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EFFECT OF SEVERAL PSYCHOACTIVE DRUGS ON THE MONOAMINE-CONTAINING NEURONS OF ADULT AND DEVELOPING RAT BRAINS

by

Hillel TAUB, B.Sc., M.Sc.

A Thesis submitted to the School of
Graduate Studies in partial fulfilment of
requirements for the degree of
Doctor of Philosophy

University of Ottawa
Ottawa, Ontario, Canada
August, 1978

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor Dr. D.A.V. Peters for his guidance, generosity, understanding and friendship extended to me these past few years.

Sincere appreciation is also expressed to my friend and able mentor Dr. David R. Usher who was instrumental in guiding my project in its infancy.

I wish to acknowledge the capable assistance of Mrs. Rainy Tang in some of the studies. I am most grateful to Mrs. Cherrill Boyer for the excellent typing of the manuscript, Mrs. Eva Szabo for her high quality drafting of some figures, and Mr. S. Klosevych and his staff for expertly preparing and photographing various illustrations. I also wish to thank many members of the department for having made my stay in Ottawa memorable, and who in their own way have contributed to the completion of this thesis.

Sincere gratitude is extended to Professor G.S. Marks (Queen's University) for supporting my applications to Ottawa University and a number of granting agencies, and for his continued confidence in me.

I would like to gratefully acknowledge the financial support provided by the Ontario Mental Health Foundation in the form of a Research Studentship award, and the University of Ottawa for the Research Fellowship.

This thesis is dedicated to my wife, JULIE whose confidence, support, encouragement and numerous sacrifices for which I am fully indebted, has allowed for the completion of this work.

CONTENTS

LIST OF ABBREVIATIONS

Amy.; Amygdala

ATPase; Adenosine triphosphatase

Cer.; Cerebellum

CSF; cerebrospinal fluid

CNS; central nervous system

CPZ; chlorpromazine

Cing; Cingulate gyrus

4-CA; 4-chloroamphetamine

DA; dopamine

DOPA; 3-(3,4-dihydroxyphenylalanine)

5,7-DHT; 5,6-dihydroxytryptamine

$DMPH_4$; 6,7-dimethyl-5,6,7,8-tetrahydropteridine

DβH; dopamine-β-hydroxylase

Hipp.; hippocampus

Hypo; hypothalamus

5-HT; 5-hydroxytryptamine, serotonin

5-HIAA; 5-hydroxyindoleacetic acid

Li; Lithium

MAO; monoamine oxidase

med.; medulla

mid.; midbrain

mtr.; motor cortex

NE; Norepinephrine

NSD-1034; N-methyl-N-3-hydroxybenzyl hydrazine

olf.; olfactory tubercle, bulb and tract

6-OHDA; 6-hydroxydopamine

p-CPA; p-chlorophenylalanine

P/M; pons-medulla

SD; Sprague-Dawley

Sept.; septum

sp.; spinal cord

str; corpus striatum

temp.; temporal cortex

TH; tyrosine hydroxylase

thal.; thalamus

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I. INTRODUCTION

In the last two decades considerable interest has been focused on the role played by the biogenic amines norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT, serotonin) in various central nervous system functions. Evidence has accumulated to implicate these monoamines in a neurotransmitter or neuromodulator role in the central nervous system (CNS). Since many drugs are known to alter mental functions in man as well as to alter animal behavior, it has been postulated that these drugs may produce their effects, at least in part, via the biogenic amines in the CNS. Thus studies involving the mechanism controlling biogenic amine synthesis and release may be instrumental to our further understanding of the basic biochemical abnormalities that are believed to exist in various mental disorders (1).

The present dissertation is concerned with two studies involving the role of brain biogenic amines. The first study involves an in-depth examination of the effects of short term and chronic treatment of lithium on biogenic amine metabolism in discrete rat brain regions, with emphasis given to the 5-HT system. The second study involves an examination of the influence of drugs on the development of brain monoamine-containing neurons.

Lithium

Although lithium salts have been used therapeutically for a number of years as antimanic agents and more recently, prophylactically, in manic depressive illness (2, 3) their sites and mechanisms of action remain unclear (4, 5). Since disorders in the metabolism of

biogenic amines have long been postulated to play a significant role in affective disorders (6-13), considerable attention has been given to the effects of lithium salts on the steady state levels and turnover rates of these amines. However, little consideration has been given to localizing the sites at which lithium is presumed to exert its therapeutic or neurotoxic effects. There is limited information available on the distribution of lithium in specific brain regions, although there is some evidence to suggest a non-uniform distribution of lithium following its acute or chronic treatment (14-16).

While the mechanism of action of lithium remains obscure evidence to date suggest that lithium produces marked alterations in the metabolism of biogenic amines, particularly of NE (17-23), and 5-HT (24-28) in the brain. Reports in the literature reveal marked inconsistencies in the results particularly when whole brain levels were studied. Some of these inconsistencies can be attributed to a wide range of administration variable, including different routes of administration, dose levels and treatment times. However, a clear distinction can be drawn between the acute effects after a single or a few doses of lithium and the chronic effects after prolonged periods of treatment. It is apparent from the literature that more consistent results have been demonstrated with studies involving short term lithium administration than those involving chronic studies. Some of these differences can be attributed to the administration of lithium at toxic doses.

Since it has been suggested that lithium produces its therapeutic effects by acting in a number of brain regions believed to be

responsible for modulating emotionality (16, 123, 169), the regional distribution of lithium was examined following treatments which produced plasma levels similar to those found to be clinically effective in man. The time periods selected were from 5 days to 5 weeks. The 5-day treatment period was chosen because several reports note significant changes in brain levels of both 5-HT and NE metabolism, although in man therapeutic effects are not usually evident until at least 7 days of treatment. The 2-week treatment period was chosen to approximate the point at which the therapeutic effect of lithium in human patients becomes evident. The 5-week treatment schedule was chosen because chronic effects on brain 5-HT levels and/or turnover have been reported in several studies.

In order to examine the effect of short and long term lithium administration (given intraperitoneally or in rat food) on brain 5-HT metabolism, the influence of lithium on the levels of tryptophan, 5-HT and its metabolite 5-hydroxyindoleacetic acid, the activities of the associated enzymes tryptophan hydroxylase and monoamine oxidase and the 5-HT turnover were investigated in discrete brain regions. Since a relationship exists between the levels of L-tryptophan in blood and brain pools and 5-HT synthesis in the brain, it was of interest to examine whether lithium alters the distribution of tryptophan in the blood and the brain.

To examine whether short and long term lithium administration produces changes in the catecholaminergic neurons, the influence of lithium on the levels of tyrosine, NE, DA and tyrosine hydroxylase activity was investigated in a number of discrete brain regions.

Drugs and Brain Development

There is some evidence to support the view that biogenic amines have important functions in growth and development, apart from their roles in the adult animal. Exposure of experimental animals during development to drugs which are known to reduce storage or synthesis of brain biogenic amines (e.g. reserpine (45), α-methyl-p-tyrosine (40) and p-CPA (44) or drugs which destroy specific monoamine terminals e.g. 6-hydroxydopamine (29, 33-37), and the dihydroxytryptamines (30, 31, 38, 39) were shown to produce behavioral and biochemical abnormalities in later life. Psychoactive drugs which interact with the monoamine receptors either directly or indirectly such as chlorpromazine (41, 46), haloperidol and penfluridol (42, 47, 48) and LSD (43) given pre- or postnatally were shown to produce similar persistent effects. Several studies attempted to identify the "critical periods" during which the vulnerability of the brain to these agents is maximal. Indeed, it was shown that the critical period for 6-OHDA for producing long lasting effects appears to be within the first week after birth (e.g. 36, 37).

However, the pattern of the neuronal destruction was shown to be dependent on a number of variable such as the age at which the pups were injected, the dose used and the route of administration.

In a number of studies, deficiencies in the experimental designs makes the interpretation of the results difficult. In fact, it has been suggested that in addition to the direct drug effects on the fetus, a number of pre- and postnatal maternal factors may influence the overall effect of the drug on behavior and biochemical changes in the offspring (40). Since it was shown that the administration of

6-OHDA to rats in the immediate postnatal period produces long lasting effects on the catecholamine containing neurons, it was of interest to examine the time course of this effect in a large number of brain regions. In the light of recent biochemical and morphological evidence that 5,6 or 5,7-dihydroxytryptamine and 4-chloroamphetamine may be selective neurotoxins acting on the serotonergic neurons (30-32, 49) an attempt was made to demonstrate biochemically, terminal or axonal degeneration similar to that shown for 6-OHDA.

There is substantial evidence that chlorpromazine (CPZ) produces adverse effects on brain development with concomitant behavioral deficits (40, 41, 46). It was therefore of interest to investigate whether changes in biogenic amine metabolism produced by prenatal treatment could be produced when the drug was given directly to the offspring in the period immediately following birth (1-6 days). It has been suggested that the brain of rats is most vulnerable to environmental insults during this period, which coincides with the period during which nerve cell formation occurs most rapidly (44). Therefore, the levels of 5-HT, 5-HIAA, tryptophan, and the activity of monoamine oxidase were determined at 20, 40 or 60 days of age to assess the development of the 5-HT containing neurons, while the levels of tyrosine, NE, DA and tyrosine hydroxylase activity were measured to examine the development of the catecholamine-containing neurons.

The effect of CPZ on the development of monoamine-containing neurons was then compared with another psychoactive drug with similar pharmacological properties, namely haloperidol. It was of interest

to also examine the possibility that lithium a drug with structural and pharmacological properties different from that of chlorpromazine and haloperidol would produce long lasting alteration in the development of monoamine-containing neurons.

2. LITERATURE REVIEW

I. NEUROPHARMACOLOGY OF BIOGENIC AMINES

A. General Characteristics of Catecholamines and 5-Hydroxytryptamine in the Central Nervous System

With the advent of new biochemical, morphological and histological techniques it became possible to more precisely study the involvement of brain biogenic amines with specific CNS neuron systems. Highly sensitive assays of the monoamines, their precursors, metabolites and associated enzymes combined with histofluorescence and radio-immunological techniques for visualization of the catecholamines and 5-HT in neurons enabled the identification and characterization of major monoamine-containing cell bodies and terminal networks in large brain areas.

It became readily apparent that the monoamines were unevenly distributed throughout the brain regions. Norepinephrine (NE) was found to occur in highest concentrations in the hypothalamus (51) whereas dopamine (DA) was found to concentrate in the basal ganglia (51, 51a). 5-Hydroxytryptamine (5-HT, serotonin) was also found to be unevenly distributed, with the highest concentrations present in hypothalamus, brain stem, neostriatum and some areas of the limbic system (52, 53, 54).

B. Monoamine Pathways

The monoamine neurons in the CNS have been mapped out, and the major neural pathways were shown to arise from well localized cell body groups mainly within the brain stem regions. The axons branch profusely to give rise to terminal networks with a widespread distribution to many regions of the brain and the spinal cord. Forebrain

catecholamines arise from terminal projections of NE cell bodies in the pons and medulla, and from DA cell bodies in the midbrain.

i. Norepinephrine-containing neurons

The major ascending tracts are the dorsal and ventral NE pathways. The dorsal bundle was shown to arise from the locus coeruleus (A6 nuclei groups as described by Dahlstrom and Fuxe (59)) and to innervate cerebellar, and cerebral cortices, the hippocampus and other major areas (Fig. 1; 55). The ventral NE pathway was shown to arise from cell groups A1, A2, A5 and A7 in the medulla and pons and sends axon terminals to the stria terminalis progressing rostrally through the medial forebrain bundle. Two other pathways have been identified using a more sensitive histofluorescence technique (56), the dorsal and ventral periventricular systems. The dorsal periventricular system appears to arise from diffusely distributed cell bodies in the pons, medulla and caudal thalamus which project to thalamic, hypothalamic and septal regions. The ventral periventricular system extends along the periventricular grey of the hypothalamus to innervate the dorsomedial and periventricular hypothalamic nuclei.

ii. Dopamine-containing neurons

The dopaminergic system arise mainly from a large collection of cell bodies in the ventral midbrain (groups A8, A9, A10) which forms a continuous sheet of cell bodies extending across the midline over the interpeduncular nucleus and into the pars compacta of the substantia nigra (A9 cell group) (Fig. 2). These nuclei give rise to a dense network of terminals in the corpus striatum, forming the nigrostriatal pathway. The second identified system, meso-limbic DA pathway, arises from the ventral tegmental mesencephalon cell bodies

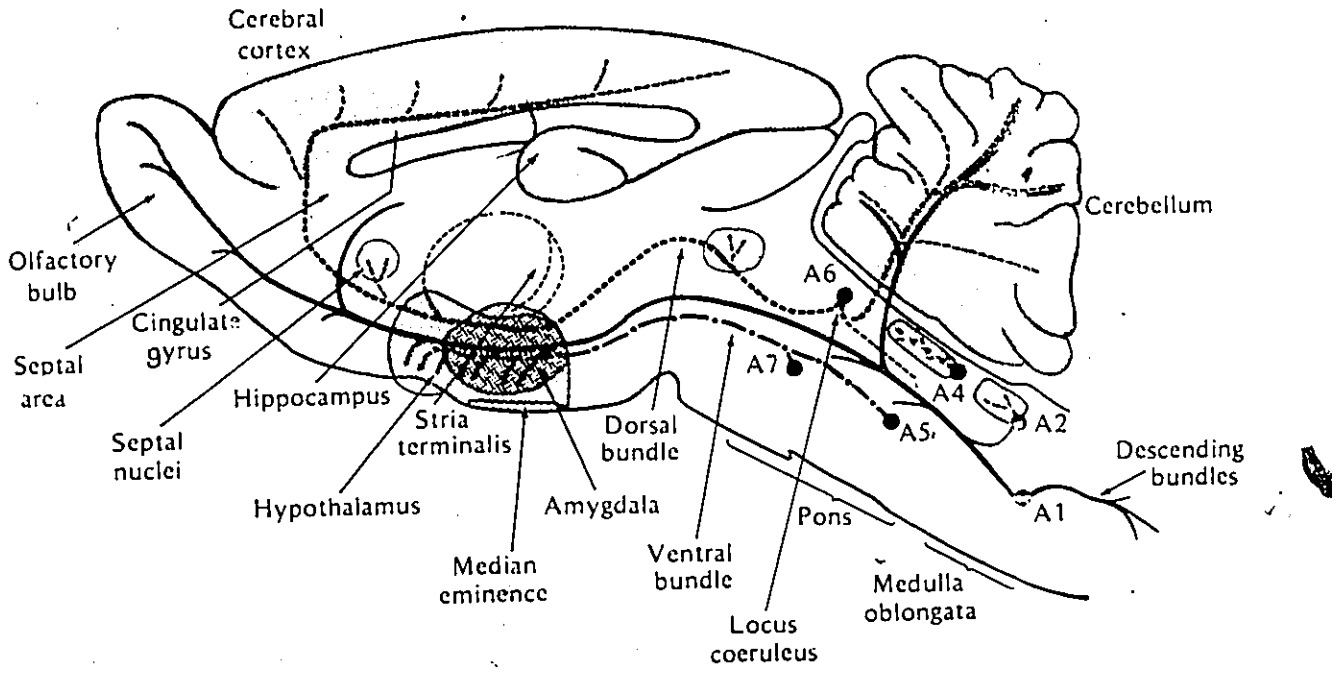

Fig. 1. Schematic representation of the projections of ascending norepinephrine pathways.

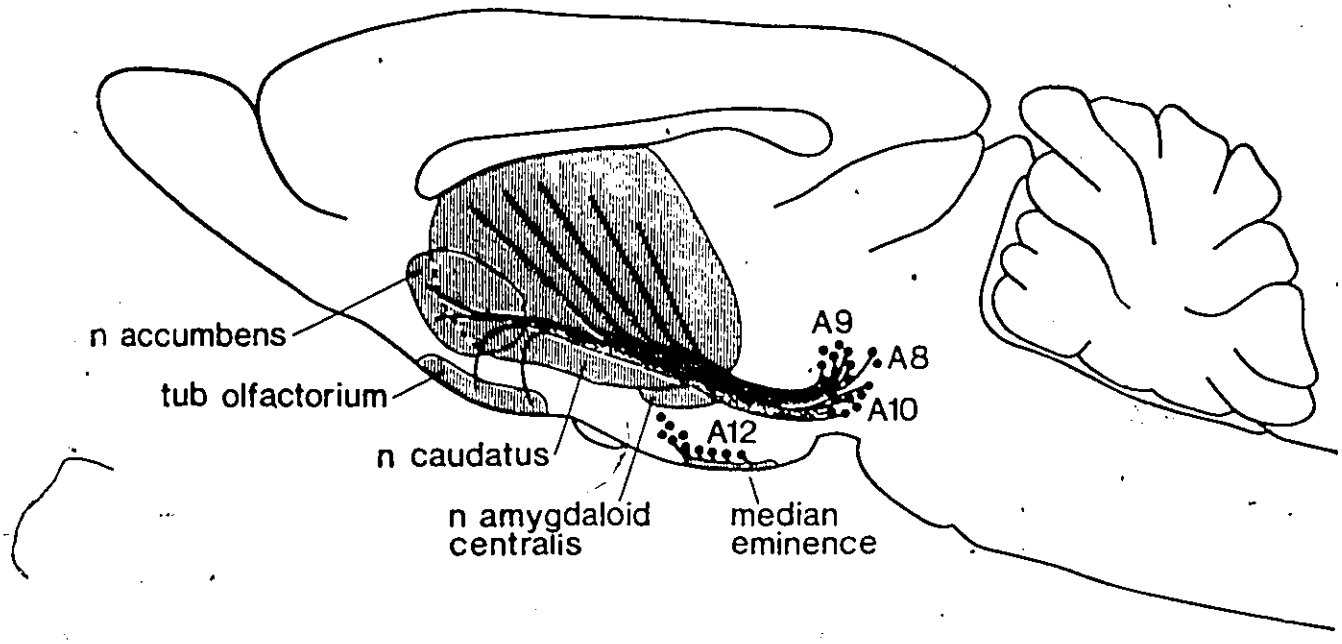

Fig. 2. Schematic representation of the projections of dopamine pathways. Stripes indicate nerve terminal areas.

