong linear relationship existed between the drug-induced increases in GABA and the anticonvulsant effects of these drug treatments. Their results have helped to establish GABA's role in "modulating" brain excitability.

GABAergic behaviors: dopaminergic relationships. An increase in the tonic activity of GABA on dopaminergic nerve cells in the SN results in decreased axonal DA firing (Delini-Stula, 1979). However, recent pharmacologic, electrophysiological, and behavioral data question whether GABA-mediation of DA is exclusively inhibitory and whether it is entirely direct (Cattabeni et al., 1979; Cheramy et al., 1978; Cools, 1979; Delini-Stula, 1979; Di Chara et al., 1979; Walters et al., 1980).

Di Chara et al. (1979), noted that the SN is essential for expression of dopaminergic behaviors such as stereotypy, turning behavior, and catalepsy, which are attributable to striatal DA activity. However, these authors also suggested that certain behavioral responses, such as catalepsy, may involve other GABA-DA interactions, including activation of nigro-thalamic GABAergic neurons. Delini-Stula found that selective stimulation of striato-nigral GABAergic pathway by

muscimol produced differential behavioral responses in rats, yielding both excitatory and inhibitory effects (Delini-Stula, 1979). She suggested that activation of the striato-nigral GABAergic pathway results in two different and distinct behavioral patterns: one pattern reflects the classic tonic inhibition of DA nigral neurons, while another appears to be independent of dopaminergic axonal innervation. This second pattern may be related to an indirect excitatory GABAergic influence on postsynaptic DA receptors in the striatum.

Recent studies investigating the inhibitory mediation of neurotransmitter pathways in the basal ganglia by GABA have centered around GABA's relationship to the striatal-DA system (Eng and Waszczak, 1978; McKenzie and Hansen, 1980; van der Heyden, Venema and Korf, 1980; Waddington and Cross, 1980; Walters, Lakoski, Eng and Waszczak, 1978). Although GABA has been shown to exert an inhibitory neuronal influence on DA function and other transmitters, excitatory actions of GABA are also emerging in recent studies (Delini-Stula, 1979; Di Chara, Porceddu, Morelli, Mulas and Gessa, 1979). Studies manipulating DA function in vivo have found that DA is related to many overt behavioral responses. DA has been linked to circling behavior (Dankova, Boucher and Poirier, 1977; Scheel-Kruger et al., 1977; Waddington, 1977; Waddington and Cross, 1980), hyperactivity and facilitated locomotion (Bernardi, Souza and Neto, 1981; Costall, Naylor, Cannon and Lee, 1977a,b; Dourish and Cooper, 1981), grooming (Lassen, 1977), reduced exploratory activity (Issacson, Yongue and McClearn, 1978), drinking behavior (Dourish and Cooper, 1981), and the manifestation of various stereotypic behaviors, including rearing, sniffing, licking, biting,

and gnawing (Bernardi, Souza and Neto, 1981; Costall, Naylor, Cannon and Lee, 1977a,b; Fray, Sahakian, Robbins, Koob and Iverson, 1980; Sahakian, Robbins, Morgan and Iverson, 1975).

It is important to note that most investigators report that the effects of DA-agonists and other DA-enhancing treatments depend on the mode of DA manipulation, site of injection, dosage, method of behavioral assessment, time course of observation, and nature of the experimental environment. In the following section the GABA-mediation of some dopaminergic behaviors will be reviewed. These include rotational behavior, stereotypy, and effects on motor behavior.

GABAergic behavior: rotational behavior. It has been established that unilateral injection of GABA and GABAergic drugs into the substantia nigra reticulata of rats induces contralateral rotational behavior (Scheel-Kruger et al., 1977; Waddington, 1977). In 1980, Waddington and Cross summarized evidence which indicates that these rotational responses are independent of the function of nigro-striatal DA neurons, although they are similar to rotational behavior induced by unilateral striatal DA receptor stimulation. DA receptors in the SN are thought to be mediated by nigral GABA receptors located on a population of non-dopaminergic neurons (Di Chara et al., 1977; Waddington and Cross, 1978; Waddington and Cross, 1979). These nigral GABA receptors are thought to be innervated by the well-known striato-nigral GABA pathway (Dray, 1976), an initial link in the striatal efferent effector system mediating the functional response to striatal dopaminergic transmission (Garcia-Munoz et al., 1977; Marshall and Ungerstedt, 1977; Olanas et al., 1978; Waddington and Cross, 1978).

In 1978, Waddington and Cross used neurochemical lesions to destroy the neurons whose axons form the striato-nigral GABA pathway. The lesions were produced by intracranial injections of Kainic acid, a neurotoxin which is thought to selectively destroy cell bodies while leaving fiber tracts undamaged. Administration of the GABA-agonist, muscimol, into the ipsilateral substantia nigra reticulata of these lesioned rats induced contralateral rotational responses. These rotational responses were positively correlated with elevations in ³H-GABA binding and depletion of GABA content region. Thus the GABA model of rotational behavior is similar, apomorphine-induced (DA related) with respect to both behavioral and neurochemical parameters, to the classical 6-OHDA lesion (DA-dependent) model of rotational behavior (Waddington and Cross, 1980).

In 1979, Thiebot and Soubrie reported that microinjection of high concentrations of GABA and muscimol unilaterally into the substantia nigra of rats induced activity of nigral neurons and potentiated amphetamine-induced spontaneous circling behavior. Kaakkola and Kaariainen (1980) obtained corroborating data, demonstrating that nigral injection of muscimol was even more potent than GABA in causing contralateral turning in rats. These researchers concluded that intranigral injection of GABA and muscimol elicits contralateral turning that seems to be dependent on the activation of the dopaminergic nigro-striatal system and on nondopaminergic nigral output systems.

GABAergic behavior: dopamine stereotypy. The GABA-DA negative feedback model predicts that increased firing of GABAergic neurons on DA neurons in the SN will result in the appearance of electrophysio-

logical and behavioral responses indicative of postsynaptic DA-receptor blockade (Delini-Stula, 1979). Electrophysiological data has shown that the discharge rate of nigral cells can be inhibited when the GABA-agonist, muscimol, is injected parenterally, or applied onto nigral cells by iontophoretic technique (Olpe and Koella, 1978). Behavioral data in agreement with this evidence includes the demonstration that systemic muscimol can augment haloperidol catalepsy while also increasing an antagonistic action against amphetamine-induced stereotypy (Delini-Stula, 1979).

However, data exist which are not consistent with the predictions of the classic GABA-DA negative feedback model. In mice muscimol was found to increase stereotypic behaviors induced by dopaminergic agonists, including cocaine and apomorphine; injected directly into the SN of rats produced stereotypic behaviors (Scheel-Kruger, Cools and Wel, 1977). The apparent inconsistency of the behavioral data has prompted Delini-Stula to propose that activation of selective GABAergic pathways can lead to both inhibitory and excitatory behavioral responses within GABA-DA systems (Delini-Stula, 1979).

In 1977, Scheel-Kruger, Cools and Wel investigated the effect of muscimol injection into nucleus accumbens of rats that had been pretreated with the DA-agonist, apomorphine (Scheel-Kruger, Cools and Wel, 1977). They found that muscimol treatment attenuated apomorphine-induced locomotor activity while increasing DA-mediated stereotypies, including licking and gnawing. The injection of muscimol per se did not elicit stereotypy in this procedure. At high dosages of muscimol, continuous licking and gnawing were observed in apomorphine-pretreated

animals, who were often in a crouched position. Apomorphine treatment alone (.25 mg/kg) caused walking and rearing, some continuous sniffing, discontinuous licking, and no incidence of gnawing or biting. Scheel-Kruger et al. (1977) concluded that their results indicate that GABA in the nucleus accumbens differentially influences apomorphine-related behavior: locomotor activity is diminished while stereotypy is increased.

Research has demonstrated that DA mechanisms within the nucleus accumbens regulate locomotor activity and stereotypy (Pijnenburg, Honigand, Rossum, 1975). It has also been observed that muscimol-induced effects differ from those associated with antipsychotic neuroleptics: the application of neuroleptics to this region of the basal ganglia blocks DA function and antagonizes DA-dependent locomotion and stereotypy (Costall, Naylor, Cannon and Lee, 1977). The results of Scheel-Kruger et al. (1977) utilizing muscimol correlate well with studies in which GABA itself was injected into the nucleus accumbens, resulting in similar behavioral responses.

In 1980, McKenzie and Hansen used dexamphetamine and apomorphine to produce behavioral activation and stereotypy in rats. Muscimol (0.5-2.0 mg/kg, r.p.) produced dose-related inhibition of neural activity in the striatum, blocking the electrophysiological activation caused by dexamphetamine and apomorphine, however, muscimol significantly increased drug-induced stereotypy. These results suggest that activation of striatal neurons may not be an essential component of stereotypic responses. These authors also elevated brain-GABA by administering aminooxyacetic acid (AOAA), a GABA-T inhibitor. Elevation

at GABA levels resulted in behavioral effects similar to those produced by muscimol (McKenzie and Hansen, 1980). In light of their findings, McKenzie and Hansen proposed that enhanced GABAergic synaptic activity may increase or decrease, neural traffic in inhibitory afferents to the striatum.

Thus, other nuclei which receive mesolimbic DA inputs, such as the olfactory tubercle, amygdala, and striae terminalis, may actually be responsible for the manifestation of stereotypy which are mediated by GABA (McKenzie and Hansen, 1980). Cools (1979) has presented evidence suggesting that the CM-PF complex (thalamic center median-parafascicular nuclear complex) may be subject to neostriatal GABAergic influenc. Cools has suggested that this thalamic complex controls the expression of apomorphine-like responses (stereotypic sniffing and walking), behaviors which he believes to be independent of changes in DA activity in either the nucleus accumbens or the neostriation (Coos, 1979).

GABAergic behavior: opiate-related effects. It has been reported that striatal neurons containing enkephalin (an endogenous opiate) have a role in the regulation of feedback loops between the caudate nucleus and SN (Moroni, Peralta and Costa, 1979). The CM-PF complex contains very high concentrations of enkephalin (Simantor, Kuhar, Uhl and Snyder, 1977); the complex is also susceptible to mediation by neostriatal GABA neurons (Cools, 1979). In addition, opiate receptors may regulate GABAergic interneurons via inhibitory action in the caudate nucleus (Moroni et al., 1979). Thus, the interaction of GABA and endogenous opiates is of interest.

Contreras, Tamayo and Quijida (1979) evaluated the consequences of gamma-acetylenic GABA and gamma-vinyl GABA administration in mice, with respect to analgesic response to morphine, morphine tolerance, and physical dependence to morphine. They found that these irreversible GABA-T inhibitors enhanced morphine-induced analgesia while increasing brain GABA content. Facilitation of analgesia was greater in morphine-tolerant mice than naive mice, and may have indicated a decrease in the intensity of morphine tolerance. Physical dependence was also attenuated by treatment with these agents. The authors note that gamma-acetylenic GABA and gamma-vinyl GABA per se were ineffective in producing analgesia when measured by the hot plate method. These results indicate a possible relationship between some opiate effects and GABA concentrations in the CNS.

Buckett (1980) conducted research with gamma-acetylenic GABA and gamma-vinyl GABA in mice and rats, finding that treatment with these GABA-T inhibitors resulted in antinociceptive (analgesic) response (hot-plate test for paw lick and escape latencies) which was not accompanied by ataxia. The analgesic effect produced correlated well with the temporal elevation of GABA levels due to treatment (maximal effect 4-6 h posttreatment). The GABA-antagonist, bicuculline, attenuated hot-plate analgesia, but the opiate-antagonist, naloxone, had no effect in reversing the analgesia. Buckett also found corroborating evidence for the enhancement of morphine-induced analgesia by these GABA-T inhibitors, although they were not able to reverse or alter naloxone-precipitated morphine-withdrawal syndrome which included diarrhea, squealing, writhing, "wet dog" shaking, ptosis and teeth

chattering. These data were interpreted by the authors as suggesting that gamma-acetylenic GABA and gamma-vinyl GABA can produce distinct analgesic effects in rodents related to elevated brain-GABA, but that these effects do not appear to be opiate-like in nature (Buckett, 1980).

De Boer, Bartels, Metselaar and Bruivels (1980) evaluated the usefulness of i.p. injection of di-n-propylacetate (DPA) in eliciting GABAergic behaviors in rats. DPA is an inhibitor of succinic semialdehyde-dehydrogenase (SSADH), an enzyme thought to be rate-limiting in GABA degradation (deBoer et al., 1980). De Boer and his colleagues succeeded in elevated GABA levels in rat brain by i.p. injection of 300 mg/kg DPA. The characteristic behavioral effects of DPA administration were termed the "DPA-induced abstinence syndrome". Elicited behaviors included: high incidence of body and forelimb shaking, digging, hunchback posture, piloerection, ptosis (partial closing of eyelids), increased horizontal and vertical locomotor activity (measured by photobeam instruments), a 50% incidence of catalepsy (measured by maintenance of forelimbs over a 7-cm-high bar for 15 s), and increases in swallowing and teeth chattering behaviors. Administration of the specific GABA-antagonist, bicuculline, at subconvulsant doses reduced the abstinence syndrome behaviors initiated by DPA treatment. Picrotoxin, another GABA-antagonist, also reduced DPA-induced abstinence syndrome behaviors, as did morphine treatment.

DeBoer et al. concluded that the behavioral syndrome caused by i.p. DPA treatment can be characterized as quasi-morphine abstinence behavior because it resembled the syndrome observed during morphine

withdrawal and abstinence in rats (Cowan and Watson, 1978; De Boer et al., 1977). Since DPA is known to increase endogenous GABA content in the brain, and since GABA-antagonists can suppress the DPA-induced behavioral syndrome, De Boer suggested that this syndrome may be evoked by an increase in GABA at its receptor sites (de Boer et al., 1980).

Body shaking behavior which some have described as "wet dog" shaking, is associated not only with morphine and opiate withdrawal and the injection of narcotic antagonists, but also with the cerebral injection of endogenous opiate peptides (endorphin and enkephalin), tactile stimulation around the ears, placement of the animal in cold water, administration of carbachol chloride, and cerebral injection of serotonin (as cited in Bedard and Pycocock, 1977; Drust, Sloviter and Connor, 1979; Drust, Sloviter and Connor, 1981; Turski, Czuczwar, Turski and Kleinrok, 1981). With the exception of the De Boer et al. (1980) study there has been no literature report of any GABA treatment or manipulation of GABAergic function which induces body shaking or "wet dog" shakes (WDS) in rodents. Recent studies investigating the nature of and neuropharmacologic actions responsible for WDS behavior tend to discard a proposed serotonergic mechanism in favor of an opiate-related mechanism that may involve a kappa-opiate receptor (Drust, Sloviter and Connor, 1981; Lanthorn, Smith and Issacson, 1979; Turski, Turski, Czuczwar and Kleinrok, 1981).

GABAergic behavior: systemic treatment with GABA agents. Behavioral studies involving pharmacological manipulation of GABA levels are proliferating in the literature (Scheel-Kruger, Arnt, Broestrup,

Christensen and Magelund, 1979). These studies have used either GABA-agonists (muscimol) or other GABA-facilitating agents (such as GABA-T inhibitors). Dysfunction of GABAergic CNS function has been implicated in the etiology of numerous human diseases (including Parkinson's, tardive dyskinesia and schizophrenia). Animal behavioral models which use GABA agents offer a useful approach for pre-clinical studies in these areas of CNS pathology. Systemic injection of GABA agents is known to affect CNS function, although the behavioral consequences of various agents have not produced a consistent pattern of action (Delini-Stula, 1979). Although it has generally been assumed that GABA and its precursor, glutamic acid do not readily cross the peripheral blood-brain barrier in mammals (McGeer, Eccles and McGeer, 1978), there is evidence which suggests that GABA may indeed enter the CNS following systemic administration. In 1977, it was reported that i.p. injection of GABA in rodents resulted in changes in the brain content of neurotransmitter substances (Biwas and Carlsson, 1977). Intraperitoneal injection of a wide range of GABA dosages resulted in elevated DA content, depressed noradrenaline levels, and variable change in serotonin levels and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA); however, GABA brain content was not significantly altered (Biwas and Carlsson, 1977). This biochemical complements research suggesting that systemic injection of GABA may cause changes in behavior similar to those reported after i.v.t. GABA injection and to those following other procedures which elevate brain GABA (Benton and Rick, 1976; Biwas and Carlsson, 1977; File, 1977).

Smialowski, Smialowski, Reichenberg, Byrska and Vetulani (1980)

reported that GABA injectioned i.p. into mice, rats and rabbits resulted in distinct behavioral changes; differences in interspecies reactivity were found. An i.p. dose of 10 mg/kg GABA did not produce any apparent behavioral change or affect locomotor activity in mice. However, doses of 500 and 1000 mg/kg produced ptosis, decreased locomotor activity, loss of interest in novel stimuli placed in the cages; no incidence of head twitching or abnormal gait were observed.

In rats, Smialowski et al. found that GABA dosages as low as 100 mg/kg produced decrements in locomotor activity, ptosis, grooming, incidental head twitching, bursts of locomotor activity, and periods of sedation (animals assumed sleeping postures). Head twitching was most evident following treatment with 500 mg/kg GABA, and this behavior was inhibited by picrotoxin, morphine, cyproheptadine (a specific serotonin blocking agent), and clonidine (a noradrenergic receptor agonist). Behavioral results in rabbits were similar to those observed in rats, although a dose of 500 mg/kg produced elevated locomotor activity in rabbits (Smialowski et al., 1980). In summary, these results indicate that systemically administered GABA can cross the blood-brain barrier and produce some CNS effects in vivo. The ability of systemically administered GABA to induce head twitching, a behavior that is morphine-reversible, suggests that i.p. GABA treatment may be influencing endogenous opiate function.

Systemic administration of cetyl-GABA, which is a transport form of GABA, has been described to cause numerous behavioral changes in mice and rats and to increase brain GABA content (Frey and Loscher, 1980). In addition to the anticonvulsant effects of cetyl-GABA treat-

ment, other reported behavioral changes include the lack of spontaneous and induced locomotion, general behavioral depression, ptosis and rapid, "jerky" respiration. At higher dosages, subjects become atactic exhibiting "hopping" movements, loss of righting reflex, uncoordinated and bizarre movements of the legs and body, anorexia and adipsia, and dyspnea prior to death. A "writhing" syndrome was also observed in some subjects which lasted between 30-90 minutes depending on dosage (Frey and Loscher, 1980).

Cooper, Howard, White, Soroko, Ingold and Maxwell (1980) demonstrated that intracisternal and i.p. administration of the GABA-agonist, ethanolamine-0-sulfate (EOS), resulted in long lasting, dose-dependent anorexia and loss of body weight in rats. The resultant anorexic effects of EOS corresponded both in time course and magnitude to the elevation of brain GABA resulting from EOS's selective inhibition of GABA-T activity. A reduction in locomotor activity was also observed with high doses of EOS, which correlated with peak elevations of GABA. In a second experiment which examined the effects of muscimol injected i.p., these researchers found that muscimol produced a dose-related reduction in food intake (sweetened milk) of up to 50% inhibition vs. control. These data support the hypothesis that GABA may function in the neural regulation of hunger and satiety mechanisms in rats.

GABAergic behavior: anxiolytic effects and benzodiazepines.

Neuro-pharmacologic studies suggest that benzodiazepines exert their effect by facilitating GABAergic transmission (Costa and Guidotti, 1978). The benzodiazepines have a wide spectrum of action: they act as anticonvulsants, muscle relaxants, hypnotics and especially anxiolytics

(anti-anxiety agents). All of these roles have been researched in considerable depth (Costa, Guidotti, Mao and Suria, 1975; Gallagher et al., 1979; Haefely, Polc, Schaffner, Keller, Pieri and Mohler, 1979; Mallagora, Hamburg, Tallman and Gallagher, 1980).

Data have been reported which demonstrate that diazepam enhances presynaptic inhibition in spinal cord (Haefely et al., 1979) and cuneate nucleus (Polc and Haefely, 1976), and has depressant effect on the spontaneous firing rate of cells in the SN (Wolf and Haas, 1977). Benzodiazepines are thought to facilitate GABAergic transmission at nigral DA synapses, reduce striatal DA turnover, and prevent some of the effects of GABA-antagonists (Haefely et al., 1979).

Lal, Shearman, Fielding, Dunn, Kruse and Theurer (1980) investigated the action of benzodiazepines in rats and found support for the interpretation that anxiolytic properties of benzodiazepines are mediated by GABA. These authors utilized two behavioral paradigms to compare the anxiolytic actions of diazepam and the GABA-mimetic agent, valproic acid. In the first paradigm, reduction of response suppression by "conflict" stimuli was measured, and in the second, the effect of diazepam and valproic acid on a previously learned state-discrimination task using the anxiolytic agent, pentylenetetrazol, was determined. In both tests, diazepam and valproic acid showed anxiolytic activity, strongly suggesting that GABA mechanisms may mediate at least some of the anxiolytic action of benzodiazepines. Further behavioral support for this conclusion was also obtained from Thiebot, Jobert and Soubrie (1980) who found that diazepam antagonized the suppression of bar pressing induced by foot shock in a discriminative-

stimulus paradigm. These recent studies are in contrast to many earlier reports which failed to find an anxiolytic effect of GABA agonist administration. The reason why GABA agonists and the benzodiazepines have some but not all of their behavioral effects in common, is best explained at the receptor level.

In the late 1970's, research indicated that binding of GABA to specific recognition sites in synaptic membrane preparations was a process regulated by other membrane proteins which participate in GABA receptor function (Mazzari, Massotti, Guidotti, and Costa, 1981). With the use of radiolabeled ^3H -diazepam studies, it has been shown that high affinity binding sites for benzodiazepines exist in the brain which fulfill the criteria for pharmacologic receptors (Tallman, Paul, Skolnick and Gallagher, 1980). In fact, recent evidence suggests that high affinity benzodiazepine binding sites may actually regulate GABA receptor function, and in turn, that GABA receptors can regulate the affinity of binding sites for benzodiazepines (Mazzari et al., 1981). Although the exact nature of GABA-benzodiazepine receptor interaction has not been fully explained, the influence of benzodiazepines on GABAergic transmission, inhibitory activity, activation of GABA receptors, and antagonism of GABA-mediated inhibition has been reported in the literature (Tallman and Gallagher, 1979).

Work by Tallman and Gallagher (1979) demonstrated that GABA-agonists enhance and antagonists inhibit GABA binding to specific benzodiazepine binding sites. Their study suggested the existence of a GABA/chloride ionophore/benzodiazepine binding complex in the brain (Tallman and Gallagher, 1979). Other research support the notion that the

protein which recognizes benzodiazepines is part of the GABA receptor, and possibly a regulatory subunit of this receptor molecule (Mazzari et al., 1981). Although the benzodiazepine binding protein(s) may be a component of, or subpopulation of some or all GABA receptors, the degree of correlation of behavioral effects associated with GABA and benzodiazepines remains unclear.

GABAergic behavior: summary. Thus, the literature concerned with the in vivo manipulation of endogenous GABA levels and the behavioral modifications associated with enhanced GABAergic function is rapidly expanding. Facilitation of CNS GABAergic activity can elevate convulsion thresholds, induce dopaminergic behaviors such as rotational turning and exhibition of stereotypies, initiate opiate-related behavioral effects and analgesia, and cause anxiolytic responses. Interpretation of a wide body of GABA literature suggests that facilitation of CNS GABAergic activity causes many GABA-specific effects in vivo, including characteristic behavioral changes. In addition, it is evident that a complex interactive relationship exists between GABA and other neurotransmitters, which at present is not fully understood.

Putrescine (1,4-diaminobutane) and the Polyamines

Background. The existing literature concerned with the polyamine, putrescine (1,4-diaminobutane), is limited in scope, although interest in the compound and its possible neuropharmacological significance has increased in recent years. It is known that putrescine is the primary precursor of the polyamines, spermidine and spermine, in addition to being a minor precursor of the GABA. However, in Lehninger's (1978) biochemistry text, only a few short paragraphs are devoted to putrescine

and the polyamines, detailing little more than their structural aspects. There is a similar paucity of information in other texts and also in the neurobiology literature, in general. Much of the research dealing with putrescine or the other polyamines is related to various aspects cellular metabolism and replication. However, some studies have been published on the behavioral effects of the polyamines, and putrescine's role as a minor precursor of GABA.

Nature of polyamines. In 1885, a basic compound named neuridine was isolated from brain tissue and was subsequently determined to be identical to spermine. The polyamines, putrescine, spermidine, and spermine are ubiquitous in all mammalian body tissues, and are found in highest concentrations of nervous system tissues (Kremzner, 1970; Michaelson, Coffman and Vedral, 1968; Shaw and Pateman, 1973; Shimizu, Kakimoto and Sano, 1964). Putrescine and the polyamines are thought to have an important role in the regulation of tissue growth and metabolism, since polyamines can stimulate cellular proliferation of both normal and neoplastic tissues. Putrescine has been shown to have growth promoting effects in bacterial and mammalian cell cultures. Alterations in the concentrations of polyamines are associated with infection, tumor growth, and nerve injury (Giorgi, Field and Joyce, 1972; Kremzner, Barrett and Terrano, 1970; Seiler and Schroder, 1970).

Other proposed roles of the polyamines include physiological regulation of cellular tRNA (transfer-ribonucleic acid) methylation, amino-acylation of tRNA, RNA synthesis, and cyclic AMP synthesis in the nucleus (Atmar, Westland, Garcia and Kuehn, 1976; Doctor, Fournier and Thornsvar, 1970; Gumpert and Weiss, 1969; Leboy, 1970). In fact,

the polyamines have been shown to modulate at least nine different reactions in the metabolic sequence: DNA RNA protein, where the synthesis of polyamines is one of the initially altered metabolic events after stimulation of cellular metabolism (Kremzner, Hiller and Simon, 1975). Increase in polyamine synthesis is mediated by the induction of ornithine decarboxylase (ODC; EC 4.1.1.17) activity which initiates putrescine conversion from ornithine. The literature suggests that ODC activity and polyamine metabolism may both have a regulatory interaction with GABA metabolism in mammalian CNS tissues (McCann, Hornsperger and Seiler, 1979; McCann, Tardif, Hornsperger and Bohlen, 1979; Seiler, Bink and Grove, 1979).

Although literature in neuropharmacology dealing with the significance of putrescine in the CNS is sparse, recent evidence does indicate the importance of polyamines in neurotransmitter function. Concentrations of polyamines that are higher than concentrations of catecholamines and indoleamines (known neurotransmitter families) have been found in certain regions of the brain (Kremzner, 1970). The polyamines may activate and inhibit acetylcholinesterase activity, mediate single neuron transmission, and have additional ontogenetic roles in the vertebrate CNS (Anand, Gore and Kerket, 1976; Seiler, Bink and Grove, 1980; Sobue and Nakajima, 1978; Wedgwood and Wolstencroft, 1977).

Polyamine neurochemistry. The main pathway for polyamine synthesis is regarded as being the same in peripheral and central nervous system tissues, although enzymatic activity and utilization may vary on a regional basis. Putrescine is synthesized from ornithine by

ornithine decarboxylase and converted to spermidine by a specific synthase which utilizes decarboxylated S-adenosyl methionine as a propylamine donor. A specific spermine synthase converts spermidine to spermine using the same donor species (Raina and Janne, 1975). Figure 5 depicts this biosynthetic pathway of the polyamines. Factors which increase the activity of ornithine decarboxylase include: stimulation by growth hormone, prolactin, human placental lactogen, nerve growth factor, and insulin (Shaw, 1979). In contrast, ODC activity is inhibited by administration of opiates in newborn rat pups, ethanol administration and withdrawal, and removal of rat pups from their mother for periods as short as one hour (deprivation of normal "mothering" behavior (Butler, Suskind and Schainberg, 1978). It appears that transformation of putrescine to GABA is the primary degradative pathway of this polyamine (to be denoted as the putrescine GABA shunt) (Seiler, Bink and Grove, 1979).

The evidence which initiated research into putrescine catabolism was obtained when detection of incorporation of putrescine carbon into molecules of GABA was made in rat liver and brain (Seiler et al., 1971), mouse brain (Seiler and Knodgen, 1971) and liver (Seiler and Eichertopf, unpublished) and in fish brain (Salmo irideus gibb, Seiler, Al-Therib and Kataok, 1973). These studies utilized radiolabeled ^{14}C -putrescine and demonstrated that putrescine transformation into GABA occurs via a direct pathway that does not have glutamic acid as an intermediate or use GAD as a catalyzing enzyme (Seiler and Al-Therib, 1974; Seiler, Al-Therib and Kataok, 1973; Seiler and Knodgen, 1971; Seiler, Wiechman, Fischer and Werner, 1971).

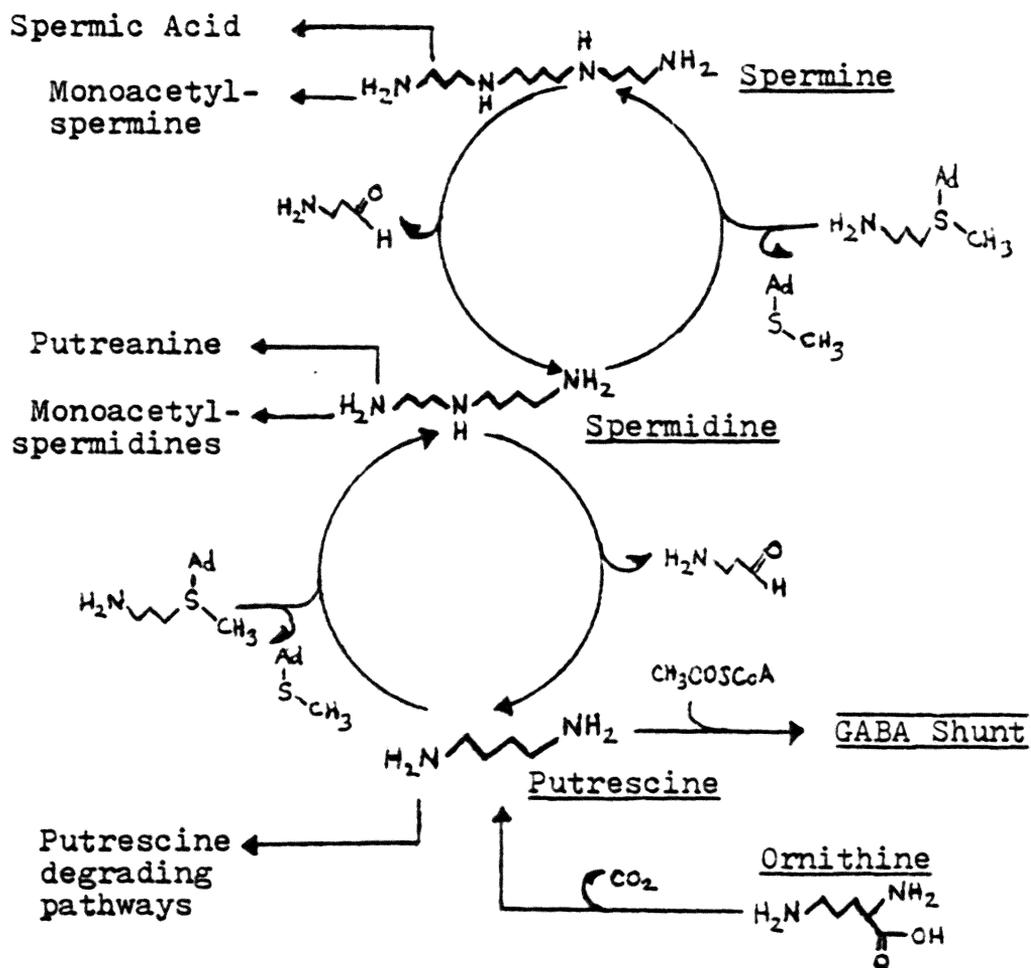


Figure 5. Biosynthetic pathway of the polyamines putrescine, spermidine and spermine (adapted from Antrup and Seiler, 1980).

In 1974, Seiler and Al-Therib described the putrescine catabolic pathway in mammalian brain using mice and rats. They hypothesized that GABA was a general metabolic intermediate of the catabolic pathway which channels putrescine carbon (and carbon atoms of the putrescine moiety of spermidine and spermine) into the Krebs (tricarboxylic acid) cycle. In their elaborate study, Seiler and Al-Therib were able to demonstrate that putrescine catabolism could lead to GABA formation via two different pathways. The initial oxidative deamination of putrescine has generally attributed to diamine oxidase (EC 1.4.3.6), although diamine oxidase activity in the brain is relatively low (Burkard et al., 1963). Thus, Seiler and Al-Therib reasoned that diamine oxidase was probably not exclusively responsible for putrescine degradation in the mammalian CNS. In fact, Kremzner (1973) had found that monoamine oxidase (MAO) inhibitors attenuated low activity putrescine oxidation in sheep brain homogenates, whereas a diamine oxidase inhibitor did not. Aided by the detection of monoacetyl-putrescine in the brain and the discovery of active putrescine acetylation by brain tissue preparations, Seiler and Al-Therib (1974) suggested and formally characterized a putrescine catabolic pathway in which the initial acetylation of putrescine is followed by oxidation of monoacetylputrescine by MAO. Figure 6 depicts the catabolic pathway of putrescine using these initial reaction mechanisms. The pathway can be summarized as follows: (1) acetylation of putrescine using acetyl-CoA-1,4-diaminobutane N-acetyltransferase; (2) oxidative deamination of monoacetylputrescine to N-acetyl-gamma-amino butyric acid using MAO; (3) transformation of N-acetyl-gamma-amino butyric acid to gamma-amino butyric acid (GABA)

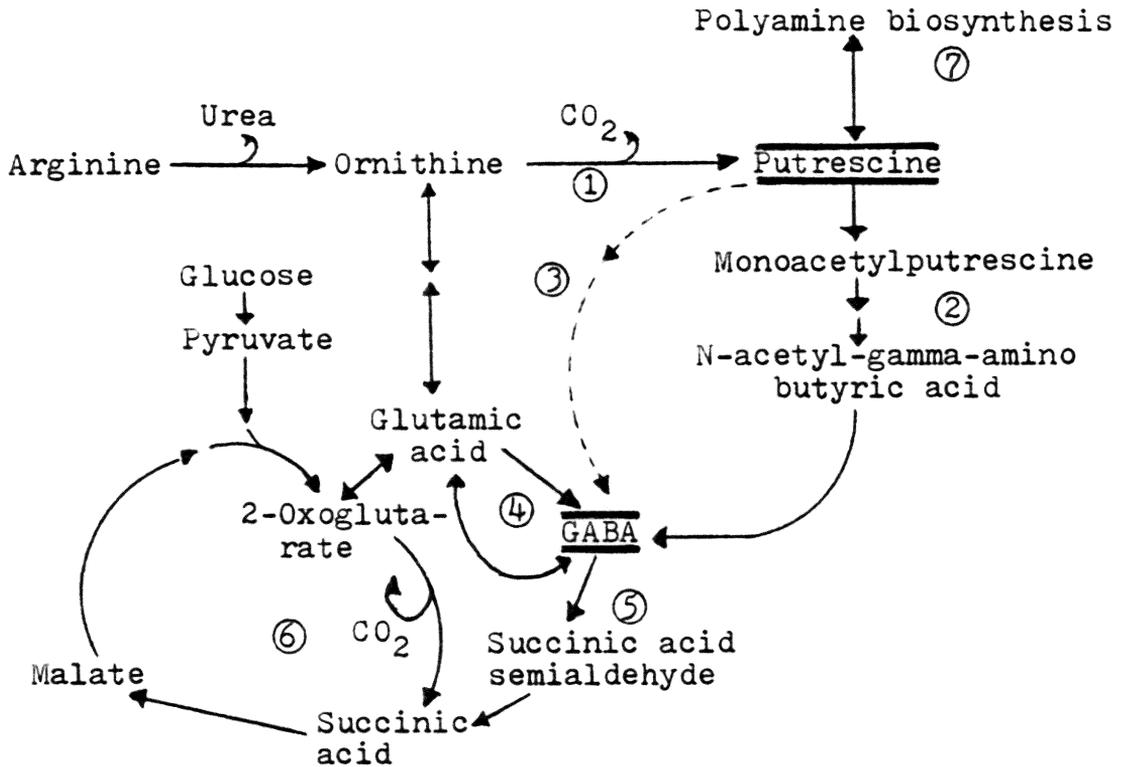


Figure 6. Schematic representation of putrescine catabolic pathway and gamma-aminobutyric acid (GABA) metabolic cycle; (1) putrescine synthesis from ornithine using ornithine decarboxylase; (2) the primary putrescine-GABA pathway using monoamine oxidase (Put-GABA shunt); (3) the secondary putrescine-GABA pathway using diamine oxidase (direct conversion); (4) the major synthetic pathway for GABA, with glutamic acid precursor and using glutamic acid decarboxylase (Glu-GABA shunt); (5) catabolic pathway for GABA using GABA-2-oxoglutarate aminotransferase; (6) the citrate cycle which results in CO₂ expiration; and (7) the polyamine metabolic cycle, where spermidine and spermine are synthesized from their putrescine precursor (a reversible system which can replenish putrescine pools) (adapted from Seiler and Al-Therib, 1974).

using GABA deacetylating enzyme; and (4) degradation of GABA to CO_2 via the Krebs cycle which is initiated by gamma-amino butyric acid-2-oxoglutarate aminotransferase (GABA-T) (Seiler and Al-Therib, 1974). This MAO-dependent putrescine catabolic pathway, the putrescine-GABA shunt has been demonstrated using in vivo and in vitro experimental procedures with both mice and rats (Seiler and Al-Therib, 1974). In addition to this primary degradative pathway of putrescine, Figure 6 also depicts the proposed secondary metabolic pathway for putrescine catabolism to using GABA diamine-oxidases and the primary synthetic pathway for GABA via glutamate.

In 1975, Seiler and Eichentopf substantiated the data of Seiler and Al-Therib (1974) by demonstrating that the catabolism of putrescine to CO_2 in vivo (mice) does occur along two different pathways in both of which have GABA as a common intermediate. These authors extended the earlier report by showing that three possible degradative pathways of putrescine exist, two of which utilize diamine oxidase, and the other, monoamine oxidase (Figure 7). This study demonstrated that oxidative deamination of putrescine by diamine oxidase in extra-mitochondrial fractions of CNS tissue resulted in derivatives of 4-aminobutyraldehyde. Seiler and Eichentopf showed that another diamine oxidase-dependent pathway exists in mitochondria. In this second the oxidative deamination of pathway, putrescine is coupled with an aldehyde dehydrogenase to form GABA directly. The third catabolic pathway of putrescine outlined by these authors is the putrescine-GABA shunt which involves MAO and is mitochondrial-dependent. Thus, several pathways are involved in the complex and dynamic regulatory interrelation-

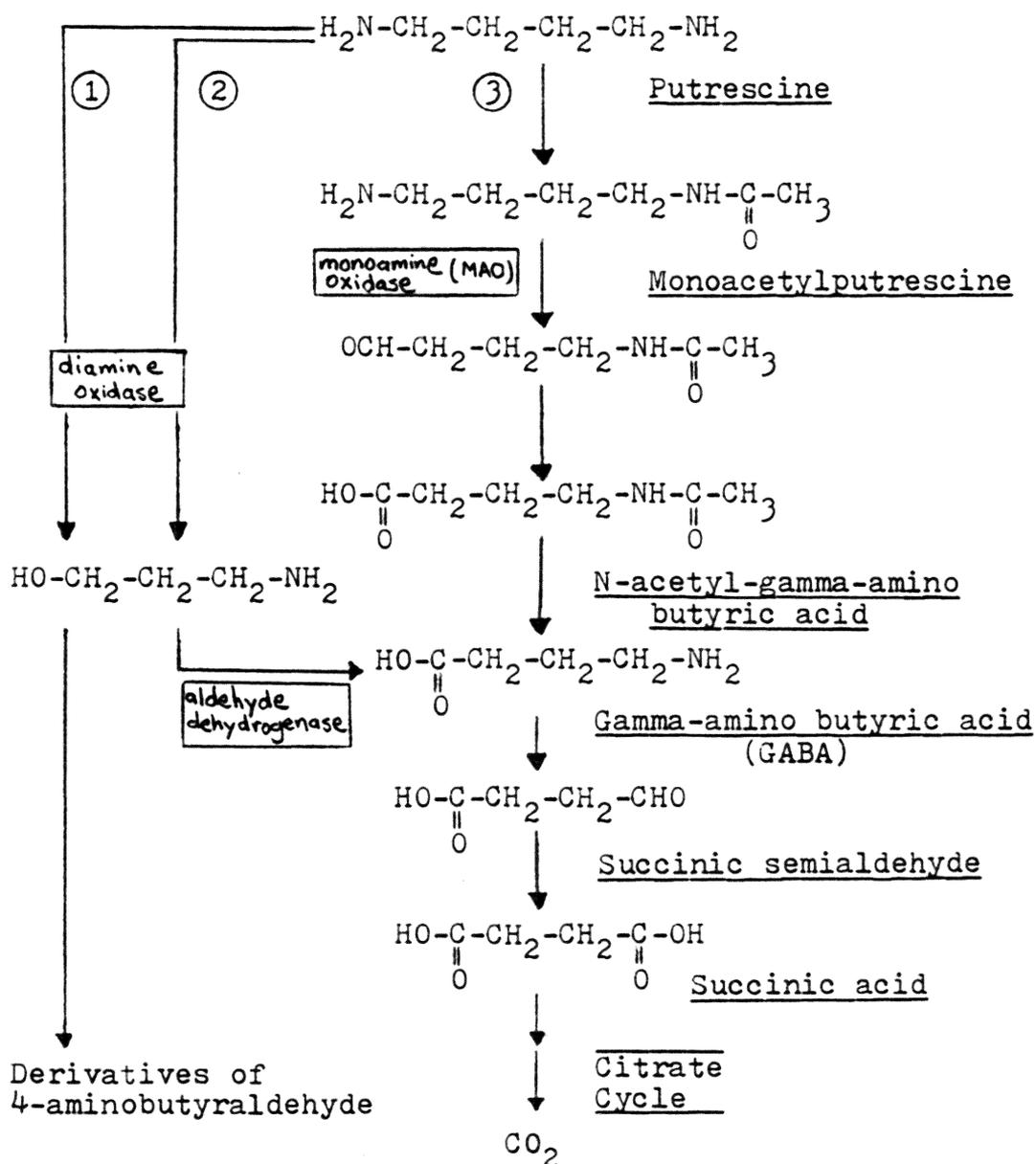


Figure 7. Scheme of the three catabolic pathways of putrescine; (1) extramitochondrial pathway using diamine oxidase yielding derivatives of 4-aminobutyraldehyde, (2) mitochondrial pathway using diamine oxidase yielding GABA, and (3) mitochondrial pathway using monoamine oxidase which is the primary synthesis mechanism for GABA formation from putrescine (Put-GABA shunt)(adapted from Seiler and Eichtopf, 1975).

ship between the polyamines and GABA in mammalian CNS tissues, pathways by which putrescine can contribute to the maintenance of brain GABA pools.

In the mid-1970's, the results of several studies concerned with putrescine activity and its relationship to the levels of brain GABA in developing organisms offered further evidence of the importance of polyamines to CNS function and neurotransmission. Seiler, Lamberty and Al-Therib (1975) reported that the enzyme acetyl-CoA: 1,4-diaminobutane N-acetyltransferase (putrescine acetylase; EC 2.3.1.5) catalyzes the initial step of mitochondrial-dependent putrescine catabolism in brain, and most likely, peripheral tissues as well. They also found that putrescine acetylase has higher specific activity in brain homogenates of immature rat brains compared to homogenates of mature ones. This observation was substantiated by the finding that microsomes and purified nuclei from brains of two-day-old rat pups exhibited considerably higher putrescine acetylase activities than was found in corresponding subcellular organelles from adult brains. These authors described a steady decline of putrescine acetylase activity from birth until approximate adult levels are reached at day 30 in rats. Thus, Seiler, Lamberty and Al-Therib (1975) concluded that putrescine acetylase activity and putrescine catabolism processes have ontogenic significance in brains of immature rats, where concentrations of putrescine and its acetylating enzyme decline after birth at a rate that parallels differentiation of nerve cell components such as nuclei and glial cells.

De Mello, Bachrach and Nirenberg (1976) isolated GABA in chick

embryonic retinal tissue. These investigators were able to demonstrate that significant amounts of GABA exist in developing chick retina prior to synaptogenesis embryonic (day 13), when GAD activity is first measurable. They noted that the rate-limiting enzyme for putrescine synthesis from ornithine, ornithine decarboxylase was high in concentration during the first 12 days of development and then decreased inversely to the rise in concentration of glutamic acid decarboxylase activity, the enzyme required for primary GABA synthesis via the Glu-GABA shunt. Retinal GABA synthesis prior to embryonic day 13 is thus attributable to putrescine catabolism. The authors summarize the discussion of their data by commenting that GABA synthesis via the Put-GABA shunt may play a significant developmental role in regulation of gene expression for GAD, while also providing a pathway for GABA synthesis in cells that lack GAD activity.

In 1977, Sobue and Nakajima reported on the metabolism of putrescine in mouse neuroblastoma and glial cell cultures. By comparing the rates of putrescine conversion to GABA and other polyamine metabolites, such as spermidine, Sobue and Nakajima were able to conclude that spermidine formation is closely related to proliferation of neural cells in general, while GABA formation via the putrescine-GABA shunt is related to differentiation or maturation of neuroblastoma cells. These data support the hypothesis that putrescine catabolism has an important role in CNS cellular development.

Sobue and Nakajima (1978) utilized in vivo procedures with chicks to study the ontogenetic metabolism of putrescine to GABA and spermidine. The synthesis of GABA via the putrescine-GABA shunt was maximal

between days 6-8 of incubation. Using radioactive glutamic acid, these authors showed that formation of GABA via the Glu-GABA shunt was not detectable until day 10 of development, after which it increased rapidly. These findings indicate that active formation of GABA from putrescine in developing chick brain occurs and may represent neuroblast differentiation. Since GAD-dependent GABA synthesis was not observed until day 10 of incubation, the authors proposed that the Glu-GABA shunt is intimately associated with neural synaptogenesis (approximately day 12).

In 1980, Seiler, Bink and Grove increased brain levels of GABA in developing rat pups by the in vivo administration of the GABA-T inhibitor, gamma-vinyl GABA. The resulting increases in GABA concentrations paralleled the developmental activity of the GABA synthesizing enzyme, GAD. In 25 day old rats, treatment with gamma-vinyl GABA resulted in a marked increase in ornithine decarboxylase activity, putrescine concentration, and GABA concentrations. Seiler and his associates also found that these effects of gamma-vinyl GABA, were not altered by treatment with either a GABA agonist (muscimol) or a GABA antagonist (bicuculline). As the authors suggest, these data may be only preliminary in nature in view of the complex relationships between polyamine and GABA metabolism that are emerging in the literature.

Metabolism of Exogenous Putrescine. Complementing the developmental studies dealing with polyamine and GABA interrelationships are several recent papers concerned with in vivo putrescine metabolism and polyamine turnover. These reports, using radioactive putrescine provide

and Sano, 1977).

In 1978, Tsuji and Nakajima published an important in vivo study demonstrating ^{14}C -putrescine conversion to radiolabeled GABA in rat brain and organs following intraperitoneal putrescine administration. ^{14}C -GABA was detected in all organs, as well as in brain tissues. The characteristics of the GABA-synthesizing enzyme indicated that it was probably diamine oxidase, histaminase. When diamine oxidase enzymatic co-factors were added to reaction mixtures, the rate of formation of GABA from putrescine increased. Although other investigators have proposed that the putrescine-GABA shunt is the primary pathway for GABA formation from putrescine, Tsuji and Nakajima suggest that the direct conversion of putrescine utilizing diamine oxidase is the major putrescine-GABA metabolic route. This conclusion was reached since the highest diamine oxidase activity was in the small intestine where the highest concentrations of radiolabeled GABA was also measured. Ontogenetic evidence supports this interpretation, since GABA formation via putrescine has been correlated with high levels of diamine oxidase activity (Sobue and Nakajima, 1978; Tsuji and Nakajima, 1978).

Antrup and Seiler (1980) evaluated the biological half-lives of spermidine and spermine in mouse organs, including brain, in a longitudinal study (62 days). Endogenous polyamine pools were radiolabeled with isotopes of the polyamine precursors (^{14}C -putrescine, ^{14}C -methionine and ^3H -methionine) using repeated systemic injections to ensure labeling of fast and slow pools over 25 days. Radioactivity rapidly accumulated in liver and brain, in addition to small intestine, spleen, kidney, skeletal muscle, and heart. Labeled monoacetylputrescine (a

primary intermediate of the proposed putrescine-GABA shunt utilizing monoamine oxidase) was also found in certain tissue preparations. Antrup and Seiler (1980) commented that actual turnover rates of polyamines under physiological conditions could not be accurately determined because of the active interconversion between spermine and spermidine, and between spermidine and putrescine in vivo. However, the biological half-lives of brain spermine and spermidine synthesized from putrescine were estimated at 42 days. Very low levels of radioactive putrescine and GABA were also present in the brain after the cessation of radiolabeled putrescine i.p. injection. Thus, utilizing extremely small tracer dosages of putrescine (.0004 mg/kg i.p.), the enzymatic mechanisms for polyamine catabolism to their putrescine precursor was demonstrated. These authors concluded that putrescine may reenter the polyamine cycle (synthesis and degradation as spermidine and spermine) at least four or five times before it is finally eliminated and catabolized to GABA and other metabolites.