(A10 cell group) to ascend dorsal to the medial forebrain bundle to innervate the nucleus accumbens, striae terminalis, the olfactory tubercle, septum, frontal and piriform cortices. The tuberoinfundibular DA pathway is a diencephalic system linking cell bodies from the arcuate nucleus to the median eminance.

iii. Epinephrine-containing neurons

Major advances in technology have provided useful techniques for determining the presence of epinephrine in brain and for localizing the enzymes responsible for its formation. Phenylethanolamine-N-methyltransferase, the enzyme involved in the biosynthesis of epinephrine from NE in the adrenal, has recently been identified in the mammalian brain and was shown to be unevenly distributed (56a) with highest activity observed in the rostral brain stem regions. Using an immunohistological technique, Hökfelt et al. (56b) provided evidence for the existence of epinephrine-forming neurons in the rat brain, and gas liquid chromatography/mass spectometry (GC/MS) technique provided quantitative data on epinephrine content in specific rat brain nuclei (56c). Thus epinephrine may serve as a neurotransmitter in the brain although its role in brain function is unknown.

iv. 5-Hydroxytryptamine-containing neurons

Histofluorescence and immunological techniques and biochemical assays have been used to localize 5-HT in the brain. These methods showed that most of the cell bodies of the 5-HT containing neurons were localized in the raphe nuclei of the midbrain, pons and medulla. 5-HT containing fiber terminals have been detected in restricted areas of the hypothalamus, limbic system, and other forebrain and brain stem structures. Three major cell body groups have been

described: the B7 which occupies the area of the dorsal raphe nucleus, the B8 localized in the medial raphe nucleus and the paired B9 groups in the ventral midbrain tegmentum. 5-HT terminals are diffusely distributed to the corpus striatum and many forebrain regions including the globus pallidus, habenula, septal region, amygdaloid complex, cingulate gyrus, hippocampus, and various hypothalamic nuclei (57, 58, 59). The precise terminal distributions arising from each of the cell bodies remains unclear. The caudal group of cell bodies (B1-3), which include the relatively large nucleus raphe magnus (B3) appear to give rise to descending pathways to the spinal cord.

C. Biosynthesis and Metabolism of Catecholamines

The synthesis of catecholamines proceeds from L-tyrosine which is hydroxylated to form L-DOPA (Fig. 3). This step is normally rate limiting in the synthesis of both NE and DA, and is catalyzed by the tyrosine hydroxylase (TH) enzyme (60). TH was shown to occur in both soluble and particulate forms with a high proportion of the soluble form in regions containing cell bodies, while a high concentration of the particulate form in the nerve terminal regions (61). The enzyme is believed to be synthesized within the cell bodies and is transported to the terminals via a slow axonal transport mechanism (62). There is considerable evidence for an end-product feedback mechanism responsible for controlling catecholamine biosynthesis (63), as well as a receptor-mediated feedback regulation operating via allosteric activation of the enzyme (64), or by a change in the physical state of the TH enzyme. Long term regulation of the enzyme activity was recently suggested to involve a trans-synaptic induction resulting in an increased amount of the enzyme (65, 67).

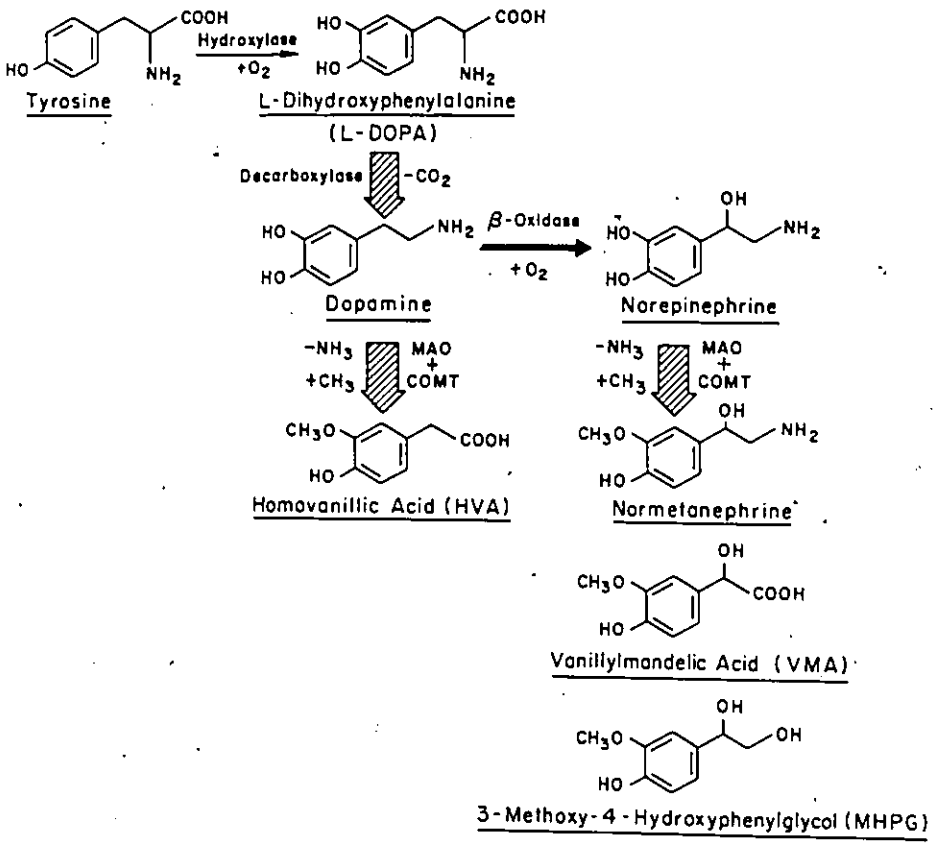

Fig. 3. Schematic representation of the biosynthesis and metabolism of catecholamines.

The enzyme involved in the conversion of L-DOPA to DA, is dopa decarboxylase, which may be identical to the 5-hydroxytryptophan decarboxylase enzyme. It is therefore often described as L-aromatic amino acid decarboxylase (66).

The hydroxylation of DA to NE by dopamine-β-hydroxylase is the final step in the synthesis of NE. The enzyme was shown to require molecular oxygen, ascorbate, fumarate and Cu^{++}. Like tyrosine hydroxylase (TH), it is an inducible enzyme and its activity was shown to be increased by stress and drugs such as reserpine (67, 68).

The catecholamines are metabolized by either deamination by monoamine oxidase (MAO) or o-methylation by catechol-o-methyl transferase (COMT). Monoamine oxidase is widespread in the CNS, where it is bound to mitochondria. Both glial cells and nerve cells contain MAO.

The initial product of deamination is an aldehyde which is rapidly metabolized by either an aldehyde dehydrogenase or aldehyde reductase to corresponding carboxylic acid or alcohol (69). MAO is known to exist in at least two forms which show different substrate specificities and physical properties (70). Both MAO and COMT contribute to the metabolism of both NE and DA giving rise to many known metabolites. DA is metabolized to 3-methoxytyramine (3-MT) by the action of COMT or deaminated to 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylethanol (DOPET) by MAO. The second stage of reactions yields 3-methoxy-4-hydroxyphenylacetic acid (Homovanillic acid, HVA) and 3-methoxy-hydroxyphenylethanol (MOPET). NE is converted either to normetanephrine by COMT or to 3,4-dihydroxymandelic acid (DOMA) and 3,4-dihydroxyphenylglycol (DOPEG) by MAO. Further metabolism results in the formation of 3-methoxy-4-

hydroxyphenylglycol (MHPG) and 3-methoxy-4-hydroxymandellic acid (vanillmandelic acid, VMA). DOPEG and MHPG are considered to be the main metabolites of NE and HVA the main metabolite of DA (70a). There is some experimental evidence to suggest that intraneuronal catabolism of catecholamines occurs mainly via MAO, while COMT is largely responsible for extracellular or extraneuronal metabolism (71, 72).

D. 5-Hydroxytryptamine Biosynthesis and Metabolism

The synthesis of 5-HT commences with the hydroxylation of L-tryptophan to 5-hydroxytryptophan (5-HTP) which in turn is decarboxylated to 5-HT (Fig. 4). The conversion of L-tryptophan to 5-HTP catalyzed by the enzyme tryptophan hydroxylase is considered to be rate limiting (73). The enzyme utilizes molecular oxygen and a pteridine cofactor as an electron source. The tryptophan hydroxylase enzyme is located within synaptosomes from which it can be released in a soluble form by hypo-osmotic shock (74). It is non uniformly distributed in the CNS of mamalian brain (75-77) with the highest levels revealed in the neurons of the midbrain raphe. It is presumably synthesized in the cell bodies of the raphe nuclei, from where it is transported to terminal regions via an axonal flow (78).

L-5-HTP decarboxylase the enzyme which catalyzes the formation of 5-HT from 5-HTP appears to be identical with the enzyme capable of decarboxylating a variety of aromatic amino acids. This enzyme occurs throughout the brain regions and its regional distribution parallels that of the monoamines (79).

The newly-formed transmitter amines, are largely taken up and stored in granules which are believed to play a role in the release of the transmitter induced by nerve impulses. The metabolism, storage

BIOSYNTHESIS AND METABOLISM OF SEROTONIN

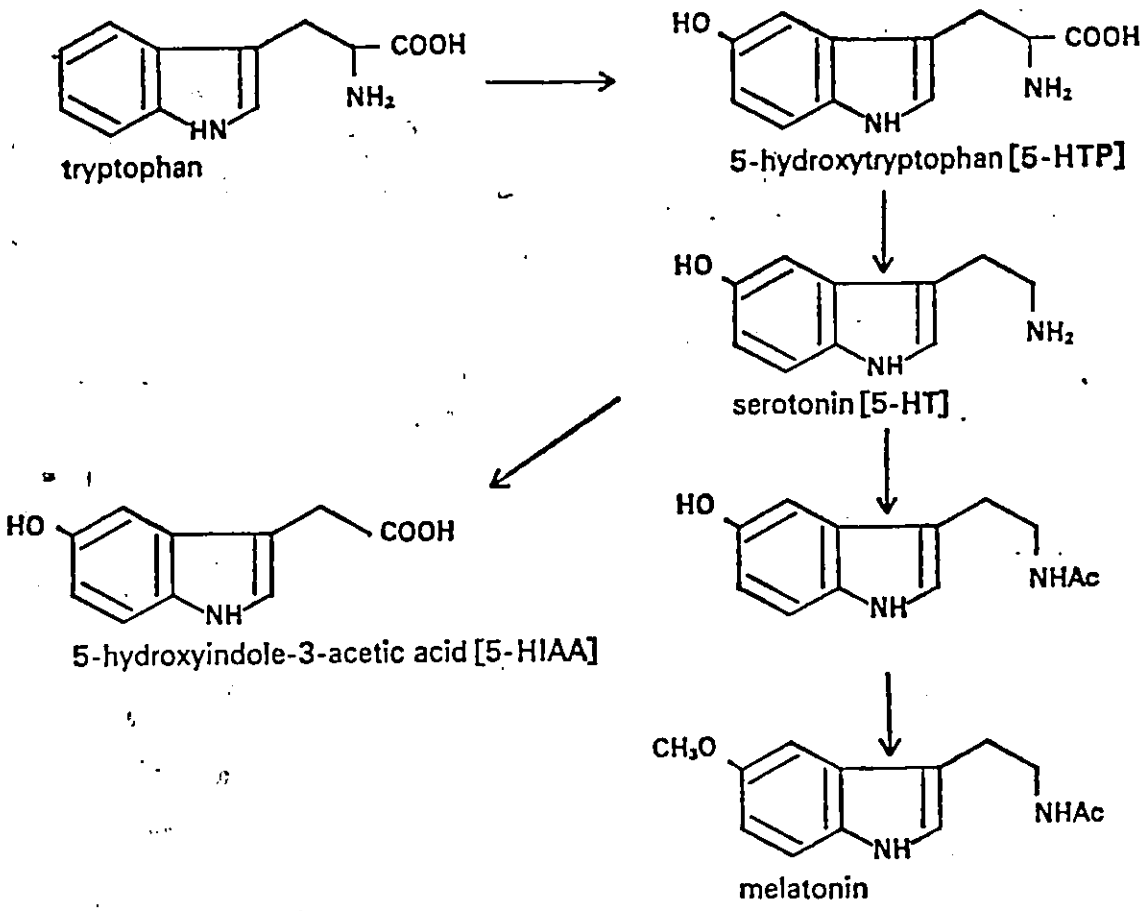

Fig. 4. Schematic representation of the biosynthesis and metabolism of serotonin.

and release of the transmitter seems to be regulated by a number of mechanisms not clearly defined. End-product inhibition of the tryptophan hydroxylase activity has not been demonstrated. However it has been suggested that 5-HT synthesis may be regulated by the availability of the substrate, since the tryptophan hydroxylase appears to be unsaturated with respect to substrate, and the indices of 5-HT in the brain appear to change in the same direction as tryptophan levels (80-84). L-tryptophan is taken up into brain and synaptosomes by means of a carrier mechanism, along with other neutral amino acids (85). Thus the uptake of tryptophan into the brain was recently suggested to be dependent on the relation between the plasma concentration of both free tryptophan and other neutral amino acids (86).

Synthesis of 5-HT was suggested not to be the important factor controlling the 5-HT stores, since at least two different storage compartments may exist, of which the newly-formed 5-HT may be the only functionally important one (87). Grahame-Smith (87) discussed the possibility that there is a more rapid 5-HT synthesis than is required to maintain the "functional" stores, whereby the surplus is degraded to 5-HIAA by a mechanism which he describes as "overflow". However, it was demonstrated by several authors that treatments of rats with a dose of tryptophan which produced a marked increase in 5-HT synthesis in the midbrain raphe neurons (88-90), results in a marked inhibition of the raphe neurons activity (91-92). Thus the increased levels of 5-HT resulting from elevated tryptophan uptake in the brain is functionally active in at least the midbrain raphe system. There is considerable evidence for the receptor-mediated feedback regulation of 5-HT synthesis, which was postulated to be activated by an increased stimulation of pre- or post synaptic 5-HT

receptors (93) or via a calcium-activation mechanism of the tryptophan hydroxylase enzyme (94-97).

5-HT is metabolized almost exclusively by monoamine oxidases to give rise to an intermediate 5-hydroxyindoleacetaldehyde which in turn is oxidized to 5-hydroxyindoleacetic acid or reduced to 5-hydroxytryptophol by an aldehyde dehydrogenase and reductase respectively. Both kinetic studies and direct measurements of 5-HT in brain tissue reveal that 5-HIAA is the major metabolite of 5-HT (69). Because of the relative simplicity of the 5-HT metabolism, both brain and cerebrospinal fluid (CSF) levels of 5-HIAA are used as a measure of 5-HT turnover in the brain.

5-HT can also be acetylated by a N-acetylase enzyme to form the immediate precursor of melatonin in the pineal. N-acetylserotonin (NAS) in turn is o-methylated by a S-adenosylmethionine-dependent enzyme, hydroxyindole o-methyltransferase (HIOMT) to form melatonin (98).

II. LITHIUM

Lithium is the lightest of the alkali metals belonging to the group IA of the Periodic Table and is known to occur in nature in at least 145 minerals and in both plant and animal tissue. Lithium is monovalent and one millimole is equal to one milliequivalent and is contained in 66 mg of lithium acetate and 37 mg of lithium carbonate.

A. Medicinal Uses of Lithium

Following the initial introduction of lithium salts into medicine by Lipowitz in 1841 and Garrod in 1859, the lithium content of mineral

springs was extensively advertised as a cure for numerous disorders including gout and rheumatism. In the 1920's, lithium was used as an antiepileptic, a tonic and hypnotic, and the early 1940's saw its brief introduction as a salt substitute for cardiac and kidney patients. The discovery of the psychoactive properties of lithium as reported by Cade in 1949 (99) led to an era of intense research culminating in the recognition of its antimanic, antidepressant and prophylactic properties (2, 3) in manic depressive disorders.

The effectiveness of lithium in the treatment of acute mania and hypomania was established by several rigorous double-blind controlled studies (100). To date more than 70 single and double blind controlled studies have conclusively shown that lithium salts produce marked improvements in nearly 80% of manic patients within 7-14 days. These effects appear to be independent of sex, age, and duration of the underlying disorder. Lithium was shown to be more specific than other antipsychotic agents, especially in cases which are dominated by pure elation-hyperactivity and pressure of speech syndrome. Lithium has been shown to be superior to chlorpromazine for highly disturbed acute manic patients (101, 102). It, however, has been shown that at the onset of an acute assaultive manic episode, the combination of lithium with chlorpromazine or haloperidol is preferable whereas after the attack has subsided lithium maintenance alone is sufficient.

While the bulk of the available data suggests that lithium is effective in the treatment of bipolar manic-depressive psychoses, it appears to be of little value against agitations of schizophrenics and neurotics. It appears to curb aggressive behavior (103, 104) and recurrent alcoholic-induced behavior manifestations (105). Experiments

on healthy subjects indicate that lithium does not impair normal intellectual activity or restrict the emotional range; yet subtle and transient changes have also been reported (106, 107).

In a moderately severe manic episode the initial daily dose of lithium carbonate required is usually from 1500 to 2100 mg in a 70 Kg patient. This dosage is given for 1 to 2 weeks and the steady state level between intake and elimination is reached within 5-6 days; thereafter serum lithium levels in blood samples drawn 12 hours after the last intake is in the subtoxic range of 0.8 to 1.5 mEq/L.

B. Pharmacokinetics of Lithium

i. Absorption and distribution

In man, as in experimental animals, lithium ion is readily absorbed following oral, subcutaneous, intramuscular or intraperitoneal routes of administration. Blood levels peak between 2 and 4 hours after a single oral dose, and intragastric absorption appears to be complete within 6-8 hours (108). Since lithium is not protein bound, it is distributed widely throughout the body water, although it crosses cell membranes at a relatively slow rate — an effect which may be related to the delay of 6-10 days in achieving full therapeutic response as well as to its continued excretion following discontinuation of treatment (121, 122).

In experimental studies involving animals, the preferred route of administration of lithium is in the diet since it ensures a fairly constant serum lithium levels throughout the day and reduces the frequency of side effects (109).

Since an equilibrium would be expected to be established between the serum and the tissue levels of lithium, a direct approach to the

study of lithium distribution has been the analysis of both tissue and serum lithium concentrations. A number of studies demonstrated that the rate of uptake of lithium into peripheral and central tissues is not uniform (15, 110, 112, 114). As in animal whole brain studies, the human experience suggests a slow rate of lithium uptake into the CNS and the cerebrospinal fluid (CSF) with a lack of equilibrium between serum and CSF levels (111). Reports on the brain regional distribution of lithium of patients and experimental animals treated with lithium has been sparse and variable, with differences in regional concentrations reported to be small (14-16, 113) or reported to be associated with particulate fractions of brain homogenates (115).

It has recently been suggested that lithium levels in the red blood cells may better correlate with the clinical state than the simple serum lithium levels (116). However, the kinetics of lithium transport across the red blood cell membrane and factors which govern its steady state are not well defined (117, 118).

ii. Excretion

The main route of lithium excretion is via the kidneys. The renal handling of lithium and the effects of lithium on renal cations reflects partial substitution for other univalent cations. After filtration through the glomerular membrane, approximately 80% of lithium is reabsorbed together with Na^+ and water in the proximal tubules. The lithium clearance has a half-life of approximately 24 hours which appears to be decreased with age and with a reduction in sodium intake (3). Following a single oral dose of lithium carbonate, 30-60% of the drug is excreted in the urine during the initial 6-12 hours, with a prolonged excretion rate extending 10-14 days (122).

C. Lithium-Induced Side Effects and Complications

There are three main types of unwanted effects of lithium: a) side effects which can be manifested at low serum levels (0.5-1.5 mEq/L) b) lithium toxicity associated with serum lithium levels greater than 2 mEq/L and c) a variety of endocrine and metabolic effects which may occur independent of serum lithium levels.