Utilizing intraperitoneal injections of ^{14}C -putrescine, Caron, Cote and Kremzner (1980) showed that differences exist in the uptake of radioactivity between neural and non-neural tissues. They found significant conversion of putrescine to GABA in rat pancreas and adrenal gland, accounting for 10% of the total endogenous GABA one hour posttreatment. Smaller, but detectable levels of ^{14}C -GABA were also found in brain, liver, kidney, heart and skeletal muscle following i.p. administration of putrescine tracer dosages (.02 mg/kg). The administration of a diamine oxidase inhibitor, aminoguanidine, resulted in a 95% inhibition of GABA formation via putrescine. In a

separate i.v.t. administration procedure, data was obtained which showed less than 1% putrescine conversion to GABA. This study lends limited support to the view that systemic administration of putrescine can be converted to measurable amounts of GABA in neural and peripheral tissues.

The precise functional role of the conversion of putrescine to GABA is still unclear. From a biochemical standpoint, it appears that putrescine conversion to GABA in intact animal systems does exist and may have a role in neurophysiological mechanisms. Further evidence for such neurobiological significance exists in limited putrescine-related research from the behavioral literature.

Putrescine and polyamine-related behavior. The behavioral literature related to putrescine and the polyamines is scant, although a few recent papers have presented data which suggest possible roles for these substances in neurotransmission. In 1972, Shaw investigated the consequences of i.p. and i.v. administrations of the polyamines, spermidine and spermine, in both mice and rats (Shaw, 1972). The behavioral changes resulting from putrescine administration depended on the route of injection. Within five minutes of i.p. injection, rats which received spermine at a dose of 25 mg/kg became slightly ataxic. Sedation was also evident in these animals together with ptosis. The animals were "disinclined" to move, although they became more active if they were disturbed. At ten minutes post injection, more pronounced ataxia appeared together with piloerection, sedation, hypothermia, respiratory embarrassment, ptosis, and slight muscle weakness. These effects were attenuated at 90 minutes post-treatment; however, slight

sedation was still evident until four hours post-injection. Rats injected with 15 mg/kg spermine exhibited similar, but less marked behavioral effects. Subjects given 5 mg/kg spermine were not distinguishable from saline controls.

Shaw also found that i.p. administration of spermidine initiated similar behavioral consequences to that of spermine, although larger doses were needed to produce equal effects. A minimum effective dose of 25 mg/kg was determined, and a dose of 50 mg/kg was needed to elicit changes of similar intensity and duration when half as much spermine was given. A 75 mg/kg dose of spermidine caused pronounced sedation and hypothermia, while at 100 mg/kg, animals were prostrate and exhibited hypothermia of 6°C or more. In studies with mice, Shaw obtained behavioral changes that paralleled those observed in rats. Evaluation of spontaneous locomotor activity in mice showed that polyamine administration caused subjects to become inactive and remain so for three hours. In addition, polyamines prolonged barbituate-induced (amylobarbitone) sleep at 20°C ambient temperature; however, this effect disappeared when ambient temperature was maintained at 35 ± 1°C.

Intravenous injection of spermidine and spermine caused several behavioral effects which contrasted with those observed following i.p. injection. A few seconds after i.v. injection of either polyamine (33 mg/kg), mice exhibited mild clonic convulsions. Most animals lost their righting reflex. After a few infrequent deep respiratory movements, a period of apnea accompanied by exophthalmos (protrusion of eyeballs) was observed, often followed by death. Cessation of respiration was the likely cause of death since cardiac function remained

after respiratory arrest. If the episodes of convulsions and apnea were survived, post-convulsive depression and tachypnea were exhibited. Smaller doses of spermine decreased the incidence of convulsions, although the lower doses did initiate jumping and myoclonic jerks in mice. The effective convulsant (CD_{50}) and lethal (LD_{50}) spermine doses in mice were: 24 mg/kg, and 25 mg/kg; and for spermidine, 55 mg/kg, and 78 mg/kg, respectively. Thus, Shaw demonstrated that both spermine and spermidine can produce either depression or excitation in rats and mice depending on the route of administration. Depressive effects were accompanied by hypothermia and sedation, whereas sedative effects may have been largely due to a pronounced hypothermic response. Although Shaw's data does not specifically include putrescine, the intimate interregulatory cycle involving putrescine, GABA, and the polyamines emphasizes the significance of this initial behavioral research.

In 1975, Anderson, Crossland and Shaw reported on the effects of intraventricular injection of spermine, spermidine and putrescine in mice and rabbits. Spermine injection (50 mg) caused marked sedation, hypothermia ($2^{\circ}C$ at two hours posttreatment) and ptosis which lasted for several hours. After apparent recovery from these effects, signs of hyperexcitability appeared in mice, including the development of tremors. Clonic convulsions then became evident for the next few hours. During the intervals between seizures, a lethal tonic spasm could be initiated by loud noise or by picking the animal up by the tail. Convulsions could also be produced with i.v.t. injection of 10 mg of spermine with an 18 hour latency, although 100 mg injections

produced convulsions within minutes (Anderson et al., 1975).

In the same study, similar behavioral effects were produced by spermidine, although spermidine was less potent than spermine. Following the injection of 50 mg spermidine (i.v.t.), ataxia appeared that was apparently related to increased limb muscle tone. The ataxia resulted in abnormally high carriage of the body. By the third or fourth day posttreatment, a flaccid paralysis developed which progressed to quadriplegia followed by a moribund state and death in one or two more days. In contrast, spermine rarely produced any paralysis. Spermidine treatment (50 mg) also resulted in immediate and prolonged fall in food and water intake, accompanied by loss of weight. This factor contributed to the deterioration of the incapacitated subjects. Spermidine-induced (50 and 75 mg) hypothermia was most evident 30-60 minutes post-treatment.

Anderson and his colleagues found that intraventricular treatment with putrescine also elicited behavioral effects in some animals, similar to those produced by spermidine, which culminated in paralysis. Putrescine was given to mice by i.v.t. injection at a dose of 140 mg. Between one and four days after injection, subjects began to exhibit behaviors similar to those observed following spermidine injection of 50 mg, including sedation, ptosis, hypothermia, ataxia, and paralysis. Other putrescine-treated subjects exhibited clonic convulsions and died without becoming paralyzed. It was found that spermidine and spermine brain content was significantly increased by approximately 30%, 24 hours following i.v.t. injection of 140 mg of putrescine. Rabbits given i.v.t. injections of spermine (250 mg) and spermidine (250 mg) had behavioral

consequences that paralleled those observed in mice. Spermine often produced paralysis in rabbits while spermidine often produced severe hind-limb muscle weakness without paralysis. A spermidine dosage of 1 mg produced convulsions in rabbits (Anderson et al., 1975).

In discussing their results, Anderson and his associates commented on the possible physiological role of the polyamines in thermoregulation. Since the polyamines were found to be dose-for-dose more potent than serotonin in eliciting hypothermia, and the polyamines are found in high concentrations in the hypothalamus. The central excitatory effects of polyamines appeared dramatic in view of the induction of convulsions and hyperexcitability which preceded death or paralysis. Also, the suppression of food and water intake suggested a direct polyamine action on hypothalamic centers. These authors did observe polyamine-induced necrotic lesions in the brainstem and spinal cord following i.v.t. polyamine injection. The lesions were most likely due to a toxic metabolite, since paralysis did not develop immediately. Thus, putrescine injected intraventricularly caused behavioral consequences similar to those seen following spermine and spermidine injection (Anderson, Crossland and Shaw, 1975).

Shaw (1977) investigated the likelihood that the central actions of polyamines in vivo are indirectly mediated via some system or systems, such as norepinephrine or serotonin. After administering spermine (30 mg/kg) or spermidine (100 mg/kg) by i.p. injection in rats, Shaw assayed brains 45 minutes posttreatment to discern any elevations in neurotransmitter concentrations. No statistically significant changes in brain concentration were found for acetylcholine,

norepinephrine, dopamine, serotonin, or GABA, using whole brain fractions. In view of these findings, Shaw proposed that any central effects caused by polyamine administration are mediated directly, rather than via some intermediate transmitter system or substance. However, these results are not conclusive, in that specific transmitter turnover rates can be modified without affecting whole brain concentrations. Also, neurotransmitter function in a certain vicinity of the brain could occur with an increase in concentration, without changing whole brain levels of the substance. Putrescine was not included in this research procedure.

Shaw, in a recent review article, emphasized the fact that a growing body of literature supports the existence of a neuropharmacological role for the polyamines (Shaw, 1979). He suggested that the polyamines may modulate or mediate central synaptic transmission (Shaw, 1979). Shaw also noted the possibility that neural responses to polyamines may be specific to these substances, and not mediated indirectly through other neurohumors or transmitter substances.

There are several articles by Japanese investigators correlating behavioral changes with brain polyamine concentrations. It has been reported that induction of aggressiveness in mice (isolation housing) is accompanied by a corresponding rise in brain spermidine (Tadano, Onoki & Kisara, 1974). This response did not occur in non-aggressive mice, and the rise in spermidine was reversed when isolated mice were returned to group housing conditions (Tadano, 1974). There are also reports that electroshock-induced aggression is correlated to an increase in brain spermine for mice (Tadano et al., 1974). Finally, destruction of olfactory bulbs (which is an animal model of depression) has been reported

to alter regional concentrations of polyamines in rat brain, a preliminary finding which is awaiting independent confirmation (Kleihues, Hossman, Kobayashi & Zimmerman, 1975).

In 1980, an important putrescine-related behavioral paper appeared, in which the effects of intraventricular administration of putrescine were studied in chicks in order to determine whether or not the acute effects were directly or indirectly mediated in vivo. Nistico, Ientile, Rotiroti and Di Giorgio (1980) found that i.v.t. injection of putrescine (25 to 150 μ g) produced dose-dependent increases in behavioral reactivity to stimulation, locomotor activity, vocalization, side to side head jerks, and tachypnoea. This symptomology appeared after a 5-10 minute period of sedation, and lasted from 15 minutes to 2 hours posttreatment, depending on dosage. The highest putrescine doses (100 to 150 μ g) elicited intense behavioral stimulation with marked postural changes, semi-squatting and wing abduction, continuous vocalization, head-neck rotation, ataxia, tachypnoea (up to 180 per minute), periodic circling or escape responses, and feather erection, all of which lasted for two hours. Frequent myoclonic movements of limbs and clonic convulsions were also observed during the first two hours post-injection. The electrocortical activity pattern of chicks treated with 150 μ g putrescine i.v.t. was characterized by continuous high voltage spiking at 10 and 110 minutes post-injection and was concomitant with the previously described symptomology. Hypothermia of up to 2^oC was observed for 1-3 hours posttreatment depending on dose. The period of hypothermia and behavioral excitation was followed by 90 minutes of behavioral sedation. Electrocortical activity

patterns showed slow wave, high amplitude potentials during the period of behavioral sedation (Nistico et al., 1980).

In this study, putrescine administration in chicks (100 μ g) caused a significant decrease in GABA (approximately 50%) in the diencephalon, but not in the cerebral hemispheres. At 30 minutes posttreatment, when symptomology was maximal, putrescine did not affect spermine content of these areas, but increased endogenous putrescine levels by 433%. The authors suggested that depletion of GABA caused by i.v.t. putrescine treatment might have been related to inhibition of the primary GABA-synthesizing enzyme, GAD, since the GABA-degradative enzyme, GABA-T, was affected (Nistico et al., 1980). The data from this study contrast with the results from mammalian studies which have suggested that the central effects of putrescine may be due to the increased formation of spermine and spermidine. In the chick study, the latency preceding overt behavioral symptomology after i.v.t. putrescine injection (approximately 15 minutes) may not correlate with the time required for spermidine or spermine synthesis from putrescine, nor was brain content of these polyamines significantly altered 30 minutes post-injection. Determination of specific polyamine metabolic rates have been difficult, with varying estimates of turnover rates (on the order of hours), and half-lives (between 3 to 42 days) reported in the literature (Anderson, Crossland & Shaw, 1975; Antrup & Seiler, 1980; Nistico et al., 1980).

Nistico and his colleagues compared their results with chicks to those reported in mammalian systems. Since i.v.t. putrescine treatment in their study caused depletion of brain GABA and induced convulsions

together with high voltage electrocortical activity resembling epileptogenic discharges, these authors suggest that polyamines may inhibit some GABAergic neurons. Also, if chicks possess similar putrescine-GABA synthesizing mechanisms to those demonstrated in mammalian systems, the amount of putrescine-derived GABA may not be sufficient to counteract the GABA pool depletion caused by GAD inhibition. They note that many of the behavioral consequences observed in chicks parallel those seen in mammalian studies following injection of putrescine into the central nervous system. They conclude that their data support a role for polyamines as neuro-modulators or neurotransmitters in the CNS (Nistico et al., 1980).

In summary, recent polyamine studies support the hypothesis that these substances may have an important role in central nervous system neurotransmission and behavior. Intraventricular polyamine administration results in initial behavioral depression followed by behavioral excitation, which in turn is followed by behavioral depression. The common effects reported, across species include sedation, hypothermia, respiratory difficulties, and clonic convulsions. Intraventricular putrescine injection in mice produced effects only after a delay of one or more days. Initial symptomatology in these animals included sedation, ptosis, and ataxia; if lethal convulsions did not occur, the effects of putrescine terminated in flaccid paralysis.

Although studies have compared the i.v.t. and systemic injection of spermine and spermidine, there is no report in the literature which has evaluated the systemic administration of putrescine. Intravenous administration of spermine and spermidine in mice and rats caused

immediate excitatory effects including clonic convulsions; this period of excitation was followed by respiratory and behavioral depression. In contrast to the effects of i.v. administration, i.p. injection of spermine and spermidine were not associated with an excitatory phase. The effects reported following i.p. polyamine injection included behavioral sedation, ptosis, ataxia, hypothermia, and respiratory depression. Thus, the elicitation of excitatory effects by the polyamines would appear to depend on the rate at which the concentration of the polyamine increases within specific structures of the central nervous system.

Thesis Purpose, Rationale, and Hypotheses

Introduction. The existing biochemical, pharmacological, and behavioral literature documents the existence of interrelationships between the inhibitory neurotransmitter, GABA, and putrescine. This diverse body of literature suggests that putrescine metabolism can influence the function of GABAergic neurons in the central nervous system, resulting in overt changes in behavior. This suggestion may infer the possibility that putrescine, administered systemically, could act as a GABA mimetic or agonist, eliciting the appearance of GABAergic behaviors in rats. In the spring of 1980, results of a series of pilot studies demonstrated that a complex of behavioral effects was induced by oral or intraperitoneal administration of putrescine in rats. These behavioral modifications appeared to be consistent with behavioral effects associated with enhanced GABA levels, facilitated GABAergic function, and in vivo polyamine administration. A summary of the initial pilot study is presented in the following

section.

Pilot study data. The aim of the study was to assess the effect of systemically administered putrescine in intact rats. Two separate experiments were completed: 1) oral injection of putrescine at doses of 0, 10, 100, 500 and 1000 mg/kg-body weight (n=4), and 2) intraperitoneal (i.p.) injection of putrescine at doses of 0, 10, 100, 500, and 1000 mg/kg (n=2). Since no literature exists on the oral toxicity of putrescine, a 100-fold dose gradient was utilized in the pilot studies.

Adult male Long Evans rats (Psychology Department colony, VPI&SU) weighing 325-425 g were used as subjects. Rats were housed two per cage and given free access to Wayne Lab Blox and tap water. During observation and testing, five animals were socially housed in large plastic tubs filled with sawdust. Social housing was utilized as a matter of convenience for single-observer evaluation, as well as allowing informal (non-quantitative) observation of social interaction. No animal was ever placed in a test tub with its cagemate.

Immediately prior to treatment, subjects were weighed and had their rectal temperatures measured. Subjects were then given a baseline startle response test. Startle response was measured by exposing subjects to a metal clicker sound (in isolation) once every 15 seconds for eight trials, and scoring them for reactivity on an arbitrary scale (body flinch, head flinch, ears twitch, no response). Treatment was administered experimenter-blind. Following putrescine injection, subjects were observed by time sampling method for 90 minutes (oral condition), or 60 minutes (i.p. condition), three categories of behaviors were scored in the time sampling procedure: activity (sitting, walking, rearing,

sleep); stereotypy (grooming, sniffing, gnawing); and abnormal behavior (abnormal posture or movement, impairment of locomotion, and miscellaneous unusual behavior). Scores were based on a 10 second observation interval each minute.

Behavioral evaluation continued with a posttreatment startle response test following the time sampling procedure. Ambulation was scored by counting the number of squares entered in an open field arena over a five minute period. A posttreatment rectal temperature was taken after open field testing. Lastly, pain responsiveness to electric shock was determined. Subjects were placed in a grid box and exposed to an ascending pulse train (.05-1.0 mA) of 40 volts. Each shock lasted 500 msec, with inter-shock intervals of 10 sec. Threshold values (mA) were recorded for the following responses: body flinch, jump (both rear paws off grid), and audible squealing. Approximate time courses for post-treatment testing were 3.5 hours (oral condition) and 2 hours (i.p. condition). The data from the oral and i.p. pilot studies were not subjected to formal statistical analysis beyond calculation of treatment group means.

In the oral putrescine experiment, it was noted that putrescine dosages of 100 mg/kg or higher seemed sufficient to cause the appearance of behaviors which differed from those seen in control animals. Time sampling observation indicated that subjects given 500 to 1000 mg/kg putrescine orally exhibited lower activity (walking and rearing) levels compared to saline controls. These subjects receiving high oral dosages of putrescine also had higher incidence of stereotypic gnawing and abnormal behavior (abnormal postures, movements, and impairment of

locomotion). Unusual behaviors observed included a stereotypic chewing (mastication), and in subjects given 1000 mg/kg putrescine, the appearance of some incomplete grooming sequences and the exhibition of body shaking similar to "wet dog" shakes. Other general effects of putrescine treatment noted were ptosis, piloerection, and sedation-like tendencies. Subjects given 10 mg/kg putrescine orally appeared similar to saline controls.

In the i.p. experiment, time sampling evaluation often yielded similar, but not identical effects to those observed in the oral procedure. The LD_{100} (100% lethal dose) for putrescine given i.p. appeared to be less than or equal to 1000 mg/kg (n=2). These subjects initially exhibited impaired motor coordination, followed by loss of upright posture; the animals then lay on their sides. Labored breathing, body twitching, and convulsions preceded death, which occurred about one hour post-injection. Subjects given 500 mg/kg putrescine i.p. also evidenced some distress and motor difficulties, but survived. Time sampling behaviors seen in subjects receiving 100 or 500 mg/kg putrescine i.p. included increased walking and rearing, increased incidence of gnawing and abnormal behaviors. Other effects observed included mastication stereotypy, ptosis, piloerection, and sedation-like tendencies. In subjects given 500 mg/kg putrescine i.p., some incomplete grooming sequences and "wet dog" shaking were seen. Subjects given 10 mg/kg putrescine i.p. were similar in appearance to saline controls.

In other tests used to evaluate systemic putrescine administration, similarities between the oral and i.p. injection conditions were observed. Startle response testing showed that both routes of administration reduced

reactivity to auditory stimuli. Open field evaluation suggested that reduced ambulation was also associated with putrescine treatment. Pain thresholds measured by electric shock grid testing were increased for putrescine-treated subjects. Finally, hypothermia was seen in subjects treated orally with 1000 mg/kg putrescine, or 500 mg/kg i.p. Informal observation of putrescine-treated subjects gave the impression that the incidence of aggressive contacts were reduced and that treated subjects were less reactive when touched.

Thus, the pilot studies suggested that behavioral effects are associated with systemic putrescine treatment. Some of the observed behaviors resembled behaviors related to GABA and polyamine manipulations which have been reported in the literature; these included ptosis, piloerection, hypothermia, abnormal postures and movement, oral stereotypies, incomplete grooming sequences, reduced startle response, decreased open field ambulation, sedation, increased pain thresholds, and elicitation of body shaking behavior. A number of these behaviors are known to be induced by experimental manipulations of central nervous system function and have not been associated with any manipulations which affect only the peripheral nervous system. Included in this category are the oral stereotypies and excessive grooming (Gilman, Goodman, & Gilman, 1980; Iverson & Iverson, 1981). It is, however, impossible to state with certainty that a particular behavior is independent of all possible changes in the peripheral nervous system.

These considerations may lead one to suspect, though it cannot be unequivocally demonstrated, that systemically administered putrescine entered the circulatory system, passed through the blood-brain barrier,

and entered the CNS of treated subjects. Comparison of the two routes of administration showed that the onset of behavioral effects in the i.p. condition was faster than in the oral condition, while the duration of observed effects was shorter in the i.p. condition. The results of the pilot studies appear to support the hypothesis that the systemic administration of putrescine could alter the appearance and behavior of intact rats. These studies indicated that a comprehensive behavioral evaluation of systemically administered putrescine in rats was warranted.

Statement of purpose. This thesis has two objectives: 1) to investigate the behavioral effects of systemic administration of putrescine in rats; and 2) to determine the extent to which the behavioral effects associated with treatment are GABA-related.

Experimental rationale. The importance of GABA's role as the major inhibitory mediator of other central transmitter systems is well accepted. Evidence linking putrescine and the polyamines to GABA metabolism is rapidly accumulating. Recent data suggests that putrescine and the polyamines may either modify GABAergic function, or have a direct CNS modulating action of their own. Although the polyamine and GABA behavioral literatures share some common aspects, there has been no comparison of these literatures reported. Initial pilot studies done in this lab suggested that systemic putrescine treatment caused behavioral effects in rats, some of which seemed to be similar to GABA-related behaviors. This thesis attempts to evaluate quantitatively the effects of systemic putrescine administration, and also to compare these effects to those produced by GABA agents. Research

concerned with the behavioral consequences of polyamine manipulations and the relationship of these effects to GABAergic behaviors may provide a new method of modulating GABA-dependent behaviors.

Synopsis of experimental procedures. In order to adequately address the purpose of the thesis, three separate experiments were designed. Each experiment attempts to characterize the behavioral effects observed following systemic putrescine administration, or attempts to determine whether these effects are related to GABAergic function.

The first two experiments are designed to describe and compare the behavioral effects resulting from (p.o.) and intraperitoneal (i.p.) administration of putrescine. The third experiment evaluates the results of putrescine and muscimol administration in subjects treated with a known dopamine agonist, apomorphine (subcutaneous). This last procedure is designed to determine whether systemic putrescine can modify apomorphine behavior in a way similar to that which has been reported for muscimol.

The three experiments comprising this master's thesis are summarized as follows:

Experiment 1: oral (p.o.) administration of putrescine at three dosages (1000 mg/kg, 250 mg/kg, 50 mg/kg) in order to describe the in vivo behavioral effects observed in comparison to saline controls; also to determine whether such effects are dose-related (N=28).

Experiment 2: intraperitoneal (i.p.) administration of four putrescine dosages (250 mg/kg, 100 mg/kg, 50 mg/kg, 25

mg/kg) in order to characterize observed effects and contrast them with those obtained in Experiment 1 (p.o.) (N=30).

Experiment 3: evaluation of the relative effectiveness of putrescine (75 mg/kg i.p.), muscimol (.75 mg/kg i.p.) and saline in modifying apomorphine-induced (.25 mg/kg subcutaneous (s.c. injection) behaviors (N=42).

Table 1 summarizes the experimental matrices for each of the three thesis paradigms.

Behavioral evaluation: subjects. A brief description of the protocols used and definition of the criteria used in each testing procedure will be presented in this section. Descriptions of all experimental procedures are presented in each separate experimental chapter.

Animal subjects were drug naive, male adult Sprague-Dawley rats, each of which was used in only one test session of any experiment. Animals were destroyed following experimental use. All drug treatments were administered experimenter-blind. Routes of administration included oral injection (intubation), intraperitoneal injection, or subcutaneous injection. Following drug treatments, subjects were evaluated and tested by direct time sampling observation for varying time durations depending on paradigm design, route of treatment administration, and length of drug effectiveness.

Behavioral evaluation: time sampling procedures. The behavioral categories used in the time sampling procedures were arbitrarily designated, based on the results of initial pilot studies and definitions used by others in quantitating the

Table 1

Experimental Matrices for Experiments 1, 2, 3

Experiment 1				
<u>Saline</u> (.85%)	<u>Putrescine, p.o. (mg/kg)</u>			
	50	250	1000	
n=7	n=7	n=7	n=7	N=28
Experiment 2				
<u>Saline</u> (.85%)	<u>Putrescine, i.p. (mg/kg)</u>			
	25	50	100	250
n=6	n=6	n=6	n=6	n=6
				N=30
Experiment 3				
	<u>Saline</u> (.85%)	<u>Putrescine</u> 75 mg/kg	<u>Muscimol</u> .75 mg/kg	
<u>Saline</u> (.85%)	n=7	n=7	n=7	
<u>Apomorphine</u> .25 mg/kg	n=7	n=7	n=7	N=42

behavioral effects of GABA, polyamine, and dopamine administration.

In Experiments 1 and 2, subjects were time sampled for observation periods lasting between 45-90 minutes. In these procedures, each subject was observed for a 10 second interval every minute, and the overt behavior recorded on a list of arbitrary behavioral categories. Definitions of these categories are given in Table 2. These arbitrary categories were designed to evaluate three general behavioral responses: general activity (sitting, walking, rearing, sleep), stereotypy (sniffing, grooming, licking, gnawing, mastication), and abnormal behaviors (abnormal posture/movement, motor impairment, head down posture, wet dog shaking). Categories were obtained from initial pilot study observations and GABA and polyamine behavioral literature. Definitions of these categories were arbitrary; some behavioral categories were modeled on behavioral definitions proposed by Fray, Sahakian, Robbins, Koob and Iverson (1980) for quantifying behavioral effects of the dopamine agonists, d-amphetamine and apomorphine (Fray et al., 1980).

In Experiment 3, subjects were only evaluated by time sampling technique, and not subjected to further behavioral analysis. This abbreviation of the testing procedure was necessitated by the short duration (45 minutes) of behavioral effects elicited by apomorphine given at .25 mg/kg s.c., as determined by pilot testing. The time-sampling procedure was chosen in order

Table 2
 Definition of Behavioral Criteria for Time
 Sampling Evaluation in Experiments 1 and 2

Category	Definition
SIT (sitting)	Typical sitting posture, awake and alert with no movement
WALK (walking)	All four legs moving for more than 3 s
REAR (rearing)	Both front limbs off of cage floor
SLEEP (sleep)	Prone or curled up posture with eyes closed for at least 10 s
SNIFF (sniffing)	Head raised with sniffing for more than 3 s
GROOM (grooming)	Grooming for more than 3 s
LICK (licking)	Non-body licking for more than 3 s
GNAW (gnawing)	Gnawing sawdust for more than 3 s
MAST (mastication stereotypy)	Stereotypic mouth/jaw movement that is rapid and continuous for more than 3 s
ABN (abnormal posture and/or abnormal movement)	All postural deviations from normal four paw stance; also, any motor activity which is bizarre
IMPAIR (motor impairment)	Impairment of normal motor activity, including slow, exaggerated locomotion
HD (head down posture)	Immobile four paw stance with head lowered to cage floor for at least 5 s; accompanied by ptosis and lack of alert appearance
WDS (wet dog shake)	Sudden shaking of entire body with side to side rippling of musculature from head to tail (episodic occurrence with 1-2 s duration)

Note. Behavioral scoring is based on 10-s observation periods.

to allow comparison of the results obtained herein with those reported previously using muscimol (Scheel-Kruger, Cools & van Wel, 1977). Table 3 lists the behavioral categories used for time sampling in Experiment 3, and their definitions. These categories were designed to include behaviors known to be induced by dopamine. Two general types of behavior were evaluated: 1) general locomotor activity (stationary, walking, rearing), and 2) specific activity (sleep, awake/mostly immobile, mobile/non-stereotypic behavior, grooming, head down posture, mastication, discontinuous sniffing, continuous sniffing, licking/mouthing sawdust, gnawing). Designation of the behavioral categories was arbitrary, and represented a compilation of a number of dopaminergic locomotor and stereotypic behaviors defined in the literature (Scheel-Kruger, Cools & van Wel, 1977; Fray, Sahakian, Robbins, Koob & Iverson, 1980).

Behavioral evaluation: other experimental procedures. In addition to the time sampling evaluation, subjects in Experiments 1, and 2 were subjected to a variety of other behavioral tests. These other tests included evaluation of startle response magnitude and initial startle magnitude, open field ambulation, grid hang-time coordination, change in rectal temperature over time, food and water intake after deprivation, and determination of response thresholds to electric shock. Although the ordering of test procedures was essentially identical in each experiment, the overall time course of each procedure varied. The determination

Table 3
 Definition of Behavioral Criteria for Time
 Sampling Evaluation in Experiment 3

Category	Definition
General Locomotor Activity	
Stationary (ST)	A nonlocomotive posture that is maintained for more than 5 s; includes sitting, head down, and sleep postures
Walking (WA)	All four legs moving for more than 3 s
Rearing (RE)	Both front limbs off of cage floor
Specific Activity	
Sleep (SL)	Prone or curled up posture with eyes closed for 10 s
Awake, mostly immobile (AW)	Awake mostly nonlocomotive posture that is maintained for more than 5 s
Mobile, non-stereotypic behavior (NON)	Subject is alert and engages in movement and exploratory activity that is not stereotypic
Grooming (GR)	Grooming for more than 3 s
Head Down (HD)	Immobile four-paw stance with head lowered to cage floor for more than 5 s; accompanied by ptosis and lack of alert appearance
Mastication (MAST)	Stereotypic mouth and jaw movements that are rapid and continuous for more than 3 s
Discontinuous sniffing (DS)	Sniffing behavior that is not continuous but dominates activity; sniffing directed to cage floor and walls

Table 3 (continued)

Category	Definition
Specific Activity (continued)	
Continuous sniffing (CS)	Sniffing behavior that is continuous and dominates activity; sniffing directed to cage floor and walls
Licking or mouthing sawdust chips (L)	Discernable licking of nonbody surfaces in a rapid and stereotypic manner for more than 3 s' also, any incidence of mouthing sawdust chips in a stereotypic manner without actual chewing or gnawing
Gnawing (GN)	Gnawing sawdust for more than 3 s

Note. Behavioral scoring is based on 10-s observation periods.

of experimental time course was arbitrary and took into consideration the duration of treatment-induced behavioral effects. Final time course durations were estimated from preliminary pilot study evaluations of putrescine (p.o. and i.p.), muscimol (i.p.) (McKenzie & Hansen, 1980), and apomorphine (s.c.) (Scheel-Kruger, Cools & van Wel, 1977) dosages given alone or in combination. Approximate time course durations for each experiment were as follows: Experiment 1 - 5 hours; Experiments 2 - 3.5 hours; and Experiment 3 - 45 minutes.

Responsivity to acoustic startle stimuli is a measure of reflex excitability, and was used in this thesis to evaluate the effects of drugs on intertrial habituation and initial startle magnitude (refer to Tables 4 and 5 for procedural explanation and definitions). In 1980, Davis published a comprehensive review article on acoustic and tactile startle response, proposing that acoustic startle reflex is well-suited for analyzing how drugs affect reflex behavior. Its appropriateness is due to the fact that acoustic startle is under stimulus control and has a "non-zero baseline" which allows detection of either excitatory or inhibitory drug effects (Davis, 1980).

Evaluation of ambulation in a novel open field environment was measured by recording the number of open field squares entered by a subject every minute over a five minute test period. This measure of ambulation was included since data has suggested that putrescine-treated subjects showed a decrease in the number

Table 4
Startle Response Scoring

Response Scale	
<u>Score</u>	<u>Response</u>
3	whole body flinch
2	head flinch only
1	ears flinch only
0	no discernable response seen

Determination of Total Score

<u>Subject</u>	<u>Trials</u>								<u>total score</u>
	1	2	3	4	5	6	7	8	
#00	S1	S2	S3	S4	S5	S6	S7	S8	Sum of scores over 8 trials

Table 5

Definition of Startle Response Terminology	
SR I (Baseline)	- first set of 8 trials; scored before drug administration
SR II (Posttreatment-1)	- second set of 8 trials; scored after drug administration
SR III (Posttreatment-2)	- third set of 8 trials; also scored after drug administration
Initial Startle Intensity (ISI)	- Score on trial 1 of a given set
Attenuation of Initial Startle Intensity	- Baseline Line ISI minus the given posttreatment ISI; also referred to as the difference in ISI
Startle Magnitude	- Baseline total score minus given posttreatment total score (a large number is associated with facilitated habituation)

of squares entered over time (pilot studies, 1980). A square was scored having been entered if the animal placed at least two paws in that square.

The grid test is a simple, straightforward test of gross motor coordination and endurance. The animal is placed on a horizontal metal grid; the grid is then rotated 90° to the vertical plane (5 sec rotation). The ability of the animal to cling to the grid during rotation gives a crude index of motor impairment. The ability of the subject to hang onto the grid in a vertical position is a measure of endurance.

Repeated measurement of rectal temperature allows the discernment of hypo- or hyperthermic responses to drug treatments. Allowing subjects access to food and water for a limited time, following a period of deprivation, allows evaluation of drug treatment on ingestive behavior. Finally, testing for threshold responsivity to electric shock can reveal drug-induced changes in reaction to painful stimuli, including analgesic effects (Spiaggia, Bodnar, Kelly & Glusman, 1979). Table 6 lists and defines the pain threshold criteria utilized in pain response testing.

Behavioral evaluation: statistical analysis of data. Data will be subjected to statistical analysis with one of the following tests of significance: analysis of variance (ANOVA), Duncan's multiple-range test, Dunnett's test, Chi-square, and linear regression analysis using Pearson's r correlation

Table 6

Definition of Behavioral Criteria for Electric
Shock Response Thresholds in Experiments 1 and 2

Category	Definition
Body flinch	Noticeable rippling of the entire body musculature
Jumping	Both rear paws leave the shock grid at the same time
Squealing	Elicitation of readily audible squeaking sound

Note. A behavior is scored when it is elicited during the presentation of a shock stimulus.

coefficient. Graphic representation of all significant main drug treatment effects (treatment), drug interactions (Treatment 1 x Treatment 2), and drug by time interactions (Treatment x time, and Treatment 1 x Treatment 2 x time) will be presented in relation to evaluated behaviors. Data for main drug effects in which time interaction was significant are not present in the main text; these graphs are presented in Appendix B.

Behavioral evaluation: experimental hypotheses. The general hypothesis of this thesis is the proposition that systemic administration of the minor GABA precursor, putrescine, elicits behavioral effects in rats, some of which are similar to GABAergic behaviors. Support for this hypothesis may be derived from the results of the three experimental procedures comprising this thesis.

The general hypothesis would be supported by the substantiation of the following experimental hypotheses: 1) oral administration of putrescine produces significant behavioral effects in rats, some of which resemble behaviors associated with GABAergic function; 2) intraperitoneal administration of putrescine, at doses lower than given orally, produces behaviors which are similar to those effects obtained following oral putrescine treatment; 3) behaviors induced by putrescine (i.p.) resemble certain behaviors observed following intraperitoneal treatment with the known GABA agonist, muscimol; and 4) behaviors induced by pretreatment with the known dopamine-agonist, apomorphine

(s.c.) are altered in a similar manner by both putrescine (i.p.) and muscimol (i.p.) administration.

Thesis format. The experiments which comprise this thesis are presented as three separate research procedures. Each experiment's chapter will include its own introduction, materials and methods, results and discussion. A general discussion section will be presented at the end of Experiment 3.

EXPERIMENT 1

Introduction

A biosynthetic pathway of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) from putrescine (1,4-diaminobutane), has been well established in mammalian organisms and is independent of the major synthetic GABA pathway which utilizes glutamic acid (Seiler, & Al-Therib, 1974; Seiler, Al-Therib & Katak, 1973; Seiler, Bink & Grove, 1979; Seiler & Eichertopf, 1975; Seiler & Knogden, 1971; Seiler, Lamberty & Al-Therib, 1975). Biochemical evidence has demonstrated that systemic (i.p.) injection of putrescine results in measurable in vivo conversion to GABA in both peripheral and central nervous system (CNS) tissues of rodents (Caron, Cote & Kremzner, 1980; Tsuji & Nakajima, 1978).

Behavioral literature concerning putrescine and the other polyamines, spermine and spermidine, is limited. Recent reports suggest that these substances may have an important role in CNS neurotransmission (Anderson, Crossland & Shaw, 1975; Nistico, Ientile, Rotiroti & Di Giorgio, 1980; Shaw, 1979). Intraventricular injections of spermine, spermidine and putrescine are known to produce marked behavioral effects in mice, rabbits and chicks (Anderson et al., 1975; Nistico et al., 1980). Systemic (i.v. and i.p.) administration of spermine and spermidine has been reported to cause behavioral changes in mice and rats (Shaw, 1972).

In rodents, intraventricular (i.v.t.) injection of polyamines generally resulted in behavioral depression (sedation, ptosis, hypothermia) followed by some behavioral excitation, and other consequences dependent on the particular polyamine administered (Anderson, Crossland

and Shaw, 1975). These effects included convulsions and lethal tonic spasms several hours after spermine injection, development of flaccid paralysis and death four of five days after spermidine injection, and the development of seizures or flaccid paralysis one to four days after putrescine injection. Although i.v.t. injection of spermine, spermidine and putrescine produced some effects which were similar, differences in latency for appearance of effects and treatment potency existed. Spermine was most potent in causing behavioral changes in rodents, spermidine was intermediate, and putrescine was least potent (Anderson et al., 1975).

Behavioral changes in mice and rats following systemic (i.v. and i.p.) injection of spermine and spermidine have been reported (Shaw, 1972). Intravenously administered spermine and spermidine produced immediate excitatory effects including clonic convulsions occurring within seconds after injection. Excitatory effects were followed by loss of righting reflex, respiratory and behavioral depression, and sometime respiratory arrest leading to death. In contrast to the results associated with i.v. injection, intraperitoneal injection of spermine and spermidine was not associated with any excitatory behavioral changes. Effects produced by i.p. administration of these polyamines lasted approximately 90 minutes and included behavioral sedation, ptosis, piloerection, ataxia, hypothermia and respiratory depression, with some residual sedation observed several hours posttreatment (Shaw, 1972).

Behavioral effects elicited by in vivo polyamine treatment appear to be dependent on the specific polyamine administered, and the rate at

which the substance increases within the brain (Anderson et al., 1975). The appearance of an excitatory behavioral phase following polyamine injection seems to be related to rapid increases in the CNS concentration of polyamines, as observed following intraventricular (spermine, spermidine, putrescine) and intravenous (spermine and spermidine) treatments. Intraperitoneal injection of spermine and spermidine did not produce excitatory effects. The lethal and morbid consequences of polyamine administration are also attenuated if the route of treatment has less immediate influence on CNS structures.

Biochemical and behavioral studies have suggested that putrescine may directly or indirectly influence CNS neuropharmacology (Nistico et al., 1980; Seiler & Al-Therib, 1974; Shaw, 1979). Although studies have compared the i.v.t. and systemic administration of spermine and spermidine, there has been no report which has evaluated the systemic administration (oral or i.p.) of putrescine. Since putrescine's relationship to GABA synthesis in mammalian brain has been demonstrated, investigation of behavioral consequences due to systemic putrescine treatment may help to specify the nature of its neuropharmacologic significance.

The purpose of this experimental procedure is to evaluate the behavioral consequences of oral administration of putrescine in rats. Eight different tests of behavior will be conducted in order to characterize the effects of oral putrescine treatment over a time course of five hours. Formal statistical analyses will be used. It is hypothesized that oral administration of putrescine in rats may cause significant behavioral effects and that these effects may be similar to

behaviors associated with GABAergic function.

Materials and Methods

Subjects. The subjects were 28 naive male Sprague-Dawley albino rats, obtained from Flow Labs (Dublin, VA), that were between 90 and 120 days of age (350-425 g) at time of receipt. Animals were housed in groups of three for two weeks before experimental use and were maintained on a 12:12 hour light-dark cycle (3 a.m.-3 p.m.) at constant room temperature (20°C). Subjects were given free access to Wayne Lab Blox and tap water.

Apparatus. Oral treatment dosages were administered with curved oral intubation needles (George Tiemann & Company, Long Island, NY). Behavioral observations were made with subjects individually housed in opaque plastic tubs (55 cm x 35 cm x 20 cm) having stainless steel cage covers and containing sawdust. Rectal temperatures were measured with a B&D rectal thermometer (°F) and the use of petroleum jelly lubricant. A metal "clicker apparatus" was used as the auditory startle response stimulus. Sonogram analysis showed that the startle stimulus had a two component, bi-phasic sonograph character (energy from $0H_2-8kH_2$), with frequency peaks at 3.6 and 7.5 kH_2 , and a duration of 440 msec (courtesy of Dr. Curt Adkisson, Biology Department, VPI & SU). The loudness of the startle stimulus was 67 db (Realistic decibel meter). Open field performance was measured in a clear plexiglass open field box (100 cm x 100 cm x 30 cm) marked into 36 interior squares. Illumination for open field evaluation consisted of two 25-watt red lights, each suspended 40 cm above the field box. Motor coordination was measured with the use of a stainless steel grid. A Grayson-Stadler shock generator and behavioral chamber with an electric grid

(30 m x 20 cm x 20 cm) were used to measure electric shock response thresholds (AC power source, 40 volts current). All subjects were viewed through the plexiglass top and end window of the grid box.

Drugs and dosages. The four treatment groups included a saline control and three putrescine groups (n=7). Solutions of putrescine hydrochloride (SIGMA Chemical Company, St. Louis, MO) were prepared in .85% saline no more than two hours prior to treatment. Putrescine dosages of 0 mg/kg, 50 mg/kg, 250 mg/kg, and 1000 mg/kg were administered in an injection volume of 1 cc/kg body weight per subject. All treatments were administered by oral intubation with each subject being used in only one test session.

Procedures

All subjects were deprived of food and water two hours prior to the beginning of the experiment. The experiment began during the last two hours of the light portion of the light-dark cycle. First, each subject was given a pretreatment startle response test for each subject. Body weights and initial rectal temperatures were measured prior to treatment. Treatment was administered experimenter-blind and by oral intubation. Animals were hand-held in a vertical position while the stainless steel intubation needles were placed far enough into the pharynx to insure delivery of dosages. Immediately prior to the start of any behavioral test, each subject was handled in order to maintain an arbitrary level of alertness. This was accomplished by picking up a subject by hand and lifting him to a height of 30 cm, repeating this sequence 2-4 times. Handling and lifting sequences were continued until the subject appeared to react and orient to his surroundings. Table 7

Table 7
Experiment 1 Protocol

Procedure	Time Course
1. Food and water deprivation begins 2 hr prior to experiment	-2:00 hr
2. Startle test I. (8 trials, 3 point intensity scale)	-0:40 hr
3. Body weights and rectal temperature I.	-0:20 hr
4. Oral putrescine treatment (0, 50, 250, 1000 mg/kg)	0:00 hr
5. Time sampling, first block (13 behavioral categories, subjects scored once per min)	+0:05 hr
6. Startle test II.	+2:05 hr
7. Time sampling, second block	+2:30 hr
8. Open field ambulation (5 min testing)	+3:00 hr
9. Grid-hang motor ability (test of motor coordination and endurance using a rotated grid)	+3:45 hr
10. Food and water ingestion (5 min)	+4:00 hr
11. Startle test III.	+4:20 hr
12. Rectal temperature II.	+4:45 hr
13. Electric shock response threshold testing	+5:00 hr
14. Retest electric shock response threshold	+21:00 hr

outlines the experimental protocol and time course of Experiment 1.

Body temperature. Pretreatment body temperatures were measured 20 minutes prior to dose administrations. Body temperatures were measured again at 4:45 hours posttreatment, and change in temperature calculated as the difference between pre- and posttreatment group means.

Startle response. The startle response test consisted of a series of clicks from the clicker apparatus with one click presented every 15 seconds for eight trials. Testing was done for each animal in isolation. The auditory stimulus was presented to each subject in his observation tub. The clicker apparatus was always held 20 cm directly above the middle of a tub. Responses to the startle stimuli were coded on an arbitrary four point scale: 3 = body flinch; 2 = head flinch; 1 = ears flinch; and 0 = no discernable response (refer to Tables 4 and 5 for further explanation). Tests of startle response were measured at three time points: prior to measurement of body weight and temperature (baseline), two-hours posttreatment, and 4.5 hours posttreatment.

Time sampling. Immediately following oral administration of treatments, subjects were directly observed for one hour by time sampling technique, with each subject monitored for 60 intervals in a repeated measures sequence. Each subject was observed for 10 seconds during every minute, and one overt behavior recorded on a list of 13 arbitrary behavioral categories. These arbitrary categories were designed to evaluate three general behavioral responses: general activity (sitting, walking, rearing, sleep), stereotypy (sniffing, grooming, licking, gnawing, mastication), and abnormal behaviors (abnormal posture/movement, motor impairment, head down posture, wet dog shaking) (refer to Table 2

for definition of categories). Time sampling was continued for an additional 30 minutes beginning at 2.5 hours posttreatment.

Open field. Approximately three hours posttreatment open field performance was measured. Subjects were individually placed in a corner of the open field enclosure. The latency to leave corner of placement was measured, and the total number of squares entered were recorded continuously during five one-minute intervals. One-minute intervals were used for assessment of open field ambulation in order to allow comparison of group means over the five minutes of testing. The open field box was thoroughly cleaned and wiped with isopropyl alcohol between subjects.

Motor ability. In order to evaluate motor coordination and endurance, the subjects' gross ability to maintain balance and position on a rotated grid was measured at 3:45 hours posttreatment. Each subject was placed on a horizontal metal grid; the grid was then rotated 90° to the vertical plane (5 sec rotation). Vertical position of the grid was maintained for 15 seconds while subjects were scored + or - for maintenance of initial posture, and hang time endurance (latency to fall). Time was measured to the nearest second (stopwatch).

Ingestive behavior. Approximately four hours posttreatment, ingestive behavior was measured (after six hours of food and water deprivation). Subjects were given simultaneous access to rat chow and tap water for a period of five minutes. Rat chow was placed in the tub cover food holders and water was given in plastic graduated cylinders fitted with water bottle stoppers. Subjects were scored "+" or "-" with respect to exhibition of food and water ingestion behavior; water volume

(ml) was also recorded for subjects which drank.

Pain response thresholds. Electric shock response thresholds were measured five hours posttreatment as the final behavioral test. Subjects were placed in the shock grid box and evaluated on an arbitrary ascending milliamp (mA) pulse train of 40 volts (.05, .06, .08, .10, .13, .16, .20, .25, .30, .40, .50, .60, .80, 1.0 mA). The pulse train values used were the smallest increments available on the apparatus. Each mA pulse lasted for 500 msec with a 10 second interstimulus interval. The mA value was recorded which produced each of the following behavioral responses: body flinch, jumping, and squealing (refer to Table 6 for behavioral criteria). At the conclusion of this shock response testing, subjects were returned to their home cages and given free access to rat chow and tap water.

Twenty-one hours posttreatment, subjects were again removed from their cages and replaced in individual observation tubs. Within five minutes, shock response thresholds were retested as previously described, in order to evaluate any residual effects of experimental treatment on pain response.

Data analysis. Statistical analysis for the data obtained in the simple one-way design included analysis of variance (ANOVA), or Student's t statistic, or Chi-square test of significance. Dunnett's Multiple Comparison Test Against Control (referred to as Dunnett's test) was used to specify significant group differences for ANOVA main effects, compared to saline controls. Duncan's multiple range test (referred to as Duncan's test) was used to characterize significant between groups differences for ANOVA interaction effects, but was not used at every

sampling interval which had significant between groups differences. Duncan's test for treatment x time interactions was used to characterize: 1) initial appearance of between groups differences; 2) the most extreme (maximum or minimum) response effect when appropriate; and 3) dissipation of between groups differences.

Linear regression analysis using Pearson's r correlation coefficient was used to determine whether a linear dose response relationship existed between statistically significant behavioral response measures and putrescine treatment. Pearson's r correlations were calculated for all behaviors which had significant ANOVA main effects using the least squares regression method (Bruning and Kintz, 1977). The response means for each treatment group were plotted against dosage (0, 50, 250, 1000 mg/kg putrescine). Dose response curves were not evaluated for food and water ingestion data (Chi-square) and the pain response threshold retesting at 21 hours posttreatment. The level of significance for all statistical analyses was $\alpha = .05$, unless otherwise noted.