During the initial periods of therapy some patients experience slight gastrointestinal irritation, nausea, abdominal pain and loose stools. They may also experience muscular weakness, weight gain, a dazed feeling, polyuria, thirst and fine hand tremor. These common symptoms often coincide with the peaks of lithium concentrations in the blood and are usually transient in nature. However, more persistent side effects are the feeling of thirst occasionally combined with polyuria and fine hand tremors which does not respond to antiparkinsonian drugs but does to propranolol (120).

When lithium is given in excess amounts or when renal mechanisms fail to eliminate it effectively, the serum lithium levels rises above 2.0 mEq/L. This rise is generally accompanied by symptoms of lithium toxicity in man notably those which involve the CNS. Early symptoms include sluggishness, slurred speech, drowsiness, course hand tremor, twitching, anorexia, ataxia, vomiting and diarrhea. Within days consciousness is severely impaired and coma may develop with hyperactive tendon reflexes, muscle tremors, fasciculations and epilepiform seisures occasionally observed (5, 121, 122).

Recent reports of lithium-related neurotoxicity at doses producing serum levels in the subtoxic range have appeared in the clinical literature. It has therefore been suggested that some of the lithium-

induced neurotoxicity may not be directly related to blood levels of lithium. These bizzare effects have been associated with electroencephalographic changes (123) and it has been suggested that lithium produces these effects directly on the brain (124, 125). However, little experimental work has been forthcoming on the effects of lithium on the CNS directly.

A number of recent studies examined the endocrine and metabolic consequences following prolonged lithium treatment in man. In general, the manifested changes are unpredictable, infrequent and occur at blood levels not usually associated with toxicity. These include neurotoxic episodes, goitre and hypothyroidism, polydipsia and polyuria, cardiological abnormalities, skin reactions and assorted metabolic effects.

i. Lithium and thyroid function

Recently Baily et al. (122) compiled and reviewed the recent literature on the effects of lithium on thyroid function both in man and experimental animals and concluded that in man, three forms of thyroid disorders are occasionally observed: transient biochemical changes (increased levels of thyroid stimulating hormone, and reduced triiodothyronine and thyroxine in the serum), compensated goitre with euthyroidism, and hyperthyroidism with or without goitre. While more consistent changes were observed following acute treatments, chronic animal experiments provided diverse and inconclusive results. However, lithium was shown to consistently inhibit the thyroid stimulating hormone — stimulated thyroid adenylate cyclase activity by a mechanism not clearly defined (126).

ii. Lithium and thirst and urine flow

Lithium-induced polyuria was shown to be consistently evoked in

experimental animals and the effects were postulated to be related to the histological changes observed in the nephron and/or altered salt content in the diet (127). The site(s) at which lithium exerts its polydipsic effect was suggested to be the lateral hypothalamus or the kidney (129).

iii. Lithium and metabolic effects

Lithium was shown to produce profound hypoglycemia and to promote tissue glucose uptake in both experimental animals and humans, which correlates with the observed rise in the serum levels of insulin (130). It has also been observed that lithium produces decreases in hepatic cholesterol and fatty acid synthesis, as well as inhibits the ACTH-stimulated adenylate cyclase activity in isolated fat cell ghosts (5). Lithium was also shown to alter carbohydrate metabolism at various sites, however since the changes observed were often after very high lithium doses, their relevance to the clinical situation remains questionable.

iv. Lithium and adrenocortical activity

The relationship between mood disorders and the pituitary-adrenal axis prompted investigations into the possible role of lithium on this system; however the published results are widely conflicting. Several reports claim elevated corticosteroid levels after lithium carbonate administration to normal volunteers and manic-depressive patients (131, 132) which were not confirmed by others (133). Holmi et al. (134) reported lower plasma cortisol levels in the morning and suggested that the apparent blunting of the circadian fluctuations of plasma cortisol by lithium may be due to altered secretion of ACTH rather than diminished responsiveness of the adrenal cortex to the trophic

hormone. Recently Usher (135) reported that high doses of lithium given in the diet of two strains of rats for 5 weeks caused increased adrenal *in vitro* corticosteroid output and diminished ACTH response in both strains, with no changes in plasma corticosteroid levels. The lack of uniformity of the experiments does not allow proper comparison of results, and the available evidence do not offer firm conclusions as to the effects of lithium on this regulatory axis.

v. Lithium and placental transfer

Since the reintroduction of lithium into medicine, several reports have been published documenting the effects of mothers and infants of mothers treated with lithium during pregnancy. Nassr (136) described a case of lithium-treated female in late pregnancy who developed a toxic condition during the delivery and the infant was cyanotic with demonstrable flaccid muscle paralysis. Indeed, it was shown that infants born to lithium-treated mothers have measurable blood lithium levels which were reported to correlate with the observed lethargy and cyanosis (137). Additional support for this suggestion was derived from the study of Weinstein *et al.* (138) who reported that lithium was detectable in breast milk at the level of 25-33% of that found in the maternal blood, such that it is likely that infant exposure to lithium may be both prenatal and postnatal in origin.

The possible teratogenic effects of lithium were investigated in experimental animals and humans. Several reports of lithium-induced teratogenicity in mammalian and submammalian species have been described, although the International Register of Lithium Babies having assessed nearly 150 cases (up to 1975) reported only 13 of

the born infants to manifest any malformations (139). The incidence was considered to be higher than would be expected to occur in a normal population. Although the literature on lithium teratogenicity in mammalian studies is relatively sparse, the available data provide inconsistent results possibly stemming from variable dose schedules and variable serum levels which was achieved in these studies (140-145).

D. Lithium and Electrolyte Distribution

Electrolytes are known to be involved in maintaining the normal membrane potential and in carrying the current responsible for action and synaptic potentials. They are also known to be intimately involved in the synthesis, storage, release and inactivation of putative neurotransmitters. It was therefore postulated that altered electrolyte metabolism in concert with interaction of monoamines would be responsible for altered neuronal function in psychiatric disorders. Indeed, altered electrolyte distribution has been reported in association with mood changes, and clinical depression was shown to be consistently associated with sodium retention (146). These early observations prompted studies into the possible role of lithium in modifying electrolyte metabolism. It is generally agreed that lithium characteristically alters electrolyte balance in man (147). The mechanism(s) responsible for the fluid and electrolyte changes are complex but appear to be independent of the glomerular filtration rate yet may be related to the renin-angiotensin-aldosterone mechanism and/or the displacements of Na^+ and K^+ in extra- and intracellular compartments of the body (148, 149). Lithium has also been shown to produce effects on other bivalent cations — Ca^{++} and Mg^{++}, both in

man and experimental animals (150, 151). However, the magnitude and the direction of changes of these ions following lithium treatment remains highly inconsistent.

The speculative interpretation of the available data lean towards a membrane effect as the primary site of lithium action. Naylor et al. (152) reported that subjects whose erythrocyte Na^+-K^+-ATPase activity rose in lithium responded best to lithium. Mendels and Frazer (153) suggested that there is an abnormality of electrolyte flow across the cell membranes of depressed patients, and that lithium may interact with membrane processes. Rafaelson et al. (54) also postulated that lithium may act by membrane stabilization. However, other studies suggested that lithium inhibits the Na^+-K^+ dependent-ATPase activity in rat vagus nerve membranes (155), or several brain regions (156) and Mg^{+2}-dependent-ATPase activity in some brain regions (156).

The fluid and electrolyte effects of lithium remain complex, but its inability to completely substitute for Na^+ in many body systems may parallel its actions in the CNS such that replacement of electrolytes by lithium in cells and its inefficient outward pumping could alter electrical transmission or possible electrolyte-transmitter interaction at critical central locations.

E. Lithium Distribution in the Central Nervous System

The distribution of lithium in the brain is not clearly established following its acute and chronic treatment. There is little information on the distribution of lithium in specific brain regions. There is however some evidence which suggests a non-uniform distribution of lithium in the CNS following both acute and chronic treatments but in a limited number of brain parts (16, 114, 157-159), although

several reports to the contrary have also been published. It has been reported that following chronic administration the concentration of lithium in whole brain closely approximates that of blood plasma (14, 110, 160-163). However, the concentrations may differ in discrete brain regions both in animals (15, 16, 114, 157) and in man (113, 164), and may be localized in the particulate fraction of rat brain homogenates (115).

The regions of the brain which were reported to contain the highest levels of lithium include the hypothalamus and white matter in rats (114, 166), the pituitary of rats and rabbits (157), the posterior hemisphere (159), and the basal ganglia and the pituitary of rat and primate brain (15, 164). Since the majority of the above studies were acute, with the administration of lithium salts in large doses, the importance of these findings when compared to long term studies becomes questionable. Furthermore, previous studies were conducted following different routes of administration with variable and inconclusive results reported. For example, Davenport (161) studied the distribution of lithium following acute (1, 24 and 96 hours) intraperitoneal injections, and Schou (110) used intravenous lithium chloride administration whereas Morrison et al. (167) studied the distribution of lithium following stomach intubation and Birch and Jenner (162) reported results based on the addition of lithium to the drinking water, Taub and Usher (16) and Bond et al. (14) and Edelfors (114) added lithium salts to dry food. Thus few comparable studies have been published on the distribution of lithium in specific brain regions which precludes any definitive comparison of results.

Two recent studies provided evidence for a temporal relationship between lithium administration and regional distribution, and a more comprehensive distribution of the ion in some 32 brain regions in a primate following long term oral lithium treatment (164, 166). Sprites (164) described the regional brain distribution of lithium in the brain of monkeys following 3-6 weeks of oral lithium carbonate treatment. Lithium levels were significantly higher in 7 regions out of 32 (in decreasing order), the anterior thalamus, caudate nucleus, fornix and the cingulum, putamen, posterior hippocampus, and olfactory tract. The lowest levels were obtained in the cerebellum and cortical regions (temporal, occipital and frontal lobes), as well as in the pons and midbrain. The study of Mukherjee et al. (166, 168) although representing an acute study, was one of first to demonstrate a time-course of lithium concentrations in different brain parts over a 24 hour period. It was reported that peak levels were obtained by 8 hours in most brain regions (cortex, hypothalamus, diencephalon, and midbrain) with the exception of the caudate nucleus which exhibited peak levels 12 hours after a single intraperitoneal injection. Furthermore, the highest concentrations were maintained in the caudate nucleus and diencephalon between 8 and 24 hours following lithium administration.

The apparent non-uniform concentrating ability or retention of lithium in discrete brain regions is compatible with the well-known specific functional roles that different regions of the brain exert on the possible modulation of emotional behaviour (169).

F. The Effect of Lithium on Central Biogenic Amines

Although lithium salts have been used therapeutically for a number of years, their site(s) and mechanism(s) of action remain

unclear (4, 5, 122). It is well recognized that the biogenic amines, may play a role in the etiology of manic-depressive disorders (6-10). Considerable attention has therefore been given to the effects of lithium on the steady state levels and turnover rates of NE, DA, and 5-HT.

A clear distinction can be drawn between the acute effects after a single or few doses of lithium over a short period of time (up to 4-5 days) and the chronic effects after longer periods of time (2-6 weeks or more). It is apparent from the literature that more consistent results have been demonstrated with the short term lithium administration than its chronic studies, although at least some of the the differences may be attributable to the use of a variety of methods and dosing schedules.

i. Effect of lithium on catecholamine-containing neurons

a. short term studies

A number of studies involving the measurements of steady state levels and turnover rates of norepinephrine (NE) and dopamine (DA) in whole brain of experimental animals treated with lithium salts suggest that lithium does not modify the brain levels of NE and DA. However, the turnover rate of NE was consistently shown to be increased. Indeed, Corrodi et al. (170) and Stern (171) and Poitou and Bohuon (22) showed no change in the levels of NE following a single intraperitoneal injection of lithium chloride at doses of 2.5-3.75 mEq/Kg. However, when a tyrosine hydroxylase inhibitor was given, brain NE turnover was shown to be increased by up to approximately 95%.

Several authors have investigated the effect of lithium on the metabolism of intracisternally administered [^{3}H]-NE in the rat.

Early studies (172) showed decreased levels of [^{3}H]-normetanephrine and increased levels in deaminated metabolites. Schildkraut et al. (173) found that following LiCl (2.4 mEq/Kg, i.p.) for 7 days, lithium increased the rate of disappearance of intracisternally administered [^{3}H]-NE from the rat brain, and the production of [^{3}H]-deaminated catechol metabolites and free deaminated-o-methylated metabolites. These authors suggested that lithium treatment led to increased turnover of NE in the brain, with a shift in the metabolism towards increased deamination. It is therefore interesting that Murphy and Weiss (174) have reported a lower monoamine oxidase activity in platelets from depressed bipolar patients, whereas Bockar et al. (175) showed an increase in the platelet monoamine oxidase activity during lithium therapy. However, in vitro lithium had no significant effect on monoamine oxidase activity in either rat brain (172), or human platelets (175). In a more recent study, Schildkraut (17) reported that both single (1.2, 2.4, or 4.8 mEq/Kg LiCl, i.p.) and repeated (2 mEq/Kg, LiCl once daily for 3 days) treatments led to increased turnover of NE in the brain as indicated by a more rapid rate of disappearance of intracisternally administered [^{3}H]-NE. However, Schubert (176) reported that 7 days LiCl treatment did not affect the disappearance of labelled DA formed from [^{14}C]-tyrosine in the brain. In contrast, Persson (177) reported a lack of significant rise in [^{3}H]-DA in the caudate nucleus following intravenous [^{3}H]-tyrosine injections to lithium carbonate (150 mg/Kg, 4 hours)-treated rats.

Recently, the focus of attention has shifted to examining the effect of short term lithium treatment on the levels of NE and DA in several discrete brain regions. Friedman and Gershon (20) reported

that 60 minutes following acute LiCl treatment (2-4 mEq/Kg, i.p.) to male Sprague Dawley rats there was no alteration in either DA levels or the synthesis rate of [^{3}H]-DA in striatal slices from 3,5-^{3}H-tyrosine. Segal et al. (178) provided evidence to suggest that the dopaminergic nigrostriatal pathway rather than the dorsal norepinephrine pathway (locus coeruleus and hippocampus-cortex regions) was selectively modified by lithium. They observed slight increase in the tyrosine hydroxylase activity in the substantia nigra and caudate nucleus following acute administration (1.5 mEq/Kg, s.c.) and significant elevations (30-40%) following 24 hours and 8 days of repeated daily treatments.

b. Long term studies

Prolonged lithium treatment was shown to cause decreased DA synthesis which appears to be consistent with the data of some investigators (19, 20, 22) but at variance with those reported by others (18, 178, 179). Moreover, the lack of effect of lithium on NE system following chronic lithium is consistent with the reports of some (18, 19, 22, 178, 179) but not all investigators (180). When lithium was given in dry food for 3 weeks achieving blood lithium levels of 0.5-1.5 mEq/L, Corrodi et al. (19) observed no changes in either the absolute levels of NE or its rate of depletion following tyrosine hydroxylase inhibition. However, they reported a significant decrease in whole brain DA turnover without changes in DA concentrations. In contrast, Ho et al. (179) could find no alteration in DA turnover in the cortex, cerebellum, hypothalamus, diencephalon and the brainstem following 4 weeks of lithium treatment (2 mEq/Kg, i.p.). Greenspan et al. (180) showed that under certain experimental conditions lithium

increases the turnover of NE in rat brain. They administered lithium carbonate in two daily doses of 1-3 mEq/Kg for 10 days. On the 11th day DL-[^{3}H]-NE was intraventricularly injected into the animals. They observed that 3 mEq/Kg of lithium more than doubled the rate of efflux of labelled NE from the brain while increasing the relative amounts of tritiated-o-methylated deaminated metabolites. Using another measure of turnover, (rate of NE accumulation after monoamine oxidase inhibition), it was shown that the rate of increase of NE was significantly lower in the lithium pretreated rats.

c. Uptake and release studies

A number of studies examined the effect of lithium treatment on the processes of NE and DA uptake. Colburn et al. (181) studied the effect of lithium carbonate given to rats in food for 5-7 days and achieved blood levels of 1-2 mEq/L. They reported that the uptake of [^{3}H]-NE by synaptosomes prepared from the brains of these rats was increased by 30%. Baldessarini and York (182) confirmed the small increase in the rate of uptake of NE by synaptosomes from rats given lithium carbonate, although they found the dose of lithium (2 mEq in food each day) to be toxic and often fatal. Similarly, Kuriyama and Speken (183) observed a small increase (18%) in synaptosomal uptake of [^{3}H]-NE following lithium treatment in mice (3.75 mEq/Kg LiCl, i.p., twice daily for 5 days). However, this effect was not seen when the uptake of [^{3}H]-NE into intact brain after either acute or repeated (7 days) dosage of lithium was studied (184). Furthermore, there was no effect on uptake of [^{3}H]-NE by slices from brains of rats given lithium chloride for 3 days, even though the stimulated release of [^{3}H]-NE from the slices was decreased (186).

More recently, Stefanini et al. (185) provided evidence to suggest that lithium produces a temporally-dependent change in the in vitro [^{3}H]-DA uptake into synaptosomes derived from caudate nuclei. In in vitro studies, lithium at concentrations of 1 to 10 mEq/L in the bathing medium was shown to decrease [^{3}H]-DA uptake by 13 to 31% into synaptosomes. However, lithium was shown to cause increased [^{3}H]-DA uptake (23%) into synaptosomes isolated from caudates of rats chronically treated with LiCl (2 mEq/Kg, twice daily, i.p.) for 20 days. Thus in vitro lithium appears to inhibit DA uptake into synaptosomes and in vivo to enhance DA uptake following chronic treatment.

Studies on the release of radioactive NE from slices of brain striata following electrical stimulation showed that lithium chloride when given intraperitoneally for 3 days (2.5 and 7.5 mEq/Kg), or when added to the incubation medium (0.8, 1.2 or 2.4 mEq/L) caused a decrease in the electrically-stimulated efflux of NE (21, 186). Based on these observations and the added report that calcium was able to prevent the lithium effect, it was postulated that lithium may interfere with the calcium-stimulated release of NE from slices in vitro. Later it was also reported that the release of [^{14}C]-NE from the perfused cat spleen induced by splenic nerve stimulation was reduced by the addition of 4 mM lithium to the perfusion medium (187). It was therefore suggested that the observed increased turnover rate in vivo may result from an increase in the intraneuronal release and deamination of NE rather than increased extraneuronal discharge of this monoamine (17).

d. Clinical studies

The limited studies in man do not demonstrate marked effects of lithium on catecholamines. Several studies demonstrated increased urinary excretion of catecholamines and deaminated O-methylated metabolites, although the changes were more consistent with the clinical state than to the high lithium levels (17). A recent report (188) suggested that during acute lithium treatment there was no clear pattern of change in the urinary MHPG levels. Similarly it was reported that following lithium treatment no changes in the excretion of VMA (usually elevated in mania) was noted whereas DA excretion was shown to return to normal levels (189, 190). In studies involving the estimation of metabolites in the cerebrospinal fluid of patients undergoing lithium treatment, the levels of DA metabolite, HVA was reported to be either elevated (191, 192), or unchanged (193).

ii. Effect of lithium on 5-hydroxytryptamine-containing neurons

The results of studies on the effects of lithium on indoleamine metabolism in the brain of rats are controversial but in a number of studies lithium has been found to increase consistently the whole brain levels of 5-hydroxyindoleacetic acid, (5-HIAA) the deaminated metabolites of 5-hydroxytryptamine (5-HT) (18, 23-25, 176). Again, a distinct separation between the short versus the chronic treatment of lithium can be made in the studies on 5-HT metabolism.

a. Short term studies

Reports of no significant changes in the levels of 5-HT and turnover rates in brain (170, 194), decreases in the turnover rate with increases in the levels of 5-HIAA (173) or decreases in the levels of 5-HT, 5-HIAA and L-tryptophan (195) following the acute

administration of lithium salts characterize the controversial nature of these studies.

Several reports describe the effects of subacute (4-5 days) treatment with lithium salts on the metabolism of 5-HT in the brain. Lithium was shown to cause an 80% increase in 5-HIAA and 15-20% increase in the 5-HT levels in the rat brain following LiCl (85 mg/Kg, i.p., twice daily for 5 days)(24). It was suggested that these changes were due to an increased rate of synthesis of 5-HT, and appear to depend on the dose of lithium administered. Perez-Cruet et al. (24) reported that brain tryptophan was elevated by 70% and this effect was postulated to be the mechanism by which the increased 5-HT synthesis was achieved. Other investigators reported comparable increases in the brains of rats given a similar dose of lithium (194, 196). Sheard and Aghajanian (23) also reported increased turnover of 5-HT in rat forebrain following a single lithium injection or 4 days of 5 mEq/Kg LiCl. The increased hydroxyindole levels could be explained either by increased synthesis and breakdown or due to decreased rate of 5-HIAA efflux from the brain. To distinguish between these two possibilities they administered probenecid, which blocks the active 5-HIAA efflux from the brain. Following electrical stimulation of the dorsal raphe nucleus, the level of the hydroxyindoles was significantly elevated in the lithium treated rats in the forebrain. Sheard and Aghajanian (23) concluded that increased turnover rate is not necessarily associated with increased release of 5-HT. It is however, not clear whether the increased synthesis is due to increased release of the amine from 5-HT-containing neurons or due to increased intraneuronal deamination by monoamine oxidase.

Several authors have investigated the effect of lithium on the metabolism of intravenously infused [^{3}H]-tryptophan in the rat. Schubert (176) reported that 7 days of lithium chloride treatment when given in the diet produced significantly increased accumulation of labelled 5-HT (56%) and 5-HIAA (44%) following [^{3}H]-tryptophan infusion. They observed no changes in the level of the hydroxyindoles in the brain, but noted a 32% increase in brain tryptophan levels with no changes in total and serum-free tryptophan. In two studies, Schubert (176), and Shaw and Ratcliffe (26) examined the rate of disappearance of the labelled 5-HT and 5-HIAA from the brain formed from [^{3}H]-tryptophan or [^{3}H]-5-HT and observed that lithium retarded the rate of disappearance by about 47%, or reduced the levels of labelled 5-HT. The authors interpreted the data to suggest that lithium may operate via two separate mechanisms: a) increase the brain tryptophan by altering the amino acid uptake resulting in augmented intraneuronal synthesis and metabolism and b) impulse-induced release of 5-HT is reduced by lithium. This idea is compatible with the observed results of several investigators (19, 23, 24, 186).

It was suggested by Collard and Roberts (25, 197) that lithium increased the deamination of free cytoplasmic 5-HT by a mechanism which inhibits transport of newly synthesized 5-HT into or the binding within the storage granule. They observed that following a tryptophan load (100 mg/Kg, i.p.) the rise in 5-HT was reduced in the forebrain of lithium-pretreated rats (LiCl 0.75 mEq/Kg, i.p. for 10 days) while the rise in 5-HIAA levels was correspondingly increased. The authors interpreted their results to suggest that lithium reduced the capacity of 5-HT storage or reduced the rate of newly synthesized transmitter

that could be incorporated into the storage pool.

Tryptophan distribution and metabolism is also affected by lithium. An increase in plasma tryptophan levels in rats given lithium carbonate has been reported (196), although the plasma free tryptophan of recovered manic-depressives appeared unaffected by lithium treatment (199). The compartmentation of tryptophan was altered by lithium (81, 200), and the high affinity tryptophan uptake system in several regions of rat brain is stimulated by lithium in vitro (27, 198).

b. Long term studies

A number of studies have investigated the effects of prolonged treatment of lithium salts using varying dose schedules and routes of administration. When lithium was given in dry food for 3 weeks Corrodi et al. (19) observed no changes in the absolute levels of 5-HT, however the administration of a tryptophan hydroxylase inhibitor produced a smaller depletion of 5-HT in lithium-pretreated animals than in controls. These results were interpreted as an indication that repeated administration of lithium over a period of 3 weeks leads to either a lowered activity of the serotonergic neurons or to an inhibition of impulse-stimulated release of 5-HT at nerve terminals. Bliss and Ailion (18) administered lithium in the food of rats for two weeks and achieved plasma levels of 0.5-1.0 mEq/L. They observed no change in the levels of 5-HT although a slight rise of 5-HIAA was noted. Following the administration of pheniprazine, a potent monoamine oxidase inhibitor, to study the synthesis rate of 5-HT, the rate of accumulation of 5-HT as well as the rate of loss of 5-HIAA in whole brain of lithium treated rats was similar to those produced by control rats. The authors interpreted their results to suggest that lithium

did not alter the rate of synthesis and metabolism of 5-HT. However, when lithium was injected intraperitoneally for 4 weeks (2 mEq/Kg) Ho et al. (179) reported a significant reduction in 5-HT levels in the hypothalamus and brainstem with no changes in the cerebral cortex, cerebellum, or the diencephalon. The 5-HT turnover rate as determined by the rate of accumulation of 5-HT following pargyline was changed only slightly in the whole brain, whereas in the cerebellum there was a 37% increase and in the hypothalamus a 51% decrease and lower decreases in the cortex, diencephalon and the brainstem. However, following 1-4 weeks of lithium treatment (given in the diet) no changes in 5-HT levels was seen in the hypothalamus and white brain matter, while 5-7 weeks of treatment produced a significant rise in the levels of 5-HT in the hypothalamus (19%) (201).

c. Uptake and release studies

Recently, Knapp and Mandell (27, 198) studied the effects of lithium (5-10 mEq/Kg LiCl for 5, 10 and 21 days) on both synaptosomal uptake of L-tryptophan and the activity of tryptophan hydroxylase. They concluded that lithium administration increased the high affinity uptake of tryptophan in synaptosomes, which in short term study led to increased synthesis of 5-HT. Following 10 days of treatment the decreased tryptophan hydroxylase activity led to a decrease in 5-HT synthesis. Mandell and co-workers (28, 198) claim that the delayed alteration in tryptophan hydroxylase activity corresponds in time to the axoplasmic flow rate of the enzyme and interpret their results by suggesting that this mechanism is a compensation to the effects which occurred at earlier time periods. This study is particularly important in that it is one of the few which attempt to account for the

different effects reported with acute and chronic lithium administration.

Studies on the release of radioactive 5-HT from slices of rat brain following electrical stimulation showed that lithium, either when given intraperitoneally for 3 days, or when added directly to the incubation medium caused a decrease in the electrically-stimulated efflux of 5-HT (186). Sheard and Aghajanian (23) showed, however that the increase in the levels of hydroxyindoles following stimulation of the midbrain raphe was enhanced by short term treatment of rats with lithium (2.5-7.5 mEq/Kg for 2-4 days).

d. Clinical studies

A number of clinical studies demonstrated increased levels of 5-hydroxyindoleacetic acid in the cerebrospinal fluid (CSF) of patients undergoing lithium treatment (191, 192). However, no changes or even decreases in the levels of 5-HIAA in the CSF were reported by others (193, 203, 204). In a study of the levels of total and free tryptophan in the plasma of depressed patients, no difference was found in plasma free tryptophan levels between patients on lithium and recovered patients not treated with lithium (199). The uptake of 5-HT into platelets from patients with depression or manic-depressive psychosis was decreased in patients treated with lithium (202), although no effect was seen when lithium (1, 2 and 5 mEq/L) was added to the incubation medium (205) containing platelets from control patients. Monoamine oxidase activity was shown to be low in depressed bipolar patients (174) and markedly elevated following lithium carbonate treatment (175).

The results of many studies on 5-HT metabolism in experimental animals are seemingly inconsistent, and the duration of lithium

administration appears to be one of the factors which contribute for the discrepancies. However, a body of evidence suggests increases in 5-HT synthesis and turnover following short term lithium (24, 27, 198, 206). In addition, increased levels of 5-HIAA in rat brains formed either from endogenous or intracisternally administered radioactive 5-HT has been reported (18, 23, 24, 26, 176, 184). However treatment with lithium for longer periods of time appear to have little effect on 5-HT synthesis and turnover rates (18, 19, 27, 179, 198). It remains to be determined whether the increased hydroxyindole levels in animal brain following lithium treatment reflect increased turnover of 5-HT, altered disposition and metabolism or altered rates of efflux from specific brain loci.

iii. Effect of lithium on the cholinergic neurons

A number of effects of lithium on acetylcholine (ACh) metabolism were reported with substantial emphasis given to the synaptic transmission mechanism. It was reported (207) that toxic doses of lithium (137 mM) depressed ACh release from the cortex by a presumed postsynaptic mechanism (208). However studies using the abdominal ganglion neurons of Aplysia provided evidence to suggest that toxic levels of lithium (10-20mM) reduced ACh release by a presynaptic mechanism (209). In contrast, pharmacological studies using cortical slices derived from lithium-treated and control rats did not produce significant changes in the ACh release (210). Haas and Ryall (211) using microiontophoretic procedures showed increased firing rates of cholinoceptive neurons in the spinal cord (Renshaw cells) and the brain (cerebral cortex, thalamus, caudate nucleus, hypothalamus and brain stem) of cats and rats. Recently, Krell and Goldberg (212) reported that acute

administration of LiCl to mice (5 mEq/Kg) produced a modest but significant decrease in whole brain ACh without changes in the levels of its precursor choline. However, chronic administration of LiCl at doses of 1 or 2 mEq/Kg/day for 6 days produced no significant changes in the levels of steady state levels of brain choline or ACh.

The administration of lithium to manic depressive patients was shown by Lee et al. (213) to reduce choline transport into the erythrocyte to 10% of control values, effects which were shown to be extensive and irreversible (214). However while choline transport in human erythrocytes is inhibited by incubation with lithium-containing medium in vitro, this effect could not be demonstrated in erythrocytes of rats and rabbits following prolonged lithium administration.

While lithium inhibits a number of events related with synaptic transmission, the evidence is not conclusive especially in the light of the fact that these changes were observed at supra-therapeutic doses which produced a corresponding change in the levels of other cations.

iv. Effect of lithium on γ-aminobutyric acid (GABA)

Several reports have been published on the effects of lithium on GABA and glutamic acid. Decreased levels of glutamic acid were reported in whole brains of mice following a single high dose of LiCl (215). In vitro studies showed that in the presence of lithium, the uptake of GABA by neural tissue was either unaltered (216) or inhibited (217), and the stimulus-induced release of GABA as well as glutamic acid from brain slices was greatly reduced (218). In rats both acute and short term lithium salt treatment resulted in increased levels of glutamate in the amygdala and hypothalamus, and GABA in the hypothalamus (219) but not in other brain regions (220). The

increase in hypothalamic GABA levels in lithium-treated rats did not appear to be due to changes in either GAD (glutamic acid decarboxylase) the synthesizing enzyme, or the metabolizing enzyme, GABA-T (GABA-aminotransferase) (221, 222). However decreases in the levels of glutamate, aspartate and glutamine in snail central ganglia were observed on incubation in vitro with lithium-containing media.

G. Lithium and Animal Behavior

In spite of many investigations in the last few years, the mechanism(s) of action of lithium on behavior remains unknown. A wide variety of experimental approaches yielded information with demonstrable effects of lithium on nonassociative behavior, and aversively motivated behavior induced by drugs in both rats and mice (Table 1). Lithium was reported to typically depress spontaneous motor activity in mice and rats (168, 223-225, 252). Some of these studies employed a variety of methods which include both rearing and locomotive movements in a novel environment (226), spontaneous activity in activity wheel cages, a jiggle cage and open field, swimming to exhaustion tests (227), or Aminex activity meter over 24 hour period (228).

Since pharmacological manipulations of the monoamine-containing neurons are known to produce profound alterations in animal behaviour, it appears that NE, DA and 5-HT neurons play a role in modulating behavior (229). In order to elaborate upon the possible neurochemical mechanisms which subserve the components of lithium-induced behavior, several investigators explored the effect of lithium following pharmacological manipulations with agents which modify brain monoamine levels. Lithium has been reported to alter drug-induced hyperactivity (225, 230-233) in a number of investigations. For example, Cox et al.

TABLE 1

SUMMARY OF SELECTED STUDIES ON THE EFFECT OF LITHIUM ON ANIMAL BEHAVIOR

	NONASSOCIATIVE					MOTIVATED
Duration of Treatment (dosage)	Basal		Drug Induced			
	-	o	-	o	+	-
Acute (1-3 mEq/Kg, i.p.)	222**		230		180	237
	223		232		240	238
	168		250		242	244
	226					
Short Term (5-8 days) (40-120 mEq/Kg food)	227*	226	231**	178		235**
	228*	235	243*			236
		227	247*			239
						245
Long Term (14-28 days) (30-70 mEq/Kg food)	252*		225	225	241*	251**
			234	232		

* Lithium given in diet

** dose >3 mEq/Kg

behavioral changes rated as (-) reduced; (o) unchanged; (+) increased. Numbers indicate reference numbers.

(232) reports that amphetamine plus chlordiazepoxide-induced hyperactivity was reduced by acute but not chronic lithium treatment to rats. In mice, behavioral activation by morphine was reduced by lithium (4 days on 5 mEq/Kg, i.p.) (231), as was the cocaine induce activity (234). In several studies lithium was shown to depress foot-shock induced aggression (235-238), or following long term isolation (251) and reduce the rate of self-stimulation (239, 244).

In contrast to its activity suppressing effect, lithium was shown to also potentiate the hyperactivity produced in rats by drugs believed to increase the availability of catecholamines in the brain. These drugs include tranylcypromine (240, 241) pargyline (17, 180), amphetamine (228, 243), clonidine and nialimide plus L-Dopa (242).

Several investigators have suggested a possible relationship between 5-HT and the physiological and behavioral effects of lithium. An effect of lithium on 5-HT metabolism has been suggested from an observation of a decrease in foot-shock jump threshold in rats given lithium (1 or 2 mEq/Kg) daily for 14 days (245). Treatment with 5-hydroxytryptophan (5-HTP), to raise 5-HT levels, restored the normal threshold but did not affect control animals not receiving lithium. Tenen (246) found a similar decrease in jump threshold reversible with 5-HTP after depletion of brain 5-HT with p-chlorophenylalanine. Shorter periods of lithium treatment (7 days at 1 or 3 mEq/Kg/day) produced no effect in jump threshold even when the lithium dose was increased to 5 mEq/Kg/day, for 5 days (235). However, several reports (223, 227, 247) suggest that both the interval between lithium treatment, testing and social setting may affect the behavioral changes due to lithium. Smith (247) recently reported that pargyline,

which elevates brain 5-HT by inhibiting MAO, counteracted the effects of short term lithium administration on exploratory and emotional behavior in rats. Curiously, p-chlorophenylalanine (p-CPA) which blocks brain 5-HT synthesis also prevented the behavioral effects produced by 1.5 mEq/Kg dose lithium given twice daily for 5 days (247). However, it has been suggested that the anomalous effects of p-CPA may be attributed to an active metabolite whose actions may not involve 5-HT depletion (248, 249).

When a comparison of the effects of lithium on animal behavior is made, short term or chronic studies report either persistent reductions or exaggerated suppression (180, 228, 237) while others report a "presumed development of tolerance" to the early lithium-induced behavioral suppression (178, 232, 233).

Although there is some evidence to implicate lithium induced-behavioral changes with the catecholamine and 5-HT containing neurons, it is not known at the present time whether the therapeutic effect of lithium is mediated by a mechanism which involves central biogenic amines. It is possible that lithium may exert its behavioral effects by acting at specific sites known to subserve emotional behavior. Indeed, certain of the limbic regions, notably the amygdala, the septum, cingulate gyrus and the hippocampal formation appear to function as certain modulators of emotion (169). Moreover Delgato and DeFeudis (250) produced high voltage 1/second sharp waves with a localized electrical after-discharge and a decrease in spontaneous restlessness after an injection of 10 µl of isotonic lithium chloride into the amygdala-hippocampus region of unanaesthesized rhesus monkeys. Based on these results the authors suggested that the

therapeutic effects of lithium may indeed be related to its specific action in the limbic system.

III. DEVELOPMENT OF CATECHOLAMINE AND 5-HYDROXYTRYPTAMINE-CONTAINING NEURONS IN THE CENTRAL NERVOUS SYSTEM

A. Morphological Development

The morphological, biochemical and functional features which characterize the development of the brain of many mammals are extremely complex, but represent a synchronized series of events associated with cellular growth and differentiation. At birth, the brain of most mammalian species is underdeveloped and undergoes maturational process in the early postnatal period. Neuronal maturation has been described to include cell division and cellular migration of neurons and glial cells, along with the extension of axons from the cell bodies and the consequent growth and arborization of dendritic processes, and the formation of functional synapses. Biochemically functional aspect of the developing brain include the formation of synaptic vesicles and their transmitter substance, neurosecretory granule, and increases in the specific activity of enzymes, and the active deposition of myelin.

In the rat, maturity is achieved in the first 5-6 weeks of life, during which the brain size increases six times, the neurons increase in size with marked proliferation of axonal and dendritic processes, and the deposition of myelin. The rate of change of DNA content in the brain suggests that cellular multiplication is maximal at about the 10th postnatal day. Most of the neurons are formed at birth in the forebrain but cell division is more active postnatally in the cerebellum where some 97% of the final number of cells is acquired

during the first three weeks (253). Histological studies reveal increases of dendritic arborization and synaptic contanct formation when the myelination process begins. The increase in synaptosomal weight from 5-15 days correlates with the increased number of nerve terminals. Synaptogenesis in many brain regions extend for longer than the first 20 postnatal days since only 80% of adult synapses are found in the striata, 70% in the cerebellum but only 40% in hippocampus (254). Electrical activity in the brain of rat was reported to occur 5-6 days after birth with the adult pattern achieved by the third postnatal week (255). The high rate of increase in brain weight in the first 14 days, represent a developmental stage called the "critical period", which coincides with morphological observations of marked growth of axons and dendrites from neurons and an active myelin formation (256).