Results

Analysis of the data demonstrated that systemic administration of putrescine by oral route produced observable behavioral effects in adult male rats. Using linear regression analysis, a dose response relationship was found for the majority of significant ANOVA effects obtained.

Formal Observations

Time sampling. Time sampling testing yielded significant analysis of variance (ANOVA) effects for five of 13 arbitrary behavioral categories. Two general activity categories (sleep and rearing), two stereotypy categories (grooming and gnawing), and the abnormal behavior category (wet dog shaking) were found to have significant treatment main effects or treatment x time effects.

A significant ANOVA main effect for the incidence of sleep behavior was found ($F(3,24)=3.5$, $p < .03$), with putrescine treatment depressing the observed incidence of sleeping behavior (Figure 8). The saline group had the highest incidence of sleep over the 90 minute period. The putrescine-treated groups were significantly different from saline and statistically similar to each other (Duncan's $p < .05$) (Table 8). Saline subjects were observed to be sleeping nearly one third of the time during time sampling and putrescine groups exhibited a sleeping incidence of less than half that amount. A linear dose response relationships was not found (Table 17).

A significant ANOVA treatment x time interaction was found for the incidence of rearing activity ($F(24,192)=2.11$, 192 , $p < .005$) (Figure 9). The results obtained the first 60 minute period were difficult to interpret. During the first 10 minutes posttreatment, only in

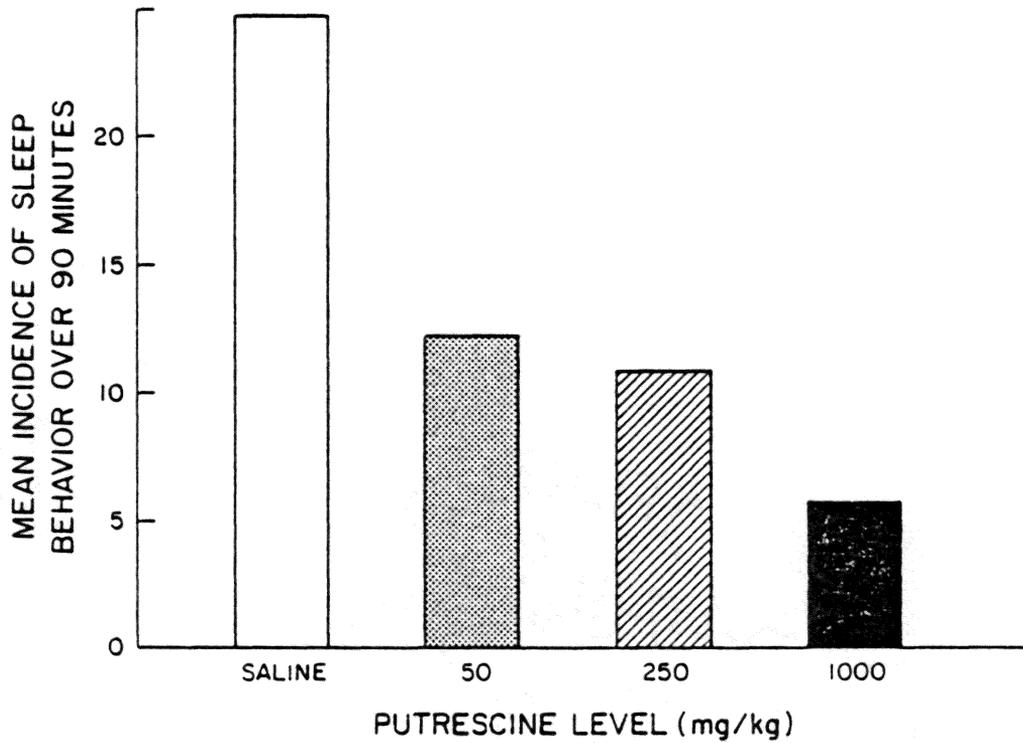


Figure 8. Effect of oral putrescine treatment on the incidence of sleep behavior.

Table 8
Duncan's Multiple-Range Test Results
for Putrescine (p.o.) Treatment Effect
and Incidence of Sleeping Behavior

Treatment	Grouping ^a
Saline	*
50 mg/kg	**
250 mg/kg	**
1000 mg/kg	**

^aTreatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 90 minutes.

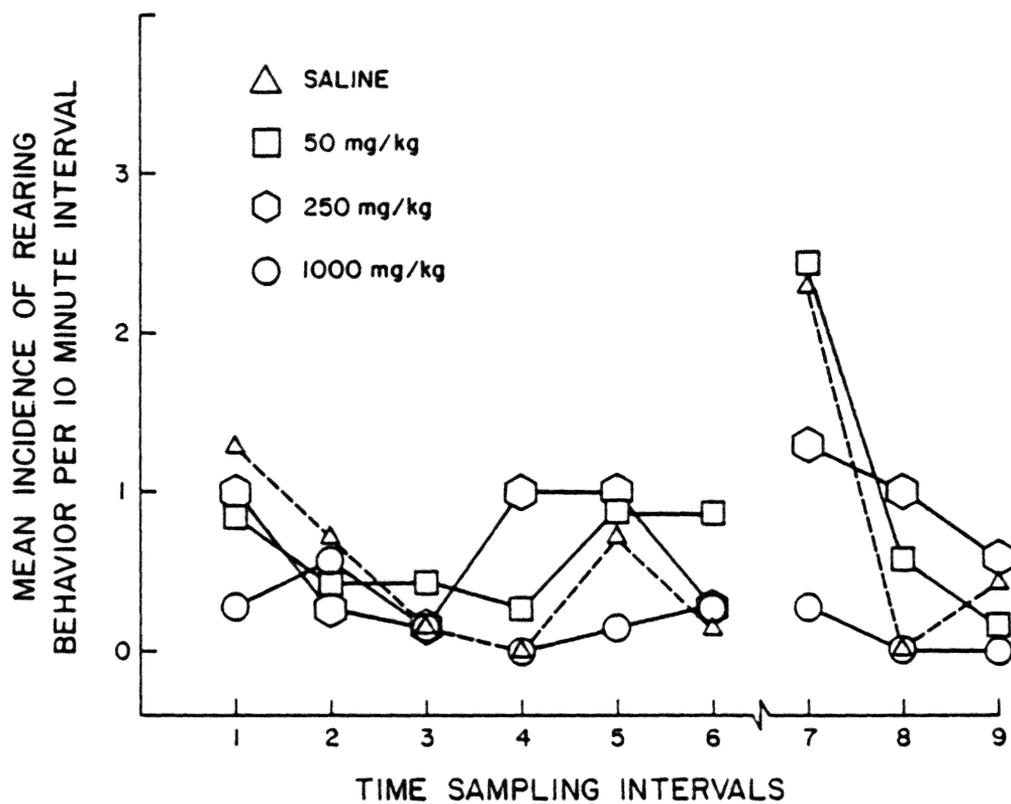


Figure 9. Oral putrescine treatment x time interaction and the incidence of rearing behavior.

the 1000 mg/kg group was the incidence of rearing significantly lower than that in the saline group (Duncan's $p < .05$) (Table 9). During the second and third time intervals following treatment, no significant differences existed between groups. At 40 to 60 minutes posttreatment, no consistent trends in rearing behavior occurred. At $t = 4$, subjects given saline and 1000 mg/kg putrescine exhibited a low frequency of rearing, while subjects given 250 mg/kg putrescine had significantly higher rearing incidence. A putrescine treatment-related decrement in rearing incidence was most clearly observed at the beginning of the second time sampling block ($t = 7$, 2.5 hr posttreatment). At $t = 7$, the 1000 mg/kg putrescine group was significantly lower than all other groups (Duncan's test $p < .05$). At that same time interval, subjects given saline or 50 mg/kg exhibited significantly higher rearing incidence, than subjects receiving 250 mg/kg putrescine. At $t = 8-9$, differences between groups were lost. Thus, the most reliable observations about rearing incidence was at $t = 7$, a time immediately following handling. The results at $t = 7$ were similar, though not identical, to those observed at $t = 1$. Differences between treatment group means immediately following handling suggests that treatment decreased the rearing behavior that normally occurs during reorientation.

Putrescine treatment was also related to a reduction in the incidence of grooming behavior, yielding a significant ANOVA main effect ($F(3,24) = 7.86$, $p < .0008$) (Figure 10). Duncan's analysis ($p < .05$), indicated that the 1000 mg/kg putrescine group had a significantly lower incidence of grooming behavior compared to all other groups, and that the other putrescine treatment groups were not significantly different from saline

Table 9
 Duncan's Multiple-Range Test Results
 for Putrescine (p.o.) Treatment x Time Interaction
 and Incidence of Rearing Behavior^a

Time Sampling Interval	Group Comparison	$p <^b$
T = 1	Saline vs 1000 mg/kg	.05
T = 4	250 mg/kg vs Saline	.05
	250 mg/kg vs 1000 mg/kg	.05
T = 7	50 mg/kg vs 250 mg/kg	.01
	50 mg/kg vs 1000 mg/kg	.01
	Saline vs 250 mg/kg	.01
	Saline vs 1000 mg/kg	.01
	250 mg/kg vs 1000 mg/kg	.05

^aEach time sampling interval is 10 minutes in duration; total testing time = 90 minutes.

^b p indicates significant difference between comparison groups.

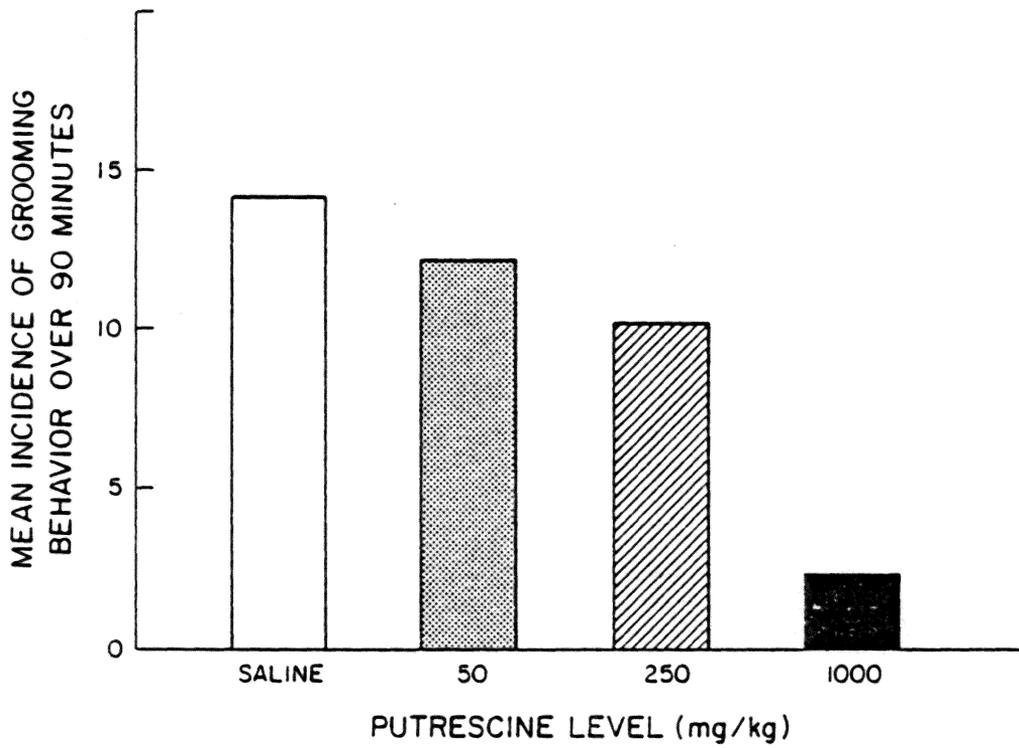


Figure 10. Effect of oral putrescine treatment on the incidence of grooming behavior.

(Table 10). Saline subjects had an incidence of grooming behavior which was approximately three-fold higher than that of animals given 1000 mg/kg putrescine. The linearity of the dose response correlation was highly significant ($p < .01$) (Table 17).

As shown in Figure 11, putrescine treatment was related to an increase in gnawing behavior yielding a significant ANOVA treatment x time interaction ($F(24,192)=2.19$, $p < .002$) (see Figure 42 in Appendix B for ANOVA main effect data). A gnawing response was characterized by a subject holding sawdust chips in his mouth with his forepaws, and exhibiting actual chewing of sawdust for at least three seconds. Subjects treated with saline did not exhibit any appreciable gnawing behavior during the entire time sampling session. Twenty minutes posttreatment, subjects given 1000 mg/kg putrescine were found to show a significantly higher incidence of gnawing compared to that of saline and 50 mg/kg putrescine groups (Duncan's test $p < .05$) (Table 11). At $t = 3$, the maximum incidence of gnawing was observed for the 1000 mg/kg putrescine group, which was statistically higher than the other three groups. The incidence of gnawing by the 250 mg/kg group was significantly higher than the very low levels seen in subjects treated with 50 mg/kg putrescine or saline. Incidence of gnawing for the 1000 mg/kg group decreased thereafter. At $t = 5$, the maximum incidence of gnawing in the 250 mg/kg putrescine group was observed. At $t = 6$, the 250 and 1000 mg/kg putrescine groups were statistically similar in relation to gnawing, and both were significantly different from saline subjects only. At two and one-half hours after treatment ($t = 7$) no between groups differences were seen for gnawing behavior. Thus, putrescine treatment appeared to increase the incidence of

Table 10
Duncan's Multiple-Range Test Results
for Putrescine (p.o.) Treatment Effect
and Incidence of Grooming Behavior^a

Treatment	Grouping
Saline	*
50 mg/kg	*
250 mg/kg	*
1000 mg/kg	**

^aTreatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 90 minutes.

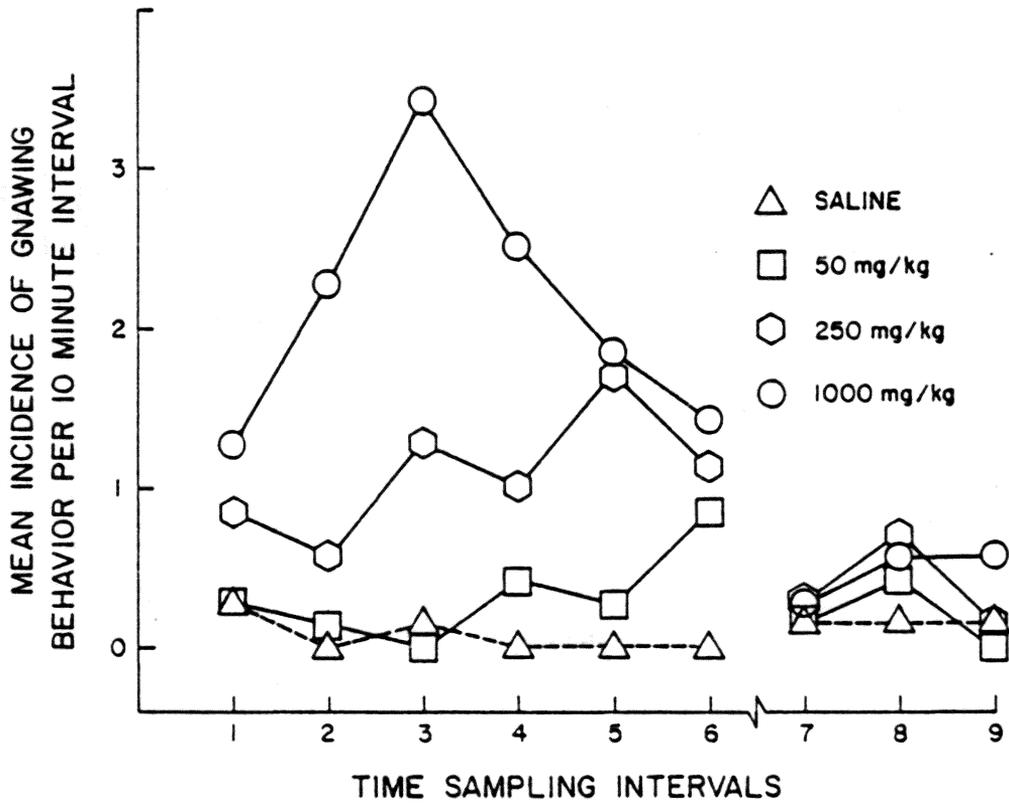


Figure 11. Oral putrescine treatment x time interaction and the incidence of gnawing behavior.

Table 11
 Duncan's Multiple-Range Test Results
 for Putrescine (p.o.) Treatment x Time Interaction
 and Incidence of Gnawing Behavior^a

Time Sampling Interval	Group Comparison	$p <^b$
T = 2	1000 mg/kg vs 250 mg/kg	.01
	1000 mg/kg vs 50 mg/kg	.01
	1000 mg/kg vs Saline	.01
T = 3	1000 mg/kg vs 250 mg/kg	.01
	1000 mg/kg vs 50 mg/kg	.01
	1000 mg/kg vs Saline	.01
	250 mg/kg vs 50 mg/kg	.05
	250 mg/kg vs Saline	.01
T = 6	1000 mg/kg vs Saline	.05
	250 mg/kg vs Saline	.05

^a Each time sampling interval is 10 minutes in duration; total testing time = 90 minutes.

^b p indicates significant difference between comparison groups.

gnawing behavior during the first 60 minutes after administration, in subjects given 250 or 1000 mg/kg putrescine p.o. A linear dose response relationship based on ANOVA main effects was found ($p < .05$) (Table 17).

"Wet dog shaking" behavior (WDS) was characterized by slight flinching and sudden movement of the head, followed by rapid side-to-side shaking of the head, neck, and trunk regions of the body, with an episode lasting approximately 2-3 seconds in duration. At the conclusion of a WDS episode, subjects often had a splayed leg posture, accompanied by ptosis and the appearance of not being oriented to their surroundings. A significant ANOVA treatment x time interaction was found for the incidence of WDS behavior over 90 minutes of sampling, as shown in Figure 12 ($F(24,192)=3.56$, $p < .0001$) (see Appendix B for main effect data). During $t = T-3$, no significant incidence of WDS was seen in any treatment group. However, an increase of WDS incidence over time was observed in the 1000 mg/kg putrescine group, and not any other group (beginning at $t=4$). The incidence of WDS in the 1000 mg/kg group was significantly higher than that in all other groups at $t = 5$ (Duncan's $p < .01$) (Table 12). The incidence level of WDS in all other groups remained at a level very close to zero at all time points. After 2.5 hours posttreatment ($t = 7$), the incidence of WDS increased in subjects given 1000 mg/kg putrescine. This effect continued through $t = 8$, while other groups failed to show any significant difference from saline control subjects. At $t = 9$, observation of WDS in animals given 1000 mg/kg putrescine remained high. During peak incidence of

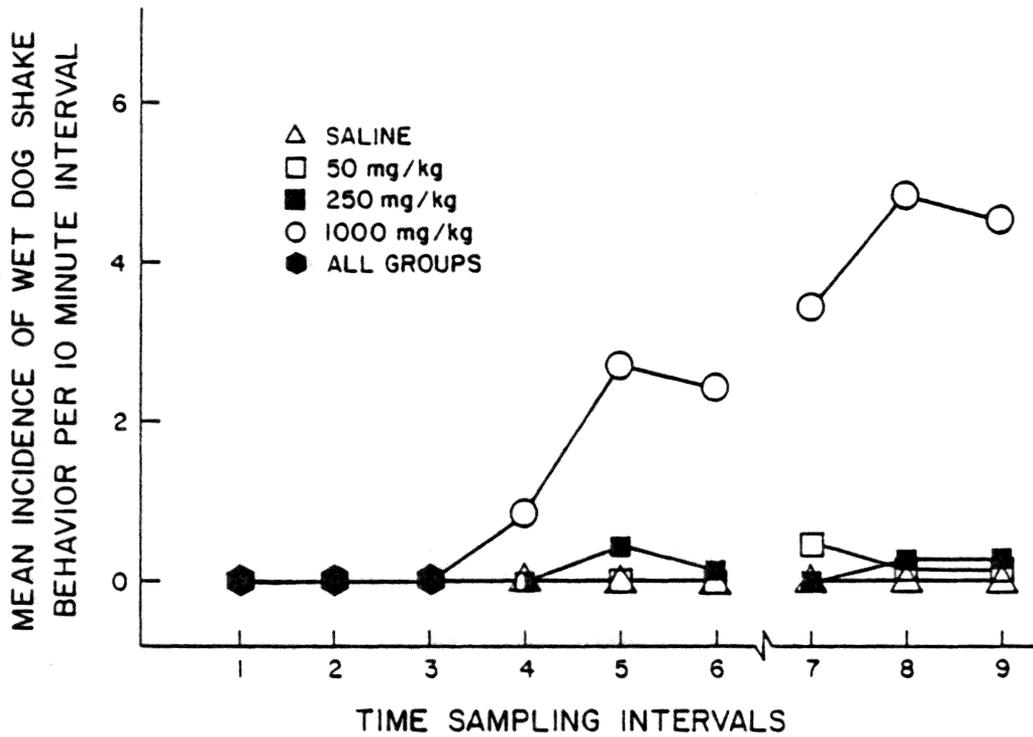


Figure 12. Oral putrescine treatment x time interaction and the incidence of wet dog shaking (WDS) behavior.

Table 12
 Duncan's Multiple-Range Test Results
 for Putrescine (p.o.) Treatment x Time Interaction
 and Incidence of Wet Dog Shake Behavior^a

Time Sampling Interval	Group Comparison	$p <^b$
T = 5	1000 mg/kg vs 250 mg/kg	.01
	1000 mg/kg vs 50 mg/kg	.01
	1000 mg/kg vs Saline	.01
T = 8	1000 mg/kg vs 250 mg/kg	.01
	1000 mg/kg vs 50 mg/kg	.01
	1000 mg/kg vs Saline	.01
T = 9	1000 mg/kg vs 250 mg/kg	.01
	1000 mg/kg vs 50 mg/kg	.01
	1000 mg/kg vs Saline	.01

^a Each time sampling interval is 10 minutes in duration; total testing time = 90 minutes.

^b p indicates significant difference between comparison groups.

WDS in a given subject, an incidence rate of 2-4 WDS/min was estimated. Although significant incidence of WDS was only observed in subjects receiving 1000 mg/kg putrescine, a significant linear dose response was found ($p < .02$) (Table 17).

Of 13 arbitrary behavioral categories which were time sampled, eight categories did not yield significant ANOVA effects (62%). These nonsignificant categories included sitting and walking (general activity), sniffing, non-body licking and mastication behavior (stereotypy), and abnormal posture/movement, motor impairment, and head down posture (abnormal behavior).

Intensity of initial startle response. The effect of oral putrescine treatment on the first startle response (ISI) of test sets at two hours and 4.5 hours posttreatment is shown in Figure 13 (a and b, respectively). Comparison of baseline means for initial startle magnitude yielded no significant ANOVA differences between groups ($F = 3,24, .73$ $p > .20$). Baseline means are given in Appendix B. At two hours posttreatment, a significant ANOVA main effect was found, with putrescine tending to decrease ISI response ($F = 3.55$, $df = 3,24$, $p < .03$). Using Dunnett's test, the lower ISI exhibited by the 1000 mg/kg group was significantly different from saline ($p < .05$); no other treatment group was different from saline. However, a linear dose response curve was obtained ($p < .01$) (Table 17).

At 4.5 hours posttreatment, analysis yielded a significant ANOVA main effect for the intensity of initial startle response ($F(3,24) = 5.20$, $p < .01$). Although ISI for the 50 and 250 mg/kg putrescine groups was lower than that seen in the saline group, Dunnett's test indicated that these groups were not significantly different from saline. The

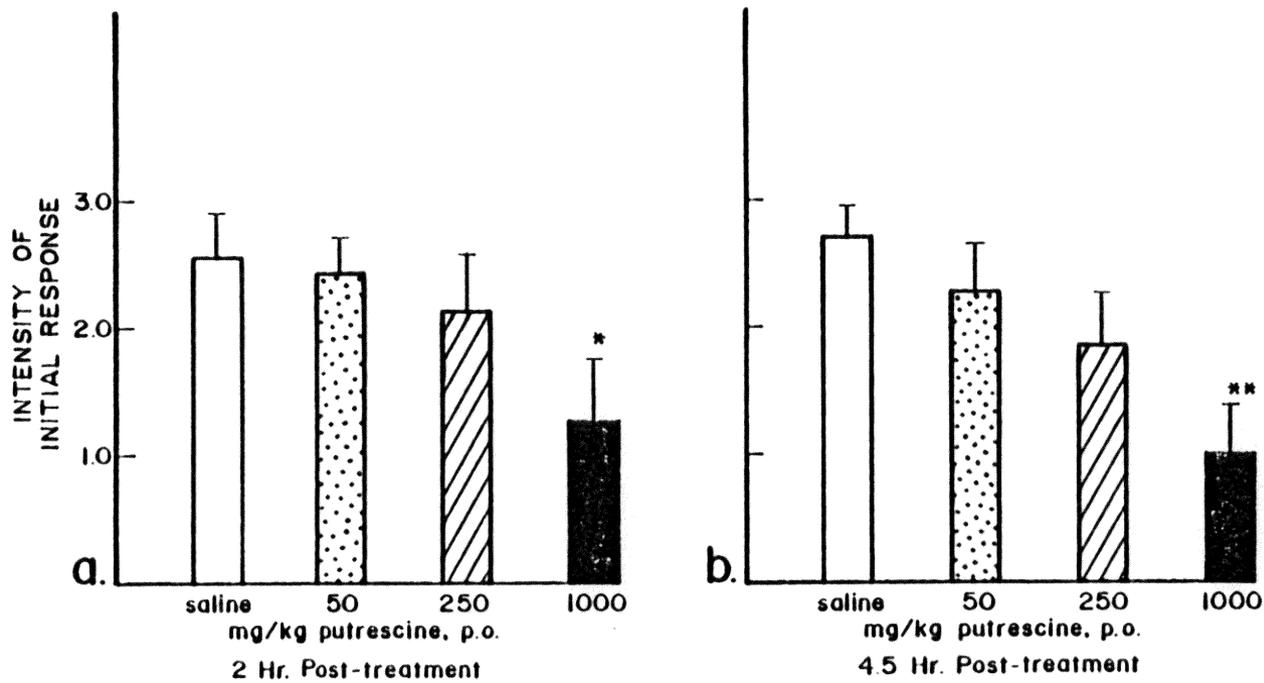


Figure 13. Effect of oral putrescine treatment on the intensity of initial startle response magnitude at 2-hr (a) and 4.5-hr (b) posttreatment. (Significant difference from saline using Dunnett's test denoted by * $p < .05$, or ** $p < .01$).

linearity of the dose response curve was nonetheless significant ($p < .05$) (Table 17). The 1000 mg/kg putrescine group was significantly different from saline (Dunnett's $p < .01$), with the average initial startle response being only an ear twitch (arbitrary score = 1). Thus, putrescine treatment appeared to decrease ISI for subjects treated with 1000 mg/kg putrescine at two hours and 4.5 hours after administration compared to treatment with saline.

Startle response magnitude. The effect of putrescine treatment on the change in startle response magnitude at two hours and 4.5 hours posttreatment is shown in Figure 14 (a and b, respectively). Two hours after putrescine administration, no significant ANOVA effect was found for change in startle response magnitude ($F(3,24)=2.58, p > .05$). At 4.5 hours posttreatment, a significant ANOVA main effect was found ($F(3,24)=3.14, p < .03$). The change in startle magnitude for the saline group was very small, in contrast, the 1000 mg/kg putrescine group showed significantly larger change in startle magnitude ($p < .05$). Although the 50 and 250 mg/kg groups exhibited some change in SR magnitude scores, these groups were not reliably different from saline using Dunnett's test. The dose response curve was not found to be linear (Table 17). The data suggests that change in acoustic SR magnitude occurs 4.5 hours posttreatment, but only for subjects given putrescine in amounts greater than 250 mg/kg.

Open field performance. A significant ANOVA main effect was obtained for open field ambulation only ($F(3,24)=14.38, p < .0001$), with putrescine treatment decreasing ambulation three hours post-treatment (Figure 15). Duncan's analysis (Table 13) showed that

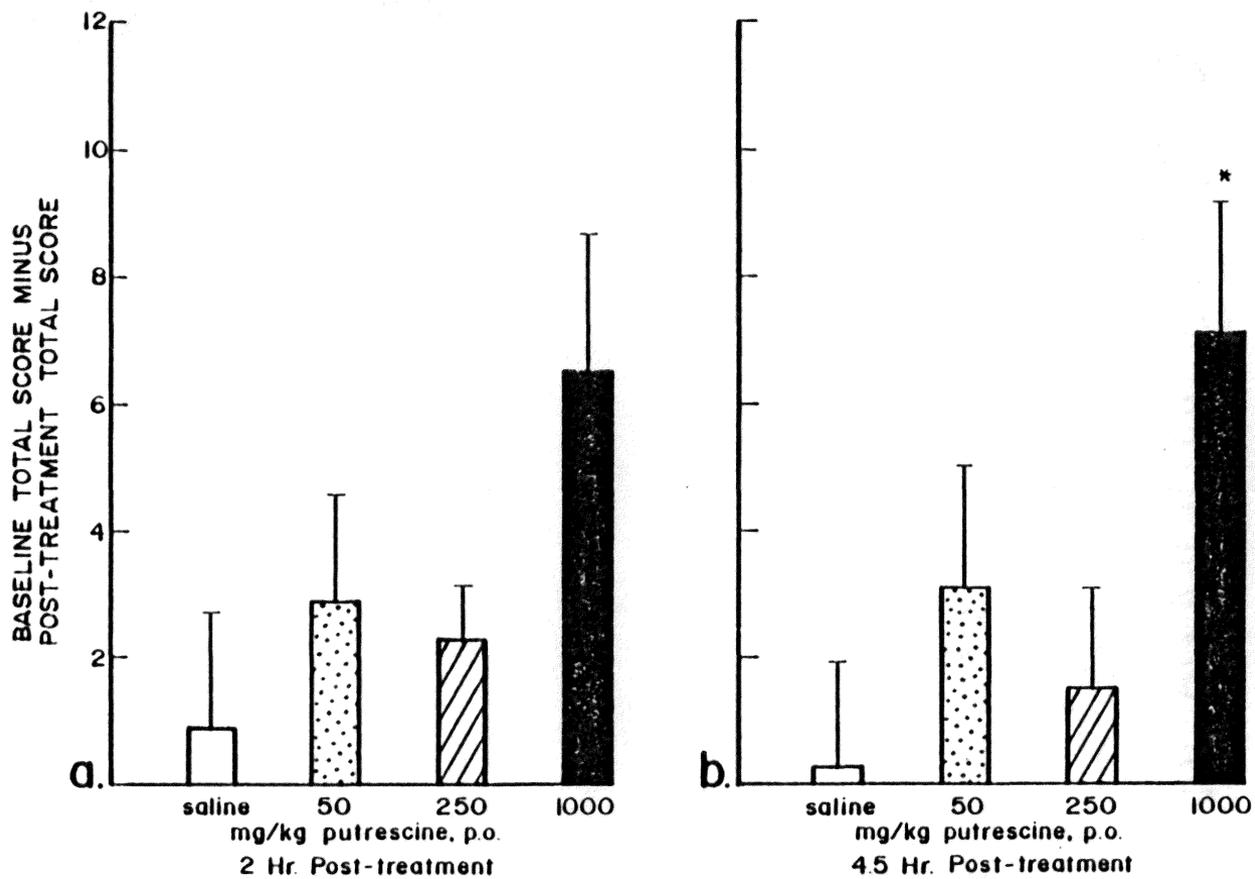


Figure 14. Effect of oral putrescine treatment on startle response magnitude at 2-hr (a) and 4.5-hr posttreatment. (Significant difference from saline using Dunnett's test denoted by * $p < .05$).

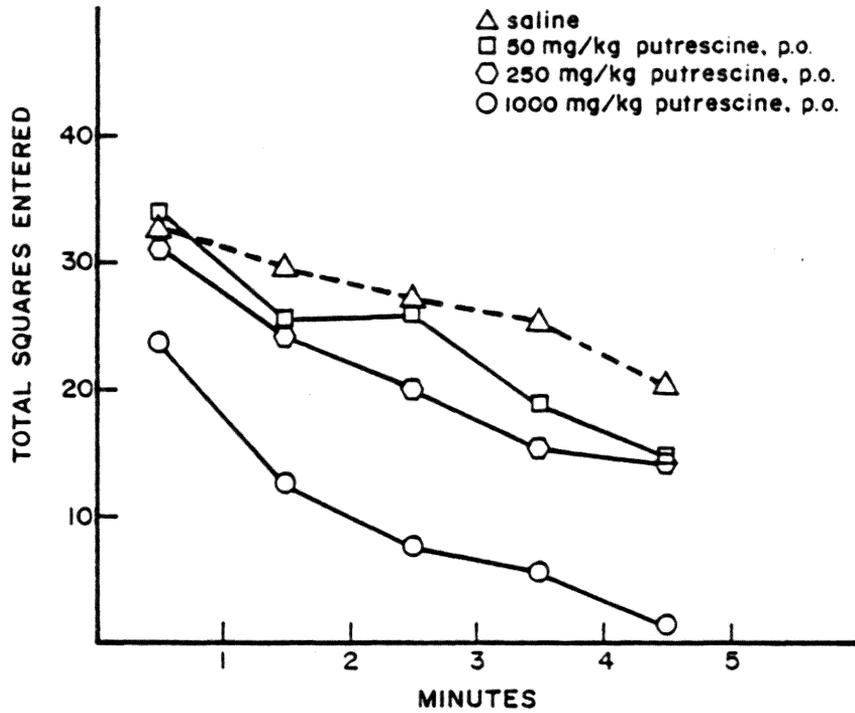


Figure 15. Effect of oral putrescine treatment on open field ambulation at 3-hr posttreatment.

Table 13

Duncan's Multiple-Range Test Results for Putrescine (p.o.)
Treatment Effect and Open Field Ambulation^a

Treatment Group	Grouping ^b
Saline	*
50 mg/kg	*
250 mg/kg	**
1000 mg/kg	***

^a Open field evaluation occurred over five one-minute intervals.

^b Treatment with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over five minutes.

saline and 50 mg/kg putrescine groups had ambulation levels which were statistically similar. The 250 mg/kg putrescine group had ambulation which was significantly lower than saline control ($p < .05$), and the ambulation of the 1000 mg/kg group was significantly lower than that of the 250 mg/kg groups ($p < .05$).

The treatment x time ANOVA interaction was not significant for open field ambulation ($F(12,96)=1.01$, $p < .43$), as all groups showed a decline in ambulation during the five-minute evaluative procedure. The decline in ambulation exhibited by the 1000 mg/kg group was particularly marked. The dose response curve was found to be linear ($p < .02$) (Table 17).

Subjects receiving 1000 mg/kg putrescine showed little ambulation by $t = 5$, with the mean number of squares entered for this group approaching zero/min. These high-dose subjects were active during minute one, but became less active and less prone to engage in sniffing, rearing, grooming, and exploratory activity as time progressed. High-dose subjects also exhibited WDS behavior, mastication stereotypy, ptosis, or piloerection. No between groups differences existed with respect to the latency to leave corner of initial placement (Student's $t(3)=1.50$, $p < .05$).

Grid coordination. Testing of coordination during grid rotation demonstrated that all groups exhibited comparable ability in maintaining initial grid-hand posture, 3:45 hours posttreatment. Only one of 28 subjects did not successfully hang onto the grid during rotation (a subject from the 1000 mg/kg group). As shown in Table 14, no

Table 14

Grid Coordination and Endurance Evaluation^a
Four Hours Posttreatment with Putrescine (p.o.)

Treatment Group	Mean Grid Hang Time \pm SEM (s)
Saline	12.1 \pm 0.9
50 mg/kg	14.0 \pm 1.0
250 mg/kg	12.3 \pm 1.4
1000 mg/kg	8.9 \pm 2.1

^aMaximum score possible = 15.0 second hang time.

significant ANOVA treatment effect was found for endurance hang time (latency to fall) ($\underline{F}(3,24)=2.27$, $p > .10$).

Shock response thresholds. Figure 16 shows the shock response thresholds at five hours posttreatment. Significant ANOVA main effects were obtained for all threshold criteria, including flinch ($\underline{F}(3,24) = 6.42$, $p < .005$), jump ($\underline{F}(3,24)=6.05$, $p < .005$), and squeal responses ($\underline{F}(3,24) = 4.94$, $p < .01$). Putrescine treatment was associated with higher shock thresholds for all three response criteria, although the 1000 mg/kg group was the only group found to be statistically different from the saline group (flinch, $p < .01$; jump, $p < .01$; squeal, $p < .01$) using Dunnett's test. While the other putrescine treatment groups (50 and 250 mg/kg) showed in shock thresholds, no reliable difference were found between these groups and saline for flinch, jump, and squeal criteria (Dunnett's test). However, dose response curves were found to be linear for flinch ($p < .02$), jump ($p < .02$), and squeal ($p < .05$) criteria (Table 17).

Figure 17 shows the shock response thresholds at 21 hours post-treatment. A significant ANOVA main effect was obtained only for flinch ($\underline{F}(3,16)=3.37$, $p < .05$), and not for jump ($\underline{F}(3,16)=2.69$, $p < .10$) or squeal responses ($\underline{F}(3,16)=2.55$, $p > .10$). With respect to flinch, the 1000 mg/kg group was found to have a higher shock threshold compared to saline (Dunnett's $p < .05$). All other treatment groups were not statistically different from saline (Note: the 21-hr shock response thresholds had $n = 5$ instead of $n = 7$, due to experimental oversight). In summary, putrescine administration resulted in higher

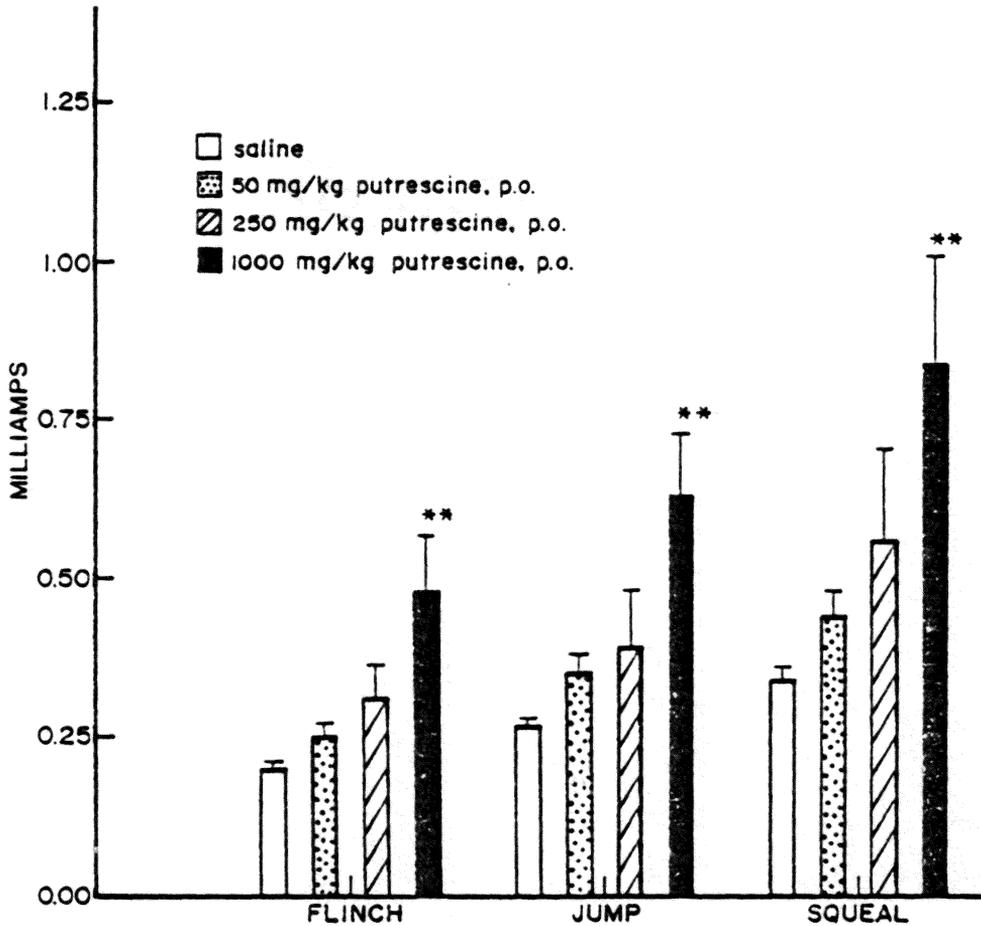


Figure 16. Effect of oral putrescine treatment on shock response thresholds for flinch, jump, and squeal criteria at 5-hr posttreatment. (Significant difference from saline using Dunnett's test denoted by ** $p < .01$).

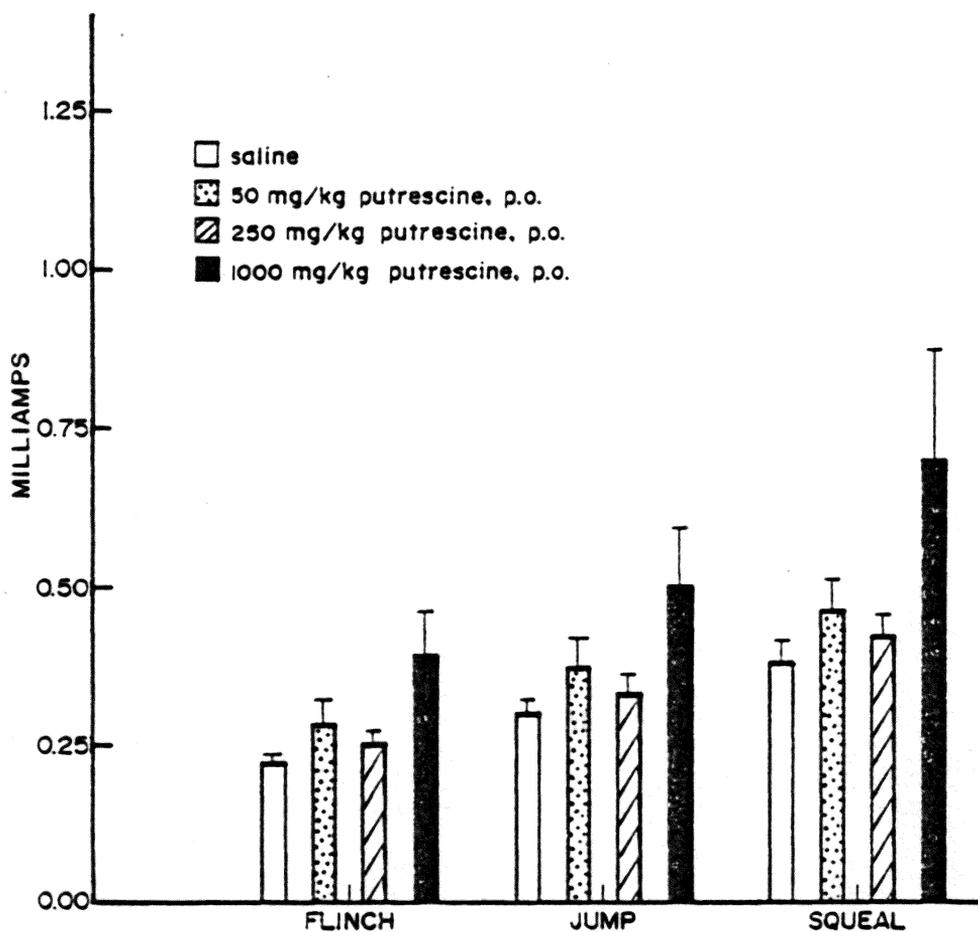


Figure 17. Effect of oral putrescine treatment on shock response thresholds for flinch, jump, and squeal criteria at 21-hr posttreatment.

response thresholds to electric shock five hours following treatment compared to saline control. At 21 hours posttreatment, the residual analgesic-like effects found were attributable to the group receiving 1000 mg/kg putrescine.

Body temperature. Table 15 gives the body temperature recorded prior to treatment, five hours posttreatment, and the resulting change in body temperature (means in °C ± SEM). No differences were observed between group means prior to treatment administration (Student's $t(3) = .59, p < .05$). Computation of the difference between posttreatment and baseline body temperature means for all treatment groups resulted in a significant ANOVA main effect ($F(3,24) = 11.15, p < .001$). As shown in Table 15, treatment with saline resulted in an increase in body temperature while treatment with 50 mg/kg putrescine resulted in only slight temperature elevation. In contrast, treatment with 250 mg/kg putrescine apparently depressed body temperature slightly, while a dose of 1000 mg/kg caused a more pronounced decline in posttreatment body temperature (-1.54 ± 0.23 °C). All putrescine treatment groups were found to have mean changes in body temperature that were statistically different from saline (Dunnett's $p < .01$). The linearity of the dose response curve was significant ($p < .02$) (Table 17). Thus, putrescine treatment was associated with a decline in body temperature five hours posttreatment compared to saline treatment. Depression of body temperature was most evident for subjects receiving the higher putrescine dosages, with marked hypothermia observed following oral administration of 1000 mg/kg putrescine.

Table 15

Change in Body Temperature

Five Hours Posttreatment With Putrescine (p.o.)

Putrescine Group	Mean Pretreatment Temp \pm SEM ($^{\circ}\text{C}$)	Mean Posttreatment Temp \pm SEM ($^{\circ}\text{C}$)	Δ Temperature \pm SEM ($^{\circ}\text{C}$)
0 mg/kg	36.41 \pm .13	36.84 \pm .24	+0.43 \pm .28
50 mg/kg	36.61 \pm .15	36.72 \pm .27	+0.11 \pm .26
250 mg/kg	36.63 \pm .14	36.52 \pm .31	-0.11 \pm .26
1000 mg/kg	36.26 \pm .14	35.32 \pm .16	-1.54 \pm .23

Food and Water ingestion. The results of food and water ingestion four hours after treatment administration (six hours of deprivation conditions) are shown in Table 16. Food ingestion was scored "+" if a subject successfully place a piece of lab chow into his mouth and began chewing it; "-" if actual chewing of the food was not observed. Water ingestion was measured only if licking of the drinking tube was observed. Water ingestion was scored "+" in milliliter quantities during the five minute access period. The percentage of subjects which either engaged in successful food or water ingestion was used for statistical analysis.

A significant Chi-square difference between group proportions was obtained for food ingestion ($p < .01$, $df = 3$). Saline subjects showed an 86% incidence of food ingestion (5 out of 7 subjects). The same incidence of food ingestion was observed for subjects given either 50 or 250 mg/kg putrescine (86%). In contrast, the 1000 mg/kg group did not exhibit any incidence of food ingestion during testing (0% subjects ingested food). High-dose putrescine subjects gave the impression that they were aware of the presence of food. Some of these subjects appeared to have some difficulty in trying to obtain food pieces from the feeders in the cage covers (overhead) than did other groups. These results indicated that subjects treated with 1000 mg/kg putrescine did not exhibit significant food intake, four hours posttreatment.

A significant Chi-square difference was also found for water ingestion ($p < .01$, $df = 3$). The percentage of subjects which drank water in the high-dose putrescine group was higher than all other

Table 16
 Food and Water Intake^a Four Hours
 Posttreatment With Putrescine (p.o.)

<u>Food Intake (+ or - score)</u>								
<u>Putrescine (mg/kg)</u>	<u>Subjects</u>							<u>%Subjects Ingested Food</u>
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	
0	+	+	+	+	+	-	+	86%
50	+	+	+	+	+	+	-	86%
250	+	+	+	+	+	-	+	86%
1000	-	-	-	-	-	-	-	0%

<u>Water Intake (ml volume if +; - if no intake)</u>								
<u>Putrescine (mg/kg)</u>	<u>Subjects</u>							<u>% Subjects Ingested Water</u>
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	
0	-	3	-	-	-	4	-	29%
50	3	2	-	-	-	-	-	29%
250	4	-	-	-	-	-	-	14%
1000	-	-	2	5	1	1	6	71%

^aFive minutes of food and water access was given simultaneously after six hours of deprivation conditions (deprivation began two hours prior to treatment).

groups. Seventy one percent of subjects receiving 1000 mg/kg putrescine showed drinking behavior, with two subjects in this group drinking 5 and 6 ml of water during the five minute access period. No more than two of seven subjects (29%) in the saline and other putrescine groups exhibited an incidence of actual water ingestion. Thus, treatment with 1000 mg/kg putrescine apparently increased the incidence of water intake, four hours posttreatment.

Linearity of dose response curves. Of 12 significant ANOVA behavioral measures, 10 measures yielded significant linear regression relationships (83%) using Pearson's r correlation coefficient at $p < .05$, or less (Table 17). Of the two categories in the time sampling protocol which showed a decline in the incidence of response due to treatment (sleep, grooming), only grooming yielded a significant Pearson's r ($p < .01$). The two behavioral categories which were associated with an increase in incidence of response due to treatment, gnawing and WDS, both yielded significant Pearson correlations ($p < .05$ and $p < .02$, respectively).