The first appearance of NE neurons was shown to occur on the 14 day of gestation in the rodent (257). Using [^{3}H]-thymidine autoradiography and histochemical studies it was revealed that NE neurons in the locus coeruleus, and DA neurons in the substantia nigra exhibit a period of cell division between 12-14 days of gestation, while the differentiation of cerebellar Purkinje cell commenced on days 14-15 (258a). The cells are capable of synthesizing the transmitter substances prenatally, and begins to proliferate soon after (257, 258).

Loizou (259) documented the postnatal proliferation of the catecholamine-containing neurons in the rat brain, and suggested that at birth the monoamine fluorescence was restricted to cell bodies in the pons-medulla, and the DA cells of the substantia nigra. With time, the fluorescence intensity was shown to progress to regions containing

terminal populations, to achieve an adult pattern by the 4-5 postnatal week. A number of studies provided evidence to suggest that NE containing neurons matured earlier than did the DA neurons (259-261).

The pharmacological manipulation of the fluorescence of monoamine neurons during development provided evidence on the functional maturity of the neurons. Agents known to deplete storage granules or inhibit the tyrosine hydroxylase enzyme activity (e.g. reserpine and α -methyl-para-tyrosine) caused a depletion of fluorescence which appeared to be related to the neuronal activity (262). Therefore it was suggested that functional maturity has been attained by these neurons prior to morphological maturations.

Evidence was provided that 5-HT containing neurons develop early in prenatal life and achieve the adult pattern by the 3rd postnatal week. Histofluorescence techniques demonstrated 5-HT terminals in brain stem regions and the spinal cord at the end of the first postnatal week, the distribution and density achieving adult pattern several weeks later (259). The maturation studies of Loizou (259) and the prenatal studies of Olsen and Seiger (257) and seiger and Olson (258) indicated an active 5-HT metabolism in 5-HT containing neurons during development and maturation phase of rat brain morphogenesis. They suggested that a mature biochemical system is present early, and neuronal outgrowth and terminalization is the controlling factor in 5-HT metabolism.

B. Biochemical Development

i. Catecholamines

Although the concentrations of brain amines are low in the

neonatal period, they progressively increase to adult levels (261, 263, 264), and the rate of increase in the monoamines differs among parts of the developing rat brain (265, 266). NE content, low at birth, is lower in forebrain regions than brain stem regions, and with time progressively increases to reach adult values by 5th week after birth with the highest rate of increase occurring during the first 14 days after birth (267).. Kellog and Lundborg (271, 271a) demonstrated that NE neurons matured prior to DA neurons. The maturation of NE and DA neurons in rat brain has been examined in detail by Coyle and Axelrod who measured the development of the major enzymes (TH and DβH) involved with the catecholamine synthesis and the neuronal uptake of [^{3}H]-NE (268-270). They observed that these 3 indices of NE neuronal development increased in co-ordinated fashion from the 15th day of gestation to adult life; regions which contained NE and DA cell bodies (pons-medulla and the midbrain) acquired TH activity at earlier stages of development than those regions in which nerve terminals of these system predominate (e.g. striatum, cortex, cerebellum). It was also observed that over 90% of DβH and TH and uptake and storage capabilities of the rat brain are developed after birth. In addition, based on their uptake studies, Coyle and Axelrod (268) suggested a lag between the development of uptake mechanism and storage granule mechanism.

ii. 5-Hydroxytryptamine

Although the levels of brain 5-HT are low in the neonatal period, they rapidly increase during the first 3-4 weeks of life (263, 267). Similarly the low monoamine oxidase activity (up to 30% of adult values) in the neonate progressively increases to adult levels (272). While 5-HTP decarboxylase activity is relatively high in the neonate (80%

of adult values) (273), tryptophan hydroxylase activity was shown to be low at birth but dramatically increases between 7-30 days after birth (274, 275). The concentration of tryptophan in the immature brain is much higher in the young neonate than in the adult rat (276), and is mostly in the free form in the plasma of newborn animals (277). The rate of [^{3}H]-5-HT uptake into synaptosomes derived from immature cortical regions was shown to be 50% of that which occurs in the brain stem, and only 10-30% of the synaptosomes appear to be mature (278). In contrast, Nomura et al. (267) found that the affinity of [^{3}H]-5-HT to synaptosomes did not increase with age but the density of synaptosomes increased in each brain region with increasing age.

Maturation of the functional responses of brain 5-HT turnover to isolation, immobilization and treatment with agents known to block 5-HT re-uptake (e.g. imipramine and desmethylimipramine) was shown to occur simultaneously and correlated with the morphological maturation of the terminals of 5-HT neurons and of the synaptic junctions of the brain (259). Mechanisms which regulate changes in amine turnover in 5-HT neurons are absent from rat brain until later stages of neonatal development. Their appearance lags behind that of the normal subcellular distribution and content of 5-HT and related enzymes, as well as that of storage and transport of 5-HT into synaptosomes (278, 279).

IV. EFFECT OF NEUROTOXINS ON THE DEVELOPMENT OF BRAIN BIOGENIC AMINES

The popularity in the use of neurotoxic agents as pharmacological tools stems from the discovery that 6-hydroxydopamine (6-OHDA) was capable of selectively destroying the catecholamine-containing neurons. The search for analogous agents with neurotoxic specificity towards the 5-hydroxytryptamine-containing neurons led to the discovery of three agents which can fulfill such a role to varying degrees. These include the 5-HT analogues, 5,6-dihydroxytryptamine (5,6-DHT) and the less toxic 5,7-dihydroxytryptamine (5,7-DHT), and the halogenated derivative of amphetamine, 4-chloroamphetamine (4-CA) (30-32).

Although histochemical and histological techniques have been used to study the cytotoxic effects of these agents, a number of biochemical measures specifically associated with catecholamines and 5-HT containing neurons offer a simpler and more rapid approach for estimating the relative degeneration that is produced on selective brain regions.

A. 6-Hydroxydopamine

Once taken up by the nerve terminals, 6-OHDA is believed to destroy the catecholamine-containing neurons by a mechanism which most likely involves the action of hydrogen peroxide (280). In general, nerve terminals are found to be more vulnerable to the actions of 6-OHDA than the axons and cell bodies. Following peripheral administration of 6-OHDA to mature animals, the catecholamine-containing neurons in the CNS appear to be little affected. However, following intracranial (intraventricular, intracisternal and intraregional) route of administration, 6-OHDA was shown to produce profound destruction of

central catecholamine neurons with varying degrees of non-specific cell damage as assessed histochemically or biochemically (29, 281).

A number of investigators examined the actions of 6-hydroxydopa or 6-OHDA oh the development of catecholamine-containing neurons (33, 35, 36, 282, 283). It has been shown that peripheral administration of these agents during the first days after birth produces a long-lasting degenerative effect in forebrain regions characterized by massive reductions in the levels of NE, uptake and tyrosine hydroxylase activity (35, 36, 284-286). These effects have been explained in terms of the immature blood brain barrier which allows the 6-OHDA to selectively destroy the dorsal noradrenergic system (284). In addition to the marked depletion of NE in most forebrain regions, consistent increases in the NE levels and tyrosine hydroxylase activity in the midbrain, pontine and/or cerebellar regions were reported (35, 36, 284, 287). These effects have been explained by suggesting that NE terminals arising from the locus coeruleus degenerate followed by a regenerative "sprouting" in the region of the cell bodies, or collateral sprouting from adjacent neurons (282).

Several reports suggest that following intraventricular or intracisternal injections of 6-OHDA different effects are produced. Indeed, the reduction in catecholamine levels and tyrosine hydroxylase activity occurs in the forebrain regions (37, 286, 288, 289), while the NE level is reported either unchanged or reduced in the brain stem (288). Recent reports by Peterson and Laverty (290) and Peters et al. (37) suggest that changes in NE levels in certain regions are not always accompanied by similar changes in the enzyme activity; rather NE levels may be better related to the physical

state of the tyrosine hydroxylase enzyme (291).

The pattern of neuronal destruction was shown to be dependent on a number of variables such as the age at which the pups were injected, the dose and the route of administration (36, 37, 292). In general, the critical period for 6-OHDA to produce long lasting effects appears to be between days 1-8 after birth (36, 292).

A number of theories have been advanced to explain the regional variations in the effects of neonatal 6-OHDA administration. The destruction of forebrain nerve terminals may result in "anomalous sprouting" in the regions containing the cell bodies (282; 283) with resulting increases in NE levels and TH activity presumably attributable to increased number of synaptic sites. Alternatively, both the enzymes and storage granules may accumulate in the cell bodies of destroyed terminals. While convincing evidence is available to support the first suggestion following peripheral injections, it does not adequately explain the lack of effect on TH activity in the brainstem regions following intraventricular injections (37). It is possible that in addition to sprouting, damage to certain catecholamine-containing neurons may result in reduced feedback inhibition at the level of the cell bodies, leading to enhanced firing rate and compensatory increase in the synthesis of NE in regions containing the cell bodies (37, 294).

The effect of neonatal 6-OHDA on 5-HT-containing neurons was shown to be minimal with reports of no changes in the levels of 5-HT (284, 293), or the ^{3}H-5-HT uptake into cerebral cortex (282), although the 5-HIAA content and tryptophan hydroxylase activity are significantly increased in both the hypothalamus and the brainstem (35, 293).

B. Dihydroxytryptamines

Since the discovery of the selective toxicity of 6-OHDA towards catecholamine-containing neurons in the periphery and CNS (34), efforts were made to identify compounds that would produce similar selective effects on 5-hydroxytryptamine-containing neurons. A variety of morphological, biochemical and functional evidence indicates that the uptake of 5,6- or 5,7-dihydroxytryptamine into 5-HT containing neurons of the CNS elicits cytotoxic effects leading to degeneration of both axons and neuronal terminals (30, 31, 295-297). Such cytotoxic effects are not limited to the 5-HT-containing structures, but also occur to a lesser extent in catecholamine-containing neurons (39, 297, 298). Selectivity of action of these agents appears to result from higher affinity for uptake processes of 5-HT-containing terminals than catecholamine terminals. However, while 5,6-DHT, the first drug to be extensively studied, was shown to be relatively specific for 5-HT, 5,7-DHT produces some depletions of NE which can be prevented by the pretreatment with desipramine before the 5,7-DHT injection (39, 299). The usefulness of 5,6-DHT as a tool was shown to be limited since it produced extensive non-specific tissue damage when given in doses greater than 75 µg (296, 298). In contrast, 5,7-DHT has gained in popularity as a better tool of investigation.

It has been shown that intracranial administrations of dihydroxytryptamines to mature animals produces long lasting degerative effects in forebrain regions characterized by marked decreases in the levels of the 5-hydroxyindoles, the tryptophan hydroxylase activity and ^{3}H-5-HT uptake (31, 39, 300, 301). In general, these reductions

are greater in terminal regions, and least in the cell body rich areas of the brain. Decreases in the 5-hydroxyindoles in the brain and spinal cord are not irreversible, since regeneration of terminals can occur (302, 303). Indeed, it was reported that the newly formed terminals, although regionally diverse, can normalize the function of 5-HT synapses (303, 304). The most active and extensive regeneration after 5,7-DHT appears to occur in the lower brainstem regions assessed biochemically as a recovery in the levels of 5-hydroxyindoles and tryptophan hydroxylase activity (305), and the ^{3}H-5-HT uptake capacity in these brain regions (304). These effects have been explained by suggesting that 5-HT terminals arising from 5-HT containing cell bodies degenerate to cause intensive sprouting in the region of the cell bodies, (31). It has also been suggested that the increase in the levels of 5-hydroxyindoles possibly reflects increased synthesis in the surviving terminals, or decreased rate of metabolism of 5-HT (38). Like 6-OHDA, it is conceivable that the increases in certain brain regions following 5,7-DHT may reflect a reduced feedback at the level of the cell bodies, leading to enhanced firing rate and compensatory increase in 5-HT synthesis in regions containing the cell bodies (111).

Few studies were reported on the effect of dihydroxytryptamines on the development of 5-HT-containing neurons. When 5,7-DHT was given intracisternally early in development in low doses, it was shown to produce a long lasting and profound decreases in the levels of 5-HT and 5-HIAA which persisted for at least 240 days (38, 39). There was a small decrease in the level of NE while the DA levels remained unaltered (38, 39). The reductions in 5-hydroxyindoles

levels were shown to be dose-related, and the extent of the decrease varied between regions. Lytle et al. (38) reported that the cortex and spinal cord were more sensitive, while the hypothalamus and the midbrain were less sensitive to the destructive effects of the neurotoxin.

Systemic administration of 5,7-DHT to newborn rats at a dose of 100 mg/Kg subcutaneously was reported to either reduce the ^{3}H-5-HT uptake into cerebral cortex by up to 50%, or increase it by the same magnitude in the pons-medulla when determined at 7 days of age (29, 300). In addition, neonatal 5,7-DHT produced only slight decreases in the ^{3}H-NE uptake into the cerebral cortex and the corpus striatum.

C. 4-Chloroamphetamine

4-Chloroamphetamine (4-CA), the halogenated derivative of amphetamine, was shown to cause marked and long lasting depletions of brain 5-HT. Parameters specifically associated with 5-HT neurons were shown to be reduced: tryptophan hydroxylase activity, 5-HT and 5-HIAA content, 5-HT turnover rate, and a decreased 5-HT uptake into synaptosomes (49, 50, 306, 307). The persistence of the impairment of 5-HT function for several months has led investigators to speculate that 4-CA may be a toxic agent (49, 308).

Recently Harvey et al. (32) provided morphological evidence for the degenerative changes in the brainstem of rats pretreated with 4-CA. This neurotoxic effect was suggested to be localized in an area of the ventral midbrain tegmentum corresponding to cell groups B9 of Dahlstrom and Fuxe (59). Biochemical evidence provided support to the suggestion that 4-CA is not toxic to the major 5-HT cell bodies projecting to the forebrain (i.e. groups B7 and B8) (309). However,

after comparing the effects of B9 lesions with the effects produced by 4-CA, it was concluded that the prolonged biochemical effects of 4-CA in the forebrain cannot be explained by a selective neurotoxic action on this cell group (310,311). Neckers et al. (312) reported differences in the effect of 4-CA on various terminal regions both in the extent and the duration of the depletion of 5-HT and the tryptophan hydroxylase activity. They interpreted their results to suggest that the nerve terminals are the primary site of the cytotoxic actions of 4-CA. This interpretation is consistent with the ultrastructural study of McGeer et al. (313) who showed that 4-CA produced degeneration of axon terminals in the striatum. More recently, based on histological and biochemical data Massari et al. (314) suggested that the cytotoxic action of 4-CA resides in the 5-HT axon terminals, sparing the 5-HT axons. In fact they observed increases in axonal 5-HT levels which was similar to the axonal swelling and accumulation of 5-HT fluorescence reported to occur following large doses of a similar drug, p-chloromethamphetamine (315).

After the administration of 4-CA, there are striking regional differences in the degree to which 5-HT is reduced. While the hippocampal and cortical regions were depleted of 5-HT by as much as 90% hypothalamic and spinal cord 5-HT levels were reduced by about 35% after 5 or 10 mg/kg of 4-CA (307, 312). The regional pattern in the reductions differ from that produced by the dihydroxytryptamines which are believed to selectively destroy nerve terminals and spare the perikarya (30). It was suggested by Massari et al. (314) that the regional difference may be accounted for by the relative proportion of 5-HT present in axons and axon terminals.

When 4-CA was given at a dose level of 50 μmol/Kg to one-day-old rats and measured 5-HT content at 56 days of age, it did not produce significant change in the levels of 5-HT in the pontine raphe nuclei, and area B9, as well as in the forebrain (312). Similar results were obtained after a single dose of 250 μmol/Kg when given to 2 day old rats (312). However, insufficient data on the effect of 4-CA on the development of the 5-HT neurons is available to interpret these observations.

V. EFFECT OF PSYCHOACTIVE DRUGS ON POSTNATAL DEVELOPMENT OF BRAIN MONOAMINES

A. General Considerations

There are many reports that various psychoactive drugs administered to pregnant rats have significant effects on the postnatal behavior of the offspring (40). Moreover, recent survey of the literature indicates that perinatal drug exposure can result in persistent biochemical changes which may be associated with an altered adult behavior (41, 42, 45). Exposure of experimental animals during development to drugs which are known to reduce storage and synthesis of brain biogenic amines (e.g. reserpine, α methyl-p-tyrosine and p-chlorophenylalanine) also produce behavioral and biochemical abnormalities in later life (316-318). Research in the field progressed with the main strategy of pharmacological manipulations of the biogenic amines in the developing brain. It became the goal of some to relate more closely the maturation of the neuronal system with the altered behavior, as well as to provide evidence for the postulate that the biogenic amines play an important role in growth and development apart from their neurotransmitter

role in the adult animals.

A number of psychoactive drugs alter mood and behavior by acting at central receptor sites of the monoamines, or by interacting with storage, release or uptake of these amines. Therefore several studies attempted to provide evidence for the effects of neonatal administration of these drugs on brain biogenic amines, and identify the "critical periods" at which vulnerability of the brain is at a peak.

B. Chlorpromazine

The effect of perinatal chlorpromazine (CPZ) administration on behavior in the adult has been studied by several investigators. Evidence of adverse effects of learning in rats treated prenatally or during infancy with CPZ has accumulated (319). Treatment with CPZ during early pregnancy resulted in slower maze acquisition in the offspring, whereas treatment during mid pregnancy resulted in a faster maze acquisition (319, 320). Rats whose mothers received CPZ on gestational days 12-15 took longer to acquire a bar press response (320). Golub and Hornetsky (46) found that the offspring of rats treated with CPZ in pregnancy showed higher avoidance scores in shock avoidance learning, higher activity and greater seizure susceptibility than the offsprings of controls. Moreover, significantly lowered adult maze performance was observed in both male and females given CPZ in early life (321).

CPZ injections to pregnant female rats and mice have been shown to reduce body weight gain in the offspring when given at a daily dose of 10 mg/Kg or more (41, 322), although lower dose levels (1-8 mg/Kg) have usually been reported to have no effect on body weight (46, 320, 323). Nair (41) reported that the suppression of

normal developmental increases in body weight was maximal in animals receiving CPZ during the last few days of pregnancy.

Several reports suggest that the persistent alterations in brain biogenic amines occurs following perinatal CPZ treatment. Tonge (324, 325) administered CPZ to pregnant rats in drinking water throughout the gestation and suckling period and studied the brain monoamines in the offspring at maturity. Both 5-HT levels and turnover rates were significantly elevated in most brain regions, with little or no change in the levels of 5-HIAA. In contrast, Nair (41) found reduced 5-HT and elevated 5-HIAA levels in whole brain at 40 and 80 days old rats following CPZ treatment (10 mg/Kg, s.c.), the effects being maximal when the drug was given either throughout pregnancy or during gestational days 18-21. The brain norepinephrine level at 40 and 80 days was increased in the group receiving the drug on gestational days 18-21. Tonge (326) reported increased turnover of NE in the cortex, hippocampus, hypothalamus and midbrain and reduced turnover in the amygdala following CPZ administered in the drinking water as assessed at the age of 90 days.