Significant Pearson correlations were also found for initial startle response intensity measured at 2-hr ($p < .01$) and 4.5-hr ($p < .05$) posttreatment. Startle response magnitude at 4.5-hr was not significant. Other behavioral measures which yielded significant correlations included open field ambulation ($p < .02$), shock response thresholds for all criteria (flinch $p < .02$, jump $p < .02$, squeal $p < .05$), and change in body temperature ($p < .02$).

Table 17

Dose Response Correlations for
Significant Behavioral Effects With Putrescine (p.o.)^a

<u>Behavioral Response</u> ^b	<u>Pearson's r</u>	<u>α Level</u>
Grooming	.991	p < .01
Sleep	.749	NS
Gnawing ^c	.965	p < .05
Wet dog shaking ^c	.982	p < .02
Initial startle response 2-hr	.996	p < .01
Startle response habituation 4.5-hr	.906	NS
Open field ambulation	.988	p < .02
Shock response flinch thresholds 5-hr	.986	p < .02
Shock response jump thresholds 5-hr	.985	p < .02
Shock response squeal	.977	p < .05
Change in body temperature	.989	p < .02

^aDose response correlations were calculated by linear regression analysis using Pearson's r correlation coefficient.

^bBehaviors analyzed were those having significant ANOVA main effects.

^cBehavioral response also having significant ANOVA treatment x time interactions.

Informal Observations

In subjects given the highest putrescine dosages (250 and 1000 mg/kg), behavioral effects were observed within five minutes post-treatment. Subjects receiving 1000 mg/kg putrescine exhibited several behaviors which were not observed in saline control subjects. These behaviors which were not quantified, included postures (laying on side with rear paws raised off of floor), movements (stretching of limbs and body), motor impairment during ambulation, respiratory difficulties (wheezing, congestion, labored breathing, tachypnea), frequent frontal grooming, paw-flicking, slow and exaggerated ambulatory activity, partial grooming sequences, yawning behavior, and a "sliding/burrowing activity" (episode duration of 10-30 seconds) characterized by extended forelimbs and pushing through sawdust in a tunneling motion. These behaviors were observed for 45 to 90 minutes following treatment administration. Hypomobility, ptosis, piloerection, hunchback posture, incidental gnawing of sawdust, and a "mastication" stereotypy (chewing motion of jaws with nothing in mouth) was also observed in the higher dose subjects, with these behaviors lasting up to three hours post-treatment.

A putrescine dose of 1000 mg/kg also produced the isolated occurrence of a "flaccid immobility" in which subjects exhibited a sedate, cataleptic like state and would appear to "freeze" in a certain non-quadrapedal postures for variable durations (5-15 minutes, or until aroused by handling). An example of this occurred during frontal/genital grooming sequences in which the subject stopped grooming

activity during mid-sequence, freezing still in a hunched-over posture with eyes closed (as if cataleptic). All putrescine-treated subjects (even those given 1000 mg/kg) would react and orient to his surroundings and appear like a saline subject upon manual handling. However, animals given higher putrescine dosages would lapse back into an apparent sedated state within two minutes after replacement into observation tubs.

A "head down" posture was often seen in subjects given 250 and 1000 mg/kg putrescine. These subjects sat in a four-paw (quadrupedal) stance with head lowered nearly to the floor. This lowered head posture was accompanied by ptosis and sedation-like tendencies which often persisted until three hours posttreatment. Subjects exhibiting this head down posture did not appear to be oriented to their surroundings. Animals receiving 50 mg/kg putrescine sometimes showed effects resembling those observed in subjects given higher dosages of putrescine, but any effects exhibited by the 50 mg/kg group were of lower intensity and shorter duration. Frequently, subjects receiving 50 mg/kg putrescine appeared similar to saline control subjects in appearance and activity.

Discussion

Oral administration of the polyamine, putrescine, was found to result in a complex of changes in the behavior of the adult male rat. During five hours of evaluation, comparison of subjects who received either saline or putrescine (50, 250, 1000 mg/kg) demonstrated that putrescine treatment was associated with statistically significant changes in motor behavior, decreased ambulation, changes in the incidence of stereotypy, decreased sensory reactivity, and depression of body temperature. Certain effects suggested that oral putrescine treatment may have influence the expression of food and water ingestive behavior. Several behavioral effects (WDS, other unusual motor behaviors) appeared to be specific to the putrescine dose of 1000 mg/kg, and were not observed in other treatment groups, including saline. Although no significant impairment of motor coordination was seen, several anecdotal behaviors may have indicated activation of peripheral ANS responses.

For the 12 behavioral measures which yielded significant ANOVA main effects, the majority (83%) were found to have reliable dose-response relationships to putrescine treatment using linear regression analysis. Significant changes in behavior were most obvious for subjects receiving 1000 mg/kg putrescine. Although the significance of most ANOVA effects were statistically attributable to the 1000 mg/kg putrescine group, the apparent dose-response nature of oral putrescine treatment suggests the existence of quantitative gradations of behavioral

change as a function of putrescine dose. Discussion of the results in relation to existing research concerned with putrescine and the polyamines, GABA, other CNS substances, and behavior, follows. Directional effects mentioned in any synopsis of treatment effects on behavior, are made in comparison to the behavior of saline-treated subjects.

Effects on Motor Behavior

Oral putrescine treatment was associated with changes in the motor behavior of rats, affecting ambulation and locomotor activity, the incidence of abnormal behaviors and stereotypy, but not coordination. Putrescine administration appeared to reduce activity levels compared to controls, and at higher dosages, seemed to produce many changes in motor behavior not observed in subjects given saline.

General activity. Measurement of open field ambulation three hours posttreatment showed that putrescine decreased ambulation in a dose-dependent manner, although the treatment effect was not time-dependent. No significant impairment of motor coordination was observed in any putrescine group at 3:45 hours posttreatment. Time sampling data showed that the incidence of rearing behavior was depressed for groups receiving higher putrescine doses at 2.5 hours posttreatment. While no between groups differences were seen for sitting and walking behaviors, the incidence of sleep over 90 minutes was reduced for subjects receiving putrescine. No dose response relationships were found for treatment effects on rearing and sleep behavior.

Stereotypy. Contrasting effects of putrescine administration on the incidence of grooming and gnawing behaviors were observed, while sniffing, non-body licking, and mastication stereotypy did not yield

significant statistical effects. A dose-dependent decrease in the incidence of grooming over 90 minutes of time sampling was observed for putrescine treatment, with saline subjects exhibiting a grooming incidence which was three-fold higher than the level of subjects given 1000 mg/kg putrescine. A dose- and time-dependent increase in gnawing behavior was seen for putrescine groups only. Subjects receiving the highest putrescine doses showed an increase in gnawing 20-30 minutes posttreatment, with the effect lasting up to one hour posttreatment.

Abnormal behavior. Time sampling results showed that a dose- and time-dependent putrescine treatment effect existed for the incidence of wet god shaking behavior (WDS), and not abnormal posture/movement, motor impairment, or head down posture. Subjects given 1000 mg/kg putrescine began to exhibit WDS episodes 40 minutes after oral treatment, with significant incidence of this behavior continuing up to three hours posttreatment (maximum incidence = 2-4 WDS/min).

Informal observations. Impressions derived from anecdotal observations during time sampling complimented statistical evidence which showed that oral putrescine treatment significantly affected motor behavior of rats. Subjects given higher putrescine doses often exhibited unusual behavioral effects not seen in saline controls, while subjects receiving 50 mg/kg putrescine sometimes exhibited comparable effects of lesser magnitude and duration. Many effects appeared to indicate some abnormal function of motor abilities, locomotor difficulties, and deficit in general activity. Observation of cataleptic-like "flaccid immobility" in some subjects given 1000 mg/kg putrescine was an additional behavior suggesting that treatment affected aspects of normal motor function.

Some behaviors informally surveyed included behaviors which appeared to be stereotypic in nature. Examples of such behaviors included excessive frontal grooming, paw-flicking, yawning behavior, sliding/burrowing behavior, and mastication stereotypy.

Motor behavior and polyamine literature. Changes in rodent and chick behavior associated with in vivo polyamine treatment are dependent on the polyamine administered and rate at which the substance increases within the brain (Anderson et al., 1975; Nistico et al., 1980; Shaw, 1972). Short latencies for polyamine increases in the brain (after i.v.t. or i.v. polyamine treatment with putrescine, spermine, or spermidine) have been associated with brief initial behavioral depression, followed by a period of behavioral excitation, and the prolonged behavioral depression (Anderson et al., 1975; Nistico et al., 1980; Shaw, 1972). Motor symptomologies characteristic of i.v.t. and i.v. polyamine administration include such effects as decreased locomotor activity, development of unusual movements (tremors, convulsions, myoclonic jerking of the body), marked postural changes, flaccid paralysis, and other changes described in the Introduction (Anderson et al., 1975; Nistico et al., 1980).

In contrast to effects on motor behavior following i.v.t. or i.v. polyamine administration, longer latencies for brain increases of polyamines after intraperitoneal injection of these substances have been associated with primarily depressant effects, and not excitatory effects (Shaw, 1972). Motor symptomologies reported following intraperitoneal injection of the polyamines, spermine and spermidine, include behavioral sedation, ataxia, hypomotility (disinclination to move), and prolonged

sedation lasting several hours posttreatment (Shaw, 1972; Shaw, 1979).

Few studies concerned with the in vivo administration of putrescine exist. The results of this study suggest that some motor effects observed following oral putrescine administration are similar to certain effects associated with cerebral (i.v.t. putrescine, spermine, spermidine) and systemic (i.v. and i.p. spermine, spermidine) polyamine administration (Anderson et al., 1975; Nistico et al., 1980; Shaw, 1972). Oral putrescine treatment was associated with the observation of primarily depressant effects on behavior, including decrement in ambulation and activity, appearance of abnormal and unusual motor behaviors, and changes in the incidence of other behaviors. No statistically significant impairment of motor coordination was observed with oral putrescine treatment. Excitatory motor effects characteristic of i.v.t. and i.v. polyamine administration (tremors, convulsions, myoclonic jerking) and death were not observed during behavioral testing following oral putrescine treatment. The motor effects seen after oral administration of putrescine (50, 250, 1000 mg/kg) most resemble the depressant behavioral changes associated with i.p. spermine and spermidine treatment in rodents.

Motor behavior and GABA literature. Manipulation of endogenous GABA activity has been accomplished by cerebral and systemic injection of GABA and GABA agonists (muscimol), and inhibition of GABA metabolism with systemic injection of GABA-transaminase (GABA-t) inhibitors. GABA is regarded as the major inhibitory neurotransmitter in the vertebrate CNS (McGeer, Eccles & McGeer, 1978), with highest GABA concentrations found in the hypothalamus, basal ganglia, colliculi, and dentate nucleus

(Leibowitz, 1980). GABA is known to exert an inhibitory influence on excitatory CNS neural activity postsynaptically, as well as presynaptically (Cattabeni, Bugatti, Groppetti, Maggi, Parenti & Racagni, 1979; Moroni, Peralta & Costa, 1979). Presynaptic inhibition of spinal cord motoneuron activity which is mediated by GABAergic interneuron function has also been reported (Naftchi, Schlosser & Horst, 1979). GABAergic activity in the striatopallidal forebrain system may also play a role in controlling posture and behavioral stimulation (Scheel-Kruger, Arnt, Broestrup, Christensen & Magelund, 1979). Thus the importance of GABAergic function for CNS maintenance of basal motor function and modification of excitatory afferent motoneuron activity has been well established. Until recently, the major effect of increasing in vivo GABA was described as the depression of motor activity (Benton & Rick, 1976; Biswas & Carlsson, 1978; Smialowski, Smialowski, Reichenburg, Byrska & Vetulani, 1980), however, many other behavioral effects have now been reported.

Behavioral depression and reduction or lack of spontaneous and induced locomotor and exploratory behavior as a result of GABAergic manipulation has been widely reported (Biswas & Carlsson, 1978; Cooper, Howard, White, Soroko, Ingold & Maxwell, 1980; Frey & Loscher, 1980; Smialowski et al., 1980). Other motor and behavioral consequences produced following enhanced GABAergic activity include bizarre movements of the legs and body, respiratory irregularities (rapid and "jerky" respiration), and loss of the righting reflex (Frey & Loscher, 1980), body and forelimb shaking, hunchback posture, and incidence of catalepsy (De Boer, Bartels, Metselaar, & Bruinvels, 1980), ptosis, incidental head twitching, grooming, bursts of locomotion, and periods of sedation

(Smialowski et al., 1980). Behaviors which are similar to those associated with opiate abstinence and withdrawal have also been seen with increased GABAergic CNS activity, including wet dog shake-like behavior, shaking of body and extremities, teeth chattering, "hopping" movements, and a writhing syndrome (De Boer et al., 1980).

Comparison of the GABAergic behavioral literature with the experimental results of this study show that oral putrescine treatment was associated with many motor and behavioral effects also described as being GABAergic effects. Behavioral similarities include the depressant effect on locomotor function and sedation, the exhibition of mutual abnormal behaviors (catalepsy, WDS-like behavior, hunchback posture, abnormal movements of body and extremities), and exhibition of some behaviors which appear to be stereotypic in nature. Although experimental subjects did not show gross motor impairment or loss of the righting reflex after oral putrescine treatment, these GABAergic effects may be similar to effects related to i.v. spermine and spermidine administration (Shaw, 1972).

Some reported GABAergic effects have stereotypic characteristics, such as head twitching, shaking, and grooming (Smialowski et al., 1980). Certain behavioral effects observed after oral putrescine treatment may be similar to these reported GABAergic stereotypies, including paw-flicking, mastication stereotypy, yawning, and gnawing behaviors. Observation of stereotypic-like behaviors suggest that oral putrescine treatment may be affecting other neurotransmitter substances either directly or indirectly, including dopamine. The interaction of GABAergic pathways with other neurotransmitters has been well accepted in biochemi-

cal and neurophysiological literature (Dray, 1980). Thus, discussion of motor behavior and other CNS substances in regards to the experimental results seems warranted.

Motor behavior and other CNS substances. Drug manipulations of central cholinergic activity have shown that nicotinic and muscarinic blocking agents tend to increase motor function, while acetylcholinesterase (AChE) inhibitors appear to decrease motor activity (deFeudis, 1974). It is generally accepted that brain catecholamines influence CNS control of motor function (Lloyd & Hornykiewicz, 1975), affecting reflex regulation of movement and posture, motor coordination and cerebellar function, and extrapyramidal mechanisms associated with motor pathologies (tremors, catalepsy, seizures) (deFeudis, 1974). Intrastriatal dopamine (DA) has a primary role in the maintenance of normal locomotor function, while noradrenaline plays a secondary role in modifying locomotion (Lloyd & Hornykiewicz, 1975). Depletion of brain catecholamines in nigrostriatal regions which control motor output results in the attenuation of spontaneous locomotion (Lloyd & Hornykiewicz, 1975) and exhibition of catalepsy (Pycock, Horton & Carter, 1978). Stimulation of DA activity in extrapyramidal systems can cause hyperexcitability and appearance of stereotypies (Pycock et al., 1978; Randrup & Munkvad, 1974).

Stereotypy has been defined as the exhibition of invariant sequences of movements in a repetitive manner (Fray, Sahakian, Robbins, Koob & Iverson, 1980), and as a characteristic feature of a pattern of behavior which appears aimless and lacks variation (Randrup & Munkvad, 1974). Behavioral elements of rodents which are manifested as stereotypic activity include sniffing, rearing, licking, grooming, and gnawing (Fray et al.,

1980). Stereotypic locomotion is thought to be mediated by DA mechanisms, although such mechanisms may be different from those which influence the expression of stereotypic behaviors that do not involve gross locomotor activity (Lloyd & Hornykiewicz, 1975).

Serotonin (5-hydroxytryptamine, or 5-HT) is a transmitter substance which causes an inhibitory response in neurons receiving 5-HT input, and which exerts influence on central DA function (Pycock et al., 1978). Reduction of brain 5-HT by lesioning of the median raphe nucleus results in increased locomotion (Steranka & Barret, 1974), cerebral injection of 5-HT into discrete brain regions can abolish DA-related hyperactivity, while facilitation of 5-HT activity can enhance catalepsy and potentiate stereotypy produced by DA agonist treatment (Pycock et al., 1978).

Opiate peptides (enkephalin and endorphin) can inhibit spontaneous neuronal firing, firing induced by glutamate, and nociceptive (analgesic) nerve stimulation (Beaumont and Hughes, 1979). In hippocampal pyramidal cells and spinal primary afferent neurons, opioids elicit excitatory responses resulting from attenuation of GABAergic input (disinhibition of inhibitory interneuron excitability) at presynaptic and/or postsynaptic sites (Beaumont & Hughes, 1979; Nicoll, Alger & Jahr, 1980). Opioids are known to inhibit acetylcholine and norepinephrine release in the peripheral nervous system, while inhibiting dopamine, acetylcholine, norepinephrine, and substance P release in the CNS (Beaumont & Hughes, 1979). In rats, in vivo administration of beta-endorphin causes facilitation of passive behavior, decrease in general motor activity, excessive grooming, abnormal sexual behavior, stimulation of food intake, rigid catatonia, loss of righting reflex, and appearance of wet dog

shaking (Beaumont & Hughes, 1979). Dose response profiles of beta-endorphin treatment have shown that low doses (5 μ g i.v.t.) cause initial hyperactivity, oral stereotypies, and WDS behavior, followed by muscular rigidity of short duration (20 min) and then hyperactivity (without WDS) (Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling & Guillemin, 1978). Higher endorphin doses (50 μ g i.v.t.) cause a brief period of WDS behavior within 30 minutes posttreatment, followed by muscular rigidity (accompanied by stiff tail) and immobility (catatonia) lasting up to four hours (Bloom et al., 1978).

Review of recent behavioral research allows the tentative suggestion that some effects of oral putrescine treatment are related to the function of various CNS substances. Decrement in ambulation and locomotor activity after putrescine treatment may be related to changes in motor behavior resulting from increased cholinergic activity, depletion of catecholamines, enhanced 5-HT function, or changes in endogenous opiate peptide levels. The observation of stereotypic behavior following oral putrescine treatment (gnawing, and anecdotally-observed mastication, frontal grooming, paw flicking) may be related to behaviors seen with direct or indirect stimulation of the nigrostriatal DA system (Wallach, 1974). Catecholaminergic-specific stereotypies have been shown to be modifiable by other CNS substances, including noradrenaline (Lassen, 1977), serotonin (Kutscher & Yamamoto, 1979), opiates (narcotic analgesics) (Langwinski & Niedzielski, 1980), and opiate peptides (Bloom et al., 1978).

Studies have shown that stimulation of DA function using different DA agonist manipulations (d-amphetamine, apomorphine) results in differ-

ential behavioral profiles, including dose- and time-dependent variations in locomotor and stereotypic activities (Fray et al., 1980). In this study, grooming behavior was significantly decreased, while other stereotypies apparently increased in incidence. This result is not inconsistent with data which has demonstrated that amphetamine-induced grooming may be mediated by noradrenaline release which accompanies the increase in DA turnover due to amphetamine treatment (Lassen, 1977). Thus, noradrenaline activity may have specifically decreased grooming following putrescine treatment, while still allowing expression of other DA-related effects. Such interpretive consistencies seem important, especially since functional interactions between cholinergic, DA, and GABA neurons in the extrapyramidal system appear to exist (Ferkany & Enna, 1980).

Anecdotal observation of experimental subjects gave the impression that oral putrescine treatment produced motor abnormalities and deficits, although only the incidence of WDS behavior was statistically related to treatment. WDS behavior has been reported following many antecedent conditions (Bedard & Pycock, 1977; Drust, Sloviter & Connor, 1979), including morphine and opiate withdrawal, injection of narcotic antagonists, systemic administration of opiate peptides (beta-endorphin) (Beaumont & Hughes, 1979; Bedard & Pycock, 1977; Bloom et al., 1978), and cerebral administration of melanocyte-stimulating hormone (MSH) (Yamada & Furukawa, 1981). Recent studies favor an opiate-related mechanism for WDS behavior that may involve a kappa-opiate receptor (Drust, Sloviter & Connor, 1981; Lanthorn, Smith & Issacson, 1979; Turski, Turski, Czuczwar & Kleinrok, 1981). Thus, it is possible that putrescine-induced WDS

behavior is related to the activation of endogenous opiate peptide activity as a result of putrescine treatment.

Other abnormal behavioral consequences were anecdotally-observed following putrescine treatment, including flaccid immobility, yawning behavior, head down posture, and unusual movements (stretching/burrowing). Behavioral states of immobility include flaccid-catalepsy associated with neuroleptic treatment, and rigid-catatonia which accompanies in vivo opiate manipulations (narcotic analgesic or opiate peptide administration) (Beaumont & Hughes, 1979; Costall & Naylor, 1974; Myslobodsky & Mintz, 1981). Both catalepsy and catatonia have been associated with cerebral DA function (Costall & Naylor, 1974) and involvement of extra-pyramidal mechanisms (Myslobodsky & Mintz, 1981), although the DA-systems responsible for each type of immobility may be distinct, or subject to mediation by different CNS substances (Costall & Naylor, 1974; Bloom et al., 1978). Involvement of cholinergic systems in the expression of neuroleptic-induced catalepsy, and not for morphine/opiate-induced catatonia, suggests that flaccid immobility observed after putrescine treatment may be similar to the flaccid-catalepsy associated with haloperidol treatment. This interpretation seems reasonable since substantia nigra pars reticulata DA and GABA neurons appear to be essential for exhibition of haloperidol-induced catalepsy (Morelli, Porceddu, Imperato & Di Chara, 1981).

Stretching and yawning syndrome (SYS) has been reported in rodents following the cerebral and systemic administration of various drugs. Cerebral injection of adreno-corticotrophic hormone (ACTH) (Rees, Dunn & Iuvone, 1976) or melanocyte-stimulating hormone (MSH) (Yamada & Furukawa,

1981) have been reported to induce significant SYS in rats. SYS has also been observed after i.p. injection of glutamate di-ethyl ester (GDEE, an antagonist of glutamate and aspartate receptors) (Lanthorn & Issacson, 1979), apomorphine and piribedil (DA agonists), and cholinergic agents, physostigmine (antagonist) and pilocarpine (agonist) (Yamada & Furukawa, 1980, 1981). While some studies have suggested that SYS involves glutamate-mediated CNS mechanisms (Lanthorn & Issacson, 1979), more recent evidence shows that a neural link involving the reciprocal balance of "serotonergic activation-DA inhibition-cholinergic activation" mechanisms may mediate SYS behavior (Yamada & Furukawa, 1981). The anecdotal-observation of stretching and yawning behavior in subjects treated with high oral doses of putrescine may be related to SYS behavior associated with manipulations of CNS substances, including serotonin, DA, glutamate, and cholinergic transmitters. This SYS-like behavior could also be related to the exhibition of gustatory mimetic responses produced by oral reactivity to putrescine treatments (Berridge, Grill & Norgren, 1981).

Observation of head down posture in rats following systemic administration of the DA agonists, d-amphetamine and apomorphine, suggest that this behavior is a sensitive indicator of facilitated dopaminergic function (Fray et al., 1980). Although d-amphetamine and apomorphine produce differential stereotypy profiles, treatment with either substance causes significant dose-dependent head down posture in rats (Fray et al., 1980). Thus, extended anecdotal observation (up to three hours posttreatment) of head down behavior in some subjects given higher putrescine doses may indicate stimulation of DA activity as a result of putrescine treatment.

A "stretching response" has been associated with activity of visceral afferent neurons and tonic contractions of abdominal, back, and limb musculature caused by stimulation of abdominal and pelvic viscera (Downman & McSwiney, 1946; Eble, 1960). This marked response is accompanied by elongation of the body, extension of hind limbs, indentation of abdominal wall, and has been reported to occur in female rats when induced by vagino-cervical stimulation (Naggar, Toner & Adler, 1980). Whether the response is due to direct viscerosomatic reflex or activity in other visceral organs (Naggar et al., 1980), the experimentally-observed stretching postures seen in this study appear to resemble literature reports of this visceral behavior.

Paw pushing activity (simultaneous and alternating extension of one forelimb and retraction of the other in a rubbing motion), chin rubbing (lowering head and bringing mouth in contact with floor with body projection forward by flexion of neck, limb musculature), and limb flailing (rapid, alternating flailing of paws immediately after face washing) are gustatory mimetic responses characteristic of oral reactivity to specific taste stimuli (Grill & Norgren, 1978). While putrescine treatment-induced paw flicking behavior is similar to limb flicking associated with face washing sequences, head down posture is not similar to chin rubbing, and sliding/burrowing behavior is distinctly different from descriptions of paw pushing responses related to oral reactivity and taste stimuli. Sliding/burrowing behavior was a marked response which did not resemble any activity exhibited by subjects treated with saline, and was only seen in subjects given 1000 mg/kg putrescine.

Motor behavior: summary. Putrescine treatment effects related to

motor function were found to be similar to behavioral changes reported after i.p. polyamine (spermine and spermidine) treatment in rats. These predominantly depressant effects included deficits in locomotor ability and ambulation, appearance of unusual postures and movements, and observation of stereotypies, but no excitatory behavioral effects were seen, such as those associated with i.v. or i.v.t. polyamine administration. Review of GABA behavioral literature suggests that many putrescine-related motor effects resemble behaviors reported to be GABAergic in nature. Certain experimental effects also support the interpretation that putrescine treatment may be directly or indirectly producing motor consequences related to the function of other CNS substances, including the catecholamines (dopamine and noradrenaline), central cholinergic activity, endogenous opiate peptides, and possibly serotonin. Finally, consideration of some anecdotally-observed behaviors suggest that oral administration of putrescine may affect glutamate CNS activity, stimulate visceral responses of the musculature, provoke gustatory mimetic responses characteristic of oral reactivity, or produce motor effects which may be unique to systemic putrescine treatment.

Effects on Sensory Reactivity

Oral putrescine treatment was associated with changes in apparent sensory reactivity of rats, affecting startle response to acoustic stimuli and electric shock response thresholds. Putrescine administration appeared to depress acoustic startle response, while producing increased pain response thresholds to electric shock.

Initial startle intensity. Measurement of initial startle response magnitudes showed that putrescine treatment resulted in significant

depression of initial startle response at two and 4.5 hours posttreatment. Although no between groups differences existed prior to treatment, this effect was found to be dose-dependent by linear regression analysis.

Startle response magnitude. Measurement of startle magnitude to acoustic stimuli at two hours posttreatment did not yield a statistically significant treatment effect. However, at 4.5 hours posttreatment, an ANOVA main effect was obtained, showing that 1000 mg/kg putrescine produced significantly larger change in startle magnitude compared to baseline. This treatment effect was not dose-dependent.

Shock response thresholds. Measurement of shock response thresholds at five hours posttreatment for flinch, jump, and squeal criteria showed that putrescine significantly increased pain response thresholds for all criteria compared to saline controls. Linear regression analysis found that this apparent analgesic effect due to putrescine administration was also dose-dependent for all criteria. Existence of residual analgesic-like effect for subjects given 1000 mg/kg putrescine was observed 21 hours posttreatment, indicated by a significant ANOVA main effect obtained for flinch response only.

Informal observations. Subjects treated with 250 or 1000 mg/kg putrescine gave the impression that sedation tendencies resulted in these subjects as a result of oral treatments. Characteristics of this sedated state included a lack of appearing oriented to surroundings, however, all putrescine subjects would react and behave in a manner similar to saline controls when subjected to handling. When left undisturbed after handling, these subjects would lapse back into an apparent sedated state. Anecdotal impressions of these behaviors suggest that

putrescine treatment was associated with some degree of decreased behavioral reactivity to stimulation. Such impressions seem consistent with the experimental results obtained while testing startle response and shock response thresholds.

Sensory reactivity: polyamine and GABA literature. Observation of behavioral sedation, attenuated motor activity, and other depressant effects associated with systemic polyamine and GABAergic manipulations have already been discussed. While the polyamine behavioral literature does not consider effects upon acoustic startle response, there has been indirect evidence that GABAergic mechanisms are important to startle response reflex. It has been reported that benzodiazepine administration (diazepam, flurazepam) prior to testing, can cause a dose-dependent reduction of potentiated startle effect (acoustic startle reflex augmented by conditioning where eliciting stimulus is paired with a cue previously paired with aversive shock) (Davis, 1979). This data is important since benzodiazepines apparently exert their effects by facilitating GABAergic transmission (Costa & Guidotti, 1978), while also enhancing presynaptic inhibition in the spinal cord (Haefely, Polc, Schaffner, Keller, Pieri & Mohler, 1979).

Acoustic startle reflex is a motor behavior which is manifested through activity of spinal motor neurons (Astrachan & Davis, 1981). Since GABA is the putative inhibitory neurotransmitter in the spinal cord, and is known to mediate spinal motoneuron activity (Naftchi et al., 1979), decrement in acoustic startle response is an effect consistent with the facilitation of GABAergic inhibitory function in the spinal cord. Thus, the reduced magnitude of initial startle and significant

change in startle magnitude effects associated with oral putrescine treatment may be related to behavioral effects mediated by spinal GABAergic activity.

While the polyamine literature does not cite specific analgesic effects produced by systemic or cerebral polyamine administration, it has been reported that polyamine treatment can pr-long barbituate-induced sleep in rodents (Anderson, Crossland & Shaw, 1975). In contrast, GABA literature suggests that GABAergic mechanisms may be important to antinociceptive (analgesic) responses. In addition to the well-known anti-convulsant and anxiolytic (anti-anxiety) effects related to increased GABAergic activity, elevation of brain GABA also results in antinociceptive effects in rats following systemic administration of GABA-t inhibitors (gamma-vinyl GABA) (Buckett, 1980). This analgesia, measured by hot-plate test for paw flick and escape latencies, correlated well with temporal increases in brain GABA, and was attenuated by treatment with the GABA antagonist, bicuculline (Buckett, 1980). Analgesia produced by GABA-t inhibitors has also been reported to enhance morphine analgesia, which indicates a possible relationship between some opiate effects and GABAergic function (Buckett, 1980; Contreras, Tamayo & Quijida, 1979). Thus, the apparent analgesic effects (increased shock response thresholds) observed after oral putrescine treatment may be related to GABAergic analgesia.

Sensory reactivity and other CNS substances. Startle reflex to acoustic stimuli is regarded as a useful behavioral measure for evaluating drug effects on sensory-motor activity (Astrachan & Davis, 1981), with the primary startle neural circuitry organized in the brain stem

and spinal cord (Davis, 1980). In a review paper of neuromodulation and drug effects on startle reflex, it was reported that literature pertaining to 50 drugs supported the conclusion that dopamine, serotonin, noradrenaline, and acetylcholine may not be involved in neural circuitry which directly mediates startle reflex (Davis, 1980). Instead, these neurochemicals may be important in modulating startle response mechanisms (Davis, 1980).

Davis (1980) proposed a theory which suggested that serotonin exerts a dual-antagonistic effect on acoustic startle, where forebrain 5-HT has a tonic inhibitory effect, and spinal 5-HT has an excitatory effect (Davis, 1980). Recent data has shown that 5-HT and noradrenaline applied directly onto the spinal cord increases acoustic startle reflex, although DA and the DA agonist, apomorphine, applied in a similar manner did not (Astrachan & Davis, 1981). Depending on the balance between the two 5-HT systems influencing startle reflex, changes in 5-HT activity can either increase, decrease, or have no effect on startle (Davis, 1980).

The modulating effects of other CNS substances on startle show that increased dopamine transmission increases startle, noradrenaline may exert an excitatory effect on startle, and acetylcholine plays a minor or indirect role in modulating startle (Davis, 1980). Opiate studies have indicated that systemic morphine treatment slightly depresses startle, but morphine produces explosive startle responses when given directly into central grey matter, or is injected by i.v.t. route (Davis, 1980). While no drug has been shown to prevent within-session startle habituation, clonidine markedly increases within-session habituation, but probably not by exclusive action as a noradrenaline agonist (Davis,

1980). It has been proposed that some region within or close to periaqueductal grey exerts tonic inhibitory effects on startle reflex, and possibly controls general stimulus reactivity of the CNS (Davis, 1980). Consideration of the experimental results prompt the notion that oral putrescine treatment may be affecting CNS systems which mediate or modulate startle reflex, indicated by the reduction of initial startle intensity and increased startle magnitude associated with treatment.

Various assessment techniques have been used to measure nociceptive responses to painful stimuli, including the flinch-jump response to electrical shock (with ascending and/or descending stimulus presentations) (Evans, 1961; Lints & Harvey, 1969; Spiaggia, Bodnar, Kelly & Glusman, 1979), and the hot-plate method measuring analgesic responses such as paw licking, escape behavior, tail flick, and jumping (latencies for observation of criterias) (Ankier, 1974; Buckett, 1980; Filibeck, Castellano & Oliverio, 1981; Woolfe & Macdonald, 1944; Zetler, 1980). These assessment methods have allowed investigation of CNS mechanisms responsible for analgesia, and have shown that many CNS substances can produce nociceptive responses.

Narcotic analgesics are opiates which decrease sensitivity to painful stimuli, including opium alkaloids and purified derivatives (morphine), and synthetic opiates (levorphanol, methadone) (Buxbaum, Yarbrough & Carter, 1973). Endogenous opiate peptide activity (endorphin) has been linked to the mediation of stressor-induced analgesia, since beta-endorphin and adreno-corticotrophic hormone (ACTH) are released by the pituitary following presentation of painful challenge or severe trauma (Rossier, Guillemin & Bloom, 1977; Spiaggia et al., 1979).

Recent evidence has suggested that distinct behavioral and analgesic patterns exist for exogenous (morphine) and endogenous (endorphin) opioid activity in mice, related to strain differences, receptor characteristics, or actions of agonists-antagonists (Filibeck, Castellano & Oliveri, 1981). Different endogenous opiate peptide systems have also been shown to mediate analgesia and catalepsy in rats, demonstrating that these two systems possess different neuropharmacologic characteristics, and the possibility that other non-opiate systems may underly the differential expression of opiate peptide-related analgesia and catalepsy (Urca, Yitzhaky & Frenk, 1981).

It has been suggested that 5-HT is involved with nociceptive CNS mechanisms, although some controversy has existed in the literature concerning 5-HT and its exact influence on analgesia (Harvey & Yunger, 1973; Tenen, 1968). The catecholamines have also been shown to play some role in opiate-induced analgesia, since haloperidol can increase morphine-analgesia, but does not cause analgesia alone (Eidelberg & Erspamer, 1975). Tricyclic antidepressants (amitriptyline, nortriptyline) have been shown to enhance morphine-induced analgesia, and such effects may be associated with alteration of noradrenaline and 5-HT CNS function, and interference with cholinergic activity (Malseed & Goldstein, 1979). Thus, from a brief literature review of nociceptive literature, it is plausible that apparent analgesic effects observed five hours after oral putrescine treatment, are related to analgesia associated with endogenous opiate peptide activity, and possibly serotonergic and catecholaminergic CNS function.

Sensory reactivity: summary. Putrescine treatment was found to

affect startle response reflex in a way which is consistent with GABAergic influence on the expression of startle in the spinal cord. Putrescine administration significantly reduced initial startle intensity in a dose-dependent manner, and increased startle magnitude in a way which was not dose-dependent according to linear regression analysis. While experimentally-observed effects on startle response may be related to the neuromodulating function of other CNS substances on startle (5-HT, catecholamines, opiates), the exact relationship between putrescine and these neurochemicals is not specifiable at present. However, putrescine treatment may be exerting some action on a region near periaqueductal grey which apparently exerts tonic inhibition on startle reflex, and may control general behavioral reactivity to stimulation. The significant dose-dependent analgesia to electric shock produced by putrescine five hours posttreatment, may be related to nociceptive effects observed in conjunction with facilitated GABA activity and brain levels following treatment with GABA-t inhibitors. Analgesia observed after oral putrescine treatment may also be associated with nociceptive systems involving endogenous opiate peptide function, other CNS substances such as 5-HT, and the catecholamines. Treatment-induced effects on startle response, manifestation of analgesia and informal observation of sedation suggests that oral putrescine administration may produce depressant effects on sensory-motor reactivity, while affecting CNS systems which mediate or modulate these behavioral patterns. Past research concerned with acoustic startle reactivity and analgesia appear to support such a proposition.

Body Temperature and Other Behaviors

Oral putrescine treatment was associated with a dose-dependent

depression of body temperature occurring five hours posttreatment. Putrescine treatment also appeared to suppress food intake while increasing water intake following six hours of deprivation conditions (four hours posttreatment). Informal observation of certain behaviors suggested that putrescine treatment activated autonomic nervous system (ANS) responses.

Body temperature. While no significant between-groups differences existed prior to treatment, the change in body temperature five hours posttreatment yielded a significant main effect of putrescine treatment. While notable hypothermia was only observed in subjects given 1000 mg/kg putrescine, decline in body temperature due to putrescine treatment was found to be dose-dependent compared to controls.

Food and water ingestion. Evaluation of food and water intake following six hours of deprivation conditions (four hours posttreatment) showed that a lack of significant food intake and increase in water intake resulted from treatment with 1000 mg/kg putrescine. All other putrescine groups were similar to saline subjects in ingestive behavior during the five minute, simultaneous-access presentation of food and water.

Informal observations. Decline in body temperature produced by putrescine treatment was accompanied by other behaviors which suggested the activation of autonomic nervous system function. Observation of ptosis, piloerection, and sedation alluded to this possibility, especially since these symptomologies were predominant in subjects given 1000 mg/kg putrescine (subjects which had marked hypothermia).

Body temperature and CNS substances. Systemic and cerebral admin-

istration of polyamines have been reported to produce significant hypothermia in mammals and chicks (Anderson et al., 1975; Nistico et al., 1980; Shaw, 1972). Hypothermic responses have been characteristic of intraventricular injection of putrescine in rodents (Anderson et al., 1975) and chicks (Nistico et al., 1980), as well as intraventricular and systemic (i.v. and i.p.) administration of spermine and spermidine in rodents (Anderson et al., 1975; Shaw, 1972). Results of Experiment 1 showed that oral putrescine treatment was associated with a slight hypothermic response compared to saline controls, which was dose-dependent. These results were consistent with previous reports of hypothermia produced by in vivo polyamine administration.

The anterior and posterior regions of the hypothalamus are known to be important to mammalian thermoregulatory mechanisms which mediate body heat production and heat dissipation (Myers, 1980). The anterior hypothalamus seems to function as a sensory integrator for thermal information from peripheral and localized thermoreceptors (located in the rostral and anterior hypothalamus) (Myers, 1980). In contrast, the posterior hypothalamus appears to be relatively insensitive to thermal displacement. The posterior region seems to process convergent efferent input from other CNS structures and apparently mediates functional effector responses involving metabolic and autonomic nervous system activity (Myers, 1980).

Research investigating the role of serotonergic thermoregulatory mechanisms have demonstrated that i.v.t. injection of low 5-HT doses results in dose-dependent hyperthermia in rats, while higher 5-HT doses can produce hypothermia (Crawshaw, 1972; Myers, 1980). A body of

research has supported the notion that 5-HT in the anterior hypothalamus controls body heat production (Myers, 1980). Catecholamine activity in the periphery has been regarded as the major response to cold stress (Fregly, Field, Nelson, Tyler & Dasler, 1977), and includes noradrenaline-mediation of nonshivering thermogenesis and colorigenic responses related to brown adipose tissue (Myers, 1980). Noradrenaline injection into the rostral hypothalamus of rats has caused dose-dependent hypothermia, while injections into the anterior hypothalamus can induce hyperthermia (Myers, 1980).

Although the neurochemical role of dopamine in thermoregulation has been difficult to demonstrate, recent studies have shown that discrete injection of DA into specific hypothalamic sites results in dose-dependent hypothermia (Ruwe & Myers, 1978). Some DA-active sites do not overlap with other catecholaminergic sites, suggesting that different catecholamine receptor types may activate heat loss, or inhibit heat production (Ruwe & Myers, 1978). In the rat, it has been demonstrated that the catecholamines mediate hypothermia in the anterior hypothalamus (Myers, 1980).

Application of acetylcholine (ACh) or cholinomimetic agents (carbachol) into the CNS apparently induces hyperthermia in rats (Tangri, Bhargava & Bhargava, 1975). Acetylcholine may also have a role in the mediation of hypothermia in rodents, although this notion is awaiting further substantiation (Myers, 1980). Other data have suggested that endogenous opiate peptides (endorphins) may produce hyperthermia following stressful stimuli (continuous foot shock) (Millan, Pzewlocki, Jerlicz, Gramsch, Holt & Herz, 1980). However, previous reports have shown that

cerebral beta-endorphin administration results in hypothermic responses in rats (Beaumont & Hughes, 1979).

Intraventricular injection of the polyamines, spermidine and spermine, have been found to be as potent as serotonin in producing hypothermia in rodents (Anderson et al., 1975), and comparatively higher concentrations of these polyamines are found in hypothalamic tissues after i.v.t. injection than serotonin. Although oral putrescine treatment resulted in measurable hypothermia, this temperature effect may, or may not be related to direct consequences in hypothalamic thermogenesis. Change in colonic temperature following the systemic administration of any drug does not allow the conclusion that the drug is affecting neurons in the hypothalamus or other brain stem regions (Lin, 1978). Many actions of the ANS mediate secondary responses for thermoregulation (Myers, 1980), and peripheral effects following oral putrescine treatment may also be influencing the appearance of hypothermic responses.

Food and water ingestion and CNS substances. Significant incidence of anorexia and adipsia have been produced in rodents following the i.v.t. injection of putrescine, spermine, and spermidine at doses lower than those which produce observable changes in motor behavior (Anderson et al., 1975). The immediacy of anorexic effects observed after i.v.t. polyamine administration have been linked to direct effects on hypothalamic mechanisms which mediate food and water ingestion (Anderson et al., 1975). Long term decline of ingestive behavior after i.v.t. polyamine administration may be due to general behavioral malaise and the development of complications (paralysis) caused by treatment, and which can result in secondary weight loss and hypothermia (Anderson et al., 1975). It may

be possible that the lack of significant food intake in subjects receiving an oral putrescine dose of 1000 mg/kg was similar to anorexia associated with i.v.t. polyamine administration. The tendency for 1000 mg/kg putrescine subjects to show increased water intake compared to controls, was in contrast to adipsia reported after i.v.t. injections of polyamines.

The hypothalamus does not control feeding and drinking behavior in an autonomous manner, but is part of a "whole-brain circuitry" which influences the expression of these specific responses (Leibowitz, 1980). The mediation of energy and fluid homeostasis is known to involve the function and integration of many neurochemical substances, including the biogenic amines, amino acids, and peptides. Monoaminergic neurons of the hypothalamus, which are rostral or at the level of the ventromedial nucleus and which contain dopamine, epinephrine, norepinephrine, or serotonin, apparently modulate and control feeding behavior (Leibowitz, 1980). Manipulations of catecholaminergic activity have shown differential effects on feeding behavior dependent on site of drug injection, type of drug used, and peripheral or cerebral routes of administration (for further explanation and detail, see Leibowitz, 1980, review article).

The hypothalamic control of fluid intake and drinking behavior has been shown to involve neurons containing acetylcholine and possibly histamine, especially those within hypothalamic structures or regions adjacent to the third ventricle (Leibowitz, 1980). More recent evidence has suggested that brain peptides (prostaglandin Es, cholecystokinin, caerulein), opiate peptides, and dopamine may be other substances which are important in the CNS regulation of drinking behavior (Dourish &

Cooper, 1981; Leibowitz, 1980; Sanger, 1981). Studies which have investigated the action of opiate peptides on energy and fluid homeostasis have suggested that endogenous opiates have an important role in controlling appetite and the expression of ingestive behavior (Sanger, 1981).

Subcutaneous administration of the opiate antagonist, naloxone, has resulted in a dose-dependent suppression of water intake in rats which were water-deprived for 24 hours (Brown & Holtman, 1981). Administration of morphine, beta-endorphin, enkephalin analogues, and other opiate receptor agonists has resulted in increased food intake (Beaumont & Hughes, 1979; Sanger & McCarthy, 1981), and variable increase in water intake (Sanger & McCarthy, 1981). Other studies have shown that brain opiates can reduce feeding behavior (King, Kastin, Olson & Coy, 1979). Reported differences in the direction of effect on ingestive behavior with endogenous opiate manipulations, may be the result of differential action of opiate drugs on different opiate receptor subtypes, general depressant action of opiates, or changes in ingestive behavior after tolerance to opiate effects develops over time (Sanger, 1981).

Data has indicated that GABA and its synthesizing enzyme, glutamic acid-decarboxylase (GAD), are found at varying concentrations within hypothalamic cells located in the anterior and preoptic areas, dorsomedial nucleus, ventromedial nucleus, paraventricular nucleus, and other hypothalamic regions (Leibowitz, 1980). Previous research has suggested that GABAergic mechanisms may influence the regulation of hunger, satiety, and feeding in rats (Cattabeni, Maggi, Monduzzi, Deangelis & Racagni, 1978; Kuriyama & Kimura, 1976). This GABAergic influence on ingestion

occur via short interneurons which modulate the activity of monoaminergic transmitters, or other metabolic signals (Leibowitz, 1980). Research has also shown that drug manipulations of GABAergic activity within different CNS structures and hypothalamic regions can result in differential effects on feeding behavior (Grandison & Guidotti, 1977; Kelly, 1978; Morley, Levine & Kneip, 1981).

Injection of the GABA agonist, muscimol, into the ventromedial hypothalamic nucleus of satiated rats has been reported to result in feeding responses which could be blocked by injection of the GABA antagonist, bicuculline (Grandison & Guidotti, 1977). These findings have been replicated by others and extended by the discovery of an antagonistic GABAergic feeding effect in different hypothalamic regions (Kelly, 1978; Leibowitz, 1980). Injection of GABA or muscimol into the medial hypothalamus has been shown to increase feeding, while injection of the GABA antagonists, bicuculline or picrotoxin, inhibited feeding (Kelly, 1978). However, contrasting effects were observed after similar treatments in the lateral hypothalamus, where injection of muscimol into this area inhibited feeding, and injection of bicuculline stimulated feeding (Kelly, 1978).

Cerebral and systemic (i.p.) administration of the GABA-t inhibitor, ethanolamine-O-sulfate (EOS), or muscimol, have been reported to cause long-lasting and dose-dependent decreases in food consumption and body weight (Cooper, Howard, White, Soroko, Ingold & Maxwell, 1980). Recent evidence has shown that dose-dependent feeding behavior can be induced in satiated rats following the i.v.t. administration of muscimol (Morley et al., 1981). This muscimol-induced feeding was found to be inhibited

by several drugs, including naloxone, haloperidol, atropine (cholinergic antagonist), and calcitonin (a potent appetite suppressant found in the hypothalamus) (Morley et al., 1981). Morley et al. (1981) have proposed a model of hypothalamic regulation of appetite, in which DA-enkephalin-ergic mechanisms (lateral hypothalamus) initiate feeding; serotonergic-cholecystokinin and adrenergic-thyrotropin releasing hormone (TRH) mechanisms (medial hypothalamus) exert inhibitory influences on feeding; and, GABAergic mechanisms can stimulate food intake by decreasing the serotonergic inhibitory effect on feeding (Morley et al., 1981).

Review of research concerning CNS substances and ingestive behavior has indicated that definitive understanding of CNS mechanisms responsible for regulating ingestive activity is presently lacking. The apparent non-exhibition of food intake and increase in the incidence of water intake following oral administration of 1000 mg/kg putrescine, may reflect some effect of treatment which indirectly influences CNS regulation of ingestive behavior, either via GABAergic inhibitory effects, or other CNS substances. The possibility that putrescine may directly influence ingestive behavior, via peripheral or CNS effects, also exists. Apparent behavioral sedation and other effects accompanying treatment with 1000 mg/kg putrescine may have inhibited the incidence of feeding behavior in these subjects. The observation of increased incidence of water intake in high-dose putrescine subjects may reflect effects resulting from oral treatment itself (taste, coating of oral cavity), and may have also precluded the exhibition of feeding activity. The lack of proper control groups which were naive to experimental proceedings has made the results and interpretations of ingestive testing preliminary and

tentative.

Other behaviors and the autonomic nervous system. The hypothalamus is the subcortical center which regulates many visceral activities and some somatic functions, including the control of autonomic nervous system responses of the brainstem and spinal cord (Myers, 1980). The anterior hypothalamus controls excitatory parasympathetic (inhibitory to sympathetic) responses, while the posterior hypothalamus has an excitatory sympathetic role, mediating such sympathetic effects as increases in heart rate and blood pressure, constriction of cutaneous blood vessels, decrease in motility, peristalsis, and exhibition of piloerection and ptosis (Seiden & Dykstra, 1977). Cholinergic and catecholaminergic neurotransmission are known to be fundamental to ANS function, while the role of GABA as the putative transmitter in the spinal cord and its inhibitory influence on synaptic activity is evidence of GABA's importance to ANS function (Haefely et al., 1979; Naftchi, 1979).

The observation of behavioral effects which may be categorized as reflecting activation of autonomic responses (ptosis, piloerection, sedation) have been reported following cerebral and systemic polyamine administration (Anderson et al., 1975; Nistico et al., 1980; Shaw, 1972), elevation of GABA levels (Buckett, 1980; De Boer et al., 1980; Smialowski et al., 1980), and manipulations of endogenous and other opiates (Beaumont & Hughes, 1979; Loh & Li, 1977; Zetler, 1980). Ptosis, piloerection and sedation associated with oral putrescine treatment may indicate direct or indirect activation of autonomic responses in the periphery. Hypothermia may produce sedation and piloerection (Lessin & Parkes, 1957), since sedation and enhancement of barbiturate sleeping

time does not occur after systemic polyamine administration in the absence of hypothermia (Shaw, 1972). However, it could be possible that these apparent autonomic behaviors produced by oral putrescine treatment are actually centrally-mediated. This is because only slight hypothermia was observed with administration of 1000 mg/kg putrescine, and the fact that most treatment-induced behaviors (including ptosis, piloerection, sedation, and even wet dog shakes) could be temporarily reversed by manual handling. Reversal of central opiate peptide- and neuroleptic-induced ptosis by exteroceptive stimulation (handling) is thought to indicate central rather than peripheral mediation of such behaviors (Tedeschi, 1967; Zetler, 1980).

Body temperature and other behaviors: summary. Oral administration of putrescine was found to depress body temperature and apparently inhibit food intake in a manner which was consistent with literature reports of similar effects produced by cerebral and systemic polyamine administration. These effects on temperature and food ingestion were most prominent for subjects receiving 1000 mg/kg putrescine, and also included an increase in the incidence of water intake. Marked hypothermia was not seen in all putrescine groups, although the depression of body temperature was found to be dose-dependent by linear regression analysis. Changes in ingestive behavior appeared to be unique to the 1000 mg/kg putrescine dosage. Observation of treatment-related behaviors which were characteristic of ANS peripheral activation included ptosis, piloerection, and sedation. It is possible that these anecdotally-recorded behaviors were centrally-mediated since they were not seen in conjunction with extreme hypothermia, and were also reversible by

exteroceptive stimulation.

Thermogenesis, regulation of ingestive behavior (energy and fluid homeostasis), and ANS activity are known to be influenced by hypothalamic mechanisms involving the function of and interaction between numerous neurochemical substances. Although GABAergic mechanism within the hypothalamus and CNS may play a role in regulation of temperature, feeding and drinking, and ANS activity, the suggestion that oral putrescine treatment may indirectly affect such physiological functions is tentative. Other factors may have contributed to the expression of putrescine-related effects on temperature, ingestive behavior, and visceral-somatic responses, including direct putrescine influence on peripheral or CNS mechanisms, effect specific to oral treatment, or artifactual data due to the inaccuracies of anecdotal observation and the lack of proper control groups for comparison (ingestive data).

General Conclusions

The results of Experiment 1 have shown that oral administration of putrescine in adult male rats was associated with statistically significant behavioral effects. Treatment-related effects included changes in motor behavior, depression of ambulation, attenuation of sensory reactivity, depression of body temperature, and possibly changes in ingestive behavior and activation of autonomic nervous system function. Of 12 behavioral measures which yielded significant ANOVA main effects, 83% were found to show reliable dose-dependency for putrescine treatment. It was suggested that oral putrescine administration may exert direct or indirect influences on the expression of several behaviors. However, the proposition that putrescine-related behavioral effects are expressed

via GABAergic systems and/or other CNS substances is tentative. Substantiation of such hypotheses will require further behavioral, as well as biochemical evidence.

The experimental design was not free of environmental and procedural limitations, and restrict the interpretation and generalization of the findings to conditions defined in the materials and methods. For example, time sampling methods measured behavior in a mutually-exclusive manner which resulted in the limited characterization of putrescine treatment-related effects based on time series observations. The presented statistical effects are selective in that they reflect certain changes in specific behaviors, and not other behaviors. Without statistical justification, no assumption or inference about any change, or lack of change in any behavioral measure is possible. However, the behavioral effects observed after oral putrescine administration appear to be robust, since many measures yielded significant statistical effects in spite of small group size (n=7).

In conclusion, the behavioral effects observed following oral putrescine treatment were found to resemble certain changes in behavior associated with in vivo manipulations of the polyamines, GABA, and other CNS substances (especially dopamine, serotonin, opiate peptides, acetylcholine). The results of Experiment 1 also supported previous findings which have suggested that exogenously administered putrescine may act as a neurotransmitter or neuromodulator, exerting centrally-mediated effects on brain function (Nistico et al., 1980; Shaw, 1979). Future behavioral and biochemical research is needed to further assess the consequences of systemic (oral and i.p.) putrescine administration, as well as investigate

any putrescine treatment-related effects on the activity and function of GABA and other CNS substances. Such research may help to clarify the apparent role of putrescine as an important neurochemical substance in the mammalian central nervous system.

EXPERIMENT 2

Introduction

Putrescine has been established as a secondary precursor substance for gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in mammalian organisms, as previously described (Experiment 1). Past research has demonstrated that systemic (i.p.) administration of putrescine results in measurable in vivo conversion of putrescine to GABA in both peripheral and CNS tissues of rodents (Caron, Cote & Kremzner, 1980; Tsuji & Nakajima, 1978).

Recent evidence has suggested that putrescine and other polyamines (spermine and spermidine) may have an important role in CNS neurotransmission (Anderson et al., 1975; Nistico et al., 1980; Shaw, 1979). As previously described, intraventricular administration of putrescine, spermine and spermidine, and systemic (i.p.) administration of spermine and spermidine have been shown to produce significant behavioral effects in rodents and other species (Experiment 1). However, differential behavioral effects produced by polyamine treatments appear to be dependent on the type of polyamine administered, dosage, route of administration, and the rate at which the polyamine increases within the brain (Anderson et al., 1975).

Rapid increases in the CNS concentrations of polyamines (associated with i.v.t. or i.v. administration) have been shown to be related to the appearance of an excitatory behavioral phase which is preceded by and followed by behavioral depression (Anderson et al., 1975; Nistico et al., 1980). In contrast, i.p. administration of spermine and spermidine has not been reported to produce excitatory effects in

rodents (Shaw, 1972).

The results of Experiment 1 indicated that oral administration of putrescine was associated with statistically significant behavioral effects in rats. A majority of the behavioral measures which yielded significant ANOVA main effects also had significant dose response relationships to putrescine treatment. Behavioral effects observed after oral putrescine treatment included changes in the following behaviors: motor behavior, ambulation, sensory reactivity, body temperature, and possibly ingestive behavior. Review of behavioral literature gave preliminary support to the suggestion that oral putrescine administration may be indirectly influencing the expression of certain behaviors via GABAergic function and activity of other CNS substances, or else directly as a transmitter or neuromodulating substance.

Previous biochemical and behavioral research has suggested that putrescine may influence CNS neuropharmacology in the rat. The results of Experiment 1 appear to be the first report of significant behavioral effects associated with oral putrescine administration in rats. There has been no extensive report which has evaluated the i.p. administration of putrescine in intact rats. Thus, further investigation of behavioral consequences due to systemic putrescine administration may help to specify the nature of its neuropharmacologic significance.

The purpose of this experimental procedure was to evaluate the behavioral consequences of intraperitoneal administration of putrescine in rats. The two questions to be addressed are 1) can significant behavioral effects be produced by the i.p. administration of lower putrescine doses than those administered orally, and 2) to what extent do

behavioral consequences produced by i.p. treatment resemble those observed after oral treatment? The experimental tests and procedures used in Experiment 2 were identical to those used in Experiment 1, although the time course of the experiments differed. Evaluation of behavior in Experiment 2 was done over a time course of 3.5 hours, since it was expected that intraperitoneal effects would be shorter in duration than oral effects (this premise was informally substantiated in pilot studies). It is hypothesized that intraperitoneal administration of putrescine in rats may cause significant behavioral effects, and that these effects may be similar to those associated with oral putrescine treatment.

Materials and Methods

Subjects. The subjects were 30 naive male Sprague-Dawley albino rats born and raised at the animal colony facilities of the Psychology Department, VPI & SU (Blacksburg, VA). Subjects were between 90 and 120 days of age (350-425 g) at time of use. Animals were housed and maintained as previously described (Experiment 1).

Apparatus. Intraperitoneal (i.p.) dosages were administered with 25 gauge needles and 1 cc syringes. Behavioral observations were made with subjects individually housed in opaque plastic tubs (40 cm x 25 cm x 15 cm) having wire mesh cage covers and containing sawdust. Apparatus used to measure rectal temperature, startle response, open field ambulation, motor coordination, and shock response thresholds were the same as those previously described (Experiment 1).

Drugs and dosages. The five treatment groups included a saline control and four putrescine groups (n = 6). Solutions of putrescine hydrochloride (SIGMA Chemical Company, St. Louis, MO) were prepared in .85% saline no more than two hours prior to treatment. Putrescine dosages of 0 mg/kg, 25 mg/kg, 50 mg/kg, 100 mg/kg, and 250 mg/kg were administered in an injection volume of 1 cc/kg body weight per subject. All treatments were administered by i.p. injection with each subject being used in only one test session.

Procedures

The experimental protocol used in this study was the same as the protocol used in Experiment 1, except for two differences. The first difference was that different putrescine dosages were administered by i.p. injection, instead of oral intubation. Secondly, while the pro-

tocol and order of testing in Experiment 2 was the same as that in Experiment 1, the time course of behavioral testing in this study was much shorter than that of Experiment 1. Table 18 outlines the experimental protocol and time course of Experiment 2.

All subjects were deprived of food and water two hours prior to the beginning of the experiment. The experiment began during the last two hours of the light portion of the light-dark cycle. Baseline startle response test, body weights, and initial rectal temperatures were measured prior to treatment administration. Intraperitoneal injection of treatments was done experimenter-blind. Handling of subjects prior to the start of any behavioral test was done in a manner as previously described (Experiment 1).

Body temperature. Pretreatment body temperatures were measured 20 minutes prior to dose administrations. Body temperatures were measured again at 3:10 hours posttreatment, and the change in temperature calculated as the difference between pre- and posttreatment group means.

Startle response. The startle response test procedure was the same as that described previously (Experiment 1). Tests of startle response were measured at three time points: prior to measurement of body weight, 50 minutes posttreatment, and 2:50 hours posttreatment.

Time sampling. Immediately following i.p. injection of treatments, subjects were directly observed for 45 minutes by time sampling techniques as previously described (Experiment 1). Time sampling was continued for an additional 15 minutes beginning at 1:10 hours posttreatment.

Open field. Approximately 90 minutes posttreatment, open field

Table 18
Experiment 2 Protocol

Procedure	Time Course
1. Food and water deprivation begins 2 hr prior to experiment	-2:00 hr
2. Startle test I. (8 trials, 3 point intensity scale)	-0:40 hr
3. Body weights and rectal temperature I.	-0:20 hr
4. Intraperitoneal putrescine treatment (0, 25, 50, 100, 250 mg/kg)	0:00 hr
5. Time sampling, first block (13 behavioral categories, subjects scored once per minute)	+0:05 hr
6. Startle test II.	+0:50 hr
7. Time sampling, second block	+1:10 hr
8. Open field ambulation (5 min testing)	+1:30 hr
9. Grid hang motor ability (test of motor coordination and endurance using a rotated grid)	+2:15 hr
10. Food and water ingestion (5 min)	+2:30 hr
11. Startle test III.	+2:50 hr
12. Rectal temperature II.	+3:10 hr
13. Electric shock response threshold testing	+3:30 hr
14. Retest electric shock response thresholds	+21:00 hr

ambulation was measured as previously described (Experiment 1). No other behaviors besides ambulation were evaluated during open field testing.

Motor ability. Measurement of motor coordination and grid hang endurance was done at 2:15 hours posttreatment as previously described (Experiment 1).

Ingestive behavior. Approximately 2.5 hours after treatment administration (4.5 hours posttreatment), food and water ingestion was measured as previously described (Experiment 1).

Pain response thresholds. Electric shock response thresholds were measured at 3.5 hours posttreatment, and again at 21 hours posttreatment as previously described (Experiment 1).

Data analysis. The statistical tests used for analysis of data were the same as those previously described (Experiment 1). The level of significance for all statistical analyses was $\alpha = .05$, unless otherwise noted.

Results

Statistical analysis of the data demonstrated that systemic administration of putrescine by intraperitoneal route produced observable behavioral effects in adult male rats. Using linear regression analysis, a reliable dose response relationship between treatment and behavioral responses was found for more than half of the significant ANOVA measures obtained. Some of the effects seen after i.p. putrescine administration were similar to effects described following oral putrescine treatment, although other effects were contrastingly different.

Formal Observations

Time sampling. Time sampling testing yielded significant analysis of variance (ANOVA) effects for nine of 13 arbitrary behavioral categories. Two general activity categories (sitting and rearing), four stereotypy categories (sniffing, grooming, gnawing, and mastication), and three abnormal behavior categories (head down posture, abnormal behavior, and wet dog shaking) were found to have significant treatment main effects or treatment x time interactions.

A significant ANOVA main effect for the incidence of sitting behavior over 60 minutes of time sampling was found ($F(4,25)=4.73$, $p < .006$), with higher putrescine dosages decreasing sitting behavior compared to saline treatment (Figure 18). Subjects given 25 and 50 mg/kg putrescine showed incidence of sitting behavior which was statistically similar to the incidence exhibited by saline controls. The 100 and 250 mg/kg putrescine groups had incidences of sitting behavior which were significantly lower than all other groups (Dunnett's test $p < .05$) (Table 19). Although the level of sitting for the 250 mg/kg putrescine

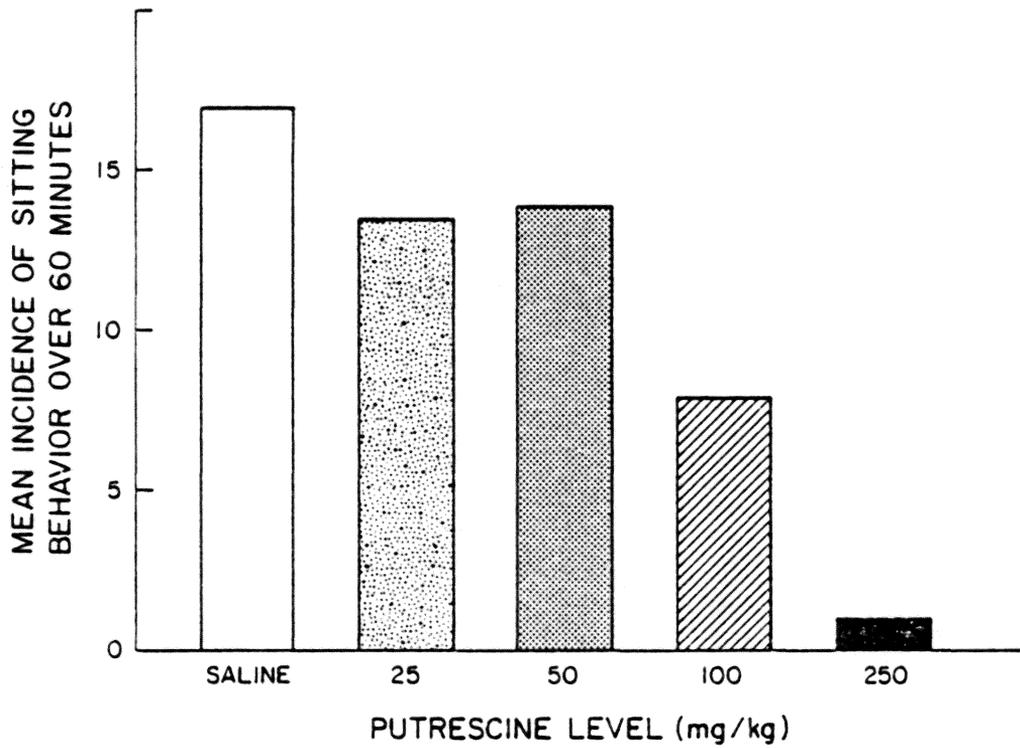


Figure 18. Effect of i.p. putrescine treatment on the incidence of sitting behavior.

Table 19
Duncan's Multiple-Range Test Results
for Putrescine (i.p.) Treatment Effect and
Incidence of Sitting Behavior^a

Treatment	Grouping
Saline	*
25 mg/kg	*
50 mg/kg	*
100 mg/kg	**
250 mg/kg	**

^aTreatments with identical grouping designations are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 60 minutes.

group was very low, the 100 and 250 mg/kg groups were not significantly different from each other. A linear dose-response relationship was found ($p < .01$) (Table 32).

A significant ANOVA main effect for the incidence of rearing behavior was found ($F(4,25)=4.87$, $p < .005$), in which putrescine treatment depressed rearing behavior compared to saline, except at low dose (Figure 19). Subjects given 25 mg/kg putrescine exhibited an incidence of rearing behavior which was statistically similar to the incidence of saline controls. The rearing incidence for 50, 100, and 250 mg/kg putrescine groups was significantly lower than the incidence for saline controls (Dunnett's test $p < .05$) (Table 20). Although a dose of 250 mg/kg putrescine apparently suppressed rearing behavior over 60 minutes of time sampling, the 50, 100 and 250 mg/kg putrescine groups were statistically similar to each other. No significant dose-response relationship was found (Table 32).

A significant ANOVA main effect for the incidence of sniffing behavior was found ($F(4,25)=9.61$, $p < .0001$), with putrescine treatment apparently decreasing the incidence of sniffing compared to saline, except at low dose (Figure 20). Subjects treated with 25 mg/kg putrescine had a sniffing incidence which was statistically similar to that of saline controls. Subjects given 50 and 100 mg/kg putrescine showed significant decrease in the incidence of sniffing compared to saline, but at an incidence level intermediate to that of 250 mg/kg group (Dunnett's $p < .05$) (Table 21). No incidence of sniffing behavior was observed over 60 minutes for the 250 mg/kg putrescine group, which was statistically different from all other groups. Linearity of the dose-

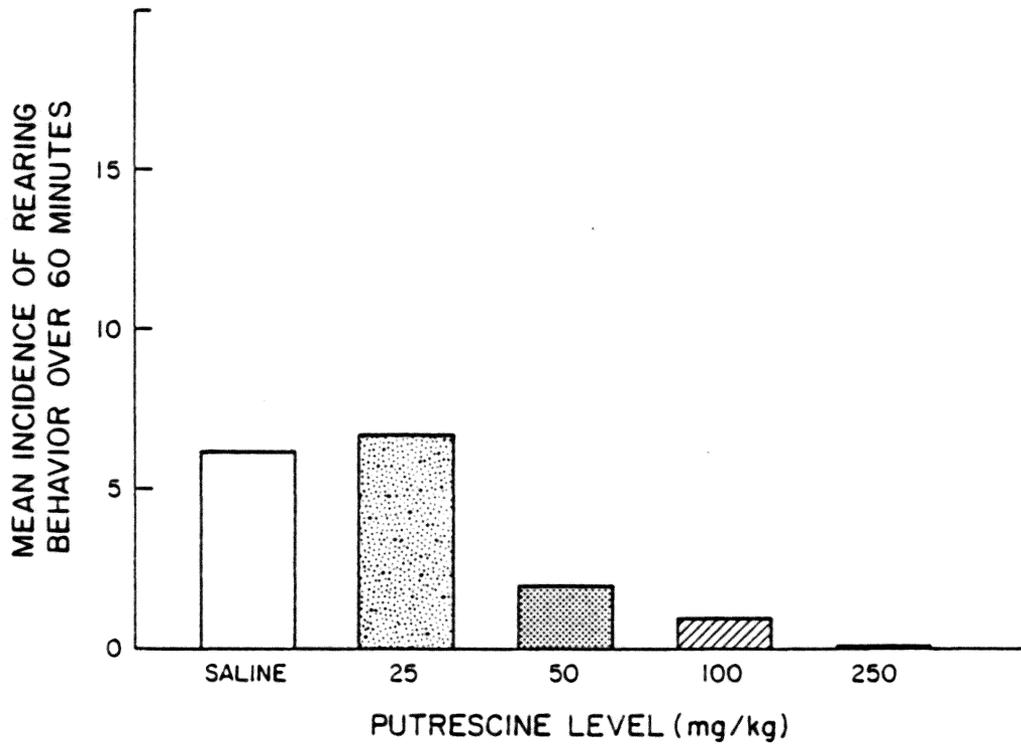


Figure 19. Effect of i.p. putrescine treatment on the incidence of rearing behavior.

Table 20
Duncan's Multiple-Range Test Results
for Putrescine (i.p.) Treatment Effect and
Incidence of Rearing Behavior^a

Treatment	Grouping
25 mg/kg	*
Saline	*
50 mg/kg	**
100 mg/kg	**
250 mg/kg	**

^aTreatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 60 minutes.

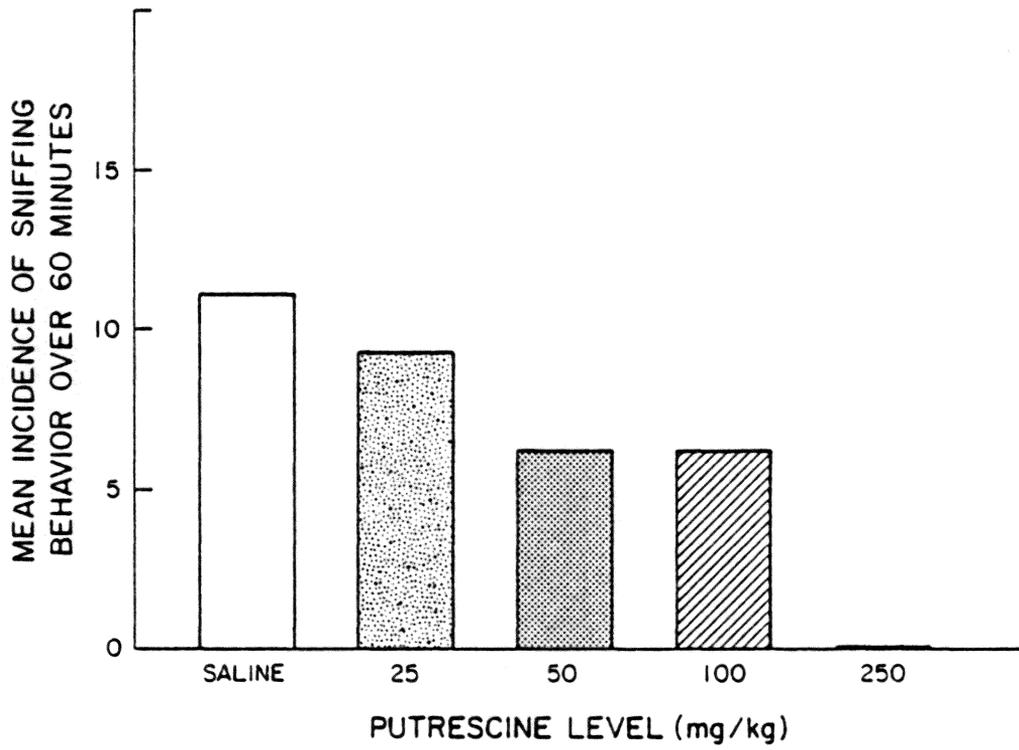


Figure 20. Effect of i.p. putrescine treatment on the incidence of sniffing behavior.

Table 21
Duncan's Multiple-Range Test Results
for Putrescine (i.p.) Treatment Effect
and Incidence of Sniffing Behavior^a

Treatment	Grouping
Saline	*
25 mg/kg	*
100 mg/kg	**
50 mg/kg	**
250 mg/kg	***

^aTreatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 60 minutes.

response curve was found ($p < .01$) (Table 32).

A significant ANOVA main effect for the incidence of grooming behavior was obtained ($F(4,25)=5.69$, $p < .003$), with putrescine treatment either increasing or decreasing grooming behavior compared to saline, depending on dosage (Figure 21). The incidence of grooming for groups treated with 25 and 100 mg/kg putrescine was statistically similar to that of saline controls, while 50 mg/kg putrescine appeared to significantly increase grooming behavior compared to saline (Dunnett's test $p < .05$) (Table 22). Subjects given 250 mg/kg putrescine did not exhibit any grooming activity over 60 minutes of time sampling, which was statistically different from all other groups. Thus, putrescine dose of 50 mg/kg significantly increased grooming behavior, while treatment with 250 apparently suppressed grooming activity. No dose-response relationship was obtained (Table 32).

As shown in Figure 22, a significant ANOVA main effect for the incidence of gnawing behavior was obtained ($F(4,25)=3.26$, $p < .03$), with higher putrescine dosages decreasing gnawing behavior compared to saline controls. Gnawing incidence exhibited by subjects given 25 mg/kg putrescine was statistically similar to the incidence of saline controls. Groups receiving 50, 100, and 250 mg/kg putrescine were statistically similar to each other, and had significantly lower gnawing incidence over 60 minutes of time sampling than saline animals (Dunnett's test $p < .05$) (Table 23). Subjects treated with 250 mg/kg putrescine did not exhibit any incidence of gnawing over 60 minutes of time sampling. A significant linear dose-response curve was found ($p < .05$) (Table 32).

Mastication stereotypy was an oral response characterized by rapid

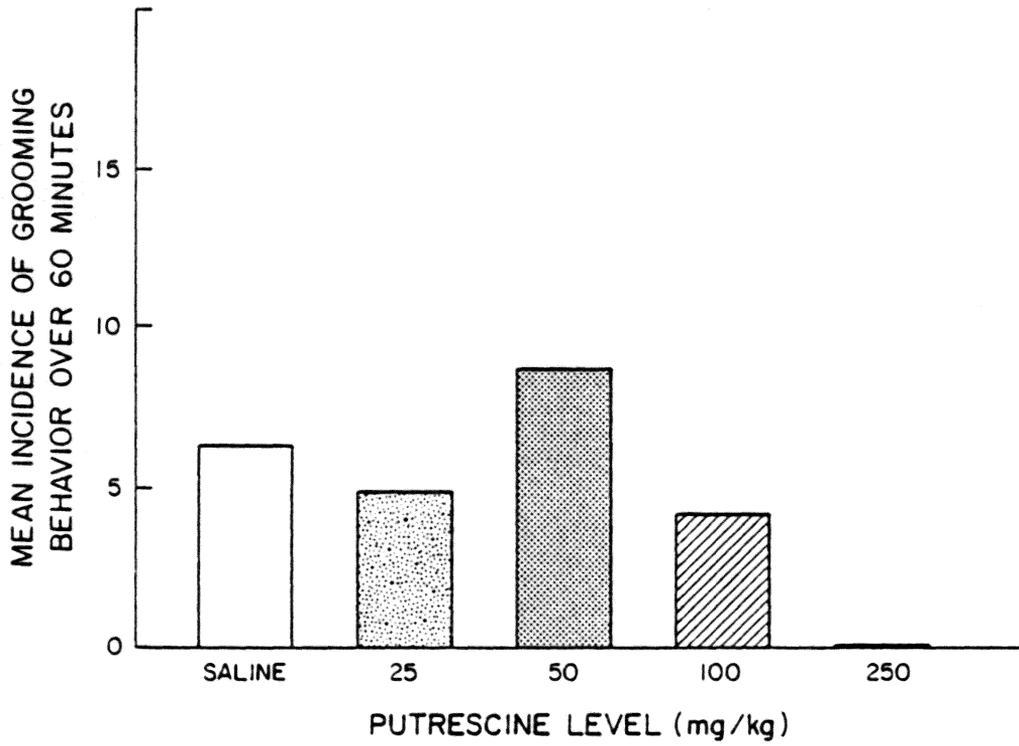


Figure 21. Effect of i.p. putrescine treatment on the incidence of grooming behavior.

Table 22
Duncan's Multiple-Range Test Results
for Putrescine (i.p.) Treatment Effect
and Incidence of Grooming Behavior^a

Treatment	Grouping
50 mg/kg	*
Saline	**
25 mg/kg	**
100 mg/kg	**
250 mg/kg	***

^aTreatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 60 minutes.

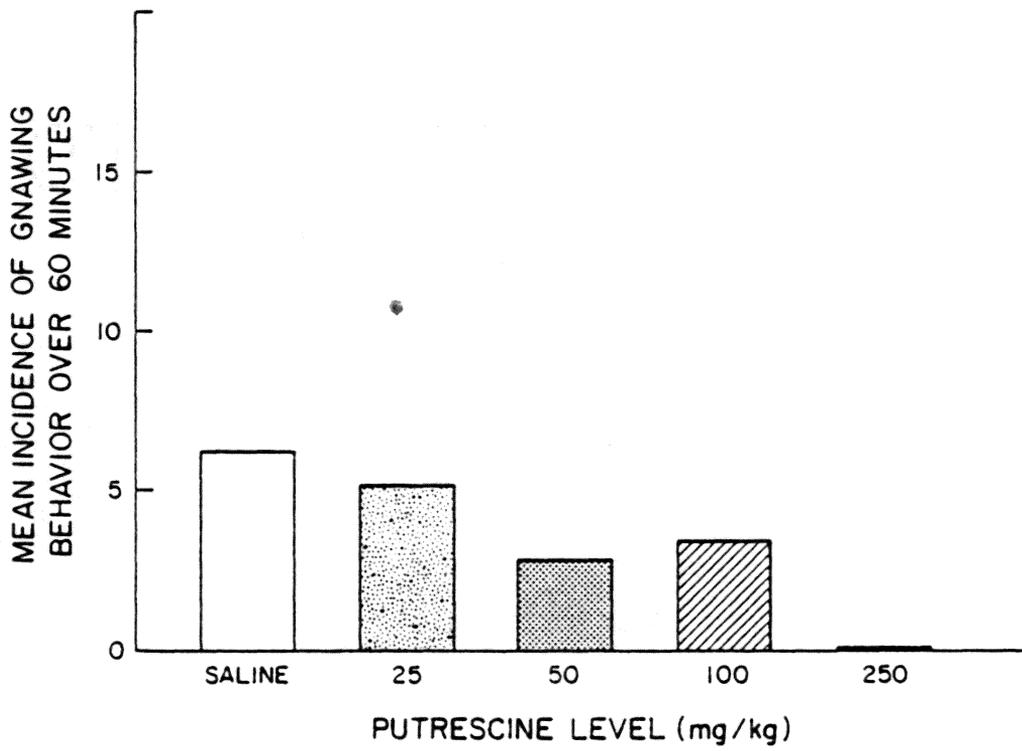


Figure 22. Effect of i.p. putrescine treatment on the incidence of gnawing behavior.

Table 23
Duncan's Multiple-Range Test Results
for Putrescine (i.p.) Treatment Effect
and Incidence of Gnawing Behavior^a

Treatment	Grouping
Saline	*
25 mg/kg	*
100 mg/kg	**
50 mg/kg	**
250 mg/kg	**

^aTreatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 60 minutes.

jaw/mouth movements in a stereotypic pattern with no actual chewing or gnawing of foreign matter (such as sawdust). A significant treatment x time interaction was found for the incidence of mastication stereotypy ($F(20,125)=2.86$, $p < .0002$), with putrescine affecting the incidence of this behavior over time, especially for a dose of 50 mg/kg (Figure 23). Saline-treated subjects did not exhibit any measurable amount of mastication until $T = 6$, with the mastication level during that 10 minute interval less than 1 response/10 minutes. Subjects given 250 mg/kg putrescine showed mastication incidence which was statistically similar to saline over all intervals. The 100 mg/kg group exhibited a low number of mastication at $T = 3$ only, but was similar to saline over all intervals. Significant incidence of mastication was observed for the 50 mg/kg putrescine group at $T = 2$, with this being significantly higher than all other groups (Duncan's test $p < .01$) (Table 24). Seventy minutes posttreatment ($T = 5$), the 50 mg/kg group still exhibited a level of mastication behavior which was significantly higher than saline and 250 mg/kg groups, but by $T = 6$, was statistically similar to levels of saline controls. While the 25 mg/kg putrescine group showed the appearance of some mastication from $T = 2-5$, the incidence of this behavior for the group was not significantly different from saline controls. Thus, significant mastication behavior was only seen in subjects treated with 50 mg/kg putrescine, beginning 20 minutes posttreatment and remaining up to 80 minutes posttreatment. A biphasic dose-response curve was found, with significant linearity for one component (saline, 25 and 50 mg/kg putrescine) at $p < .05$, and no significant linearity for the second component (50, 100, 250 mg/kg putrescine)

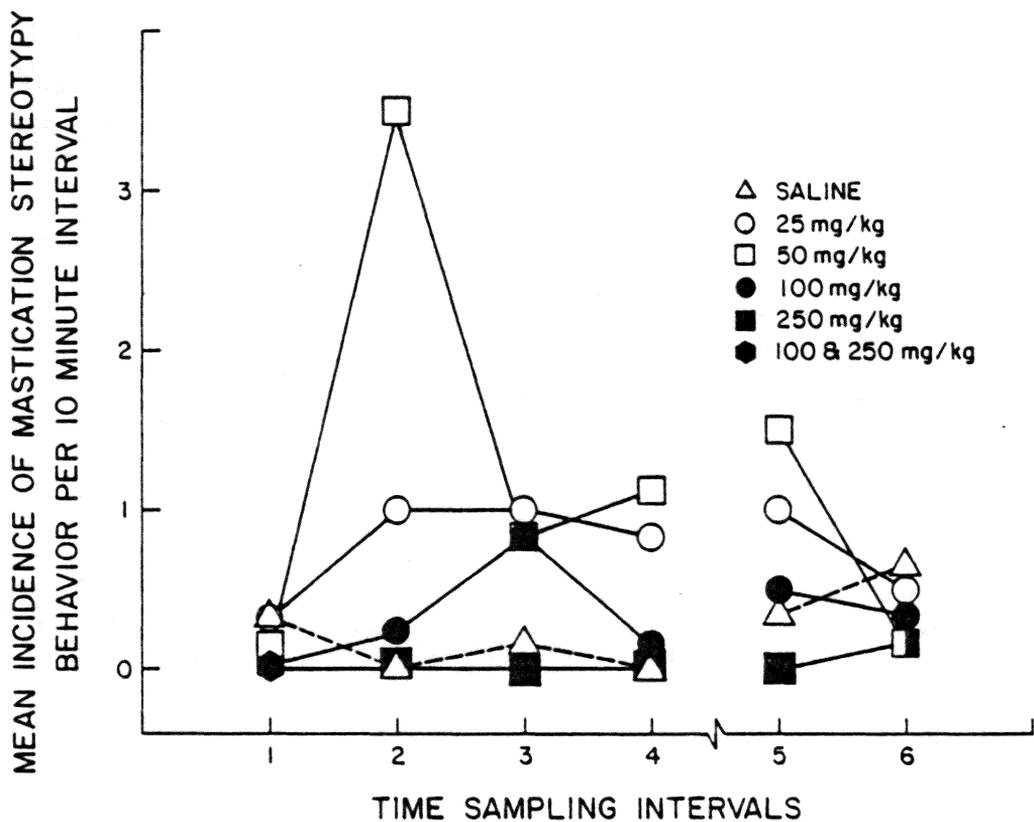


Figure 23. Intraperitoneal putrescine treatment x time interaction and the incidence of mastication stereotypy.

Table 24

Duncan's Multiple-Range Test Results
for Putrescine (i.p.) Treatment x Time Interaction
and Incidence of Mastication Stereotypy^a

Time Sampling Interval	Group Comparison	$p <^b$
T = 2	50 mg/kg vs 25 mg/kg	.01
	50 mg/kg vs 100 mg/kg	.01
	50 mg/kg vs 250 mg/kg	.01
	50 mg/kg vs Saline	.01
T = 5	50 mg/kg vs Saline	.05
	50 mg/kg vs 250 mg/kg	.01

^aEach time sampling interval is 10 minutes in duration; total testing time = 60 minutes.

^b p indicates significant difference between comparison groups.

(Table 32).

Head down posture was a response in which subjects would be sitting in a quadrupedal stance with head lowered nearly to the floor, with this positioning often accompanied by ptosis. A significant ANOVA treatment \times time interaction was obtained for the incidence of head down behavior ($F(20,125)=2.12$, $p < .007$), with this behavior observed in subjects given 100 mg/kg putrescine, and to a lesser extent for 25 and 50 mg/kg groups (Figure 24). No appreciable incidence of head down posture was observed for saline-treated subjects, except during $T = 4$ (40 minutes posttreatment). Subjects given 250 mg/kg putrescine did not exhibit any head down behavior during the first 40 minutes of sampling, but showed moderate incidence of this behavior at $T = 5-6$ (70-90 minutes posttreatment) which was significantly higher than seen for saline controls (Duncan's test $p < .05$) (Table 25). The 100 mg/kg group showed head down behavior during the first 10 minutes posttreatment which was significantly higher in incidence than saline and 250 mg/kg (Duncan's test $p < .05$). During the next sampling interval ($T = 2$), the level of head down behavior for the 100 mg/kg group was significantly higher than all groups, and was the maximum incidence for this behavior during sampling (about 6 responses/10 min). During $T = 2-4$, a decline in head down behavior occurred for subjects given 100 mg/kg, and at $T = 5$, the incidence was statistically similar to that of saline controls. However, at $T = 6$, the 100 mg/kg group showed head down incidence which was significantly higher than saline controls.

During $T = 1$, subjects treated with 50 mg/kg putrescine had very low incidence of head down which was statistically similar to saline-

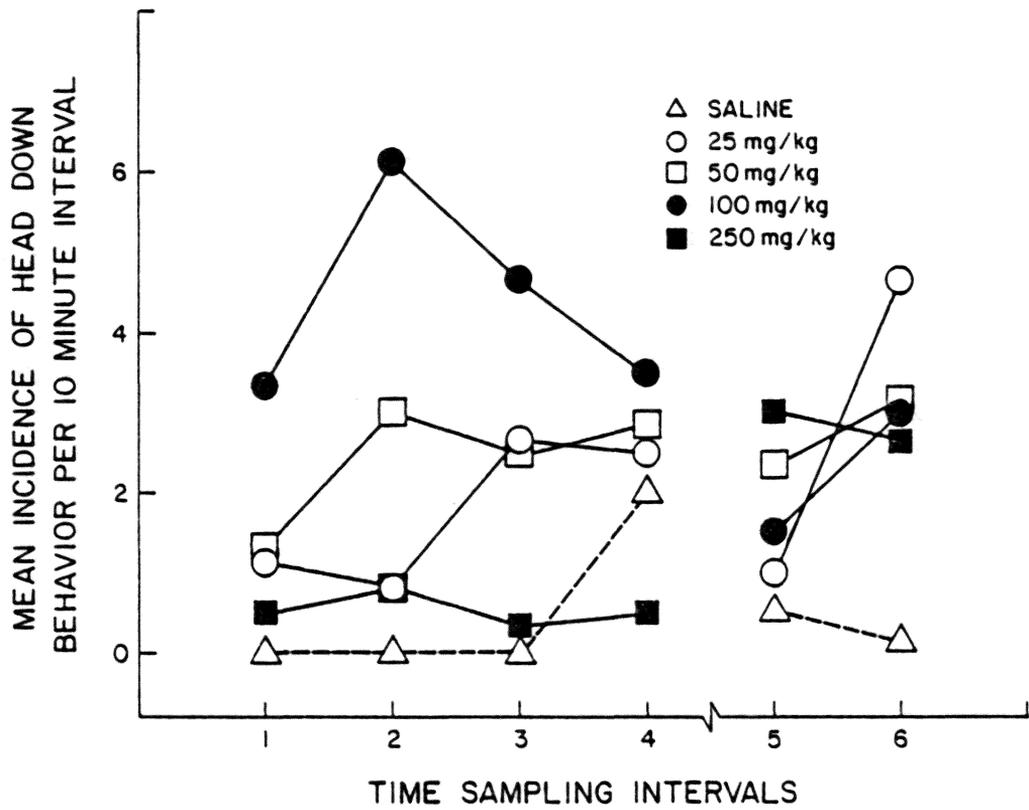


Figure 24. Intraperitoneal putrescine treatment x time interaction and the incidence of head down posture.

Table 25
 Duncan's Multiple-Range Test Results
 for Putrescine (i.p.) Treatment x Interaction
 and Incidence of Head Down Behavior^a

Time Sampling Interval	Group Comparison	p < ^b
T = 1	100 mg/kg vs 250 mg/kg	.05
	100 mg/kg vs Saline	.05
T = 2	100 mg/kg vs 25 mg/kg	.05
	100 mg/kg vs 50 mg/kg	.01
	100 mg/kg vs 250 mg/kg	.01
	100 mg/kg vs Saline	.01
	50 mg/kg vs Saline	.05
T = 6	50 mg/kg vs Saline	.05
	25 mg/kg vs Saline	.01
	100 mg/kg vs Saline	.05
	250 mg/kg vs Saline	.05

^aEach time sampling interval is 10 minutes in duration; total testing time = 60 minutes.

^bp indicates significant difference between comparison groups.

treated subjects. At $T = 2$, the 50 mg/kg group exhibited a level of head down behavior which was significantly higher than the saline group, and lower than that of the 100 mg/kg group (Duncan's test $p < .05$ and $p < .01$, respectively). Intermediate levels of head down behavior were seen during $T = 3-6$, with the 50 mg/kg group showing significantly higher incidence at $T = 6$ compared to saline controls. Subjects given 25 mg/kg putrescine had incidence of head down which was statistically similar to saline-treated subjects during $T = 1-2$, and showed an increase in this behavior during $T = 3-4$ which was similar to incidence of the 50 mg/kg group. While the level of head down for the 25 mg/kg group was the same as saline controls at $T = 5$, the incidence of this behavior increased during $T = 6$, and was significantly higher than for saline controls (Duncan's test $p < .01$). All putrescine-treated groups statistically similar to each other at $T = 6$, and had significant exhibition of head down posture compared to saline controls. A linear dose-response relationship was not found (Table 32).

Appearance of abnormal behaviors included unusual postures and movements which were not exhibited by saline-treated subjects, especially behaviors which deviated from a normal four-paw stance and appeared bizarre. A significant ANOVA treatment x time interaction was found for the incidence of abnormal behavior ($F(20,125)=13.89$, $p < .0001$), with abnormal behavior associated with the highest putrescine dosages (Figure 25). No significant incidence of abnormal behavior was observed for groups treated with saline, or 25 and 50 mg/kg putrescine over all intervals. Treatment with 250 mg/kg putrescine appeared to produce high levels of abnormal behavior during the first 10 minutes posttreat-

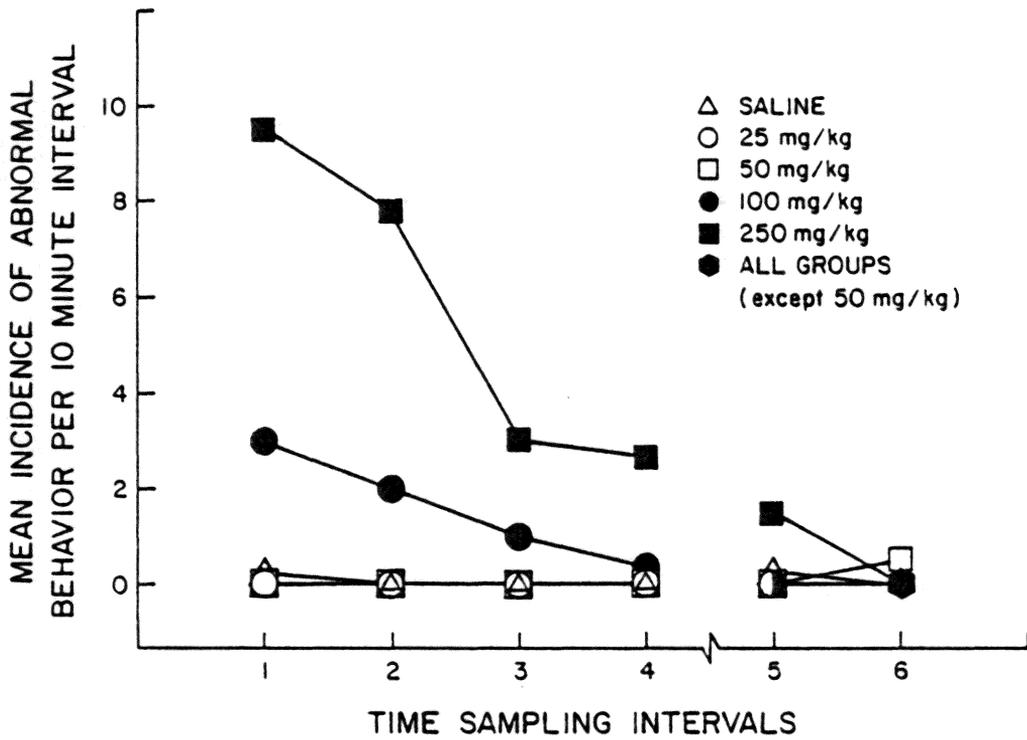


Figure 25. Intraperitoneal putrescine treatment x time interaction and the incidence of abnormal behavior.

ment, which was significantly higher than all other groups (Duncan's test $p < .01$) (Table 26). While the data show that no other behaviors but abnormal behaviors were exhibited by the 250 mg/kg group during $T = 1$, the incidence of abnormal behavior declined slightly during $T = 2$, and more sharply at $T = 3-4$, although these levels were still significantly higher than all other groups. Seventy minutes posttreatment ($T = 5$), the level of abnormal behavior was still significantly higher for the 250 mg/kg group, but declined over the last sampling interval. At $T = 6$, the 250 mg/kg group and all putrescine treatment groups were similar to saline control. The 100 mg/kg group did not exhibit statistically significant incidence of abnormal behavior compared to saline over all intervals. The linearity of the dose-response correlation was highly significant ($P < .01$) (Table 32).

Wet dog shaking behavior (WDS) was a response characterized by slight flinching and sudden movement of the head, followed by intense side-to-side shaking of the head neck, and trunk regions of the body, with an episode lasting approximately 2-3 seconds duration. At the conclusion of a WDS episode, subjects often had a splayed leg posture accompanied by ptosis and the appearance of not being oriented to his surroundings. As shown in Figure 26, a significant ANOVA treatment x time interaction was obtained for the incidence of WDS ($F(20,125)=6.88$, $p < .0001$), with the significant appearance of WDS only observed in subjects given 250

Table 26

Duncan's Multiple-Range Test Results
 for Putrescine (i.p.) Treatment x Time Interaction
 and Incidence of Abnormal Behavior^a

Time Sampling Interval	Group Comparison	p < ^b
T = 1	250 mg/kg vs 100 mg/kg	.01
	250 mg/kg vs 50 mg/kg	.01
	250 mg/kg vs 25 mg/kg	.01
	250 mg/kg vs Saline	.01
T = 3	250 mg/kg vs 100 mg/kg	.01
	250 mg/kg vs 50 mg/kg	.01
	250 mg/kg vs 25 mg/kg	.01
	250 mg/kg vs Saline	.01
T = 4	250 mg/kg vs 100 mg/kg	.01
	250 mg/kg vs 50 mg/kg	.01
	250 mg/kg vs 25 mg/kg	.01
	250 mg/kg vs Saline	.01
T = 5	250 mg/kg vs Saline	.01
	250 mg/kg vs 100 mg/kg	.01
	250 mg/kg vs 50 mg/kg	.01
	250 mg/kg vs 25 mg/kg	.01

^aEach time sampling interval is 10 minutes in duration; total testing time = 60 minutes.

^bp indicates significant difference between comparison groups.