However, these studies did not distinguish between pre- and post natal effects nor did they separate direct effects on the fetus or neonate from indirect effects operating through the mother. Circumstantial evidence exists that CPZ can influence brain maturation of biogenic amines as well as the development of normal behavioral processes.

C. Haloperidol

Haloperidol, a neuroleptic and potent dopamine receptor blocking agent when administered to immature rats at doses of 0.25 and

.50 mg/Kg caused an increase in ambulation in the open field arena, and caused a lowered maze performance when tested at 90 days of age (321). The effect of neonatal treatment with a pharmacologically similar drug, penfluridol, was studied by Lundborg and co-workers (42, 47, 48). When given to nursing rat mothers, the pups at the age of 4 and 8 weeks showed inferior conditioned avoidance response acquisition (47). The drug treated offsprings were shown to have a change in the levels of the monoamines and/or reduced rate of tyrosine hydroxylation at 28 days of age. In addition, they reported that tryptophan hydroxylation was also decreased in the limbic system (olfactory tubercle, nucelus accumbens, septum, amygdala) and brainstem of these rats (48). Furthermore, the dopamine turnover in the mesolimbic region was shown to be reduced by prenatal penfluridol treatment (42). The authors interpreted their results to suggest that the persistent effects of penfluridol may be the result of treatment with dopamine-receptor antagonist during a vulnerable period for the functional maturation of the central dopamine system. A possible relationship between these behavioral and biochemical effects was supported by the observation that d-amphetamine specifically could counteract the learning deficit in the offspring of the nursing mothers treated with a similar dopamine receptore blocking agent, pimozide (327).

D. Reserpine

Reserpine, a well known CNS depressant which depletes monoamine stores, when given prenatally or neonatally, was found to cause decreases in birth weight and brain weight which progressively increased several days later (45). The adrenal tyrosine hydroxylase and dopamine-β-hydroxylase activities of these neonates were found to be

persistently elevated, while the brain tyrosine hydroxylase activity was significantly decreased at later life. The more pronounced decreases were observed in rats 30-45 days old, and the most vulnerable group being those rats whose mothers received reserpine on days 9-11, or 12-14 of gestation (328). Recent histological and biochemical studies (329) provided evidence to suggest that reserpine (2.5 mg/Kg dose) causes marked depression in cell proliferation in the brain of 11 day old rats. This study utilized the ^{3}H thymidine incorporation into DNA as a measure of cell proliferation.

Although reserpine did not affect the learning of several behavioral tasks, the effects of susceptibility to audiogenic seizure and activity are of significance (40).

E. p-Chlorophenylalanine

p-chlorophenylalanine (p-CPA), a 5-HT synthesis inhibitor, when given during the postnatal period was shown to retard brain growth in rats (165), probably attributable to loss in cell numbers (119) and delayed myelin formation (128). Recently, p-CPA was also shown to delay the onset of neuronal differentiation in brain regions reported to receive 5-HT innervation in the adult (44). The behavioral study of Hole (165) suggests that p-CPA treatment during the first postnatal week produced animals with reduced arousal levels, with no learning deficits or clear motivational changes. In addition, the brain 5-HT levels were substantially decreased in these rats.

F. D-Lysergic Acid Diethylamide (LSD)

LSD, which is a highly potent inhibitor of 5-HT neurons in the raphe (111), when given prenatally was shown to decrease the normal developmental body weight gain. The reduction was found to be

greatest in animals exposed to LSD during the final days of pregnancy (41). In addition they reported reduced brain 5-HT levels in the offspring when measured at 40 and 80 days of age. In contrast, newborn male mice injected with LSD (5-50 ng/gm) on days 1-7 showed a small but statistically significant increase in 5-HT levels and MAO activity in the mesencephalon-diencephalon (43) when measured at 8 weeks of age.

Thus ample evidence is available to suggest that psychoactive drugs when administered during the prenatal or the perinatal period can produce behavioral deficits and/or altered brain biogenic amine levels or turnover rates at later life. Based on morphological and biochemical data it appears that these agents influence the maturation of the central nervous system, and may also alter the development of central catecholamine and 5-HT containing neurons. However, in a number of these studies deficiencies in the experimental design makes the interpretations of the results difficult. In fact, it has been suggested that in addition to the direct drug effects on the fetus a number of pre- and postnatal maternal factors may influence the overall effect of the drug on the behavior and the biochemical changes of the offspring (40). Moreover, evidence is lacking to show the effect of these agents on the maturation of specific pathways associated with catecholamines and 5-hydroxytryptamine. The present study was therefore undertaken to investigate the effects of neonatal administration of several psychoactive drugs on the metabolism of catechoalmine and 5-HT in up to 14 discrete brain regions at different postnatal ages.

3. MATERIALS AND METHODS

I. ANIMALS

A. Mature Rats

In the course of this investigation two variant strains of rats were utilized, albino Sprague Dawley rats obtained from Bio Breeding Laboratories of Canada, Ottawa, Ontario, and the brown ACI (Micro) rats obtained from Microbiological Associates, Walkersville, Md. or from a maintained breeding colony at the University of Ottawa. Unless otherwise indicated, all animals were provided with Master Laboratory Chow and water ad libitum and were kept in the faculty of Medicine, University of Ottawa animal quarters (constant environment of 24°C, 60% humidity and alternate timed cycles of 12 hours of light and darkness).

Drugs to be administered were dissolved in physiological saline and were injected intraperitoneally in a volume of 0.2 ml/100 g body weight unless otherwise stated. In the lithium studies, injections were given between 08:00 and 09:00 hours and 12 hours later on the same day and at the same time on the succeeding 4 days. When a second drug (pargyline, probenecid, or L-tryptophan) was to be administered to these animals, groups of control and lithium-treated rats were given a single injection of the second drug at approximately 12 hours after the last lithium (10th) injection. The animals were sacrificed at various time periods later.

In experiments in which lithium was given in the diet, groups of rats were injected with the second drug on the 15th or 36th day following the start of the diet and were killed at various time periods thereafter. Similarly rats given a placebo diet were given a single intraperitoneal injection of either the vehicle or test drug in a

manner identical to the lithium-pretreated group of rats.

B. Neonatal Rats

In experiments on the effects of neonatal drug administration, male and female Sprague Dawley rats were mated at approximately 100 days of age. At birth, groups of 4-6 litters born within the same 12 hour period were mixed and redistributed between mothers so that all litters consisted of 4-5 male and 4-5 female pups, and the surplus was discarded.

Drugs to be administered were dissolved in physiological saline (lithium, chlorpromazine, 4-chloroamphetamine) or saline-ascorbic acid solution (1 mg/ml) (6-hydroxydopamine, 5,7-dihydroxytryptamine) and were injected subcutaneously with a 10 µl Hamilton micro-syringe at various dose levels in a volume of 1 µl/g body weight. Control pups received the same volume of the vehicle. Injections were given as soon after the redistribution of litters as possible (09:00-10:00 hours) and repeated either once only or at the same time of the day on the succeeding 5 days. Littermates received the identical treatment of either the vehicle or the drug. Rats were allowed to grow to the age of 20 days at which time groups of pups were sacrificed for biochemical estimations. The remaining groups of rats were weaned at 21 days and kept in groups of 3-6 until 40 or 60 days of age.

II. PREPARATION OF LITHIUM-CONTAINING DIET

The lithium-containing diet was prepared by the procedure described by Corrodi et al. (19) with the recommended modifications of Olesen et al. (127). The diet was prepared by mixing batches of an emulsion and dry mixture of various components. The powder mixture

had the following ingredients: fortified skim milk powder, 1250 g; caesin, 1850 g; alfalfa powder, 450 g; whole wheat flour, 8300 g; and calcium carbonate 190 g for a total of approximately 12 Kg. The emulsion contained the following ingredients: beef extract (Nutritional Biochemical Corporation, Cleveland, Ohio), 75 g; Cod Liver Oil, U.S.P., 15 ml; sodium chloride, 35 g; glass distilled water 100-200 ml. The dry fodder was thoroughly mixed and a portion (2 Kg) was mixed with the emulsion preparation. The wet diet was then allowed to dry in a low temperature oven for several hours, and the preparation was subsequently crushed to smooth powder with the use of a blender. This preparation was then added to the bulk of the dry fodder with continuous stirring for approximately 60 minutes. The freshly prepared diet was then divided into two portions, one to serve as placebo diet and the other as the lithium-containing diet. Either lithium carbonate or sodium carbonate was added to each batch to achieve a final concentration of 9, 30 or 80 mEq/Kg food. To the lithium containing diet an additional amount of sodium chloride was added (15 gm) and the preparation was thoroughly mixed for another 30 minutes. The diet was available to animals ad libitum for the duration of the experiments, and was dispensed in special glass bowls.

III. BIOCHEMICAL DETERMINATIONS

A. Tissue Preparation and Dissection Procedure

Animals were sacrificed by decapitation, the brain was removed from the skull, rinsed with ice-cold saline, blotted and placed on a special glass plate kept on ice. In order to study the regional distribution of monoamines in the developing and mature rats following

treatment with psychoactive drugs the brain was dissected into 10-15 brain regions according to the procedure of Saari and Pappas (personal communication), with slight modifications. The regions examined were the amygdala, cerebellum, cingulate gyrus, both the motor and temporal cortecies, hippocampus, hypothalamus, medulla, midbrain, olfactory tubercle, with bulb and tract, pons or pons-medulla, corpus striatum, septum, spinal cord, and the thalamus.

The dissection of the brain was performed as follows: after the removal of the cerebellum, pons-medulla, spinal cord and olfactory tubercle, bulb and tract by separation along their natural boundaries, the hypothalamus was removed immediately caudal to the optic chiasma and cephalad to the mammalary bodies, undercutting to the thalamus. The brain was then partially split with a median sagittal section extending to the level of the midbrain, which was removed by slicing cephalad to the superior colliculi. The cingulate gyrus was bilaterally removed from the genu, cutting posteriorly along a narrow shallow strip 1 mm cephalad and 0.5 mm lateral to the medial border of the corpus callosum. The septum was removed in its entirety using the anterior commissure as antro-caudal border, lateral ventricle as lateral boundary and cutting an arc cephalo-posterior to the inner surface of the corpus callosum and along the columns of the fornix. The amygdala together with portions of the pyriform cortex was bilaterally excised making frontal sections posterior to the hypothalamic cavity and dissecting anteriorly a total of 3 mm. Finally, a bilateral dissection of the hippocampus was performed by peeling away from the cortex. The motor cortex was dissected out, and the corpus striatum was gently teased towards the midline being careful to exclude any cortical white

matter underlying the striatum. The corpus striatum was bilaterally excised and the remaining portions of the temporal cortex removed. The thalamus was dissected out bilaterally by taking the medial-lateral portions of the thalamus, teasing away any adjoining white matter, such that a square cut was taken. The dissected brain regions were weighed immediately, placed in plastic capsules, were quickly frozen by immersion in liquid nitrogen and kept frozen at -70°C until neurochemical analysis were performed.

B. Preparation of Tissue for Lithium Determination

ACI(Micro) and Sprague Dawley rats were killed by decapitation and the blood was collected from the severed neck blood vessels into heparin-containing test tubes kept on ice. The blood was spun at approximately 900g for 5 minutes and the plasma aspirated out and was kept frozen for a few days at -25°C for lithium analysis. The brain regions were dissected out following removal of the brain from the skull, were weighed and kept frozen until analysis.

i. Determination of blood and brain lithium

The concentration of lithium in plasma and brain tissue was determined by the modification of the method of Schou (110) and Admisen (108) using a Beckman Model B flame photometer at an emission wavelength of 683 nm , with slit width setting of 0.15 mm and phototube voltage set at position C. Brain tissue or blood was homogenized in 10-25 volumes of ice-cold solution of trichloroacetic acid-isopropanol mixture (5% trichloroacetic acid: 10% isopropanol) using a Potter Elvehjem glass homogenizer tightly fitted with a teflon pestle, or ground in a small ceramic crucible. The precipitated proteins were removed by centrifugation for approximately 10 minutes

at 900g and the clear supernatant carefully transferred to small glass tubes for lithium determination. Tissue blanks were the plasma and brain regional values obtained from animals which have not been treated with lithium; all values have been corrected for these blanks and the results are expressed as net lithium levels in microequivalents (μEq)/g wet weight of tissue or mEq/L of plasma.

C. Determination of Biogenic Amines and Metabolites

The extraction procedure for brain 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, norepinephrine, and dopamine were similar to those described by Curzon and Green (331) and Maickel et al. (332). Brain samples were homogenized in 2.0-3.5 ml of chilled acidified n-butanol using a Polytron PT-10 homogenizer (Brinkman Instruments Inc., New York, N.Y.) at low speed for less than 30 seconds. The homogenate was centrifuged for 10 minutes at speed setting 6 using a clinical centrifuge International Equipment Co., Boston, Mass.). The supernatant was transferred to a 13-ml glass test tube (with cap) containing 7 ml of n-heptane and 0.7 ml 0.1N HCl. After a 10 minute shaking on a Fisher Roto-Rack mechanical shaker, the tube was centrifuged and the aqueous layer removed to determine the content of NE, DA and 5-HT. The remaining organic layer was transferred to another set of tubes containing 0.7 ml 0.5M phosphate buffer pH 7.0 to extract 5-HIAA. Duplicate standards of the amines also underwent this procedure.

i. 5-Hydroxytryptamine

Brain 5-HT was determined by the method of Curzon and Green (331) 0.20 ml aliquots of the acidic extract was pipetted into a tube which contained 1.2 ml of 0.01% o-phthalaldehyde in 10 N HCl and 0.020 ml 1% cysteine. After mixing, the tube was heated for 15 minutes in a

60°C water bath, was allowed to cool to room temperature and the formed fluorescence measured in an Aminco-Bowman spectrophotofluorometer (American Instrument Co., Inc., Silver Spring, Md.) at excitation and emission wavelengths of 360 and 470 nm respectively. Blanks used were 0.1 N HCl, and a standard curve was constructed using serotonin creatinine sulphate solution from 0.1 to 1.0 µg/ml. The amount of 5-HT in the unknown sample was estimated from the freshly prepared standards and the results are expressed as ng/g wet weight of tissue.

ii. 5-Hydroxyindoleacetic acid

Regional brain levels of 5-HIAA was determined by the method of Curzon and Green (331). A 400 µl aliquot of the phosphate buffer extract was transferred to a tube containing 50 µl 1% cysteine, and the following reagents were added in sequence with mixing: 1.0 ml 12N HCl, 50 µl 0.1% o-phthalaldehyde in methanol and 50 µl 0.02% sodium periodate. The sample was heated at 60°C for 15 minutes in a water bath, allowed to cool to room temperature and the fluorescence read within one hour in an Aminco-Bowman spectrophotofluorometer at excitation and emission wavelengths of 360 and 470 nm respectively. Blank used for 5-HIAA determination was 0.5 M pH 7.0 phosphate buffer, and the amount present in unknown samples was estimated from a standard curve and results are expressed as ng/g wet weight of tissue.

iii. Norepinephrine

Regional brain levels of NE were determined by the method of Laverty and Taylor (333). A 200 µl aliquot of the acidic extract was pipetted to a test tube containing 0.6 ml 1M pH 7.0 phosphate buffer, and the following reagents were added in sequence with the indicated timing: (a) 100 µl iodine solution, wait exactly 4

minutes, (b) 500 µl alkaline sulfite solution, wait 5 minutes, and (c) 200 µl of glacial acetic acid. The sample was left at room temperature for an additional 20 minutes and the fluorescence measured at excitation and emission wavelengths of 380 and 480 nm respectively on an Aminco-Bowman spectrophotofluorometer. Aliquots of standards and unextracted blanks were treated identically, and the results are expressed as ng/g wet weight of tissue.

iv. Dopamine

The level of DA in brain was estimated by the method of Laverty and Taylor (333) with slight modifications. A 200 µl aliquot of the acidic extract was pipetted into a glass test tube containing 1.0 ml 1M pH 8.0 phosphate buffer. The following reagents were added in sequence with the indicated timing a) 100 µl of iodine solution, wait exactly 4 minutes, b) 250 µl alkaline sulfite solution, wait 5 minutes, and c) 100 µl glacial acetic acid. After the samples were boiled in a 60°C water bath for 40 minutes, the tubes were cooled to room temperature and the fluorescence measured at excitation and emission wavelengths of 330 and 390 nm respectively using an Aminco-Bowman spectrophotofluorometer. Standards and unextracted blanks were treated as samples, and the results are expressed as ng/g wet weight of tissue.

D. Determination of Amino Acids

i. Tissue preparation

(a) blood

Animals were killed by decapitation and blood was collected into heparin-containing tubes kept on ice. The samples were centrifuged for 5 minutes at 900g to separate the plasma for determination of amino

acids. Plasma ultrafiltrate for the determination of plasma free tryptophan was obtained by the method of Tagliamonte et al. (84) which involved centrifuging at least 1.0 ml of plasma in CF-50 Diaflo membrane cones (Amicon, Corp., Lexington, Mass.) at 900g for 35 minutes at room temperature. The clear ultrafiltrate collected in the cone was transferred immediately to small glass tube and kept frozen until assay.

(b) brain

Brain tissue was homogenized in 1-2 ml of ice cold 0.4M trichloroacetic acid (TCA) using a polytron PT-10 homogenizer (Brinkman Instruments, New York, N.Y.) at low speed for 30 seconds. The homogenate was centrifuged for 10 minutes at position 6 using a clinical centrifuge (International Equipment, Co., Boston, Mass.), and the clear supernatant fluid employed for determination of endogenous tryptophan and tyrosine levels.

Fresh brain tissue was used to prepare a crude synaptosomal fraction (P_2) by the method of Gray and Whittaker (334). Tissues were homogenized in 1.0-2.0 ml ice-cold 0.28M sucrose using a Polytron PT-10 homogenizer (Brinkman Instruments, New York, N.Y.). The homogenates were centrifuged at 900g for 15 minutes to sediment the nuclear fraction and then centrifuged at 20,000g for 40 minutes at $4^{\circ}C$ using a Lourdes centrifuge (Lourdes Instrument Co., Brooklyn, N.Y.). The supernatant fraction (S_2) was decanted to another set of tubes, and the pellet (P_2) was resuspended in 1.0-2.0 ml 0.02M Tris-acetate buffer pH 6.5 containing 0.2% Triton 100 and rehomogenized. Aliquots of 0.5 ml portions of the S_2 and solubilized P_2 fractions were diluted with 0.6M trichloroacetic acid, and 1.0 ml portions

following protein precipitation used for the determination of tryptophan.

ii. Tyrosine assay

Brain tyrosine levels were determined by the method of Waalkes and Udenfriend (336). To 0.5 ml extract was added 1.0 ml nitroson-aphthol reagent (1.0 ml of 0.1% 1-nitroso-2-naphthol in 95% ethanol) and 1.0 ml of a nitric acid reagent containing 2.5% sodium nitrite in 2.3N nitric acid. The tube was mixed and heated in a 60°C water bath for 30 minutes. The sample was cooled on ice, 2.5 ml ethylene dichloride was added and the tube centrifuged for 5 minutes and following a 30 second shaking. The aqueous layer was carefully transferred to a new tube and the fluorescence formed was read in an Aminco-Bowman Spectrophotofluorometer at excitation and emission settings of 460 and 570 nm respectively. Standards and blanks were subjected to identical treatment and the results are expressed as µg/g wet weight of tissue.

iii. Tryptophan assay

(a) plasma tryptophan determination

The levels of tryptophan in whole plasma or plasma ultrafiltrate were determined by the method of Denckla and Dewey (335). Duplicate samples of plasma (20 µl) were dispensed into tubes containing 1.8 ml trichloroacetic acid (TCA): ferrous chloride solution (10% TCA containing 3×10^{-4} M $FeCl_3$). The tubes were mixed and following a 10 minute centrifugation at approximately 900g, the supernatant fluid was decanted off to another set of glass stoppered tubes. Portions of 0.2 ml of 1.8% formaldehyde was added, and the reaction was continued for 1 hour in a boiling water bath kept at 100°C. Samples

were cooled to room temperature and 10% TCA solution was used to make up the 2 ml volume. The fluorescent product was subsequently assayed using the Aminco-Bowman spectrophotofluorometer at excitation and emission settings of 373 and 452 nm respectively.