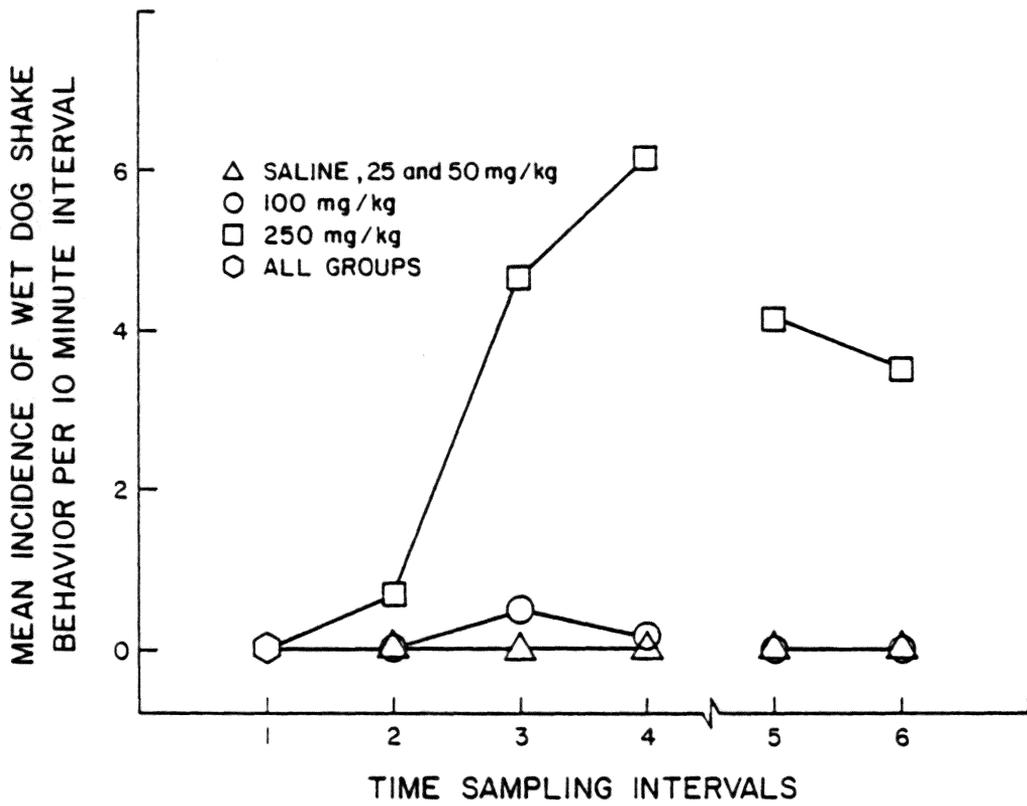


Figure 26. Intraperitoneal putrescine treatment x time interaction and the incidence of wet dog shaking (WDS) behavior.

mg/kg putrescine. No significant observation of WDS was seen in subjects receiving saline, or putrescine doses of 25, 50, and 100 mg/kg. While 250 mg/kg putrescine was associated with display of WDS behavior, significant incidence compared to all other groups did not occur until 30 minutes posttreatment (Duncan's test $p < .01$) (Table 27). The incidence level of WDS increased to a maximum at $T = 4$, showing that approximately 6 WDS responses were scored per 10 minute sampling interval for the 250 mg/kg group. During $T = 5-6$, data showed that WDS incidence declined slightly for the 250 mg/kg group, but was still significantly higher than all other groups at the end of time sampling (90 minutes post-treatment). A significant dose-response relationship was found ($p < .02$) (Table 32).

Of 13 arbitrary behavior categories which were time sampled, four categories did not yield significant ANOVA effects (31%). These nonsignificant categories included walking and sleeping (general activity) non-body licking (stereotypy), and motor impairment (abnormal behavior).

Intensity of initial startle response. Figure 27 shows the effect of intraperitoneal putrescine treatment on the intensity of startle response on the first trial of test sets at one hour (SR I) and three hours (SR II) posttreatment (a and b, respectively). Comparison of baseline means for initial startle (ISI) yielded no significant ANOVA between groups differences ($F(4,25) = 1.49, p > .20$). Baseline means are given in Appendix C, Table 45.

Table 27

Duncan's Multiple-Range Test Results
 for Putrescine (i.p.) Treatment x Time Interaction
 and Incidence of Wet Dog Shake Behavior^a

Time Sampling Interval	Group Comparison	p < ^b
T = 3	250 mg/kg vs 100 mg/kg	.01
	250 mg/kg vs 50 mg/kg	.01
	250 mg/kg vs 25 mg/kg	.01
	250 mg/kg vs Saline	.01
T = 4	250 mg/kg vs 100 mg/kg	.01
	250 mg/kg vs 50 mg/kg	.01
	250 mg/kg vs 25 mg/kg	.01
	250 mg/kg vs Saline	.01
T = 6	250 mg/kg vs 100 mg/kg	.01
	250 mg/kg vs 50 mg/kg	.01
	250 mg/kg vs 25 mg/kg	.01
	250 mg/kg vs Saline	.01

^aEach time sampling interval is 10 minutes in duration; total testing time = 60 minutes.

^bp indicates significant difference between comparison groups.

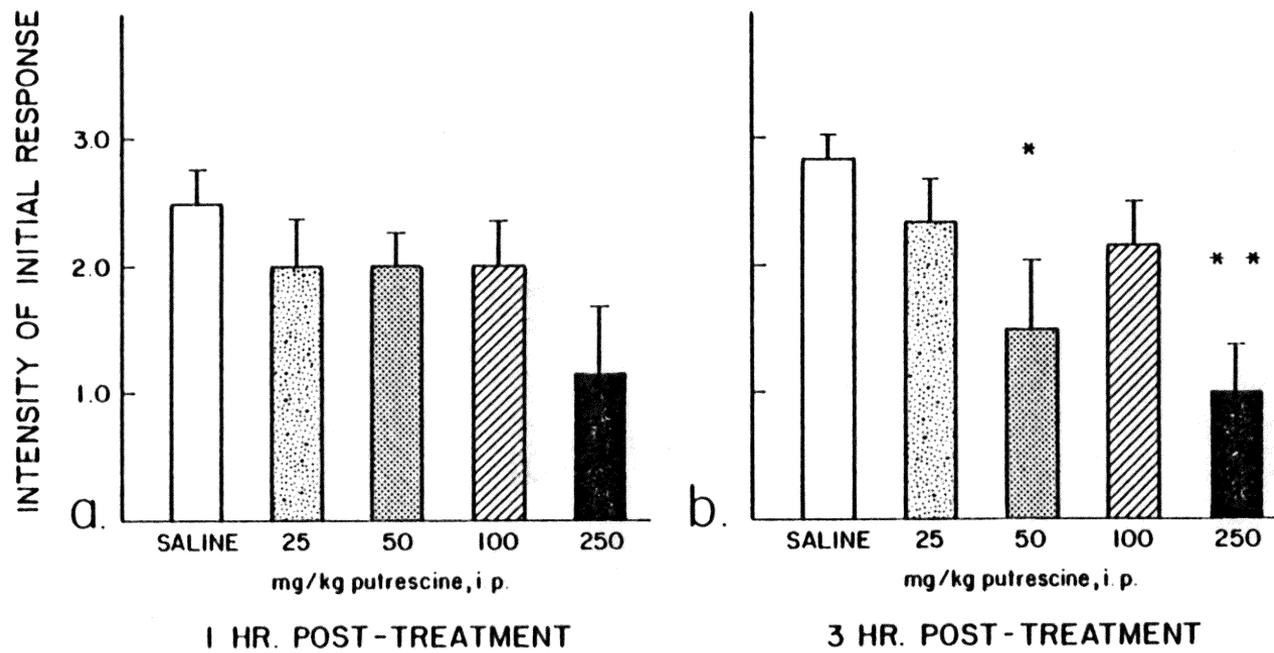


Figure 27. Effect of i.p. putrescine treatment on the intensity of initial startle response magnitude at 1-hr (a) and 3-hr (b) posttreatment. (Significant difference from saline using Dunnett's test denoted by * $p < .05$, ** $p < .01$).

At one hour postinjection, no significant ANOVA treatment effect was found for ISI ($F(4,25)=1.89$, $p>.20$).

At three hours posttreatment, a significant ANOVA main effects was obtained for ISI ($F(4,25)=4.21$, $p<.01$). Subjects receiving 25 or 100 mg/kg putrescine exhibited initial startle intensity means which were statistically similar to saline controls. Putrescine dose of 50 mg/kg appeared to significantly decrease the intensity of initial startle compared to saline controls (Dunnett's test $p<.05$). Treatment with 250 mg/kg putrescine decreased initial startle intensity even lower than 50 mg/kg, which was significantly lower than that of saline-treated subjects at $p<.01$ (Dunnett's test). The average ISI response for the 250 mg/kg group at three hours posttreatment was ears twitch. Linearity of dose-response curve was not found (Table 32).

Startle response magnitude. Intraperitoneal putrescine treatment effect on startle magnitude at one and three hours posttreatment is shown in Figure 28 (a and b, respectively). At one hour posttreatment, putrescine did not yield a significant effect for change in startle magnitude ($F(4,25)=2.09$, $p>.10$), even though the 250 mg/kg group had a higher graphic score. A significant ANOVA treatment effect was obtained three hours posttreatment ($F(4,25)=3.13$, $p<.05$), showing that a change in SR magnitude was observed in some putrescine groups. A significant change in startle magnitude was only seen for the 250 mg/kg

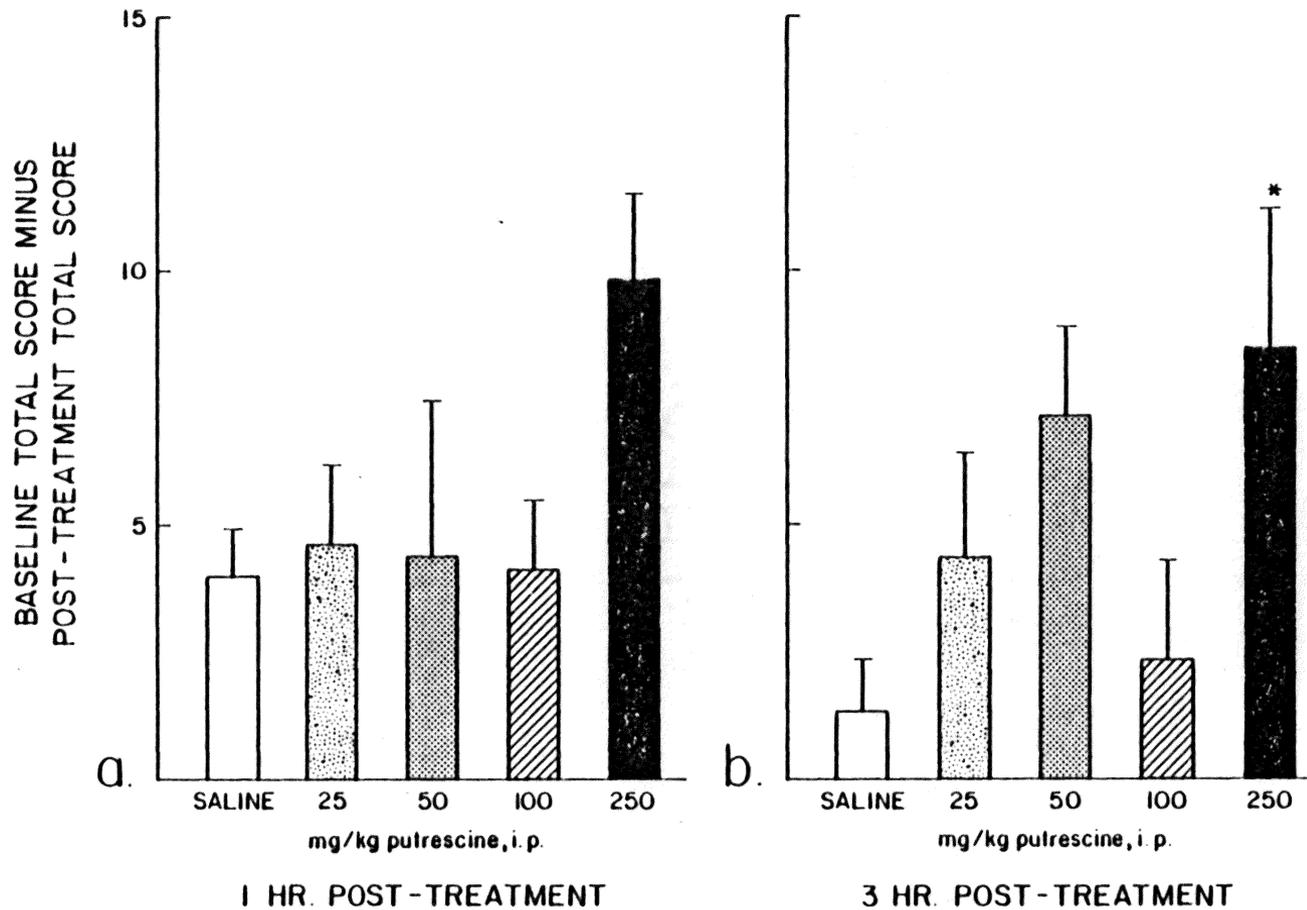


Figure 28. Effect of i.p. putrescine treatment on startle response magnitude at 1-hr (a) and 3-hr (b) posttreatment. (Significant difference from saline using Dunnett's test denoted by * $p < .05$).

group compared to saline controls (Dunnett's test $p < .05$). Although subjects given 25 and 50 mg/kg appeared to show some change in acoustic startle magnitude, these group means were not significantly different from the low startle magnitude scores of the saline and 100 mg/kg groups. A significant dose-response relationship was not found (Table 32).

Open field performance. A significant ANOVA main effect was found for open field ambulation only at 90 minutes posttreatment ($F(4,25)=9.33$, $p < .0001$), with higher putrescine dosages reducing ambulation compared to saline (Figure 29). During the first one-minute interval of testing ($T = 1$), ambulation by the saline group was statistically similar to ambulation exhibited by groups treated with 25, 50 and 100 mg/kg putrescine (approximately 32 squares entered/min). Ambulation exhibited by the 250 mg/kg putrescine group was significantly lower than that of all other groups during $T = 1$ (Dunnett's test $p < .01$), averaging about 20 squares entered/minute (Table 28). By the second test interval, ambulation of the 250 mg/kg group had decreased to less than 10 squares entered per minute, and was significantly different from saline during $T = 3$ (Dunnett's test $p < .01$). This decreasing ambulation continued over the last three test intervals, and during $T = 5$, subjects treated with 250 mg/kg putrescine were entering less than two squares per minute, which was significantly less than the ambulation of saline controls (Dunnett's test $p < .01$).

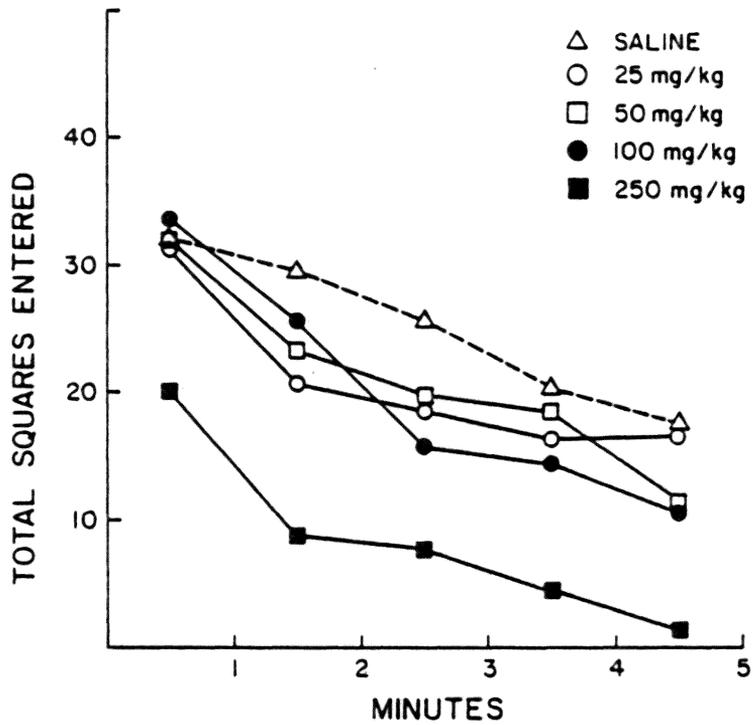


Figure 29. Effect of i.p. putrescine treatment on open field ambulation at 1.5-hr posttreatment.

Table 28
Duncan's Multiple-Range Test Results
for Putrescine (i.p.) Treatment Effect
and Open Field Ambulation^a

Treatment Group	Grouping ^b
Saline	*
25 mg/kg	*
50 mg/kg	*
100 mg/kg	*
250 mg/kg	**

^a Open field evaluation occurred over five one-minute intervals.

^b Treatments with identical grouping designations are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over five minutes.

The 25 and 50 mg/kg putrescine groups showed a similar decline in ambulation over successive intervals as saline controls, with these groups being statistically similar to saline from T = 1-4. However, at T = 5, the 50 mg/kg group had significantly lower ambulation compared to saline and 25 mg/kg groups (Dunnett's test $p < .01$). Subjects given 100 mg/kg putrescine showed similar ambulation activity as saline controls until T = 3, when ambulation for the 100 mg/kg group was significantly lower than saline (Dunnett's test $p < .05$). During the last test interval, ambulation for the 100 mg/kg group was still significantly lower than saline, but similar to the ambulation of the 50 mg/kg group. Thus, higher putrescine dosages (50-250 mg/kg) appeared to decrease ambulation compared to saline, especially with 250 mg/kg dosage, although this effect did not yield a treatment x time ANOVA interaction. Linearity of the dose-response correlation was significant ($p < .02$) (Table 32).

Grid coordination. Testing of initial coordination during grid rotation demonstrated that all groups exhibited comparable competency in maintaining initial grid posture, 2:15 hours posttreatment. No subject from any treatment group failed to hang onto the grid during initial rotation. As shown in Table 29, no reliable between groups differences existed, and no significant ANOVA treatment effect was found ($F(4,25)=1.25$, $p > .20$). Remeasurement of grid coordination and hang time endurance did

Table 29

Grid Coordination and Endurance Evaluation^a

2:15 Hours Posttreatment With Putrescine (i.p.)

Putrescine Treatment (mg/kg)	Mean Grid Hang Time \pm SEM(s)
0	13.7 \pm .88
25	15.0 \pm .00
50	14.7 \pm .33
100	13.8 \pm .83
250	12.7 \pm 1.33

^aMaximum score possible = 15.0 second hang time.

not yield significant between groups differences, 21 hours posttreatment.

Shock response thresholds. Figure 30 shows the results of measuring shock response thresholds at 3.5 hours posttreatment. Significant ANOVA main effects were obtained for jump ($F(4,25)=6.49$, $p<.001$) and squeal ($F(4,25)=7.38$, $p<.001$) criteria, but not flinch response ($F(4,25) = 2.64$, $p>.10$). Dunnett's test showed that only the 250 mg/kg putrescine group showed higher shock response thresholds which were significantly different from saline controls for jump ($p<.01$) and squeal ($p<.01$) responses. The 100 mg/kg group appeared to have a higher in squeal threshold, although this group was statistically similar to saline. However, dose-response curves were found to be linear for jump ($p<.02$) and squeal ($p<.01$) criteria (Table 32). Remeasurement of shock response thresholds at 21 hours posttreatment did not yield any significant ANOVA treatment effects for flinch ($F(4,25)=1.41$, $p>.20$), jump ($F(4,25)=1.47$, $p>.20$), and squeal ($F(4,25)=1.48$, $p>.20$) criteria.

Body temperature. Table 30 shows the results of measuring body temperature prior to treatment, 3:10 hours posttreatment, and the resulting change in body temperature (means in $C^{\circ} \pm SEM$). No differences were observed between group temperature means prior to treatment administration (Student's $t(4)=.38$, $p>.05$). Computation of the difference between posttreatment and baseline

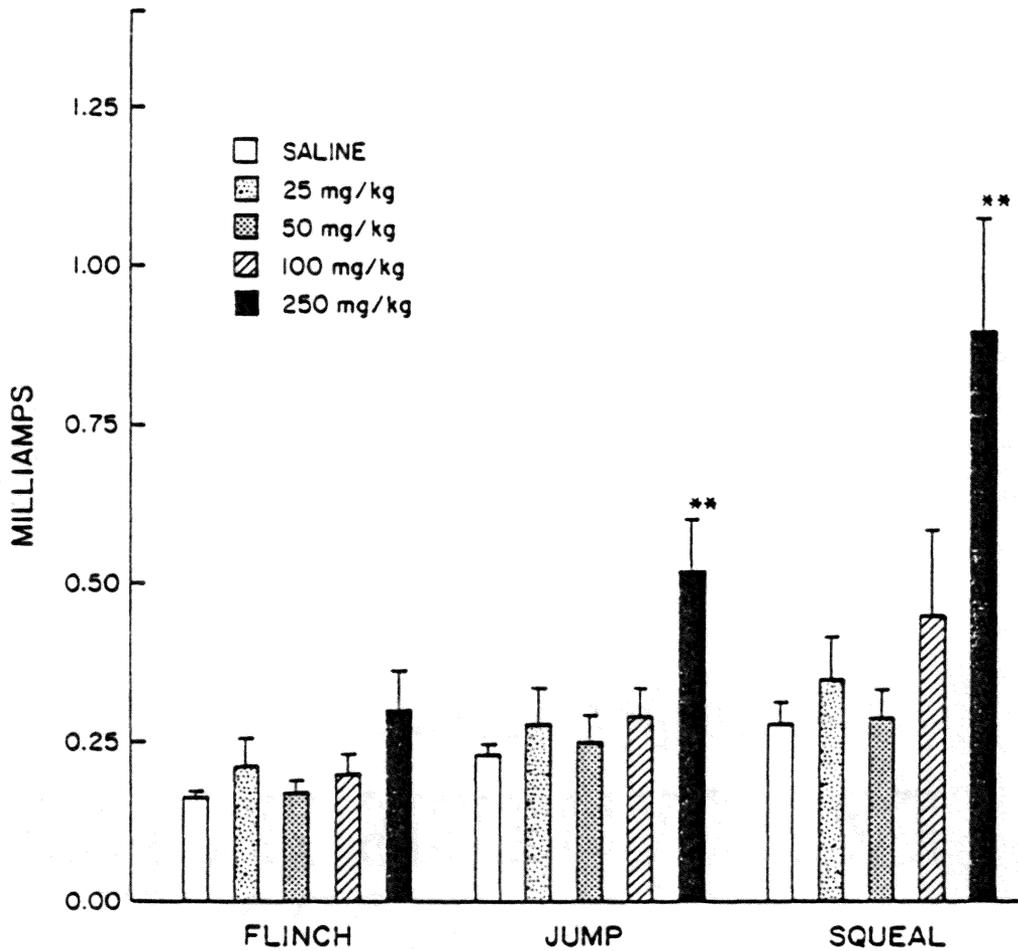


Figure 30. Effect of i.p. putrescine treatment on shock response thresholds for flinch, jump, and squeal criteria at 3.5-hr posttreatment. (Significant difference from saline using Dunnett's test denoted by ** $p < .01$).

Table 30

Change in Body Temperature

Three Hours Posttreatment With Putrescine (i.p.)

Putrescine Group	Mean Pretreatment Temp \pm SEM (°C)	Mean Posttreatment Temp \pm SEM (°C)	Δ Temperature \pm SEM (C°)
0 mg/kg	36.14 \pm .39	35.96 \pm .39	-0.18 \pm .29
25 mg/kg	36.38 \pm .30	35.76 \pm .09	-0.62 \pm .33
50 mg/kg	36.01 \pm .36	35.68 \pm .22	-0.33 \pm .17
100 mg/kg	35.99 \pm .34	35.36 \pm .15	-0.63 \pm .33
250 mg/kg	35.99 \pm .12	35.04 \pm .52	-0.95 \pm .62

body temperature means for all treatment groups did not yield a significant ANOVA main effect ($F(4,25)=1.24$, $p>.20$).

Food and Water Ingestion. Table 31 contains the results of measuring food and water ingestion behavior 2.5 hours post-treatment (4.5 hours of deprivation conditions). Food ingestion was scored "+" if a subject successfully placed a piece of lab chow into his mouth and began chewing it; "-" if actual chewing of the food was not observed. Water ingestion was measured only if licking of the drinking tube was observed. Incidence of water intake was measured in milliliter quantities during the five minute access period. The percentage of subjects which either engaged in successful food or water ingestion was used for statistical analysis.

No significant Chi-square difference between group proportions for exhibition of food ingestion was found ($p>.05$, $df = 4$). Only one subject out of 30 total subjects showed food ingestion during testing (one subject in the 100 mg/kg putrescine group). Thus, all putrescine groups were similar to saline controls, in that no groups exhibited significant food intake during testing at 2.5 hours posttreatment.

A significant Chi-square difference between group proportions was found for incidence of water ingestion ($p<.01$, $df = 4$). Both saline and 250 mg/kg groups had 67% of their subjects display water ingestion during testing, while all other groups (25,50, 100 mg/kg putrescine) had only 33% of their groups

Table 31
 Food and Water Intake^a 2.5 Hours Posttreatment
 With Putrescine (i.p.)

<u>Food Intake (+ or - score)</u>							
<u>Putrescine (mg/kg)</u>	<u>Subjects</u>						<u>%Subjects Ingested Food</u>
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
0	-	-	-	-	-	-	0%
25	-	-	-	-	-	-	0%
50	-	-	-	-	-	-	0%
100	-	-	-	-	+	-	17%
250	-	-	-	-	-	-	0%

<u>Water Intake (ml volume if +; - if no intake)</u>							
<u>Putrescine (mg/kg)</u>	<u>Subjects</u>						<u>%Subjects Ingested Water</u>
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
0	2	1	0	1	3	0	67%
25	1	1	-	-	-	-	33%
50	-	-	-	-	1	3	33%
100	-	-	1	-	6	-	33%
250	6	5	0	4	1	0	67%

^aFive minutes of food and water access was given simultaneously after 4.5 hours of deprivation conditions (deprivation began two hours prior to treatment).

display incidence of drinking. Comparison of average volumetric intake between saline and 250 mg/kg groups showed that subjects given 250 mg/kg putrescine drank more than twice the volume of water ($2.7 \pm .32$ ml) than did saline controls ($1.2 \pm .29$ ml). The data indicates that saline and 250 mg/kg putrescine groups had similar incidence of water intake at 2.5 hours posttreatment, which was significantly higher than the incidence observed in all other groups.

Linearity of dose-response curves. Of 14 significant ANOVA behavioral measures, eight measures yielded significant linear regression relationships (57%) using Pearson's r correlation coefficient at $p < .05$, or less (Table 32). One behavioral measure (mastication) showed a biphasic dose-response curve, with significant linearity for one component. Of the four categories in the time sampling protocol which showed a decline in the incidence of response due to treatment (sitting, sniffing, gnawing, rearing), three categories had significant linearity of dose-response curves (sitting, $p < .01$; sniffing, $p < .01$; gnawing, $p < .05$). No dose-response relationship was found for the ANOVA main effect on the incidence of grooming behavior.

Of the four behavioral categories associated with an increase in the incidence of response due to treatment (head down, abnormal behavior, WDS, mastication), only abnormal behavior and WDS yielded significant linearity of dose-response curves ($p < .01$ and $p < .02$, respectively). No dose-response relationship was

Table 32

Dose Response Correlations for Significant
Behavioral Effects With Putrescine (i.p.)^a

<u>Behavioral Response</u> ^b	<u>Pearson's r</u>	<u>Level</u>
Sitting	.975	p < .01
Rearing	.639	NS
Sniffing	.966	p < .01
Grooming ^c	.846	NS
Gnawing ^c	.931	p < .05
Head down posture ^c	.812	NS
Mastication ^c (biphasic)		
(0, 25, 50 mg/kg putrescine)	.998	p < .05
(50, 100, 250 mg/kg putrescine)	.862	NS
Abnormal behavior ^c	.982	p < .01
Wet dog shaking ^c	.938	p < .02
Initial startle response 3-hr	.676	NS
Startle response habituation 3-hr	.445	NS
Open field ambulation	.966	p < .01
Shock response jump thresholds 3.5-hr	.965	p < .02
Shock response squeal thresholds 3.5-hr	.972	p < .01

^aDose response correlations were calculated by linear regression analysis using Pearson's r correlation coefficient.

^bBehaviors analyzed were those having significant ANOVA main effects.

^cBehavioral response also having significant ANOVA treatment x time interactions.

obtained for the incidence of head down posture. Mastication stereotypy had a biphasic dose-response curve, with one component yielding a significant Pearson's r coefficient for the groups including saline, 25 and 50 mg/kg putrescine ($p < .05$). The other curve component (50, 100, and 250 mg/kg putrescine) did not have a significant Pearson's r coefficient. The incidence of grooming behavior was differentially increased or decreased according to putrescine dose, and this measure did not have significant dose-response.

Linearity of dose-response curves for initial starting response intensity and startle habituation at 3.5 hours post-treatment was not found. Other behavioral measures which yielded significant correlation coefficients included open field ambulation ($p < .02$), and shock response thresholds for jump ($p < .02$) and squeal ($p < .01$) responses.

Informal Observations

In subjects treated with 100 and 250 mg/kg putrescine, behavioral effects became obvious within two or three minutes postinjection. Subjects given 250 mg/kg putrescine exhibited certain behaviors which were not observed in saline controls. Subjects receiving 100 mg/kg putrescine had incidence of some of these behaviors, but effects were of a lesser intensity and shorter duration. Examples of these unquantified effects included postures (laying on side with rear paw raised off floor), movements (stretching of limbs and body), motor impairment,

respiratory difficulties, slow and exaggerated ambulation, partial grooming sequences, yawning behavior, paw-flicking genital grooming, head swaying, and "sliding/burrowing" activity. These anecdotally-observed behaviors were seen for a duration of 30-45 minutes posttreatment. Hypomotility, ptosis, piloerection, and sedation were also seen in groups given 100 and 250 mg/kg putrescine, and lasted between 1.5 to two hours posttreatment. No falccid immobility was seen in subjects treated with 250 mg/kg putrescine.

Subjects treated with 25 mg/kg putrescine were similar to subjects treated with saline in appearance and activity. A dose of 50 mg/kg resulted in some behaviors not observed in other groups, including mastication stereotypy and increased grooming activity. In general, subjects given 50 mg/kg putrescine exhibited effects which were intermediate compared to behavior of the saline and 250 mg/kg putrescine groups.

Discussion

Intraperitoneal administration of the polyamine, putrescine, was found to result in a complex of changes in the behavior of the adult male rat. During three and one-half hours of evaluation, comparison of subjects receiving either saline or putrescine (25, 50, 100, 250 mg/kg) demonstrated that putrescine treatment was associated with statistically significant changes in motor behavior, decreased ambulation, changes in the incidence of stereotypy, and depression of sensory reactivity. Informal observations seemed to complement the statistical evidence in suggesting that treatment-related effects of i.p. putrescine administration were not restricted to certain specific behaviors, but instead, appeared to reflect robust changes in many aspects of behavior.

For the 14 behavioral measures which yielded significant ANOVA main effects, the majority (57%) were found to have reliable dose-response relationships to putrescine treatment using linear regression analysis. Most significant changes in behavior were obvious in subjects given 250 mg/kg putrescine, although some behaviors appeared to be specific to other putrescine dosages. These observations and the fact that dose-response relationships were found for the majority of significant behavioral measures, suggests that quantitative and qualitative gradations of behavioral change may exist as a function of putrescine dose.

Discussion of the results in relation to previously cited research (Experiment 1) concerned with putrescine and the polyamines, GABA, other CNS substances, and behavior, follows. Directional effects mentioned in any synopsis of treatment effects on behavior are made in comparison to the behavior of saline-treated subjects. Brief comparison of the

behavioral effects of Experiment 2 to those of Experiment 1 will be made, with such comparisons based only on visual inspection of the data. Thus, any interpretations of similarities and differences between behavioral consequences of these experiments are preliminary and not statistically reliable.

Effects of Motor Behavior

Intraperitoneal putrescine treatment was associated with changes in the motor behavior of rats, affecting ambulation and general activity, the incidence of abnormal behaviors and stereotypy, but not coordination. Putrescine administration appeared to reduce activity levels in subjects treated with 250 mg/kg putrescine, and subjects given higher putrescine dosages (100-250 mg/kg) often exhibited changes in motor behavior which were not seen in saline controls.

Motor behavior: polyamine and GABA literature. As previously cited in the Discussion section of Experiment 1, changes in the behavior of rodents produced by in vivo polyamine treatment are dependent on the type of polyamine administered, and the rate at which the substance increases within the brain. Although depressant behavioral effects are observed with i.v.t. and i.v. polyamine (putrescine, spermine, spermidine) administration, some excitatory behavioral effects (tremors, convulsions, myoclonic jerking) are also characteristic of such treatments. Non-excitatory behavioral effects produced by i.v.t. and i.v.

polyamine treatment include decreased locomotor activity, marked postural changes, and flaccid paralysis.

As previously discussed in Experiment 1, longer latencies for brain increases of polyamines after i.p. administration have been reported to produce primarily depressant behavioral effects, and not excitatory effects. Examples of such effects seen after i.p. spermine and spermidine treatment include behavioral sedation, ataxia, hypomotility, and prolonged sedation lasting several hours posttreatment. The results of Experiment 2 suggest that some changes in motor behavior following i.p. putrescine administration resemble certain effects reported after cerebral (i.v.t. putrescine, spermine, spermidine) and systemic (i.v. and i.p. spermine, spermidine) polyamine administration (Anderson, et al., 1975; Nitico, et al., 1980; Shaw, 1972). Intra-peritoneal putrescine treatment resulted in the observation of primarily depressant effects, including decrement in ambulation and activity, appearance of motor behaviors not seen in saline controls (movements, postures), sedation, and changes in the incidence of other behaviors. No significant impairment of motor coordination was observed with i.p. putrescine treatment, and no excitatory behavioral effects characteristic of cerebral polyamine administration were seen in any putrescine group.

The importance of GABAergic function of the CNS maintenance of basal motor function and modification to excitatory afferent motoneuron activity has been well established in mammalian

organisms, as discussed in Experiment 1. Behavioral consequences reported in the literature after manipulations of in vivo GABAergic activity include the following: behavioral depression, reduction or lack of spontaneous locomotor and exploratory activity, appearance of abnormal or bizarre movements of the body and extremities, hunchback posture, body and forelimb shaking, ptosis, incidental head twitching, grooming, and sedation, as cited in Experiment 1. Increased GABAergic activity has also been reported to cause the appearance of behaviors seen with opiate abstinence and withdrawal conditions, including wet dog shake-like behavior, teeth chattering, and a writhing syndrome (De Boer, et al., 1980).

The results of Experiment 2 suggested that many motor effects produced by i.p. putrescine treatment were similar to behaviors which have been characterized as being GABAergic behaviors. Some of these behavioral similarities include the depressant effects on locomotor activity, sedation, exhibition of mutual abnormal behaviors (WDS, hunchback and other postures, abnormal movements of body and extremities), and the appearance of some behaviors which may be stereotypic. Stereotypies cited in the literature as being related to GABAergic activity include head twitching, shaking, grooming, and teeth chattering (De Boer, et al., 1980; Smialowski, et al., 1980). Certain behaviors seen after i.p. putrescine treatment (i.e., paw flicking, yawning, head swaying, grooming, mastication stereotypy) may be similar to

stereotypies which are thought to be GABAergic in nature. The observation of significant changes in the motor behavior of putrescine-treated subjects suggested that systemic (i.p.) putrescine administration may be affecting the function of other neurotransmitters in the CNS, either in a direct manner, or indirectly via GABAergic mechanisms.

Motor behavior and other CNS substances. A comprehensive review of behavioral and biochemical literature in the Discussion section of Experiment 1 outlined the importance of many CNS substances to the control, maintenance, and modification of motor behavior in rodents. Discussion of the motor effects of i.p. putrescine administration and the function of other neurochemicals in the expression of such effects will be made in reference to literature cited in Experiment 1.

Recent behavioral research allows the tentative suggestion that some effects of i.p. putrescine treatment on motor behavior may be related to the function of various CNS substances. The attenuation of locomotor activity and reduction of ambulation seen after putrescine treatment may be related to increased cholinergic or serotonergic (5-HT) activity, depletion of catecholamines, or changes in endogenous opiate peptide levels. Other observed behaviors suggested that direct or indirect involvement of nigrostriatal dopaminergic (DA) activity may have a role in changing the motor behavior of putrescine-treated subjects.

Dopamine activity is known to modify locomotor behavior, as well as the appearance of stereotypies, in various dose- and time-dependent ways. Thus, putrescine-induced stereotypies (i.e., paw flicking, yawning, mastication, grooming, head swaying) may be related to the DA mediation of stereotypic behavior. Facilitation of endogenous DA activity has been shown to result in the exhibition of sniffing, rearing, licking, grooming, and gnawing stereotypies (Fray, et al., 1980). The results of Experiment 2 showed apparent differential effects of putrescine treatment on the appearance and incidence of motor stereotypies, which may be interpretable in regards to differential dopaminergic CNS function. Higher putrescine doses (100, 250 mg/kg) resulted in the decreased incidence of rearing, sniffing, and gnawing behaviors, although a dose of 50 mg/kg produced significant increases in the levels of grooming and mastication behaviors. At a putrescine dose of 50 mg/kg, direct or indirect facilitation of DA activity may have occurred, resulting in the exhibition of significant grooming and mastication. However, at putrescine doses greater than 50 mg/kg, this putrescine effect on DA mechanisms was either reduced, lost, or modified in a manner which decreased the incidence of rearing, sniffing, and gnawing. Since catecholaminergic-specific stereotypies are known to be modifiable by other CNS substances (noradrenaline, serotonin, opioids), putrescine doses greater than 50 mg/kg may have

activated the modifying effects of these or other neurochemicals on the expression of motor behavior.

Several behaviors seen after i.p. putrescine treatment suggested the possible involvement of serotonergic and opiate peptide functions in their expression as cited in Experiment 1. An increase in serotonin (5-HT) activity can potentiate the manifestation of stereotypy, reduce DA-related hyperactivity, and modify locomotor behavior. The in vivo administration of beta-endorphin can cause passive behavior, decrease general motor activity, and produce the appearance of grooming and wet dog shaking behaviors. Since an opiate-related mechanism has been proposed as being responsible for the expression of WDS behavior, WDS seen after i.p. injection with 250 mg/kg putrescine may be a result of the activation of endogenous opiate peptide activity. Thus, the observation of a decrement in locomotor activity and the appearance of stereotypic behavior and WDS in high-dose subjects may be related to changes in 5-HT and opiate peptide CNS function as a result of putrescine treatment.

Anecdotally-observed behaviors seen after putrescine treatment also suggested the possible role of other CNS substances in the expression of putrescine behaviors, as cited in Experiment 1. Stretching and yawning syndrome (SYS) observed in some high-dose putrescine subjects has been reported after the in vivo administration of certain drugs which modify the function of several neurotransmitters, including serotonin, DA, glutamate,

and cholinergic substances. Head down posture, characteristic of 100 mg/kg putrescine treatment, is also related to the systemic administration of DA agonists, and is thought to be a sensitive indicator of enhanced DA function (Fray, et al., 1980).

"Stretching responses" have been associated with activity of visceral efferent neurons and tonic contractions of the body musculature caused by visceral stimulation and vagino-cervical stimulation, as cited in Experiment 1. Such stretching behavior has been reported as a response to the i.p. injection of certain irritating solutions, such as hypertonic lithium chloride (Nachman & Ashe, 1973). This stretching response was often seen in subjects given 250 mg/kg putrescine, and may be a result of direct viscerosomatic reflex or activity in other visceral organs, or else a response due to the painful i.p. injection of the putrescine solution. Sliding/burrowing activity was another behavior only observed in subjects treated with 250 mg/kg putrescine, and does not appear to be similar to literature reports of gustatory mimetic responses characteristic of oral reactivity to specific taste stimuli, such as paw pushing, chin rubbing, or limb flailing (Grill & Norgren, 1978). Although paw flicking was observed in some high dose putrescine subjects, it is doubtful that this behavior was a response to taste stimuli, since all treatment doses were administered by i.p. injection. Limb flailing was similar to limb flicking associated with face washing sequences, and thus, putrescine-induced paw flicking may be the

exhibition of related face washing/grooming activities (Grill & Norgren, 1978).

Comparison of Experiment 1 and 2 Results. Some of the changes in motor behavior of rats observed after oral putrescine treatment resembled certain motor effects seen after the i.p. administration of lesser putrescine dosages. Although some behavioral similarities were obtained, distinct differences in motor effects were also seen between the different routes of treatment and putrescine dosages administered.

Comparison of the general activity results showed that both p.o. and i.p. putrescine treatment decreased open field ambulation in a dose-dependent manner, in which the 1000 mg/kg p.o. and 250 mg/kg i.p. groups exhibited similar rates of decline in ambulation over five minutes of testing. At the end of open field testing, both p.o. and i.p. high-dose groups did not have significant levels of ambulation. Both routes of administration were found to have treatment effects which decreased rearing behavior, but without dose-response relationships. Intra-peritoneal putrescine doses of 50, 100, and 250 mg/kg reduced levels of rearing, over 60 minutes of sampling, while a time-dependent decrement in rearing was found for 1000 and 250 mg/kg p.o. putrescine groups only at 2.5 hours posttreatment. Neither route of administration yielded significant treatment effects for the incidence of walking behavior, or for impairment of motor coordination. Oral putrescine treatment was found to

reduce the incidence of sleep behavior without a dose-response relationship, while i.p. treatment did not yield an effect for this behavior. A dose-dependent decrease in the incidence of sitting behavior was found for i.p. putrescine treatment, while p.o. treatment did not yield an effect of this behavior.

Comparison of the stereotypy results showed that i.p. putrescine treatment resulted in the observation of more significant stereotypy effects than p.o. treatment. Non-body licking behavior did not yield significant treatment effects for either route of administration. Significant changes in the incidence of grooming and gnawing behaviors were found for both routes of administration, although the treatment effects were different. Comparison of the levels of grooming for saline control groups in both experiments indicated that a higher incidence of grooming activity existed for p.o. saline-treated subjects. This difference may have been prompted by oral treatment techniques, resulting in grooming activity due to oral reactivity to taste stimuli. Both p.o. and i.p. high-dose putrescine groups showed a significant decrease in grooming compared to saline controls, while an i.p. dose of 50 mg/kg increased grooming activity. Contrastingly different p.o. and i.p. treatment effects on gnawing behavior were seen. Intraperitoneal-treated subjects seemed to exhibit much higher levels of gnawing behavior than those treated orally, especially saline controls. While a significant dose- and time-dependent increase in gnawing behavior

was observed for p.o. putrescine treatment, a dose-dependent decrease in gnawing was seen for i.p. treatment. The differences in the p.o. and i.p. treatment effects on the levels of gnawing behavior may be a function of route of administration, differences between subject pools, temporal effects, or other unknown factors. A dose-dependent decrease in sniffing behavior was found for i.p. putrescine treatment, as well as the significant appearance of mastication stereotypy at an i.p. dose of 50 mg/kg. No significant p.o. putrescine treatment effects were obtained for either sniffing or mastication behaviors.

Comparison of the abnormal behavior results showed that i.p. putrescine treatment resulted in the observation of more significant abnormal behavior effects than p.o. treatment. Both routes of administration yielded significant dose- and time-dependent treatment effects for the incidence of wet dog shaking (WDS) behavior, although the temporal characteristics of the WDS effects differed between experiments. Subjects treated with 250 mg/kg putrescine i.p. began to exhibit WDS at 30 minutes post-treatment, while the 1000 mg/kg p.o. group did not have significant incidence of WDS until 50 minutes after treatment. The duration of WDS incidence for the p.o. condition was marked, with WDS levels remaining high between 2.5-3.0 hours posttreatment. In contrast, the levels of WDS behavior for the i.p. condition declined between 70-90 minutes posttreatment. The occurrence of peak incidence levels of WDS differed for the i.p. and p.o.

routes of administration (0:40 and 2:40 hours posttreatment, respectively). However, the maximum intensity levels were comparable for both conditions (5-6 WDS/10 min interval).

No significant p.o. treatment effects were found for motor impairment, head down posture, and abnormal movement/posture categories. No i.p. treatment effect was found for motor impairment, however, significant time-dependent i.p. effects were obtained for the incidence of head down posture and abnormal movement/posture. Head down posture was most frequently seen at a dose of 100 mg/kg i.p. putrescine, while abnormal movements/postures were characteristic of the 250 mg/kg i.p. group. Head down behavior was most evident during the first 30 minutes posttreatment, and incidence of abnormal movement/posture was highest during the first 20 minutes after i.p. putrescine administration.

Comparison of the informal observations showed that both p.o. and i.p. putrescine high-dose groups exhibited behaviors not frequently seen in other treatment groups, especially saline controls. These effects included abnormal function of motor abilities, locomotor difficulties, and deficit in general activity levels. The anecdotal observation of stereotypic behaviors seemed to indicate that both routes of administration caused the appearance of similar stereotypies, including paw flicking, yawning (SYS), frontal and genital grooming, mastication, and sliding/burrowing behavior. Some stereotypies may have been

specific to a certain treatment condition, i.e., flaccid immobility (p.o.), and head swaying (i.p.). The appearance of mastication and increased grooming behaviors was observed with an i.p. putrescine dose of 50 mg/kg.

Motor behavior: summary. Changes in the motor behavior of rats given i.p. injections of putrescine were interpreted as being similar to some behavioral effects reported after in vivo polyamine treatment by cerebral (i.v.t.) and systemic (i.v. and i.p.) routes of administration. The i.p. putrescine treatment effects were also found to resemble some of the changes in motor behavior seen following p.o. putrescine treatment (Experiment 1), although several effects were contrastingly different. Motor behavior effects produced by i.p. putrescine were primarily depressant effects, and included deficits in locomotion and ambulation, changes in the incidence of abnormal and other behaviors, as well as the observation of effects on stereotypy. No apparent excitatory behavioral treatment effects were seen, such as those associated with i.v. or i.v.t. polyamine administration.

Comparison of the p.o. and i.p. putrescine effects on general activity found that both treatment conditions decreased open field ambulation, decreased rearing behavior, and did not yield effects for walking behavior or impairment of motor coordination. A reduction in sleep behavior was found for p.o. putrescine treatment only, while a decrease in sitting behavior was

obtained for i.p. putrescine treatment only. Intraperitoneal putrescine treatment resulted in more significant stereotypy effects than p.o. treatment, and neither condition yielded an effect for non-body licking behavior. High-dose p.o. and i.p. putrescine treatment decreased grooming behavior, while 50 mg/kg i.p. putrescine significantly increased incidence of grooming activity. A contrasting effect on gnawing behavior was observed for p.o. and i.p. conditions, in which p.o. treatment increased gnawing and i.p. treatment decreased the level of gnawing. Intraperitoneal putrescine treatment was found to increase the incidence of mastication stereotypy and also decrease sniffing behavior, while p.o. putrescine did not yield treatment effects for these behaviors. Intraperitoneal putrescine treatment resulted in more significant abnormal behaviors than p.o. treatment, although neither condition yielded effects for motor impairment. Both routes of administration were found to produce the dose- and time-dependent incidence of WDS behavior, although the duration of this effect was much longer for the p.o. condition. An i.p. putrescine dose of 100 mg/kg produced significant head down posture and an i.p. dose of 250 mg/kg produced significant abnormal movements/postures, while p.o. treatment did not yield effects for these behaviors. Many similar anecdotal motor effects were seen after p.o. and i.p. putrescine administration, including abnormal motor abilities, locomotor difficulties, and deficit in general activity. Some apparent stereotypies were

also observed after both treatment conditions (i.e., paw flicking, yawning, mastication, sliding/burrowing), however, other behaviors appeared to be specific to one treatment condition, including flaccid immobility (p.o.) and head swaying (i.p.).

The literature supports the preliminary proposition that motor behaviors produced by i.p. putrescine treatment may be related to GABAergic CNS function, or else the direct or indirect influence on the function of other CNS substances, including the catecholamines (dopamine), cholinergic activity, opiate peptides, and serotonin. Some anecdotal behaviors seemed to indicate that i.p. putrescine treatment may affect glutamate CNS activity, stimulate visceral responses of the musculature or produce pain responses, initiate gustatory mimetic response related to oral reactivity and grooming, or produce motor effects which may be specific to i. p. putrescine administration. The observation of motor effects after p.o. putrescine treatment which resembled some effects produced by i.p. administration of lesser putrescine doses was preliminary evidence for the suggestion that systemic putrescine application may exert influences on the neuropharmacologic function of the rat CNS.

Effects on Sensory Reactivity

Intraperitoneal putrescine treatment was associated with changes in apparent sensory reactivity of rats, affecting startle response to acoustic stimuli and shock response thresholds.

Putrescine administration appeared to depress acoustic startle response, while producing increased response thresholds to electric shock.

Sensory reactivity: polyamine and GABA literature. Comprehensive review of behavioral literature concerned with sensory reactivity was presented in the Discussion of Experiment 1, and will be referred to in the discussion of i.p. treatment results. The importance of the polyamines for the manifestation of acoustic startle reflex has not been addressed in the literature, although there is indirect evidence that GABAergic mechanisms may be important to startle response behavior, as cited in Experiment 1. Since decrement in acoustic startle reflex is consistent with the facilitation of GABAergic inhibitory function in the CNS, the reduction of ISI and increase in startle response magnitude produced by i.p. putrescine may be related to behavioral effects mediated by spinal GABAergic activity.

The role of polyamines in the manifestation of analgesia has not been directly addressed in the literature, although polyamine treatment can prolong barbiturate-induced sleep (Anderson, et al., 1975). However, literature exists which suggests that GABAergic mechanisms may have a fundamental role in the expression of antinociceptive (analgesic) responses, as cited in Experiment 1. The systemic administration of GABA-T inhibitors, which increase endogenous GABA levels in the CNS, has been shown to result in significant analgesia which is reversible by GABA antagonists.

Certain GABAergic antinociceptive effects have also been found to enhance morphine analgesia. Thus, it may be possible that the apparent analgesic effects indicated by increased shock response thresholds following i.p. putrescine are related to GABAergic mediated analgesia.

Sensory reactivity and other CNS substances. Understanding of the primary startle neural circuitry in the CNS is increasing, as is understanding of the roles of several neurotransmitters in the modulation of startle reflex, including dopamine, serotonin, noradrenaline, and acetylcholine, as cited in Experiment 1. Although the neurotransmitter interactions involved in the manifestation of acoustic startle response are complex, consideration of i.p. putrescine treatment effects on ISI and startle response magnitudes suggest that i.p. putrescine may be affecting CNS systems which mediate or modulate startle reflex.

Various assessment techniques have been used to measure nociceptive responses to painful stimuli resulting in the finding that many CNS substances can produce analgesic effects, as cited in Experiment 1. Narcotic analgesics and endogenous opiate peptides have been shown to have a role in the manifestation of analgesia, and there is some evidence which has suggested that 5-HT is also involved in the function of nociceptive mechanisms. Other CNS substances, i.e., dopamine and the catecholamines, noradrenaline, and cholinergic substances, have also been hypothesized as being important to analgesic behavioral

effects. Thus, the apparent analgesic effects produced by i.p. putrescine treatment may be related to nociception associated with the function of several CNS substances, including the opiate peptide, serotonin, and the catecholamines.

Comparison of Experiment 1 and 2 results. Many of the changes in sensory reactivity behaviors of rats treated with p.o. putrescine resembled the effects seen after the i.p. administration of lesser putrescine dosages. Although most sensory reactivity effects were similar between the two conditions, some temporal and specific treatment effect differences were found.

Comparison of the startle response results showed that both p.o. and i.p. putrescine treatment decreased ISI and increased startle magnitude. However, i.p. putrescine yielded significant effects for these startle response measures at three hours posttreatment only, while p.o. treatment yielded significant effects at both two and 4.5 hours posttreatment. Significant shock response analgesia was found for both i.p. and p.o. putrescine treatment conditions at 3.5 hours and five hours posttreatment, respectively, but an i.p. treatment effect was not obtained for flinch criteria. The analgesic effects for the significant shock response criteria of both treatment conditions were found to be dose-dependent, and residual analgesia was only found for the 1000 mg/kg p.o. putrescine group at 21 hours posttreatment.

Comparison of the informal observations showed that both p.o. (250-1000 mg/kg) and i.p. (100-250 mg/kg) putrescine treatment often resulted in the observation of sedation tendencies, which were reversible by handling. Anecdotal impressions of subjects given higher p.o. and i.p. putrescine doses suggested that systemic putrescine administration may have produced some degree of decreased behavioral reactivity to stimulation, which was consistent with formal sensory reactivity results for both treatment conditions.

Sensory reactivity: summary. The intraperitoneal administration of putrescine was found to affect startle response reflex in a manner consistent with GABAergic influence on the expression of acoustic startle in the CNS of rats. These i.p. effects included the dose-dependent decrease of ISI and an increase of startle magnitude at three hours posttreatment which was not dose-dependent. The observed i.p. putrescine effects on startle response may also be related to the neuromodulating function of other CNS substances on startle, although the exact relationship between putrescine and other neurochemicals is not specifiable at present. Significant dose-dependent analgesia to electric shock at three hours posttreatment may be related to nociceptive effects associated with endogenous GABAergic analgesia-mechanisms, and/or other CNS nociceptive systems which may include the function of the opiate peptides, 5-HT, and the catecholamines.

Comparison of the p.o. and i.p. putrescine effects on sensory reactivity behavior indicated that both treatment conditions significantly modified sensory reactivity, and sometimes in similar ways. Putrescine administered either by p.o. and i.p. routes modified acoustic startle response, produced analgesia to electric shock, and seemed to induce sedation. However, some temporal and specific treatment effect differences were found. Intraperitoneal putrescine treatment reduced ISI and increased startle magnitude only at one posttreatment test session, while p.o. treatment significantly modified these startle measures at two posttreatment test sessions. Both p.o. and i.p. treatment conditions resulted in significant shock analgesia, although the i.p. condition did not yield an analgesic effect for flinch criteria, or show any residual analgesia at 21 hours post-treatment. The informal observations on sensory reactivity behavior gave the impression that p.o. and i.p. putrescine administration produced sedation-like tendencies which were similar in appearance.

The literature appeared to support the preliminary notion that i.p. putrescine treatment may result in depressant effects on the expression of acoustic startle reflex, shock response thresholds, and behavioral sedation. The observation of p.o. treatment effects on sensory reactivity which were similar to effects produced by i.p. treatment with lesser putrescine dosages, was preliminary evidence for a possible neuro-

pharmacologic role of systemically applied putrescine in the rats CNS. The systemic effects of putrescine administration on sensory reactivity may reflect a direct treatment effect on the function of several CNS substances, or else as indirect effect via GABAergic CNS mechanisms.

Body Temperature and Other Behaviors

Intraperitoneal putrescine treatment resulted in the significant modification of water ingestion behavior, but did not yield any effects for change in body temperature or food ingestion behavior. Informal observations of several behaviors suggested that i.p. putrescine treatment activated autonomic nervous system (ANS) responses.

Body temperature and CNS substances. The systemic and cerebral administration of polyamines has been reported to cause significant hypothermia in rodents (i.v.t. putrescine; i.v.t., i.v., i.p. spermine and spermidine) and chicks (i.v.t. putrescine), as cited in Experiment 1. The finding that i.p. putrescine treatment did not result in significant change in body temperature at 3:10 hours posttreatment, was contrary to past literature reports of polyamine effects on body temperature. Thus, the results indicated that the i.p. putrescine doses administered were not effective in influencing thermoregulatory mechanisms of the intact rat.

Food and water ingestion and CNS substances. Significant anorexia and adipsia have been produced in rodents following the

i.v.t. injection of the polyamines at doses lower than those necessary to produce observable behavioral effects (Anderson, et al., 1975). The hypothalamic control of fluid intake and drinking behavior is known to involve neurons containing acetylcholine and histamine, as well as the biogenic amines, amino acids, and peptides (Liebowitz, 1980). Recent evidence has also suggested that importance of the opiate peptides, dopamine, and GABA in the CNS regulation of drinking behavior, as cited in Experiment 1. The results of ingestive behavior testing showed that an i.p. putrescine treatment effect was observed for drinking behavior, but not feeding behavior. A higher percentage of subjects in the saline and 250 mg/kg groups (67%) exhibited an incidence of drinking behavior compared to all other putrescine groups (33%). Subjects in the 250 mg/kg putrescine group drank more water by volume than saline controls, but the results seem to indicate that putrescine doses of 25, 50, and 100 mg/kg decrease the exhibition of drinking behavior. These results are preliminary in nature, and suggested that the i.p. treatment effect on the exhibition of drinking behavior may be related to influences on CNS mechanisms which regulate water intake behavior in rats.

Other behaviors and the autonomic nervous system. Behaviors which appear to be characteristic of autonomic nervous system (ANS) activation have been reported after the cerebral and systemic administration of polyamines, elevation of endogenous GABA levels, and manipulations of endogenous and other opiate

substances, as cited in Experiment 1. The hypothalamus is known to regulate the ANS responses of the brainstem and spinal cord, as well as control ANS activities which may involve the CNS function of cholinergic, catecholaminergic, and GABAergic systems. The observation of ptosis, piloerection, and sedation in subjects receiving higher putrescine doses (100-250 mg/kg) suggested that i.p. putrescine administration may have directly or indirectly activated autonomic nervous system responses in the periphery. However, the possibility that these apparent ANS responses were centrally-mediated was supported by the observation that these effects were reversible by exteroceptive stimulation (handling) and no significant hypothermia was seen as a result of i.p. treatment (Shaw, 1972; Zetler, 1980).

Comparison of Experiment 1 and 2. Oral administration of putrescine produced significant posttreatment temperature changes, modified ingestive behavior, and caused the appearance of behaviors suggestive of ANS activation. In contrast, i.p. putrescine treatment induced the appearance of ANS-like responses, but did not affect body temperature or produce clear effects on ingestive behavior.

A dose-dependent decrease in body temperature was seen in subjects treated with p.o. putrescine at five hours post-treatment, while no significant change in body temperature was obtained after i.p. treatment at 3:10 hours posttreatment. Comparison of ingestive behavior results indicated contrasting

treatment effects between p.o. and i.p. conditions. Oral administration of 1000 mg/kg putrescine significantly suppressed incidence of food ingestion behavior, and also increased incidence of drinking behavior. In contrast, i.p. treatment with a dose of 250 mg/kg did not significantly affect food ingestion behavior, although doses of 25, 50, and 100 mg/kg apparently decreased incidence of drinking behavior compared to saline and 250 mg/kg groups. The administration of higher putrescine doses by p.o. (250-1000 mg/kg) and i.p. (100-250 mg/kg) routes resulted in the anecdotal observation of ptosis, piloerection, and sedation effects. The appearance of such effects suggested the activation of autonomic nervous system responses due to systemic putrescine treatment, although the reversibility of these effects by handling indicated that these behaviors may be centrally-mediated.

Body temperature and other behaviors: summary. Intra-peritoneal putrescine administration did not depress body temperature or modify food ingestion behavior, which was contrary to previously reported effects produced by cerebral and systemic polyamine administration in rodents. However, i.p. putrescine doses of 25, 50, and 100 mg/kg appeared to decrease incidence of drinking behavior compared to saline and 250 mg/kg groups. Effects of p.o. putrescine treatment on body temperature and food ingestion were different from the observed i.p. effects, since a p.o. dose of 1000 mg/kg produced significant hypothermia,

suppressed the incidence of food ingestion, and increased the incidence of water ingestion behavior. Both p.o. and i.p. treatment conditions produced behaviors which suggested the activation of peripheral or centrally-mediated ANS responses.

The regulation and control of thermogenesis, ingestive behavior, and ANS activity are known to be influenced by hypothalamic mechanisms involving the function and interaction of many CNS substances. The results of p.o. putrescine administration suggested that systemic putrescine treatment may directly or indirectly modify the CNS regulation of certain physiological functions. Although i.p. putrescine treatment did not yield significant effects for change in body temperature, or produce clear effects on ingestive behavior, the contrasting p.o. and i.p. results may be due to several factors. Such considerations include the possibility that significant i.p. effects were not obtained due to differences in the effectiveness of dosages administered, effects specific to either route of administration, temporal variations of treatment effects and behavioral evaluation methods, and the observation of artifactual data or inaccuracies resulting from the lack of proper control groups (ingestive data) and/or small group size (n=6).

General Conclusions

The results of Experiment 2 appear to be the first report of statistically significant behavioral effects produced by the

intraperitoneal administration of putrescine in adult male rats. Treatment-related effects included changes in motor behavior, depression of ambulation and activity, attenuation of sensory reactivity, and possibly changes in drinking behavior and activation of autonomic nervous system function. Of 14 behavioral measures which yielded significant ANOVA treatment effects, 57% were found to show reliable dose-response relationships to i.p. putrescine treatment.

Informal comparison of the results of Experiment 2 to those of Experiment 1 suggested that p.o. and i.p. putrescine administration produced some similar effects on motor behavior, sensory reactivity, and other behaviors, although several effects were different. Intraperitoneal putrescine was found to produce more stereotypy and abnormal behavior effects than p.o. treatment, however, both conditions produced decrement in ambulation, dose-dependent incidence of WDS behavior, and some similar anecdotal motor effects. Intraperitoneal and oral putrescine treatment modified acoustic startle response, produced shock analgesia and sedation, although p.o. effects appeared to be more robust than i.p. effects. No significant changes in body temperature or food ingestion behavior was seen for i.p. putrescine, in contrast to p.o. results, but i.p. treatment may have modified drinking behavior. Informal observations from both experiments suggested that systemic putrescine treatment caused the appearance of several ANS responses.

Consideration of the consequences of systemic putrescine application indicated that some of the behavioral effects related to oral treatment resembled certain effects produced by i.p. administration of lower putrescine dosages. Observation of similarities between effects resulting from either treatment condition suggested that the bases for expression and modification of similar behaviors may have involved common CNS and peripheral mechanisms in the intact rat. Descriptive and temporal dissimilarities between several p.o. and i.p. effects may have implied that exogenous putrescine differentially influenced some common behavioral mechanisms and/or influenced different mechanisms, dependent on dosage and route of administration. The preceding observations, as well as the finding of reliable dose-response relationships for the majority of significant putrescine effects in Experiment 1 and 2, were preliminary support for a neuropharmacological role of putrescine in the rat CNS.

Many of the behavioral effects obtained in Experiment 2 resembled previously reported consequences of in vivo putrescine and polyamine administration in rodents. The existing literature has shown that differential effects on behavior result as a function of the type of polyamine administered, dosage, route of administration, and the rate at which the polyamine increases within the brain (Anderson, et al., 1975; Shaw, 1972). The i.p. administration of spermine and spermidine in rodents has been

reported to produce primarily depressant effects on behavior (Shaw, 1972), without excitatory effects characteristic of cerebral polyamine treatment. Primarily depressant effects on behavior were observed after the oral and intraperitoneal administration of putrescine in Experiments 1 and 2, which were often similar to treatment effects of i.p. spermine and spermidine administration. The observation of certain differences between several p.o. and i.p. putrescine treatment effects appeared to be consistent with reports of differential changes in behavior as a result of various in vivo manipulations of exogenous polyamines.

The limitations of the experimental design have restricted the generalization of the findings to conditions specified in the materials and methods section. The informal comparison of behavioral effects seen after p.o. and i.p. putrescine treatment were not subjected to statistical analysis, and thus, any interpretations are preliminary. However, the observation of some similar treatment effects and significant dose-response relationships after both treatment conditions suggested that the behavioral consequences of systemic putrescine treatment were robust.

In conclusion, some of the behavioral effects of intraperitoneal putrescine administration were found to resemble certain changes in behavior associated with in vivo manipulations of the polyamines, GABA, and other CNS substances. It was

suggested that systemic putrescine treatment may have exerted direct influences on the expression of behavior in the intact rat, or indirect influences via GABAergic mechanisms and/or other CNS neurochemical systems. Thus, the results of Experiment 2 supported previous research which has tentatively suggested that exogenous putrescine may act as a neurotransmitter or neuromodulator in the brain (Nistico, et al., 1980; Shaw, 1979).

Additional behavioral and biochemical substantiation of the preceding interpretations is needed, including research which will evaluate the interactions between exogenous putrescine, GABA, and other neurochemicals. Investigations which utilize agonist and antagonist agents of other CNS substances may help to clarify the behavioral and neuropharmacological significance of putrescine in the mammalian central nervous system.

EXPERIMENT 3

Introduction

The systemic (intraperitoneal) administration of physiologic dosages of the polyamine, putrescine, has been shown to result in the measurable conversion to GABA in peripheral and CNS tissues of rodents (Caron, Cote & Kremzner, 1980; Tsuji & Nakajima, 1978). GABA is the major inhibitory neurotransmitter in regions of the basal ganglia (Anden, Anden & Wachtel, 1979; Balcom, Lenox & Meyerhoff, 1975; Dray, 1980), and may have a fundamental role in the function of striatal structures, including the striato-nigral pathway (Dray, 1980; Costa, Cheney, Mao & Moroni, 1978; van der Heyden, Venema & Korf, 1980).

Recent evidence has suggested the existence of GABAergic interactions with numerous CNS transmitters and neurochemicals, including dopamine, acetylcholine, serotonin, and most recently, the endogenous opiate peptides (Cattabeni, Bugatti, Groppetti, Maggi, Parenti & Racagni; Moroni, Peralta & Costa, 1979). The two brain regions which contain the highest concentrations of GABA, i.e., the substantia nigra and nucleus accumbens of the basal ganglia, also contain the highest concentrations of dopamine (Anden, Anden & Wachtel, 1979). The existence of GABAergic/dopaminergic interactions within the basal ganglia has been supported by biochemical and physiological evidence (Ferkany, Strong & Enna, 1980; McKenzie & Hansen, 1980; van der Heyden, Venema & Korf, 1980; Waddington & Cross, 1980; Walters,

Lakoski, Eng & Waszczak, 1978; Waszczak & Walters, 1979), and behavioral evidence (Kaakkola & Kaariainen, 1980; Marshall & Ungerstedt, 1977; Scheel-Kruger, Cools & van Wel, 1977; Thiebot, 1979; Waddington, 1977; Waddington & Cross, 1978, 1980).

Various experimental manipulations have been utilized for investigation of GABAergic/dopaminergic interactions within the basal ganglia, including the in vivo administration of agonist agents such as muscimol (GABA agonist) and apomorphine (dopamine agonist). Muscimol is a potent agonist of receptor sites of GABAergic neurons, and can produce physiological and behavioral effects which are consistent with GABAergic neurotransmitter function (Scheel-Kruger, Arnt, Brostrup, Christensen & Magelund, 1979). Apomorphine is a known dopamine agonist which potentiates endogenous dopaminergic activity, and can produce behavioral effects consistent with dopaminergic neurotransmitter function (Fray, Sahakian, Robbins, Koob & Iverson, 1980). The facilitation of GABAergic function by the systemic administration of muscimol has been shown to mediate and modify in vivo dopaminergic function, as well as the exhibition and expression of dopaminergic behavioral responses (McKenzie & Hansen, 1980; Scheel-Kruger, Cools & van Wel, 1977; van der Heyden, Venema & Korf, 1980; Waddington & Cross, 1980; Waszczak & Walters, 1979). See Appendix D for supplemental information concerning muscimol and apomorphine.

The results of Experiments 1 and 2 indicated that both oral and intraperitoneal administration of putrescine could produce statistically significant behavioral effects in rats, including changes in motor behavior, ambulation, and sensory reactivity. Certain behavioral effects seen after p.o. putrescine treatment (50-1000 mg/kg doses) were comparable to some effects seen after i.p. administration of lesser putrescine dosages (25-250 mg/kg) in terms of statistical significance, response characteristics, direction of treatment effect, and dose response. However, some behavioral effects had contrasting response characteristics which appeared to be dependent on/or specific to route of administration and dosage.

Review of behavioral literature gave preliminary support to the suggestion that systemic putrescine administration may directly or indirectly influence CNS neuropharmacology in the rat, as well as modify the expression of behaviors which resemble changes in behavior following in vivo manipulations of the polyamines, GABA, and other CNS substances. While Experiments 1 and 2 appear to be the first extensive evaluations of the systemic effects of putrescine administration in rats, understanding about the basis and nature of these behavioral effects is lacking in specificity. Thus, further investigation of the consequences of systemic putrescine treatment in comparison to and in conjunction with other neuropharmacologic agents may help to specify the significance of putrescine to CNS function.

The purpose of this experiment was to evaluate the effectiveness of intraperitoneal putrescine treatment in modifying behavioral effects induced by treatment with the dopamine agonist, apomorphine, and compare those results to the modifying effects of the GABA agonist, muscimol. The two questions to be addressed are 1) can i.p. putrescine treatment significantly modify apomorphine-induced behavior, and 2) to what extent do the modifications of apomorphine-induced behavior by putrescine resemble those observed following i.p. muscimol treatment? The biochemical and behavioral literature has demonstrated that GABAergic/dopaminergic interactions exist in the basal ganglia, and that manipulations of GABAergic function using muscimol can produce changes in dopaminergic behaviors, including locomotion and exhibition of stereotypy. Thus, the finding that putrescine can modify apomorphine-induced behavior in a manner similar to that of muscimol, would be preliminary support for the involvement of GABAergic mechanisms in the expression of CNS effects produced by systemic putrescine administration.

The experimental procedures used in Experiment 3 differed from those of Experiment 1 and 2. Evaluation of behavior in Experiment 3 was done over a time course of 45 minutes due to temporal limitations and short duration of apomorphine-induced effects. It is hypothesized that intraperitoneal administration of putrescine in rats may significantly modify apomorphine-induced behaviors, and that these modifications may be similar to those produced by intraperitoneal muscimol treatment.

Materials and Methods

Subjects. The subjects were 42 naive male Sprague-Dawley albino rats born and raised at the animal colony facilities of the Psychology Department, VPI & SU (Blacksburg, VA). Subjects were between 90 and 120 days of age (350-425 g) at time of use. Animals were housed and maintained as previously described (Experiment 1).

Apparatus. The apparatus used to administer i.p. treatment dosages were the same as those previously described (Experiment 2), while subcutaneous (s.c.) dosages were administered with 26 gauge needles and 1 cc syringes. All behavioral observations were made in clear plexiglass boxes (30 cm x 30 cm x 24 cm) which had clear plexiglass tops containing 64 evenly-spaced ventilation holes of small diameter. Every box had its sides covered on the exterior by brown wrapping paper to eliminate visual-field distractions for the subjects. A small amount of sawdust was placed in one corner of every box at the beginning of a subject's test session. All observation boxes were thoroughly cleaned and wiped with isopropyl alcohol between subjects.

Drugs and dosages. The experimental design was a 3 x 2 factorial which had six treatment groups (n = 7). The Treatment 1 condition had three levels (saline, putrescine, and muscimol), while the Treatment 2 condition had two levels (saline and apomorphine). Solutions of putrescine hydrochloride (SIGMA Chemical Company, St. Louis, MO), muscimol (Tridom Chemical,

Inc., Hauppauge, NY), and apomorphine hydrochloride (SIGMA Chemical Company, St. Louis, MO) were prepared in .85% saline no more than one hour prior to treatment. For apomorphine solutions, 1 mg/cc ascorbic acid was added as an antioxidant. Putrescine (75 mg/kg), muscimol (.75 mg/kg), and apomorphine (.25 mg/kg) dosages were administered in an injection volume of 1 cc/kg body weight per subject. These treatment dosages were selected after consideration of the behavioral results of Experiment 2 and pilot studies with muscimol and apomorphine, as well as literature reports which studied the systemic administration of muscimol (McKenzie & Hansen, 1980) and apomorphine (Scheel-Kruger, Cools & van Wel, 1977). Treatment 1 doses were administered by i.p. injection, while Treatment 2 doses were administered by s.c. injection (lower neck region). Each subject was used in only one test session.

Procedures

The experimental protocol used in this study differed from all previous experiments, in that time sampling evaluation was the only assessment method utilized (Table 32). The experimental design of Experiment 3 was constrained by the short temporal duration (approximately 40 minutes) of behavioral effects produced by an apomorphine dose of .25 mg/kg, as determined by pilot evaluations and literature reports (Scheel-Kruger, Cools and van Wel, 1977).

The behavioral criteria used for time sampling testing in Experiment 3 differed from those used in Experiments 1, and 2. The behavioral categories were designed to allow evaluation of treatment effects on locomotor and stereotypic components of behavior which are known to be affected by dopaminergic manipulations in the CNS (Fray, Sahakian, Robbins, Koob and Iverson, 1980; Scheel-Kruger, Cools and van Wel, 1977). As described in the Thesis Rationale section of the General Introduction, two major types of activity were assessed in Experiment 3 (comprised of 13 behavioral categories): general locomotor activity (stationary, walking, rearing), and specific activity (sleep, awake/mostly immobile, mobile/nonstereotypic behavior, grooming, head down posture, mastication, discontinuous sniffing, continuous sniffing, licking/mouthing sawdust, and gnawing sawdust). The definitions of these categories are given in Table 3 of the General Introduction. The behavioral categories represent a list of discrete, arbitrarily-defined responses and were not used as a stereotypy scale per se.

All subjects were deprived of food and water 90 minutes prior to the beginning of the experiment. The experiment began during the last two hours of the light portion of the light-dark cycle. Body weights were measured prior to treatment. Treatment 1 doses were given first (i.p.) and were followed 15 minutes later by Treatment 2 doses (s.c.). All treatments were given experimenter-blind. Handling of subjects prior to the start of

time sampling evaluation was done in a manner as previously described (Experiment 1).

Ten minutes after the Treatment 2 dose administration, subjects were directly observed for 45 minutes by time sampling technique, with each subject monitored for 45 intervals in a repeated measures sequence. Each subject was observed for 10 seconds during every minute, and one behavioral category scored from each of the two previously described activity types, i.e., general locomotor activity and specific activity. No further testing was done at the conclusion of time sampling evaluation. The protocol for Experiment 3 is given in Table 32.

Data analysis. The statistical tests used for analysis of data included analysis of variance (ANOVA) and Duncan's multiple-range test. The level of significance for all statistical analyses was $\alpha = .05$, unless otherwise noted.

Table 32
Experiment 3 Protocol

Procedure	Time Course
1. Food and water deprivation begins 90 min prior to experiment	- 1:30 hr
2. Body weights	- 0:10 hr
3. Treatment 1 - i.p. injection (saline, putrescine, muscimol)	0:00 hr
4. Treatment 2 - s.c. injection (saline, apomorphine)	+ 0:15 hr
5. Time sampling (10 behavioral categories, subjects scored once per minute)	+ 0:25 hr
6. End of time sampling	+ 1:10 hr

Results

Statistical analysis of the data demonstrated that systemic administration of Treatment 1 and Treatment 2 dosages resulted in significant behavior effects in adult male rats. Of 13 behavioral categories comprising two major activity types (general locomotor and specific activity), nine categories yielded significant ANOVA Treatment 1, Treatment 2, Tr 1 x Tr 2, or treatment x time interaction effects (69%).

Formal Observations

Time sampling: General locomotor activity. Of three categories (stationary, walking, rearing), only walking did not yield any significant ANOVA effects (F -values are given in Table 33). The group means for the incidence of stationary and rearing behaviors are presented in Table 34. Significant Treatment 2 ANOVA main effects were obtained for both stationary ($p < .05$) and rearing ($p < .05$) behavioral categories (Table 33). Duncan's analysis of these Treatment 2 effects showed that apomorphine treatment significantly decreased the incidence of stationary behavior over 45 minutes of sampling compared to treatment with saline (Table 34). Duncan's test results also showed that apomorphine treatment increased the incidence of rearing behavior compared to saline treatment ($p < .05$). None of the general locomotor activity categories yielded any significant ANOVA treatment x time interactions.

Table 33
 ANOVA F-Values for Treatment 1, Treatment 2,
 and Tr 1 x Tr 2 Effects and
 Incidence of Time Sampling Behaviors^a

Behavioral Category	Treatment 1 ANOVA <u>F</u> -Value	Treatment 2 ANOVA <u>F</u> -Value	Tr 1 x Tr 2 ANOVA <u>F</u> -Value
Stationary (ST)	.59	4.73 ***	.25
Walking (WA)	.18	4.00	1.58
Rearing (RE)	.72	4.39 ***	.78
Sleep (SL)	.38	5.61 ***	.43
Awake, mostly immobile (AW)	1.47	86.86 *	1.44
Mobile, non- stereotypic (NON)	1.74	25.70 *	2.27
Grooming (GR)	.54	3.07	2.16
Head down (HD)	1.39	3.86	.35
Mastication (MAST)	1.53	6.81 ***	4.31
Discontinuous sniffing (DS)	1.16	64.92 *	1.31
	df = 2,36	df = 1,36	df = 2,36

* $p < .0001$, ** $p < .003$, *** $p < .05$.

^a All F-values are nonsignificant ($p > .05$), unless otherwise noted.

Table 33 (Continued)

ANOVA \underline{F} -Values for Treatment 1, Treatment 2, and
Tr 1 x Tr 2 Effects and Incidence of Time Sampling Behaviors^a

Behavioral Category	Treatment 1 ANOVA \underline{F} -Value	Treatment 2 ANOVA \underline{F} -Value	Tr 1 x Tr 2 ANOVA \underline{F} -Value
Continous sniffing (CS)	3.64	11.37 **	3.94 ***
Licking (LI)	2.76	11.04 **	2.51
Gnawing (GN)	1.54	3.56	1.82
	df = 2,36	df = 1,36	df = 2,36

* $p < .0001$, ** $p < .003$, *** $p < .05$

^a All \underline{F} -values are nonsignificant ($p > .05$), unless otherwise noted.

Table 34

Group Means and Duncan's Multiple-Range Test Results
for Behavioral Categories with Significant Treatment 2 Effect

Behavioral Category	Treatment 2 Group	Mean Incidence ^a of Behavior	Duncan's _s ^b Grouping
Stationary (ST)	sal	8.15	*
	apo	6.74	**
Rearing (RE)	apo	1.21	*
	sal	0.24	**
Sleep (SL)	sal	1.02	*
	apo	0.01	**
Awake, mostly immobile (AW)	sal	5.13	*
	apo	0.13	**
Mobile, non-stereotypic (NON)	sal	1.33	*
	apo	0.07	**
Licking (LI)	apo	1.02	*
	sal	0.04	**

^a Means represent average incidence of behavior over 45 minutes of time sampling.

^b Groups with different grouping designations are significantly different at $p < .05$ (Duncan's analysis).

Time sampling: Specific activity. Of 10 behavioral categories, only three (grooming, head down, gnawing) did not yield any significant ANOVA effects (Table 33). Significant Treatment 2 ANOVA main effects alone were found for four behavioral categories, including sleep ($p < .05$), awake/mostly immobile ($p < .0001$), mobile/nonstereotypic behavior ($p < .0001$), and non-body licking behavior ($p < .003$). The group means for the incidence of these behaviors and the results of Duncan's analysis of the significant Treatment 2 ANOVA effects are given in Table 34. Duncan's test results showed that apomorphine treatment significantly decreased the incidence of sleep (SL), awake/mostly immobile (AW), and mobile/nonstereotypic (NON) behaviors over 45 minutes of sampling compared to saline controls ($p < .05$). Subjects receiving apomorphine had a significant increase in the incidence of licking (LI) behavior compared to those receiving saline treatment (Table 34). No significant ANOVA treatment x time interactions were found for SL, AW, NON, and LI behaviors.

One behavioral category, mastication stereotypy (MAST), was found to have a significant Treatment 1 x Treatment 2 ANOVA effect ($F(2,36)=4.31$, $p < .03$), but no treatment x time interactions (Figure 31). Simple effects analysis of this Tr 1 x Tr 2 interaction for MAST showed that when Treatment 2 = saline, a significant Treatment 1 effect was obtained ($F(2,18)=5.46$, $p < .02$). Duncan's analysis showed that muscimol treatment produced the highest incidence of mastication stereotypy, while

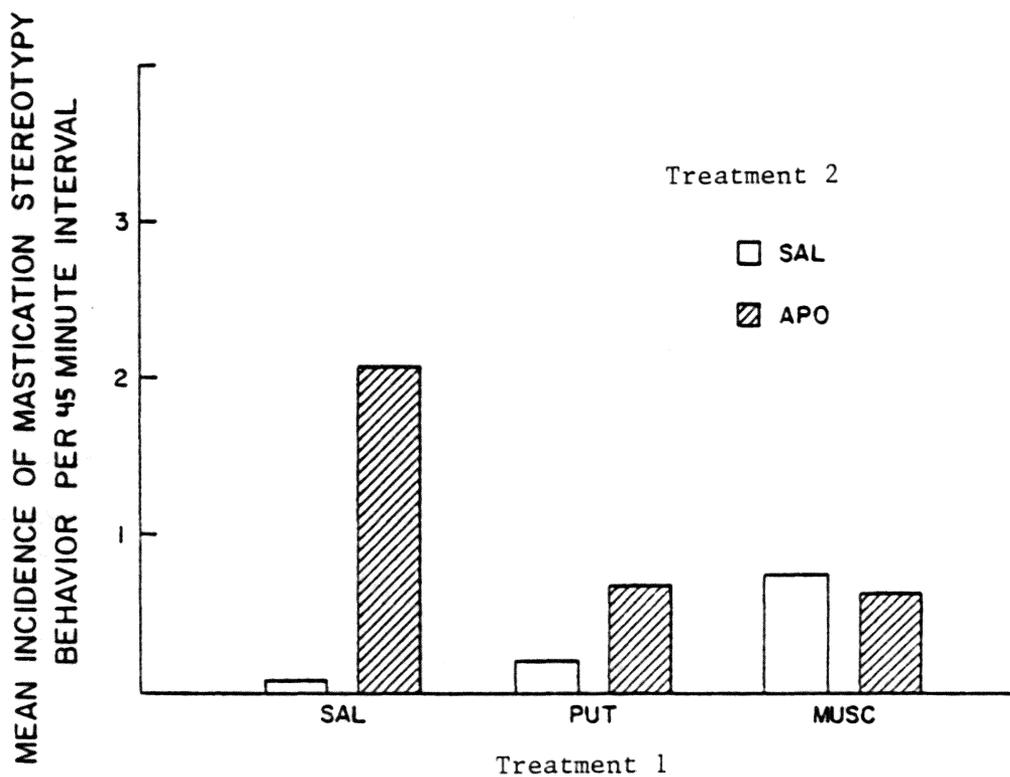


Figure 31. Treatment 1 x Treatment 2 interaction effect and the incidence of mastication stereotypy behavior.

putrescine and saline groups had significantly lower incidence of this behavior over 45 minutes of sampling (Table 35). When Treatment 2 = apomorphine, no significant Treatment 1 effect was found ($F(1,18)=2.69$, $p>.09$). A significant Treatment 2 effect was obtained only when Treatment 1 was saline ($F(1,12)=6.20$, $p<.03$), and not when Treatment 1 = putrescine ($F(1,12)=2.44$, $p>.14$) or muscimol ($F(1,12)=.16$, $p>.70$). Duncan's test results showed that apomorphine treatment produced a significantly higher incidence of MAST compared to treatment with saline (Table 35).

Two behavioral categories yielded significant Tr 1 x Tr 2 x time ANOVA interactions, including discontinuous sniffing ($F(8,144)=2.49$, $p<.02$) and continuous sniffing ($F(8,144)=2.19$, $p<.04$). Figure 32 depicts the Tr 1 x Tr 2 x time effects on the incidence of discontinuous sniffing (DS) behavior. At T = 3 (27 minutes posttreatment), a significant Treatment 1 x Treatment 2 ANOVA interaction effect was found for the incidence of DS behavior ($F(2,36)=4.86$, $p<.02$). Simple effects analysis found a significant Treatment 1 effect at T = 3 when Treatment 2 = apomorphine only ($F(2,18)=4.86$, $p<.03$). Duncan's analysis (Table 36) showed that the putrescine and muscimol groups were statistically similar at T = 3, and had a level of DS behavior which was significantly lower than that of the saline group ($p<.05$). Significant Treatment 2 simple effects for DS behavior were found when Treatment 1 = saline ($F(1,12)=43.71$, $p<.0001$) and putrescine ($F(1,12)=11.12$, $p<.006$), but not when Treatment 1 =

Table 35

Duncan's Multiple-Range Test Results for
 Treatment 1 x Treatment 2 Interaction
 and Incidence of Mastication Stereotypy^a

Treatment 1 Grouping	Treatment 2 Grouping
(when Tr 2 = sal)	(when Tr 1 = sal)
mus *	apo *
put **	sal **
sal **	
(when Tr 2 = apo)	(when Tr 1 = put)
mus *	apo *
put *	sal *
sal *	
	(when Tr 1 = mus)
	sal *
	apo *

^a Treatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 45 minutes.

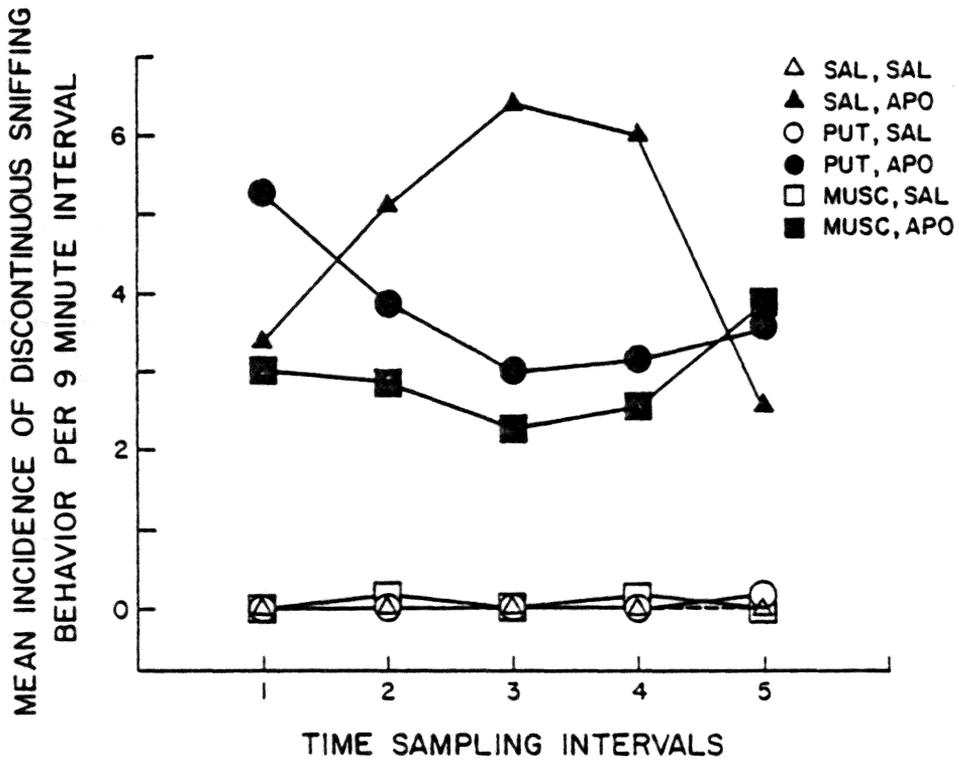


Figure 32. Treatment 1 x Treatment 2 x time interaction effect and the incidence of discontinuous sniffing behavior.

Table 36

Duncan's Multiple-Range Test Results for Treatment 1
 x Treatment 2 Interaction at T = 3 and Incidence of
 Discontinuous Sniffing Behavior

Treatment 1 Group Means ^a and Duncan's Groupings ^b	Treatment 2 Group Means ^a and Duncan's Groupings ^b
(when Tr 2 = apo)	(when Tr 1 = sal)
sal = 6.34 * put = 3.00 ** mus = 2.29 **	apo = 6.43 * sal = 0.00 **
	(when Tr 1 = put)
	apo = 3.00 * sal = 0.00 **
	(when Tr 1 = mus)
	apo = 2.29 * sal = 0.00 *

^a Means represent average incidence of behavior over 45 minutes of time sampling.

^b Groups with different grouping designations are significantly different at $p .05$ (Duncan's analysis).

muscimol ($F(1,12)=4.11$, $p>.06$). Duncan's test results (Table 36) showed that when Treatment 1 = saline or putrescine, a higher incidence of DS behavior was observed for subjects treated with apomorphine compared to those treated with saline ($p<.05$). Significant Treatment 2 effects only were obtained at sampling intervals $T = 1$ ($p<.0001$), $T = 2$ ($p<.0001$), $T = 4$ ($p<.0001$), and $T = 5$ ($p<.0001$). ANOVA F -values for these Treatment 2 effects are given in Table 37. Duncan's test results for these Treatment 2 simple effects (Table 38) showed that apomorphine treatment produced a significantly higher incidence of DS behavior ($p<.05$) than saline treatment at $T = 1, 2, 4$, and 5 .

Figure 33 is the graphic representation of the Treatment 1 x Treatment 2 x time effects on the incidence of continuous sniffing (CS) behavior. Significant Treatment 1 x Treatment 2 ANOVA interactions were found for the incidence of CS behavior at $T = 2$ ($F(2,36)=4.35$, $p<.03$) and $T = 3$ ($F(2,36)=4.09$, $p<.03$). Significant Treatment 1 ANOVA simple effects were found when Treatment 2 = apomorphine only, at $T = 2$ ($F(2,18)=4.44$, $p<.03$) and $T = 3$ ($F(2,18)=4.06$, $p<.04$). Duncan's analysis (Table 39) showed that when Treatment 2 = apomorphine at $T = 2$ and 3 , subjects receiving putrescine had a significantly higher incidence of CS behavior compared to saline control ($p<.05$), but that the putrescine and saline groups were statistically similar to the muscimol group. Significant Treatment 2 ANOVA simple effects were found when Treatment 1 = putrescine only at $T = 2$ ($F(1,12)=9.25$, $p<.02$) and

Table 37

ANOVA F-Values for Treatment 1, Treatment 2,
and Tr 1 x Tr 2 Effects and Incidence
of Discontinuous Sniffing Behavior^a

Time Sampling Interval ^b	Treatment 1 ANOVA <u>F</u> -Value	Treatment 2 ANOVA <u>F</u> -Value	Tr 1 x Tr 2 ANOVA <u>F</u> -Value
T = 1	1.51	46.80 *	1.51
T = 2	0.67	26.27 *	0.85
T = 3	4.86 **	45.33 *	4.86 **
T = 4	2.46	34.41 *	2.75
T = 5	0.38	25.01 *	0.33
	df = 2,36	df = 1,36	df = 2,36

* $p < .0001$, ** $p < .02$.

^a All F-values are nonsignificant ($p > .05$), unless otherwise noted.

^b Each time sampling interval was 9 minutes in duration; total testing time = 45 minutes.

Table 38
 Duncan's Multiple-Range Test Results
 for Treatment 2 Effects and Incidence
 of Discontinuous Sniffing Behavior

Time Sampling Interval ^a	Treatment 2 Group Means	Duncan's _s ^b Grouping
T = 1	apo = 3.90 sal = 0.00	* **
T = 2	apo = 3.95 sal = 0.05	* **
T = 4	apo = 3.90 sal = 0.05	* **
T = 5	apo = 3.33 sal = 0.05	* **

^a Each time sampling interval was 9 minutes in duration; total testing time = 45 minutes.

^b Groups with different grouping designations are significantly different at $p < .05$ (Duncan's analysis).

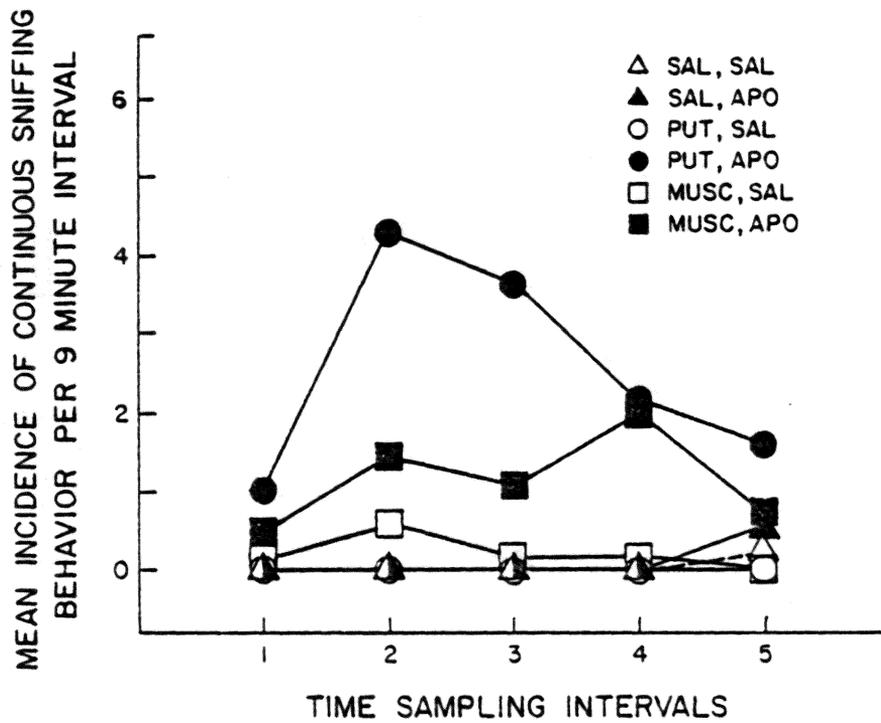


Figure 33. Treatment 1 x Treatment 2 x time interaction effect and the incidence of continuous sniffing behavior.

T = 3 $F(1,12)=9.55$, $p<.01$), and not when Treatment 1 = saline ($p>.50$) or muscimol ($p>.30$) at either sampling interval.

Duncan's test results (Table 39) showed that when Treatment 1 = putrescine at T = 2 and 3, apomorphine treatment produced significantly higher incidence of CS behavior than did saline treatment ($p<.05$).

Significant Treatment 2 effects only were found for the incidence of CS behavior at sampling intervals T = 1 ($p<.03$), T = 4 ($p<.02$), and T = 5 ($p<.005$). ANOVA F -values for these Treatment 2 effects are given in Table 40. Duncan's analysis for these Treatment 2 effects (Table 41) showed that subjects receiving apomorphine treatment had a significantly higher incidence of CS behavior ($p<.05$) than subjects receiving saline treatment at T = 1, 4, and 5.

Informal Observations

The administration of putrescine and muscimol (Treatment 1) dosages resulted in the observation of minimal behavioral effects within 10 minutes posttreatment, which were not seen in sal/sal subjects. Subjects receiving putrescine treatment exhibited some sedative behaviors (attenuated mobility and reduced rearing), head down posture, and incidental genital grooming. Subjects treated with muscimol did not readily exhibit significant behavior changes, except incidental yawning behavior. Behavioral modifications observed following the administration of higher i.p. dosages of putrescine and muscimol (Experiment 2 and pilot

Table 39

Duncan's Multiple-Range Test Results for Treatment 1
 x Treatment 2 Interaction at T = 2, 3 and Incidence
 of Continuous Sniffing Behavior

Treatment 1 Group Means ^a and Duncan's Groupings ^b	Treatment 2 Group Means ^a and Duncan's Groupings ^b
<u>At T = 2</u>	
(when Tr 2 = apo)	(when Tr 1 = put)
put = 4.29 *	apo = 4.29 *
mus = 1.43 *	sal = 0.00 **
sal = 0.00 **	
<u>At T = 3</u>	
(when Tr 2 = apo)	(when Tr 1 = put)
put = 3.43 *	apo = 3.42 *
mus = 1.29 *	sal = 0.00 **
sal = 0.00 **	

^a Means represent average incidence of behavior over 45 minutes of time sampling.

^b Groups with different grouping designations are significantly different at $p .05$ (Duncan's analysis).

Table 40

ANOVA \underline{F} -Values for Treatment 1, Treatment 2, and
Tr 1 x Tr 2 Effects on Incidence of
Continuous Sniffing Behavior^a

Time Sampling Interval ^b	Treatment 1 ANOVA \underline{F} -Value	Treatment 2 ANOVA \underline{F} -Value	Tr 1 x Tr 2 ANOVA \underline{F} -Value
T = 1	0.91	5.33 ***	0.91
T = 2	3.89 ***	7.46 **	4.35 ***
T = 3	3.98 ***	9.34 *	4.09 ***
T = 4	1.76	6.12 **	1.55
T = 5	0.71	9.16 *	0.98
	df = 2,36	df = 1,36	df = 2,36

* $p < .005$, ** $p < .02$, *** $p < .03$

^a All \underline{F} -Values are nonsignificant ($p > .05$), unless otherwise noted.

^b Each time sampling interval was 9 minutes in duration; total testing time = 45 minutes.

Table 41
 Duncan's Multiple-Range Test Results
 For Treatment 2 Effect and Incidence
 of Continuous Sniffing Behavior

Time Sampling Interval ^a	Treatment 2 Group Means	Duncan's s _b Grouping
T = 1	apo = 0.43 sal = 0.00	* **
T = 4	apo = 1.38 sal = 0.05	* **
T = 5	apo = 1.00 sal = 0.05	* **

^a Time sampling interval = 9 minutes; total sampling time was 45 minutes.

^b Groups with different grouping designations are significantly different at $p < .05$ (Duncan's analysis).

Discussion

The systemic administration of the dopamine agonist, apomorphine, was found to produce certain excitatory effects in the adult male rat, including stimulated locomotor activity and the increased incidence of stereotypic behaviors compared to saline controls. Intraperitoneal injection of putrescine per se resulted in a few anecdotally-observed depressant effects, while i.p. muscimol treatment per se only increased the incidence of mastication compared to saline and putrescine treatments. However, significant time-dependent incidence of sniffing stereotypies was obtained for subjects treated with putrescine in conjunction with apomorphine. Subjects in the mus/apo group exhibited some changes in the incidence of sniffing stereotypies, but the effects were of lesser intensity compared to the put/apo group.

Locomotor Activity

General activity. Apomorphine treatment per se decreased the incidence of sitting behavior, increased the incidence of rearing behavior, but did not modify walking behavior over 45 minutes of testing compared to saline controls. No significant Treatment 1 effects were found for any general activity category. These results suggested that apomorphine treatment produced excitatory effects on general locomotor activity, a finding consistent with previous reports of apomorphine-induced changes in locomotor function. Increased locomotor activity can be

induced in rodents by intracerebral injection of dopamine (DA) or apomorphine into the nucleus accumbens of the basal ganglia (Issacson, Yongue & McClearn, 1978), and by the systemic administration of apomorphine or d-amphetamine (Fray, et al., 1980; Lloyd and Hornykiewicz, 1975). Dose-dependent and usually monotonic stimulation of locomotor activity has been reported to be characteristic of apomorphine dosages (.1-5.0 mg/kg) given by subcutaneous (s.c.) injection (Fray, et al., 1980). However, treatment with d-amphetamine is apparently more effective than apomorphine in producing facilitation of rearing activity (Fray et al., 1980).