Plasma free tryptophan was measured as described above with the exception that aliquots of 200 µl rather than 20 µl were used. Standard tryptophan curves were constructed by serial dilutions of a stock solution with values ranging from 50 ng/ml to 15 µg/ml. Results are expressed as µg/ml plasma.

(b) brain tryptophan determination

Brain tryptophan was determined by the method of Duggan and Udenfriend (336). To 1.0 ml aliquot of deproteinized supernatant 1.5 ml 2% formaldehyde was added, and the reaction allowed to proceed for 20 minutes in loosely stoppered tubes in boiling water (100°C). A 200 µl aliquot of 3% hydrogen peroxide was then added to each tube and the resulting mixture heated for an additional 20 minutes. After cooling to room temperature, the fluorescent product was assayed in an Aminco-Bowman spectrophotofluorometer at excitation and emission settings of 365 and 440 nm respectively. Blanks and standards were run in duplicates throughout the procedure, and from a constructed standard curve, tryptophan content was estimated and is expressed as µg/g wet weight of tissue.

The levels of tryptophan in the S_2 and P_2 fractions derived from rat brain regions were determined according to the method of Duggan and Udenfriend (336). One ml portions of TCA extract was used for determination of tryptophan levels as described above.

E. Enzyme Assays

Fresh brain tissue was homogenized in 1.0-2.0 ml of 0.25M ice-cold sucrose, or 0.02M Tris-acetate buffer pH 6.0 containing 0.2% Triton X-100 using a Polytron PT-10 Homogenizer (Brinkman Instruments, New York, N.Y.) for 30 seconds. Aliquots of the homogenates were used for the estimation of tyrosine hydroxylase, tryptophan hydroxylase and monoamine oxidase activities.

i. Tyrosine Hydroxylase

Tyrosine hydroxylase was assayed by two different procedures both involving the conversion of L-[^{14}C] tyrosine to L-[^{14}C] dopa. In the first method the enzyme was assayed in the sucrose homogenates without solubilization or addition of exogenous cofactor as described by McGeer et al. (337). This method involves a complex system since the enzyme is located within the synaptosomes. Thus the conversion of precursor to dopa is limited not only by the enzyme activity but also by the uptake of the substrate into the synaptosomes and by the available endogenous cofactors within the synaptosomes. The second method was that of McGeer et al. (337) as modified by Peters and Tang (338) in that the enzyme was first solubilized and then assayed in the presence of a pteridine cofactor. The incubation mixture consisted of 2 nmol L-tyrosine containing 250,000 d.p.m., L-[U-^{14}C] tyrosine (450-520 mCi/mmol, Amersham, Oakville, Ontario); 500 nmol 6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$ Calbiochem, La Jolla, California) stabilized with 10 µmol 2-mercaptoethanol; 50 nmol N-methyl-N-3-hydroxyphenylhydrazine (NSD-1034; Smith and Nephew Research, Ware, Hertfordshire, U.K.); 100 µmol 0.28M phosphate buffer, pH 6.2, and 0.1 ml enzyme source in a total of 0.3 ml. Tissue blanks used homogenates which had been

heated for 30 minutes at 100°C prior to incubation, or homogenates with perchloric-acetic acid mixture added prior to the dispensing of incubation mixture. When the enzyme was assayed in the sucrose homogenates the incubation mixture contained all the components except for the added $DMPH_4$ and 2-mercaptoethanol.

The reaction proceeded for 30 minutes in a shaking water bath at 37°C. The reaction was terminated by the addition of 2 ml ice-cold perchloric-acetic acid mixture (0.4N perchloric acid: 0.2N acetic acid). Following centrifugation, the pH of the supernatant was adjusted to 8.8-9.2 by adding to beakers containing a mixture of 2 ml 0.2M EDTA, 3 ml 0.28M phospate buffer, pH 6.2 and 2.5 ml 2 M Tris. Following stirring, the solution was poured onto a polypropylene Econo-Columns® (0.7 x 4 cm BioRad Laboratories, Richmond, Calif.) packed with 400 mg of alumina, and the fluid allowed to run through the column. The alumina column was subsequently washed with 5 ml 0.04 M Tris, and 5 ml of distilled water, and the ^{14}C-labelled DOPA was then eluted with 4 ml 0.5N acetic acid. Twelve ml of Scintillation mixture (339) was added to the scintillation vials and the radioactivity counted in a Mark I Nuclear Chicago Scintillation Counter. The enzyme activity is expressed as nmoles DOPA produced/g tissue/hour.

ii. Tryptophan hydroxylase

Brain tryptophan hydroxylase activity was measured in homogenates by the method of Peters et al. (340). The rate of formation of labelled 5-HT produced from incubation with L-[^{14}C] tryptophan in the presence of a monoamine oxidase inhibitor was used as a measure of tryptophan hydroxylase activity. The enzyme activity was assayed by incubating a 0.200 ml aliquot of the sucrose homogenate with 0.40 ml of the incubation

mixture containing 0.10 ml of L-^{14}C-tryptophan (250,000 d.p.m.; 50 mCi/mmol); 0.10 ml of pargyline (6×10^{-3} M); 0.10 ml 0.5 M Tris-acetate buffer, pH 7.8; 0.050 ml of distilled water and 0.050 ml of 20 µg/ml 5-HT solution. Tissue blanks were the homogenates which had been heated to 100°C for 30 minutes prior to the incubation, or homogenates with perchloric-acetic acid mixture added prior to the dispensing of incubation mixture. The mixtures were incubated in a shaking water bath (American Optical Corporation, Ann Arbor, Mich.) for 30 minutes at 37°C. The reaction was terminated by the addition of 2 ml ice-cold perchloric-acetic acid mixture (1:1 mixture of 0.4N perchloric acid and 0.2N acetic acid), and the tubes left on ice for an additional 10 minutes. Following 10 minutes of centrifugation the supernatants were decanted off to another set of tubes. The ^{14}C-labelled 5-HT was isolated on CG-50 type I(100-200 mesh) Ion Exchange resin (Amberlite^{R})(Mallinckrodt Chemical Works, St. Louis, Mi.) using glass columns topped by a 50 ml reservoir, and plugged with cotton at the tip. The supernatant solution was adjusted back to pH 7.0, and immediately passed through the glass column with a resin bed of approximately 2.5 cm. The resin was subsequently washed with 35 ml of distilled water, and the labelled ^{14}C-5-HT was eluted with 4.0 ml 4M acetic acid. Twelve ml of Scintillation mixture (340) was added to each scintillation vial, and the radioactivity counted in a Nuclear Chicago Mark I liquid Scintillation Counter.

iii. Monoamine oxidase

Monoamine oxidase activity was determined by the method of Wurtman and Axelrod (341) which measures the deaminated ^{14}C metabolite of the substrate, ^{14}C-tryptamine. In a typical assay, 0.10 ml of

homogenate was mixed with 0.50 ml of incubation mixture containing 2 nmol [2-^{14}C] tryptamine bisuccinate (200,000 d.p.m., 48.5 mCi/mmol); 0.09 ml of 0.5M tris acetate buffer, pH 7.4; and 0.40 ml of distilled water, and incubated in a shaking water bath for 15 minutes at 37^{o}C. The reaction was terminated by the addition of 2 ml ice-cold 2N HCl. Tissue blanks contained homogenates which had been heated for 15 minutes at 100^{o}C prior to incubation, or homogenates which had 2 ml of 2N HCl added prior to the addition of the incubation mixture. The ^{14}C-labelled deaminated product was extracted into 10 ml of toluene by shaking for 10 minutes on a Fisher Roto-Rack mechanical shaker. Following centrifugation, 5 ml portions of the toluene phase was carefully transferred to a counting vial containing 7.5 ml of toluene/Omnifluor cocktail (New England Nuclear). The radioactivity was counted using a Mark I Nuclear Chicago Scintillation Counter. The enzyme activity is expressed as nmoles tryptamine product/g wet weight tissue/hour.

F. Chemicals

All reagents used in these experiments were of the purest grade available and dissolved in glass distilled water. Serotonin creatinine sulphate, 5-hydroxyindoleacetic acid, o-phthalaldehyde, 6,7-dimethyl-5,6,7,8-tetrahydropteridine (DMPH$_4$), L-tyrosine, L-tryptophan were purchased from Calbiochem (La Jolla, Calif.), 2-Mercaptoethanol was purchased from Eastman Kodak Co. (Rochester, N.Y.). L-Cysteine was purchased from Sigma Chemical Co. (St. Louis, Mo.). Probenecid, pargyline hydrochloride, DL-p-Chloroamphetamine Hydrochloride, and 6-Hydroxydopamine Hydrobromide, were purchased from Regis Chemical Co. Amberlite resin CG-50 was obtained from Mallinckrodt Chemical

Works, (St. Louis, Mi.). N-Methyl-N-3-hydroxybenzylhydrazine (NSD-1034) was purchased from Smith and Nephew Research, Ware, Hertfordshire, U.K.). [2-^{14}C]-Tryptamine bisuccinate was purchased from New England Nuclear (Lachine, Quebec), and L-[U-^{14}C] tyrosine, L-[methylene-^{14}C] tryptophan were purchased from Amersham (Oakville, Ontario).

Chlorpromazine Hydrochloride B.P. was provided by Poulec Ltd. (Montreal, Que.), and Haloperidol as a gift from McNeil Laboratories (Canada) Ltd., (Don Milles, Ont.). 5,7-Dihydroxytryptamine creatinine sulphate was obtained through the kind courtesy of Dr. Bruce Pappas, Department of Psychology, Carleton University, Ottawa, Ontario.

IV. STATISTICAL ANALYSIS

A. Estimation of 5-Hydroxytryptamine Turnover Rates

Brain 5-HT turnover rate was estimated by two different methods: (a) by following the rate of increase of 5-HT and decrease in 5-HIAA following inhibition of monoamine oxidase (342) and (b) by the rate of increase of 5-HIAA following inhibition of 5-HIAA transport with probenecid (343). In the first method, groups of control and lithium-treated rats were given a single intraperitoneal injection of pargyline hydrochloride (75 mg/Kg) and groups of animals were killed 0,30, 60 or 90 minutes later. In the second method, control and lithium-treated rats were given a single intraperitoneal injection of probenecid (200 mg/Kg, dissolved in minimum 1N NaOH with the pH adjusted back to 7.4 with 0.1M phosphate buffer) and groups of animals were killed 0, 60, 120 or 180 minutes later.

The tissue levels of 5-HT and 5-HIAA obtained by these two methods were logarithmically transformed for regression analysis, by the method

of least squares. The turnover values were calculated from the slopes of the derived regression lines. The fractional rate constant, K, was calculated from equation (1), and the rate of amine synthesis was calculated from equation (2), and expressed as nmoles/g wet weight of tissue/hour. The appendix contains some representative calculations resulting in the final expression of turnover rates shown in tables

Equation (1) Slope of regression line = K x 0.434

Equation (2) Rate of synthesis = steady state levels x K

B. Significance

The data was subjected to a two-tailed analysis using the Student's t-test, and statistically significant differences between mean values are indicated at $p < 0.05$.

4. RESULTS

I. EFFECT OF LITHIUM TREATMENT ON LITHIUM DISTRIBUTION IN THE CENTRAL NERVOUS SYSTEM

Male Sprague-Dawley (SD) rats housed in pairs in a standard laboratory cage received a dry diet containing 9, 30 or 80 milliequivalents of lithium carbonate per Kg dry food for a period of two or five weeks. The highest dietary lithium level used (80 mEq/Kg) proved to be toxic, with only 50% of the animals surviving the two week treatment. In contrast, all animals survived the treatments with the lower doses of lithium and did not show any apparent toxic effects. Their body weights did not differ significantly from control values. After two weeks the body weights of rats receiving 9 and 30 mEq/Kg lithium diet were 301±9 g and 275±6 g respectively compared with control values of 292±9 g.

In similar experiments male and female ACI(Micro) rats undergoing lithium treatment (2.67 mEq/Kg twice daily) for five days by intraperitoneal injection or for 2 or 5 weeks of a 30 mEq/Kg lithium-containing diet maintained body weights within 15% of the body weights of control rats (Table 2). Although lithium-treated rats appeared lethargic and sedated following 5 days of lithium injections, they were indistinguishable from the untreated controls following oral treatment with lithium for 2 or 5 weeks.

A. Distribution of Lithium in Rat Brain

i. Sprague Dawley rats

The levels of lithium in plasma and in brain regions of SD rats following 2 weeks of lithium carbonate-containing diet (9, 30 or 80 mEq/Kg food) are given in Table 3 . The plasma and brain lithium concentrations were dose-related although the higher dose levels were relatively more

TABLE 2

EFFECT OF LITHIUM TREATMENT TO ACI RATS ON BODY WEIGHTS

Results are given as mean ± S.E.M. for groups of at least 25 rats. Lithium was given intraperitoneally twice daily (2.67 mEq/Kg) for 5 days or lithium - Carbonate containing diet (30 mEq/Kg food) for 2 or 5 weeks. Control rats received saline or sodium carbonate-containing diet. The figure in parenthesis is the percentage of the control values.

Lithium Treatment	Body Weight (g)	
	Male Rats	Female Rats
Control	278±13 (100)	177±2 (100)
5 days	237±10* (85)	158±2* (89)
2 weeks	245±12* (88)	153±5* (86)
5 weeks	255±5 (92)	167±2 (94)

* $p < 0.025$ when compared to control values

TABLE 3

LITHIUM LEVELS (μEq/g) IN BRAIN REGIONS OF MALE SPRAGUE DAWLEY RATS FOLLOWING 2 AND 5 WEEKS OF LITHIUM-CONTAINING DIET

Results are expressed as mean ± S.E.M. of at least 6 determinations. Rats were given lithium carbonate containing diets (9, 30, or 80 mEq/Kg food) for 2 or 5 weeks, or a sodium carbonate containing diet.

Brain Region	Lithium Content of Food (mEq/Kg)			
	9	30		80**
	2 Weeks	2 Weeks	5 Weeks	2 Weeks
Septum	0.46±0.15	0.97±0.06*	0.92±0.09*	5.47±0.65
Cingulate gyrus	0.21±0.12	0.96±0.13*	0.63±0.03†	5.34±0.62
Hypothalamus	0.20±0.08	0.67±0.04	0.67±0.13	4.22±0.19
Olfactory tubercle bulb and tract	0.16±0.07	0.69±0.09	0.71±0.04	5.21±0.22
Amygdala	0.16±0.09	0.63+0.05	0.47±0.11	6.02±0.84
Hippocampus	0.17±0.08	0.72±0.10	0.63±0.03	5.40±0.30
Midbrain	0.20±0.06	0.70±0.07	0.54±0.08	4.23±0.46
Pons-Medulla	0.19±0.08	0.64±0.08	0.44±0.03†	3.65±0.23††
Cerebellum	0.14±0.07*	0.59±0.08	0.46±0.02	3.89±0.50
Plasma (mEq/L)	0.38±0.07	1.01±0.06	1.10±0.06	4.79±0.05

* $p < 0.05$ where compared with other brain regions of same treatment

** only 50% of rats survived this diet for the full two week treatment

† $p < 0.05$ when compared with 2 weeks of treatment

†† $p < 0.05$ when compared with septum and cingulate gyrus of same treatment.

effective in raising plasma and brain lithium concentrations. Two brain regions, the septum and cingulate gyrus, tended to have higher lithium levels than the other brain regions. In those animals receiving the 30 mEq/Kg lithium-containing diet for two weeks the lithium levels of these two regions were significantly higher ($p < 0.05$) than all other brain regions. With the 9 mEq/Kg lithium-containing diet only the cerebellum had a significantly lower lithium level than the septum whereas after 80 mEq/Kg lithium-containing diet the pons-medulla had a significantly lower lithium level than both the cingulate gyrus and the septum.

After 5 weeks of lithium treatment (30 mEq/Kg food) plasma and brain lithium levels were similar to those following 2 weeks of treatment with the exception of the cingulate gyrus and pons-medulla which showed significantly lower lithium levels than after 2 weeks treatment (Table 3). The plasma lithium level after 2 weeks and 5 weeks of treatment with 30 mEq/Kg lithium-containing diet was in the same range as the human plasma lithium after treatment of mania with lithium salts (0.8-1.4 mEq/L). Since the 30 mEq/Kg food dose level was found to produceplasma lithium levels comparable to those reported in humans undergoing lithium treatment and did not significantly affect body weight gain or produce significant mortality, this dose was used in all subsequent experiments involving oral administration.

ii. ACI (MICRO) Rats

Results presented in Table 4 demonstrate that twice daily intraperitoneal administration of LiCl (2.67 mEq/Kg weight) for 5 days which achieved plasma lithium levels of 1.4 ± 0.15 mEq/L produced a non-uniform distribution of lithium in the brain. Several brain regions, the septum, cingulate gyrus, hypothalamus, olfactory tubercle, bulb and tract and the

TABLE 4

LITHIUM LEVELS (μEq/g) IN BRAIN REGIONS OF ACI (MICRO) RATS FOLLOWING LITHIUM TREATMENT

Results are expressed as mean ± S.E.M. of at least 6 determinations. Rats were given either twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks.