Specific activity. Three out of 10 specific activity behavioral categories (SL, AW, NON) yielded significant Treatment 2 effects which were complementary to the apomorphine effects on general locomotor activity. Apomorphine treatment per se decreased the incidence of sleep behavior, awake/mostly immobile behavior, and mobile/nonstereotypic behavior over 45 minutes of sampling compared to saline controls. No significant Treatment 1 effects were found for these three behavioral categories. These results reflected an apparent excitatory effect of apomorphine treatment which produced the decline of stationary and sleep behavior, while increasing the incidence of more mobile locomotor activities. The observed change in these specific activity categories appeared to be consistent with previously cited apomorphine effects on locomotor function. The results were also

in agreement with the hypothesized involvement of brain catecholamines in the central control and regulation of motor function, including movement, posture, and coordination (deFeudis, 1974; Lloyd and Hornykiewicz, 1975).

It has been generally accepted that intrastriatal DA has a primary role in the maintenance of normal locomotor function, while noradrenaline may play a secondary role in the modification of locomotion (Lloyd and Hornykiewicz, 1975). Central cholinergic systems are also important to the reflex regulation of movement and postural mechanisms, and may have functional interactions with catecholaminergic systems which influence extrapyramidal and cerebellar activities (deFeudis, 1974). Thus, the observed effects of apomorphine treatment which appeared to stimulate locomotor activity was in agreement with literature which has proposed that stimulation of locomotion depends primarily on the stimulation of the nigrostriatal dopaminergic system (Lloyd and Hornykiewicz, 1974; Scheel-Kruger, Christensen and Arnt, 1978). Recent evidence has suggested that different DA mechanisms may exist within the nucleus accumbens which differentially influence the expression of dopaminergic locomotor activity and dopaminergic stereotypy (Costall, Naylor, Cannon & Lee, 1977; Scheel-Kruger, Arnt, Braestrup, Christensen, Cools and Magelund, 1978).

Specific Activity: stereotypy and other behaviors

Numerous definitions of stereotypy have appeared in the behavioral literature, including the following: a characteristic feature of a pattern of behavior which appears aimless and lacks variation (Randrup and Munkvad, 1974); the selective stimulation of one or more items of behavior with concurrent inhibition of others (Randrup, Munkvad, Fog and Ayhan, 1975); and, the exhibition of invariant sequences of movements in a repetitive manner (Fray, et al., 1980). Rodent behaviors often manifested as stereotypic activities following acute apomorphine administration include sniffing, licking, grooming, gnawing, and rearing (Fray et al., 1980; Kenny & Leonard, 1978). However, stereotypic locomotor activity is thought to be mediated by separate DA mechanisms than those mechanisms which express stereotypies that do not involve gross locomotor activity (Costall, et al., 1977; Lloyd and Hornykiewicz, 1975; Scheel-Kruger et al., 1978a).

Drugs which mimic the central actions of a catecholaminergic neurons are thought to produce such effects either via presynaptic or postsynaptic mechanisms (Lloyd & Hornykiewicz, 1975). The facilitation of dopaminergic activity by d-amphetamine involves presynaptic mechanisms, in which d-amphetamine treatment initiates the actual release of DA from stores in catecholamine neurons. In contrast, the site of apomorphine agonist-action appears to be the postsynaptic stimulation of DA receptors which results in facilitated

dopaminergic function (Lloyd & Hornykiewicz, 1975). Although numerous behavioral studies have drawn parallels between the effects of amphetamine and apomorphine administration on motor activity, more recent evidence has suggested that these DA agonists actually produce differential behavioral profiles, including dose- and time-dependent variations in the exhibition of locomotor and stereotypic activities (Fray et al., 1980).

Apomorphine and behavior. Systemic (s.c.) administration of apomorphine has been shown to produce the increased incidence of certain stereotypies, including sniffing, licking, and gnawing responses, but at different threshold dosages (Fray et al., 1980). Fray et al. (1980) reported that a dose of .25 mg/kg apomorphine administered in rats resulted in the elevation of sniffing stereotypy and incidence of head down posture, but not rearing behavior. Others have reported that rats receiving an apomorphine dose of .25 mg/kg exhibited mainly walking, rearing accompanied by continuous sniffing, and episodes of discontinuous licking and incidental continuous licking of short duration (Scheel-Kruger, Cools & van Wel, 1977). Larger apomorphine dosages (.5-1.0 mg/kg) given systemically to rats has resulted in the elevation of licking and gnawing stereotypies (Fray, et al., 1980), as well as an increase in sniffing, burrowing, and rearing behaviors (Kenny and Leonard, 1978). The s.c. administration of 5.0 mg/kg apomorphine doses in rats has been shown to increase locomotion, head down posture, and incidence of gnawing

stereotypy (Fray et al., 1980), while s.c. doses of 10-60 mg/kg have produced weak and non-reproducible gnawing in mice (Scheel-Kruger, Christensen & Arnt, 1978).

The time sampling results of Experiment 3 suggested that many of the behavioral categories which yielded significant apomorphine treatment effects, coincided with previously reported effects of .25 mg/kg apomorphine s.c. dosages in rats. These effects included the increased incidence of non-body licking, rearing, and discontinuous sniffing stereotypies. Apomorphine treatment also increased the incidence of mastication stereotypy, although no behavioral correlate was found for mastication in the existing apomorphine literature. No significant treatment effects were obtained for three specific activity categories, including grooming, head down posture, and gnawing behaviors. While some studies have not reported increased rearing with an apomorphine dose of .25 mg/kg, others have reported this effect (Scheel-Kruger, et al., 1977) which was similar to the obtained results of Experiment 3. The finding of no significant apomorphine treatment effects for grooming, head down, and gnawing behaviors in Experiment 3 was in agreement with evidence showing that larger apomorphine dosages are required to produce significant incidence of these behaviors.

Muscimol and behavior. The systemic administration of GABA agents, such as the potent GABA receptor agonist, muscimol, is known to affect CNS function, although the behavioral

consequences of various GABA agent manipulations have not always produced consistent patterns of action (Delini-Stula, 1979). The systemic administration (s.c.) of a 20 mg/kg muscimol dose in rats has been shown to produce anesthesia, loss of righting reflex, and a 70% increase in striatal dopamine concentration, while a dose of 10 mg/kg was found to increase striatal dopamine by 30% (Scheel-Kruger, Arnt, Brostrup, Christensen & Magelund, 1979). Muscimol doses of 1, and especially 2 and 4 mg/kg s.c. have been found to produce catalepsy in rats which was dose-dependent (Scheel-Kruger et al., 1979). Subcutaneous injection of .63 mg/kg muscimol in mice has resulted in increased activity, but no other significant changes in behavior (Scheel-Kruger et al., 1978b).

Intraperitoneal administration of muscimol (.5-2.0 mg/kg) in rats has been reported to produce dose-dependent inhibition of striatal multi-unit neuronal activity, a consequence consistent with the hypothesis that muscimol mimics GABA at inhibitory synapses in the CNS (McKenzie & Hansen, 1980). Muscimol doses of 1 and 2 mg/kg i.p. have produced noticeable sedation, ease in handling, and decreased spontaneous locomotor activity in rats, although subjects given a dose of .5 mg/kg muscimol were found to be behaviorally similar to subjects treated with saline (McKenzie & Hansen, 1980).

No significant appearance of stereotypic behavior has been reported following i.p. muscimol administration in rats (McKenize

& Hansen, 1980), s.c. muscimol administration in mice (Scheel-Kruger et al., 1978), or intracerebral injection of muscimol into the nucleus accumbens of rats (Scheel-Kruger et al., 1977). The results of mus/sal treatment in Experiment 3 yielded a significant main effect for incidence of mastication stereotypy compared to sal/sal and put/sal groups. Informal observation of yawning (SYS) behavior was also seen in the mus/sal group. The observation of these apparent stereotypies produced by mus/sal treatment suggested that muscimol per se may have been effective in producing some behaviors related to mediating effects of dopaminergic CNS function on the expression on non-locomotor stereotypy. The anecdotal appearance of yawning (SYS) behavior may imply that mus/sal treatment influence one or more CNS substances which have been linked to SYS behavior, including DA, serotonin, glutamate, and cholinergic transmitters (Lanthorn & Issacson, 1979; Yamada & Furukawa, 1981). The lack of other significant muscimol-specific locomotor and stereotypic treatment effects appeared to be consistent with literature reports of minimal behavioral changes following systemic administration of muscimol dosages between .5-1.0 mg/kg (McKenzie & Hansen, 1980; Scheel-Kruger et al., 1978). However, recent research investigating the interactive relationships between GABAergic and dopaminergic CNS function have indicated that in vivo manipulations of GABAergic activity can influence the expression of dopaminergic locomotor and stereotypic behaviors.

GABAergic/dopaminergic interactions and behavior. The manifestation of behavioral syndromes such as locomotor activity and stereotypy induced by apomorphine and cocaine are known to depend primarily on the stimulation of dopaminergic CNS mechanisms (Scheel-Kruger et al., 1978b). The significant modification of the development and/or intensity of dopaminergic locomotor and stereotypic activities is possible by manipulations of other neurotransmitters, including acetylcholine, serotonin, and noradrenaline (Scheel-Kruger et al., 1978). Several recent studies have also reported the modulating influence of GABAergic mechanisms on the expression of dopaminergic behavioral syndromes, which includes both systemic and cerebral manipulations of GABA, GABA agonists (muscimol), and other GABA agents (balcofen, imidazole acetic acid) (Delini-Stula, 1979; McKenzie & Hansen, 1980; Scheel-Kruger et al., 1977; Scheel-Kruger et al., 1978a; Scheel-Kruger et al., 1978b).

The unilateral i.c. injection of GABA into the substantia nigra has induced contralateral rotational behavior in rats which appeared to be related to the activation of the dopaminergic nigrostriatal system (Scheel-Kruger et al., 1977; Scheel-Kruger et al., 1978a; Waddington, 1977). The bilateral injection of muscimol into the substantia nigra of rats has resulted in the observation of strong stereotyped behavior (head movements, sniffing, licking, gnawing) which may be mediated by non-dopaminergic nigral output systems, including GABAergic

mechanisms (McKenzie & Hansen, 1980; Scheel-Kruger et al., 1978a).

Systemic muscimol administration, as well as cerebral muscimol injection into the nucleus accumbens of the basal ganglia, appear to exert similar influences on the expression of dopaminergic locomotor and stereotypic activities in rodents (Scheel-Kruger et al., 1978a). Bilateral injection of muscimol into the nucleus accumbens of rats (10-100 ng/kg) significantly increased stereotypies (continuous licking and gnawing) induced by apomorphine (.25 mg/kg s.c.), while also antagonizing apomorphine-related locomotor activity (Scheel-Kruger et al., 1977). The observation of similar differential effects upon apomorphine-induced (.25 mg/kg s.c.) behaviors was reported in mice following the systemic administration of non-sedative muscimol doses (1 mg/kg s.c.), including depression of locomotor activity and facilitation of stereotyped gnawing (Scheel-Kruger et al., 1978b). Subcutaneous muscimol doses (.25-2 mg/kg) have strongly increased stereotyped, gnawing, sniffing, head nodding in rats (Scheel-Kruger et al., 1979). Larger muscimol doses (2 mg/kg) given in conjunction with apomorphine (.5-2.0 mg/kg s.c.) have produced a typical oral "dyskinetic biting activity" in rats (Scheel-Kruger et al., 1979). This pronounced oral dyskinetic biting activity was not seen after apomorphine treatment per se even at very high doses (Scheel-Kruger et al., 1979).

Intraperitoneal pretreatment with muscimol (1-2 mg/kg) has been shown to significantly enhance stereotypic behavior in rats (sniffing, licking, head movements) produced by the s.c. administration of the DA agonists apomorphine (1 mg/kg) and d-amphetamine (2.5 mg/kg) (McKenzie & Hansen, 1980). The existing behavioral literature has suggested that changes in behavior resulting from in vivo muscimol treatment depend primarily on muscimol influences on GABAergic receptor function in the CNS (Scheel-Kruger et al., 1978b). These muscimol-induced GABAergic effects appear to differentially modify various dopaminergic systems and/or receptors involved in the expression of DA-dependent locomotor activity and stereotyped behavior (Scheel-Kruger et al., 1978a).

Putrescine, muscimol effects and GABA/DA behavior. The time sampling results of Experiment 3 yielded significant time-dependent Treatment 1 x Treatment 2 drug interactions for the incidence of sniffing stereotypies only. The nature of the drug interaction effects for the exhibition of discontinuous and continuous sniffing behaviors suggested that pretreatment with putrescine (75 mg/kg), and to a lesser extent muscimol (.75 mg/kg), enhanced the incidence and intensity apomorphine-induced stereotypy. These treatment effects appeared to resemble previous literature reports which have shown that the systemic administration of non-sedative doses of muscimol can modulate the expression of dopaminergic behaviors induced by systemic

apomorphine treatment, including the time-dependent potentiation of stereotypic sniffing activities (McKenzie & Hansen, 1980; Scheel-Kruger et al., 1978a). Although there has been no report of facilitated GABAergic CNS function following the systemic administration of putrescine in rats, a similarity of mus/apo and put/apo treatment effects on the incidence of DS and CS behaviors may indicate a common bases for these effects. It was suggested that such observations may be preliminary evidence of putrescine-induced influences on CNS GABAergic function and the subsequent modification of dopaminergic behavior.

Consideration of the qualitative and temporal changes in sniffing behaviors exhibited by the put/apo, mus/apo, and sal/apo groups (Figures 32 and 33) suggested that complementary treatment effects were obtained for DS and CS behaviors as a function of drug interactions and time. While sal/apo treatment was effective in producing DS behavior, this group did not exhibit significant CS stereotypy. In contrast, the level of DS behavior was initially high for the put/apo group, while the initial level of CS behavior was low. However, the incidence of DS and CS stereotypies for the put/apo group changed in an inverse relationship over time; as levels of DS decreased over time, the levels of CS increased. These observations suggested that a qualitative change in the manifestation of sniffing stereotypy (discontinuous to continuous) occurred as a result of put/apo treatment. Compared to sal/apo and put/apo groups, the mus/apo group

appeared to exhibit intermediate levels of both DS and CS stereotypies during time sampling, with the direction of mus/apo effects resembling the effects produced by put/apo treatment.

At the end of time sampling, significant levels of DS remained for the sal/apo, put/apo, and mus/apo groups, although no group had significant incidence of CS behavior. These observations suggested that the qualitative changes in levels of continuous sniffing stereotypy produced by put/apo and mus/apo treatments lasted less than 45 minutes posttreatment, while apomorphine-related DS effects lasted up to 45 minutes post-treatment.

Informal observations and CNS substances. Anecdotally-observed behaviors seen in Experiment 3 included stereotypic activities, rearing and sedation. The appearance of sedation, head down posture, and incidental genital grooming was specific to the put/sal group, and these behaviors were similar to effects obtained in Experiment 2. Exhibition of head down posture has been reported to indicate facilitated DA function (Fray et al., 1980), although this behavior was not seen in the sal/apo group. Previous research has shown that sedation can be the result of autonomic nervous system activation, as well as the manipulation of various substances, including the polyamines, GABA, catecholamines, endogenous opiates, and cholinergic transmitters, as cited in Experiment 1.

Yawning (SYS) behavior was incidentally observed in the mus/sal, sal/apo, and put/apo groups, and was similar to SYS behavior seen after the p.o. and i.p. administration of large putrescine dosages in Experiments 1 and 2. Exhibition of SYS in these three treatment groups suggested that GABA/DA interactions may have influenced the appearance of this behavior. Previous research supported this interpretation, since several CNS substances have been linked to SYS behavior, including serotonin, DA, glutamate, and cholinergic transmitters, as cited in Experiment 1.

Limb flicking and stereotypic sniffing activities were characteristic of sal/apo and put/apo groups only, while incidence of rearing was informally observed only in the put/apo group. These observations suggested that limb flicking behavior may be related to drug interaction effects involving dopaminergic mechanisms. Stimulation of rearing is known to be influenced by facilitated DA function (Lloyd & Hornykiewicz, 1975), and since rearing was only seen in the put/apo group, this may indicate that put/apo treatment enhanced DA activity. The observation of other DA-related stereotypies for the put/apo group may be further support for this apparent interaction between putrescine and dopamine.

Anecdotal observation of sniffing and licking behavior was seen in the sal/apo, put/apo, and mus/apo groups, while gnawing activity was characteristic of put/apo and mus/apo treatments

only. Facilitation of DA function by application of apomorphine has resulted in the appearance of stereotypies, including sniffing, licking and gnawng responses (Fray et al., 1980). Thus, the observation of these stereotypies in the sal/apo, mus/apo, and put/apo groups implied that enhanced DA activity may have occurred as a result of these treatments.

Gnawing behavior has been seen following the administration of large dosages of apomorphine (Fray et al., 1980), and after systemic muscimol treatment in conjunction with apomorphine (Scheel-Kruger et al., 1978b; Scheel-Kruger et al., 1979). Data such as these indicated that put/apo and mus/apo treatments were more potent than sal/apo treatment in producing dopaminergic-related gnawing behavior. Observation of genital grooming in the put/apo group may have also been related to facilitated DA and/or GABA function (Fray et al., 1980; Smialowski et al., 1980). In summary, the anecdotal behaviors associated with the various treatment groups seemed to indicate the involvement of GABAergic, and dopaminergic and possibly other CNS systems in the manifestation of these behaviors.

General Conclusions

The results of Experiment 3 demonstrated that i.p. putrescine administration effectively modified the expression of dopaminergic behaviors induced by apomorphine treatment in adult male rats. These putrescine effects included the possible attenuation of apomorphine-induced stimulation of locomotor

activity (decreased stationary behavior and increased rearing), the apparent enhancement of dopaminergic sniffing stereotypies, and the production of anecdotal stereotypies characteristic of CNS dopamine function. The i.p. administration of the GABA agonist, muscimol, was also effective in the modification of apomorphine-induced behaviors. Although the directional effects of mus/apo treatment resembled those of put/apo treatment, the put/apo effects appeared to be more robust in comparison. These observations suggested that putrescine treatment was more potent than muscimol treatment in modifying apomorphine-induced behaviors at the dosages utilized.

The temporal and qualitative variations in the exhibition of sniffing stereotypies for the put/apo and mus/apo groups was additional evidence of the putrescine and muscimol treatment effects on dopamine function. Sal/apo treatment produced discontinuous sniffing stereotypy, but not continuous sniffing. In contrast, both put/apo and mus/apo groups showed significant incidence of discontinuous and continuous sniffing behaviors during time sampling. The temporal change in levels of DS and CS for the put/apo group indicated that potentiation of apomorphine-induced sniffing by putrescine resulted in the qualitative change of DS behavior to CS behavior over the first 27 minutes posttreatment. The reversal of this qualitative change in sniffing stereotypy occurred over the last 18 minutes of sampling, which indicated that the potentiating effects of

putrescine lasted less than 45 minutes posttreatment. A similar inverse relationship for the change in DS and CS stereotypy was seen for the mus/apo group, although these muscimol effects were less potent than putrescine effects.

Review of the behavioral literature supported the preliminary interpretation that muscimol and putrescine treatment modified dopaminergic behaviors via the facilitation of GABAergic function in the intact rat. The functional link between GABA and DA has been well established in basal ganglia structures, including the substantia nigra, nucleus accumbens, and globus pallidus (Delini-Stula, 1979; Pycock, Horton & Carter, 1978; Scheel-Kruger et al., 1978a), however, activation of selective GABAergic pathways can lead to both excitatory and inhibitory behavioral responses within GABA-DA systems (Delini-Stula, 1979). Recent evidence has suggested that separate DA mechanisms in the nucleus accumbens may influence the expression of locomotor activity and stereotypy (Scheel-Kruger et al., 1978a). The finding that systemic putrescine and muscimol treatment may modify apomorphine-induced locomotion and stereotypy was consistent with literature which has shown that facilitated GABAergic function can differentially influence various dopamine systems in the CNS and the expression of dopaminergic behaviors (Scheel-Kruger et al., 1978a).

The apparent drug interactions and synergistic behavioral effects observed in Experiment 3 are interpretable in the

perspective of previously hypothesized neurophysiological models. Major GABAergic systems in the brain include short axon projections in the striatum and long axon projections which make up the striato-nigral pathway (McGeer & McGeer, 1975; Ribak et al., 1977). GABAergic mediation of behavior may be influenced by each of these systems, or an integrative combination of these systems. Muscimol treatment per se may have produced differential effects on GABAergic-mediated behavior compared to putrescine per se since muscimol is a GABA receptor agonist, while putrescine is a precursor of GABA. Thus, the effectiveness of these agents in causing similar modifications of behavior is dependent on site of action, temporal lag for effect, activation of similar GABAergic systems, and dosage considerations. The lack of significant common effects for putrescine and muscimol treatments per se acknowledges these considerations, and suggested that these agents do not produce identical consequences at the dosages utilized.

When muscimol and putrescine were given in combination with apomorphine, it was observed that these agents did produce changes in behavior which were significantly similar. A mechanistic explanation for this circumstance was that these agents had an interaction with dopaminergic CNS activity which was similar in nature. It may be suggested that certain interactive mechanisms may have involved common GABA/DA pathways in the brain which were significantly affected by either muscimol or

putrescine treatment. One possible mechanism could have been via the striato-nigral GABA pathway which is known to mediate and influence the nigro-striatal DA pathway, as well as other non-dopaminergic systems (Ribak et al., 1977). Since the concentrations of DA and GABA are very large in the nucleus accumbens, the effects of mus/apo and put/apo treatments on apomorphine behavior may have occurred via common GABA/DA mechanisms in this area of the brain. In other words, the observed drug interactions and changes in behavior in Experiment 3 are reconcilable in terms of known physiological models concerning GABA and DA function in the brain.

The results of Experiment 3 provided preliminary evidence for a neuropharmacological role of exogenously administered putrescine in intact rats. However, the proposition that systemic putrescine treatment exerts indirect influences on CNS function via GABAergic mechanisms remains tentative until additional behavioral and biochemical substantiation is obtained. It is possible that putrescine treatment exerted direct and specific influences on apomorphine-related behaviors. The major limitation of this study was the use of one dosage for all drug treatments, which has restricted the generalizability of the results.

Since a wide spectrum of behavioral consequences is known to result from different manipulations of GABA and dopamine CNS function, further research is needed to evaluate the effects of

putrescine treatment in conjunction with these neurotransmitters. The utilization of different drug interactions, dose variations, and use of several agonist and antagonist agents of GABA, DA, and other CNS substances may help to better specify the behavioral influences of systemic putrescine administration. The data gathered in these future investigations of putrescine may yield a better understanding of central nervous system function, as well as substantiate the apparent neuropharmacological significance of this minor GABA precursor.

GENERAL DISCUSSION

Investigation of the effects of the systemic administration of the minor GABA precursor, putrescine, demonstrated the oral and intraperitoneal putrescine treatment produced a complex of changes in the behavior of the adult male rat. Although descriptive and temporal dissimilarities were found between certain p.o. and i.p. putrescine treatment effects, several changes in behavior were similar in many respects. Intraperitoneal putrescine treatment was found to be effective in modifying behavior induced by the systemic administration of the dopamine agonist, apomorphine. Some of the putrescine-related effects on apomorphine behavior resembled certain effects produced by i.p. administration of the GABA agonist, muscimol. Existing behavioral literature yielded preliminary support for the interpretation that the administration of exogenous putrescine in rats may have influenced CNS function and the expression of behavior compared to saline-treated rats.

Recapitulation

Systemic putrescine effects

Evaluation of changes in behavior following oral (50, 250, 1000 mg/kg) and intraperitoneal (25, 50, 100, 250 mg/kg) putrescine treatment yielded behavioral measures which had significant ANOVA treatment effects or treatment x time interactions (12 and 14, respectively). Of these significant p.o. and i.p. measures,

the majority were found to show reliable linearity of dose-response curves (83% and 57%, respectively). The depression of ambulation and other general activity behaviors were effects common to both treatment conditions. However, differential effects on the exhibition of stereotypy effects were found, with a greater number of effects seen after i.p. putrescine. Intra-peritoneal putrescine treatment produced more abnormal behaviors than p.o. treatment, although both conditions produced significant wet dog shaking (WDS) at the highest putrescine doses, as well as several common anecdotal motor behaviors (sliding/-burrowing, yawning, paw flicking, mastication stereotypy). Incidence of flaccid immobility was observed in some high p.o. dose subjects, while head swaying was seen in a few high i.p. dose subjects.

Both p.o. and i.p. putrescine administration depressed sensory reactivity, although p.o. effects appeared to be more robust than i.p. effects. The effects on sensory reactivity included decreased acoustic startle response, increased shock response thresholds (analgesia), and sedation. Some residual analgesia was seen in subjects treated with 1000 mg/kg p.o. putrescine at 21 hours posttreatment. No i.p. treatment-related changes were found for body temperature or food ingestion behavior, however, 250 mg/kg i.p. putrescine may have modified drinking behavior. In contrast, 1000 mg/kg p.o. putrescine depressed body temperature, decreased incidence of food

ingestion, and increased incidence of drinking behavior. The anecdotal observation of ptosis, piloerection, and sedation in subjects given higher p.o. and i.p. doses suggested that systemic putrescine treatment may have activated certain autonomic nervous system effects.

Many of the behavioral effects observed following either p.o. or i.p. putrescine treatment resembled some previously reported changes in behavior produced by in vivo polyamine manipulations. Certain systemic putrescine effects obtained in Experiments 1 and 2 resembled changes in behavior seen after i.p. spermine and spermidine treatment in rodents, and were primarily inhibitory in nature (82% p.o., 64%, i.p.), as shown in Table 46 of Appendix E. Review of existing behavioral literature prompted the tentative suggestion that systemic putrescine treatment may have exerted direct putrescine-specific influences on behavior of the intact rat, or indirect influences via GABAergic mechanisms and/or other CNS neurochemical systems (DA, serotonin, opiate peptides). The informal categorization of systemic putrescine effects on behavior (Appendix E, Table 47) suggested that a majority of the p.o. and i.p. treatment effects resembled GABAergic behaviors and behaviors associated with other CNS substances. Thus, the results of Experiments 1 and 2 yielded preliminary support for a role of putrescine as a neurotransmitter or neuromodulating substance in the brain, as suggested by prior putrescine research.

Putrescine and GABA, dopamine behavior

The results of Experiment 3 showed that i.p. putrescine (75 mg/kg) administration was effective in modifying behavior produced by s.c. treatment with the dopamine agonist, apomorphine (.25 mg/kg). Certain putrescine-related effects on apomorphine-induced behavior resembled some effects produced by i.p. administration of the GABA agonist, muscimol (.75 mg/kg), although the effects of putrescine treatment appeared to be more robust than muscimol at the dosages utilized.

Apomorphine treatment per se produced significant non-body licking, increased rearing, and discontinuous sniffing stereotypy, which was consistent with previously reported apomorphine-induced behavior. The observation of several behaviors (mastication, yawning, limb flicking, licking, gnawing) suggested that the function of GABAergic and/or dopaminergic systems may have influenced the manifestation of certain sal/apo, mus/sal, put/apo, and mus/apo treatment effects. Putrescine treatment per se produced anecdotally-observed sedation, head down posture, and genital grooming behaviors only.

The modification of apomorphine-induced behavior by systemic putrescine and muscimol treatment included changes in DA-related locomotor activity and stereotypy. While apomorphine treatment per se produced excitatory effects on locomotor activity, similar effects were not obtained in the put/apo and mus/apo groups which suggested that putrescine and muscimol may have attenuated such

excitatory apomorphine effects. Significant time-dependent drug interaction effects were found for the incidence and intensity of dopaminergic sniffing stereotypies only. The results indicated that pretreatment with putrescine or muscimol enhanced the incidence and intensity of apomorphine-induced discontinuous sniffing. The time-dependent qualitative change of discontinuous sniffing to continuous sniffing stereotypy was produced by put/apo treatment, and to a lesser extent by mus/apo treatment. No continuous sniffing was seen for the sal/apo group during sampling. The reversal of the changes in DS and CS incidence levels suggested that the potentiating influences of putrescine and muscimol on apomorphine behavior lasted less than 45 minutes posttreatment.

Review of existing behavioral literature indicated that the treatment effects on sniffing behavior resembled prior reports which have shown that systemic administration of non-sedative muscimol doses facilitated GABAergic activity and modified the incidence and intensity of apomorphine-induced stereotypy. Informal categorization of the treatment effects obtained in Experiment 3 suggested that the majority of effects were excitatory in nature and were related to dopaminergic CNS function, while about 41% of the effects reflected involvement of GABAergic function (Appendix E, Table 48).

The finding that putrescine and muscimol may have antagonized apomorphine-induced locomotor activity and potentiated

stereotypy was consistent with reports showing that enhanced GABAergic function can differentially modify dopaminergic behaviors. Thus, the results of Experiment 3 yielded preliminary support for a significant neuropharmacological role of putrescine in the intact rat.

The obtained findings of the thesis are tempered by several experimental design considerations. The nature of certain evaluative tests may limit the generalizability of any derived interpretations. For instance, the lack of non-treated control groups for all experiments does not allow direct comparison to the intact rat per se. Instead, any observed treatment-related effects were made in comparison to saline-treated controls. The time sampling procedure may have had design insufficiencies which could have added substantial statistical error. Two possible insufficiencies included the limited character of behavioral assessment (limited number of recorded behavior) and a much larger number of dependent measures compared to treatment cell sizes. Thus, these design considerations should be taken in account when reviewing any interpretations of the experimental data.

Interpretations and Significance

The data of Experiments 1, 2, and 3 have provided preliminary behavioral evidence for the proposition that systemic putrescine treatment may directly or indirectly influence CNS function and the expression of behavior in the adult male rat.

The behavioral results appeared to be consistent with and complementary to the known role of putrescine as a minor precursor of the inhibitory neurotransmitter, GABA. The systemic (i.p.) administration of putrescine has been previously shown to result in significant conversion of putrescine to GABA in CNS tissues. Although the changes in behavior following p.o. and i.p. putrescine treatment were not always similar, the majority of both p.o. and i.p. effects were inhibitory in nature and resembled certain behaviors related to the function of GABA and other CNS substances.

The finding of some similarities between p.o. and i.p. putrescine effects on motor behavior and sensory reactivity suggested that the bases for manifestation of certain changes in behavior may have involved some common CNS and peripheral mechanisms. The results of Experiment 3 suggested that these common mechanisms may involve GABAergic and dopaminergic systems, since putrescine treatment was found to modify behavior produced by apomorphine treatment. All of these observations were in agreement with the fact that systemic putrescine administration can alter the synthesis and levels of GABA in the brain. Thus, the changes in behavior produced by p.o. and i.p. putrescine administration may be indirect and due to putrescine influences on the central nervous system function of endogenous GABA.

The significance of the findings are emphasized by the results of Experiment 3 which suggested that systemic putrescine

treatment may exert influences on the expression of dopaminergic behavior via GABAergic mechanisms. If definitive biochemical substantiation is obtained which demonstrates that oral and intraperitoneal putrescine administration can significantly modify GABA metabolism and CNS activity, a unique method for the in vivo manipulation of central nervous system function via intact neural mechanisms may become available. The importance of such an advance to clinical and research applications is obvious, since dysfunction and abnormalities involving GABAergic CNS function has been implicated in the etiology of several human pathologies, including Parkinson's disease, Huntington's chorea, Tardive dyskinesia, epilepsy, and schizophrenia (Grove, Schechter, Tell, Koch-Weser, Sjoerdsma, Warter, Marescaux & Rumbach, 1981; Manyam, Katz, Hare, Gerber & Grossman, 1980).

The availability of a method which could affect the normal activity of a known neurotransmitter via existing synthetic pathways could change theoretical perspectives, as well as the focus of biochemical and behavioral investigations of brain function. This possibility is underscored by the fact that GABA is the major neurotransmitter in the mammalian brain, and is thought to have interactions with most known CNS neurochemicals.

Future investigations of systemic putrescine administration should include both biochemical and behavioral studies. Biochemical substantiation of direct putrescine influences and modifications on regional and whole-brain GABA concentrations,

turnover dynamics, and utilization will help to specify the functional significance of putrescine in the CNS. The use of various neurotransmitter interactions, agonist and antagonist interactions, and other drug manipulations in conjunction with putrescine holds the potential to open a new field of research in the neurosciences. It is likely that further investigation of putrescine and its relationship to GABA and other CNS substances will enhance understanding about the mammalian brain and the expression of behavior.

General Findings

On the basis of the behavioral results and interpretations of this thesis, it is concluded that:

1. Oral and intraperitoneal administration of a minor GABA precursor, putrescine, significantly affects behavior in the adult male rat.
2. Similarities between certain changes in behavior following p.o. and i.p. putrescine treatment suggests that common mechanisms may influence the expression of some p.o. and i.p. effects.
3. The behavioral effects resulting from systemic putrescine treatment are primarily inhibitory, and certain effects resemble behaviors associated with the CNS function of GABA and other CNS substances.
4. Pretreatment with intraperitoneal putrescine modified the incidence and intensity of certain behaviors produced by s.c. administration of the dopamine agonist, apomorphine.
5. Intraperitoneal pretreatment with the GABA agonist, muscimol, modified apomorphine-induced behaviors, although putrescine appeared to be more potent in modifying sniffing stereotypies than muscimol at the dosages utilized.

6. Behavioral literature yielded support for the tentative suggestion that systemic putrescine treatment exerts indirect influences on the expression of behavior via GABAergic CNS function.

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Appendix A

Pilot study data: temporal comparisons.

The temporal duration of putrescine treatment-related effects following oral and intraperitoneal administration was estimated by the observation of subjects treated with large putrescine dosages (1000 mg/kg, p.o.; 250 mg/kg, i.p.). The resultant temporal estimations were used to design the time course of the actual pilot studies. Results of the pilot studies suggested that behavioral effects observed following i.p. putrescine injection appeared earlier after treatment than did oral putrescine-related effects. In addition, i.p. putrescine effects appeared to have a shorter overall temporal duration than did those seen after oral putrescine treatment. These temporal comparisons were readily observed for certain behavioral responses, especially time sampling behaviors such as abnormal movement/posture and wet dog shaking behavior.

Appendix B

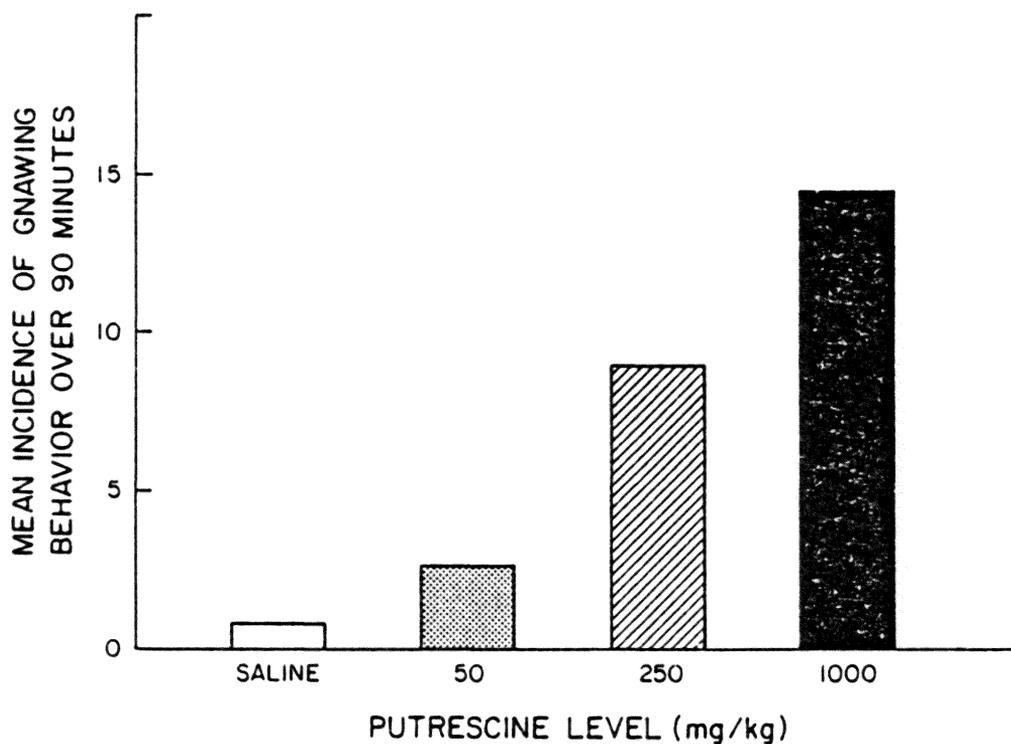


Figure 35. Effect of oral putrescine treatment on the incidence of gnawing behavior.

Appendix B (Continued)

Table 42

Duncan's Multiple-Range Test Results
for Putrescine (p.o.) Treatment Effect
and Incidence of Gnawing Behavior^a

Treatment Group	Grouping
1000 mg/kg	*
250 mg/kg	**
50 mg/kg	**
50 mg/kg	***
Saline	***

^a Treatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 90 minutes.

Appendix B (Continued)

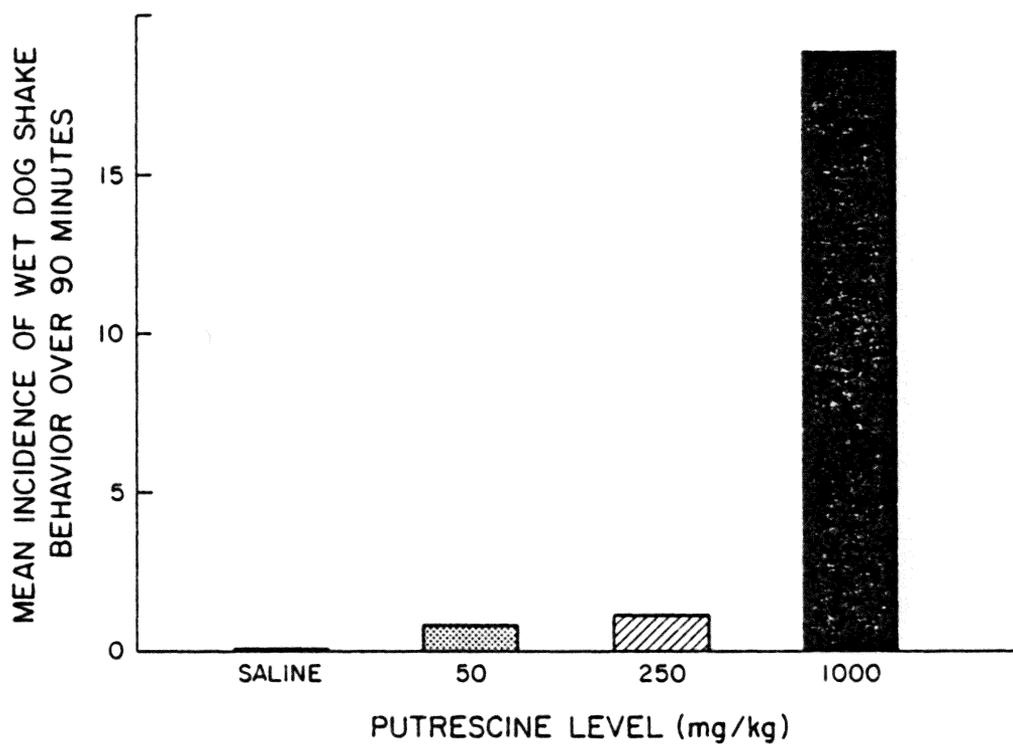


Figure 36. Effect of oral putrescine treatment on the incidence of wet dog shaking behavior.

Appendix B (Continued)

Table 43

Duncan's Multiple-Range Test Results
Putrescine (p.o.) Treatment Effect
for Incidence of Wet Dog Shake Behavior^a

Treatment Group	Grouping
1000 mg/kg	*
250 mg/kg	**
50 mg/kg	**
Saline	**

^a Treatments with identical grouping designation are not statistically different; treatments differing in grouping designation show significant difference at $p < .05$ for this behavior collapsed over 90 minutes.

Appendix B (Continued)

Table 44

Experiment 1 Baseline Mean Startle Response

ISI^a and SR I Total Score^b Data

Putrescine Group (mg/kg) ^c	Baseline ISI ± SEM	Baseline SR I Total Score ± SEM
0	2.43 ± .30	6.86 ± 0.96
50	2.71 ± .19	9.43 ± 2.15
250	2.71 ± .19	6.43 ± 0.69
1000	2.86 ± .14	9.29 ± 1.69

^a Initial startle intensity (ISI) measured as the reponse to acoustic stimuli scored using an arbitrary four point scale.

^b SR I Total Score = sum of response scores over 8 trials, 40 minutes prior to treatment.

^c Oral (p.o.) putrescine treatment groups (n=7).

Appendix C

Table 45

Experiment 2 Baseline Mean Startle Response

ISI^a and SR I Total Score^b Data

Putrescine Group (mg/kg) ^c	Baseline ISI ± SEM	Baseline SR I Total Score ± SEM
0	3.00 ± 0.0	12.00 ± 2.02
25	2.33 ± .33	11.50 ± 2.85
50	2.50 ± .22	11.83 ± 2.59
100	2.83 ± .17	9.00 ± 0.68

^a Initial startle intensity (ISI) measured as the response to acoustic stimuli scored using an arbitrary four point scale.

^b SR I Total Score = sum of response scores over 8 trials, 40 minutes prior to treatment.

^c Intraperitoneal (i.p.) putrescine treatment groups (n=6).

Appendix D

Glossary of Pharmacologic Agents

Muscimol. Muscimol is a psychotomimetic isoxazole compound isolated from the mushroom Amanita muscaria, and is a potent agonist of the bicuculline-sensitive, strychnine-insensitive receptors of spinal cord neurons characteristic of GABA CNS inhibitory sites. This GABA analogue can cause considerable psychic disturbance in man when given orally at dosages of 10 mg. Muscimol is 10 times more potent than GABA in displacing radioactive GABA or the GABA antagonist, bicuculline methiodide, bound to membranes from rat brain. When injected into rats by i.v. route, muscimol potentiates morphine analgesia, antagonizes seizures, and can alter brain activity of other neurotransmitters, including acetylcholine and dopamine (Johnston, 1978). A large body of physiological and biochemical literature exists which addresses the effects of muscimol administration and subsequent effects on GABAergic mediation of dopaminergic activity in the basal ganglia, especially the substantia nigra (Walters and lakoski, 1978; Waszczak, Eng and Walters, 1980). In addition, behavioral literature has underlined the important research benefits of muscimol utilization in characterizing in vivo GABAergic function in mammalian species (Scheel-Kruger, Cools and Wel, 1977; Scheel-Kruger, Arnt, Brostrup, Christensen and Magelund, 1979; Andersson, Ogren, De La Mora, Schwarz,

Hokfelt, Eneroth, Gustafsson and Skett, 1979; van der Heyden, Venema and Korf, 1980).

Apomorphine. Apomorphine is a known dopamine agonist which stimulates dopamine-sensitive adenylate cyclase, and also antagonizes phenothiazine blockade of dopaminergic CNS function, while decreasing dopamine turnover and reducing the firing rate of dopaminergic neurons (McGeer, Eccles, and McGeer, 1978). A large body of physiological, biochemical and behavioral literature exists which has exemplified the CNS consequences of apomorphine treatment on CNS dopaminergic function (Ernst, 1966; van der Heyden, Venema and Korf, 1980; Fray, Sahakian, Robbins, Koob, and Iverson, 1980; Issacson, Yongue and McClearn, 1978; Anderson, Ogren, De La Mora, Schwarz, Hokfelt, Eneroth, Gustafsson and Skett, 1979; Scheel-Kruger, Cools and Wel, 1977). Systemic apomorphine treatment can induce the incidence of stereotypy in rats, including sniffing and repetitive movements of the head and limbs at low doses, and the exhibition of continuous gnawing, biting, and licking behavior at higher dosages. Apomorphine also potentiates locomotor behavior (Scheel-Kruger, Cools and Wel, 1977), can induce contralateral turning behavior (Anden, Grabowski-Anden and Wachtel, 1979), and affect grooming behavior (Fray, Sahakian, Robbins, Koob and Iverson, 1980).

Appendix E

Table 46

Inhibitory and Excitatory Effects of Systemic Putrescine Administration

	<u>p.o. treatment</u>		<u>i.p. treatment</u>	
	<u>Inhib. Effects</u>	<u>Excit. Effects</u>	<u>Inhib. Effects</u>	<u>Excit. Effects</u>
<u>I. Motor Behavior</u>				
A. General Activity	Yes	No	Yes	No
B. Stereotypy	Yes	Yes	Yes*	Yes*
C. Abnormal Behavior	No	Yes	No	Yes
D. Informal Observations	Yes	Yes	No	Yes
<u>II. Sensory Reactivity</u>				
A. Startle Response	Yes	No	Yes	No
B. Shock Thresholds	Yes	No	Yes	No
C. Informal Observations	Yes	No	Yes	No
<u>III. Body Temp and Other Behaviors</u>				
A. Change in Body Temp	Yes	No	No	No
B. Ingestive Behavior	Yes	Yes	Yes	No
C. Informal Observation	Yes	No	Yes	No

	<u>Inhibitory Effects</u>	<u>Excitatory Effects</u>	<u>Mixed Effects</u>
p.o. Putrescine	9	4	0
i.p. Putrescine	7	3	1

* Denotes finding of mixed effect(s)

Appendix E (Continued)

Table 47

Systemic Putrescine Effects and Relationships to CNS Substances

	<u>Polyamines Effects</u>	<u>GABA Effects</u>	<u>Effects of Other CNS Substances</u>
I. <u>Motor Behavior</u>			
A. General Activity	Yes	Yes	Yes
B. Stereotypy	No	Yes	Yes
C. Abnormal Behavior	Yes	Yes	Yes
D. Informal Observ.	No	Yes	Yes
II. <u>Sensory Reactivity</u>			
A. Startle Response	NA	Yes	Yes
B. Shock Thresholds	NA	Yes	Yes
C. Informal Observ.	Yes	Yes	Yes
III. <u>Body Temp and Other Behaviors</u>			
A. Change in Body Temp*	Yes	Yes	Yes
B. Ingestive Behavior	Mixed	Yes	Yes
C. Informal Obsev.	Yes	Yes	Yes
	<u>Polyamine Effects</u>	<u>GABAergic Effects</u>	<u>Other CNS Effects</u>
Systemic Putrescine Treatment	5	10	10

* Applicable only to p.o. treatment effects.

Appendix E (Continued)

Table 48

Experiment 3 Effects and Relationships to CNS Function

	<u>Inhib. Effects</u>	<u>Excit. Effects</u>	<u>GABA Effects</u>	<u>DA Effects</u>
I. <u>General Locomotor</u>				
A. Stationary	No	Yes	No	Yes
B. Rearing	No	Yes	No	Yes
II. <u>Specific Activity</u>				
A. Sleep	No	Yes	No	Yes
B. Awake/Mostly Immobile	No	Yes	No	Yes
C. Mobile/Non-Stereotypic	No	Yes	No	Yes
D. Non-body Licking	No	Yes	No	Yes
E. Mastication	No	Yes	Yes	Yes
F. Discontinuous Sniffing	No	Yes	Yes	Yes
G. Continuous Sniffing	No	Yes	Yes	Yes
III. <u>Informal Observations</u>				
A. Sedation	Yes	No	Yes	No
B. Head Down, Genital Grooming	Yes	Yes	Yes	Yes
C. Yawning	No	Yes	Yes	Yes
D. Limb Flicking	No	Yes	Yes	Yes
E. Sniffing Stereotypy	No	Yes	No	Yes
F. Licking Stereotypy	No	Yes	No	Yes
G. Gnawing Stereotypy	No	Yes	No	Yes
H. Rearing	No	Yes	No	Yes
<u>Total Number of Effects</u>	<u>2</u>	<u>16</u>	<u>7</u>	<u>16</u>

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SYSTEMIC ADMINISTRATION OF PUTRESCINE INDUCES GABA-LIKE
BEHAVIORS IN RATS

by

F. David Feng

(ABSTRACT)

Putrescine is a polyamine with multiple roles in cellular metabolism. It is also a minor precursor of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA). Conversion of systemically administered putrescine to GABA in rat neural tissues has been reported. In this thesis, three experiments were conducted to characterize changes in rat behavior after putrescine administration, and determine if any effects resembled known GABAergic behaviors.

Experiments 1 and 2 investigated the behavioral consequences of oral and intraperitoneal putrescine treatment. Male adult rats were given either saline or putrescine doses and tested with seven procedures which evaluated motor behavior, sensory reactivity, body temperature, and other behaviors. Results showed that putrescine-treated subjects exhibited significant changes in behavior compared to saline controls, and that certain effects resembled behaviors related to the function of GABA and/or other neurochemicals.

Experiment 3 investigated the modifying effects of putrescine treatment on behavior induced by a dopamine agonist,

apomorphine, and were compared to effects produced by a GABA agonist, muscimol. Male adult rats were given either saline, putrescine, or muscimol, and later treated with saline or apomorphine. Locomotor and specific activities were time sampled for 45 minutes. Results indicated that putrescine and muscimol had similar interactions with apomorphine in modifying sniffing stereotypies.

From the behavioral evidence obtained in this thesis, it was suggested that systemic putrescine administration may indirectly influence behavior in the rat via GABAergic mechanisms. The results were interpreted as preliminary support for a significant neuropharmacological role of putrescine in the mammalian central nervous system.