Brain Region		Lithium Levels (μEq/g) 5 Days	2 Weeks	5 Weeks
Septum	1	2.46±0.29	1.13±0.25	1.84±0.39
Cingulate gyrus	2	1.95±0.19	1.11±0.20	0.72±0.15
Hypothalamus	3	1.41±0.26	1.08±0.26	1.04±0.20
Olfactory tubercle bulb and tract	4	1.49±0.26	0.44±0.07	0.54±0.05
Amygdala	5	1.36±0.18	0.50±0.05	0.71±0.06
Hippocampus	6	0.94±0.08	N.A.*	0.57±0.06
Corpus striatum	7	0.89±0.14	0.32±0.02	N.A.
Midbrain	8	0.54±0.08	0.21±0.04	0.51±0.04
Thalamus	9	0.51±0.08	0.22±0.05	N.A.
Pons-medulla	10	0.51±0.08	0.15±0.02	0.43±0.05
Cerebellum	11	0.56±0.07	0.10±0.02	0.32±0.05
Temporal cortex	12	0.22±0.03	0.13±0.03	N.A.
Plasma (mEq/L)		1.40±0.15	0.49±0.02	1.05±0.02

* N.A. denotes that this brain region was not assayed for lithium content.

interregional differences:

a) 5 days: 1-5 > 6-12 at $p < 0.05$
12 < 1-11 at $p < 0.02$

b) 2 weeks: 1-3 > 4-12 at $p < 0.02$
4-5 > 6-12 at $p < 0.02$

c) 5 weeks: 1 > 2-12 at $p < 0.01$
3 > other regions expect 1 and 2 $p < 0.05$
5 > 4, 10, 11 at $p < 0.05$

amygdala showed significantly higher lithium levels than the midbrain, thalamus, pons-medulla, cerebellum and the temporal cortex. The level in the temporal cortex was significantly lower than in all other brain regions studied.

When the period of exposure of lithium was extended to two weeks and the ion given in the diet as lithium carbonate (30 mEq/Kg food), plasma lithium levels obtained were 0.49±0.02 mEq/L. The regional distribution of the ion again appeared to be non-uniform with the septum, cingulate gyrus and hypothalamus showing significantly higher levels than all other brain regions. These differences were of the same relative magnitude as seen following intraperitoneal administration. Two weeks lithium treatment produced similar brain lithium levels as with the SD rats (Table 3) but the blood lithium levels in ACI rats was substantially lower. Several brain regions had significantly lower lithium levels than in the SD rats (e.g. midbrain, pons-medulla, cerebellum, amygdala, and olfactory tubercle bulb and tract) yet regions which appeared to concentrate or retain more lithium tended to have higher lithium levels than SD rats following a similar protocol (e.g. septum, and cingulate gyrus) with significantly greater values obtained in the hypothalamus.

A more prolonged treatment of rats with lithium carbonate-containing diet (30 mEq/Kg food) for 5 weeks resulted in blood and brain lithium levels in the same range as in the SD rats but again there was more uneven distribution in the brain. The septum showed a significantly higher level than all other brain regions; the level in the hypothalamus was significantly higher than other brain regions with the exception of the septum and cingulate gyrus, and the level in the amygdala was higher than the olfactory tubercle, bulb and tract, midbrain, pons-medulla and the cerebellum.

From the above data lithium appears to be unevenly distributed in the CNS in two strains of rats when the plasma lithium levels were of the magnitude similar to that observed in patients undergoing lithium therapy. A high level of lithium was observed in the septal complex and cingulate gyrus of both strains of rats, however other regions related to limbic system also showed high lithium levels, such as the hypothalamus, olfactory tubercle, bulb and tract, and amygdala.

II. EFFECT OF LITHIUM TREATMENT ON 5-HYDROXYTRYPTAMINE METABOLISM

A. Effect of Lithium on Steady-State Levels of 5- Hydroxytryptamine, Tryptophan and 5-Hydroxyindoleacetic Acid

To examine whether short and long term lithium administration given intraperitoneally or in the diet produces changes in 5-hydroxytryptamine metabolism, the influence of lithium on the levels of tryptophan, 5-hydroxytryptamine and its metabolite, 5-hydroxyindoleacetic acid was investigated in discrete brain regions.

i. Regional tryptophan distribution

In control animals, tryptophan was distributed relatively evenly throughout the various brain regions of ACI rats with the exception of a significantly lower concentration in the hippocampus (Fig. 5). There was a general tendency for the tryptophan level to be increased in all brain regions following lithium treatment although the apparent increases proved to be statistically significant ($p < 0.05$) in only some cases. Following repeated administration of LiCl for 5 days (2.67 mEq/Kg. i.p.) the tryptophan level was significantly elevated in 7 regions by 17-50% above control values, (amygdala, cerebellum, motor and temporal cortices, hippocampus, pons and thalamus). In contrast, when lithium carbonate-containing diet (30 mEq/Kg food) was given to ACI rats for 2 weeks

TABLE 5

THE EFFECT OF THREE DIFFERENT LITHIUM TREATMENTS ON TRYPTOPHAN LEVELS IN SELECTED BRAIN REGIONS OF ACI RATS

Results are expressed as percentage of control values (shown in Fig. 5-7) ± S.E.M. of at least 6 determinations. Rats were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks.

Brain Region	Lithium Treatment 5 days	2 weeks	5 weeks
		(% Controls)	
Amygdala	118±5*	117±11	117±12
Cerebellum	121±11*	170±36*	139±4*
Cingulate gyrus	N.A.†	N.A.	162±7*+
Cortex-motor	118±6*	112±10	N.A.
-temporal	150±9*	112±10	92±3
Hippocampus	131±9*	119±9*	124±7*
Hypothalamus	107±7	122±9	169±31*+
Medulla	99±4	126±11*	120±3*
Midbrain	105±6	95±5	133±13*
Olfactory tubercle bulb and tract	104±6	113±10	131±16*
Pons	129±14*	102±7	N.A.
Septum	N.A.	N.A.	130±8*+
Striatum	104±6	114±8	N.A.
Thalamus	117±6*	145±9*	N.A.

* $p < 0.05$ when compared with controls

\+ mean of only 2-3 determinations

† N.A. indicates that this region was not assayed.

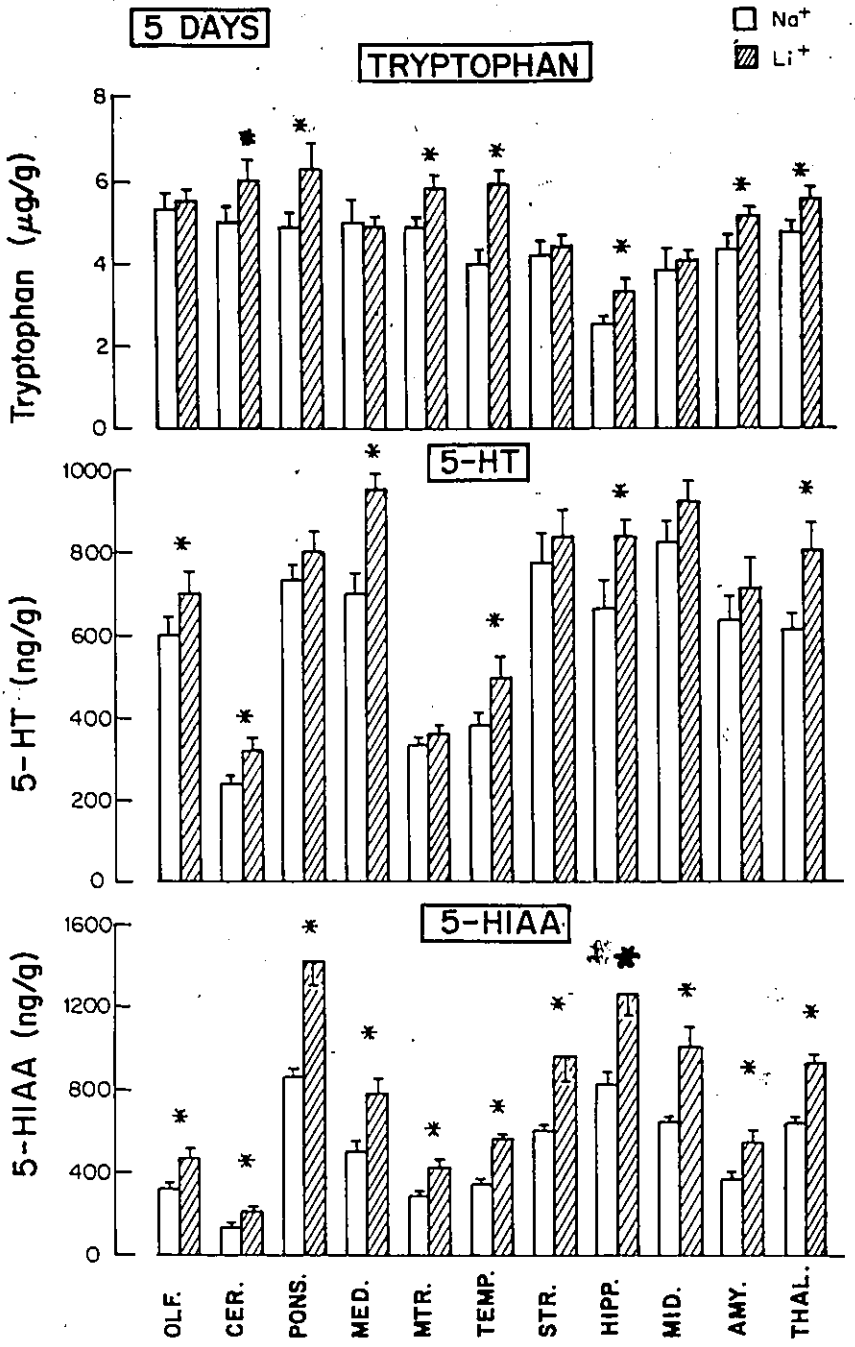

Fig. 5. Effect of 5 days LiCl treatment on the levels of tryptophan 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in rat brain regions. Each bar represents the mean ± S.E.M. of at least 6 animals. Rats were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days. Abbreviations are: olfactory tubercle (olf), cerebellum (cer), pons, medulla (med), motor cortex (mtr), temporal cortex (temp), striatum (str), hippocampus (hipp), midbrain (mid), amygdala (amy), thalamus (thal).

* Significantly different from controls at $p < 0.05$.

statistically significant lithium-induced changes in the levels of tryptophan were observed in only 4 out of 13 regions (cerebellum, hippocampus, medulla and thalamus) with 10-70% elevations above control values (Table 5 , Fig. 6). However, when the lithium-supplemented diet was extended for 5 weeks, tryptophan levels were significantly higher in all brain regions with the exception of the amygdala and the temporal cortex (Fig. 7 , Fig. 8).

ii. Regional 5-hydroxytryptamine distribution

Five days of LiCl injections (2.67 mEq/Kg, i.p. twice daily) to ACI rats was found to significantly increase brain 5-HT levels in 7 out of 14 regions (cerebellum, temporal cortex, medulla, hippocampus, hypothalamus, olfactory tubercle bulb and tract, and thalamus) with elevations of 20-35% above those in control rats given NaCl injections (Fig. 5, 8).

Following 2 weeks of oral lithium carbonate treatment, the 5-HT level was significantly elevated in the hypothalamus, midbrain, olfactory tubercle, bulb and tract, and the thalamus (Fig. 6,8). When the period of treatment was extended to 5 weeks the 5-HT level was significantly increased in the cingulate gyrus, , midbrain, olfactory tubercle, bulb and tract, pons and thalamus (Figs. 7, 8).

Whereas 5 days repeated lithium administration produced marked elevations in the levels of 5-HT in many regions, 2 and 5 weeks of chronic oral lithium treatment produced a less dramatic change. This may be the consequence of the generally higher brain lithium levels achieved in the 5 day experiment.

iii. Regional 5-hydroxyindoleacetic acid distribution

The levels of 5-HIAA were significantly increased in most brain regions following 5 days of repeated intraperitoneal administration

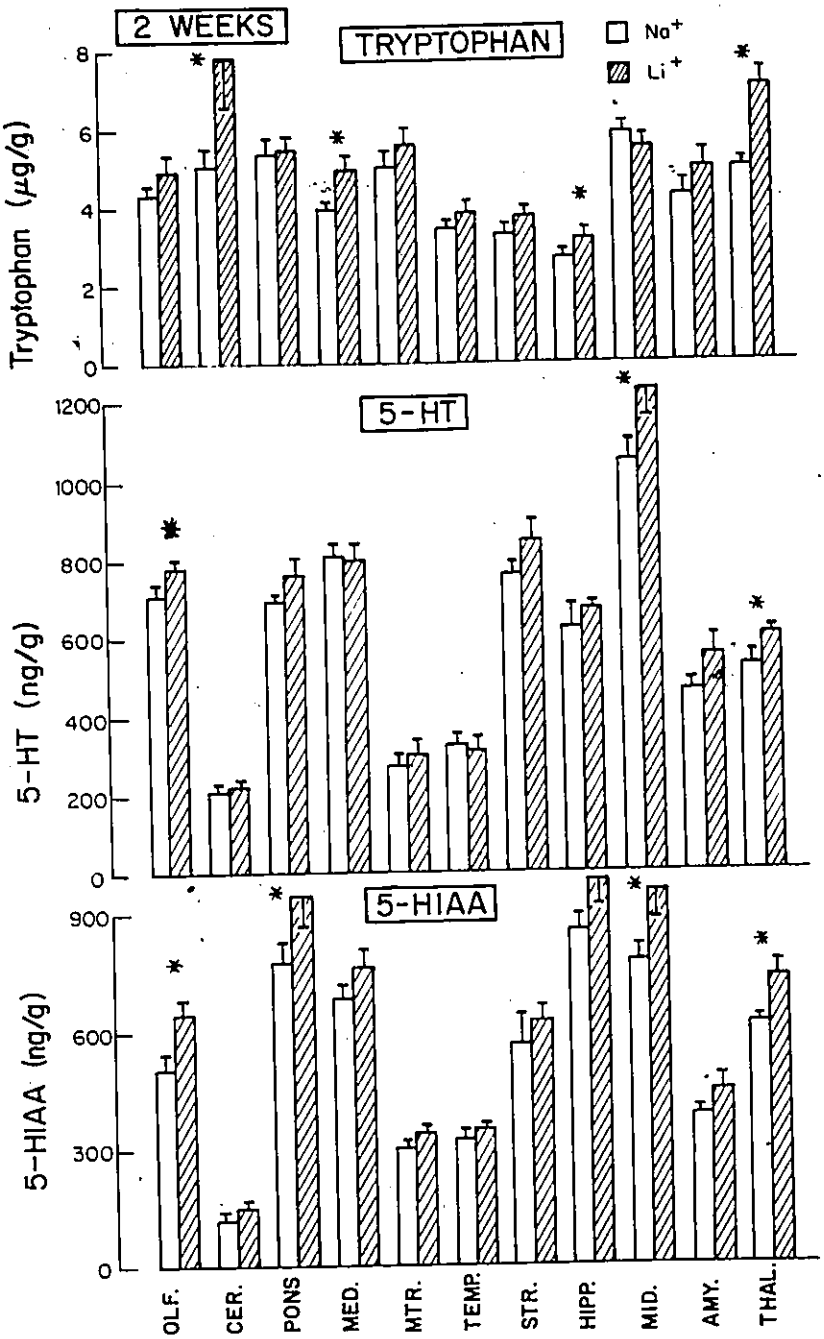

Fig. 6. Effect of 2 weeks lithium treatment on the levels of tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in rat brain regions. Each bar represents the mean ± S.E.M. of at least six animals. Rats were given Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for two weeks. Abbreviations as in Fig. 5.

* Significantly different from control values at $p < 0.05$.

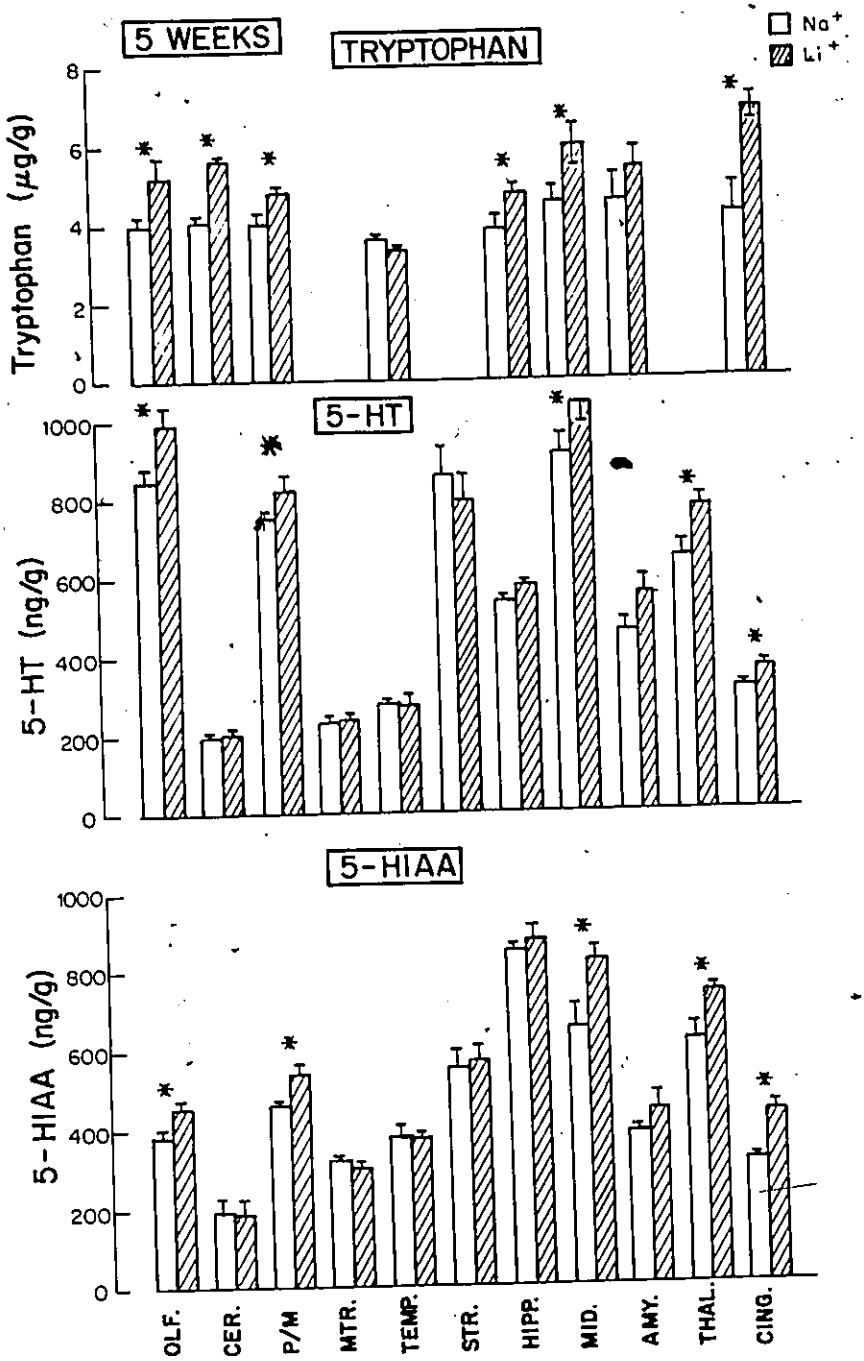

Fig. 7. Effect of 5 weeks lithium treatment on the levels of tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in rat brain regions. Each bar represents the mean ± S.E.M. of at least 6 animals. Rats were given Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 5 weeks. Abbreviations as in Fig. 5, pons-medulla (p/m), and cingulate gyrus (cing).

* Significantly different from control values at $p < 0.05$.

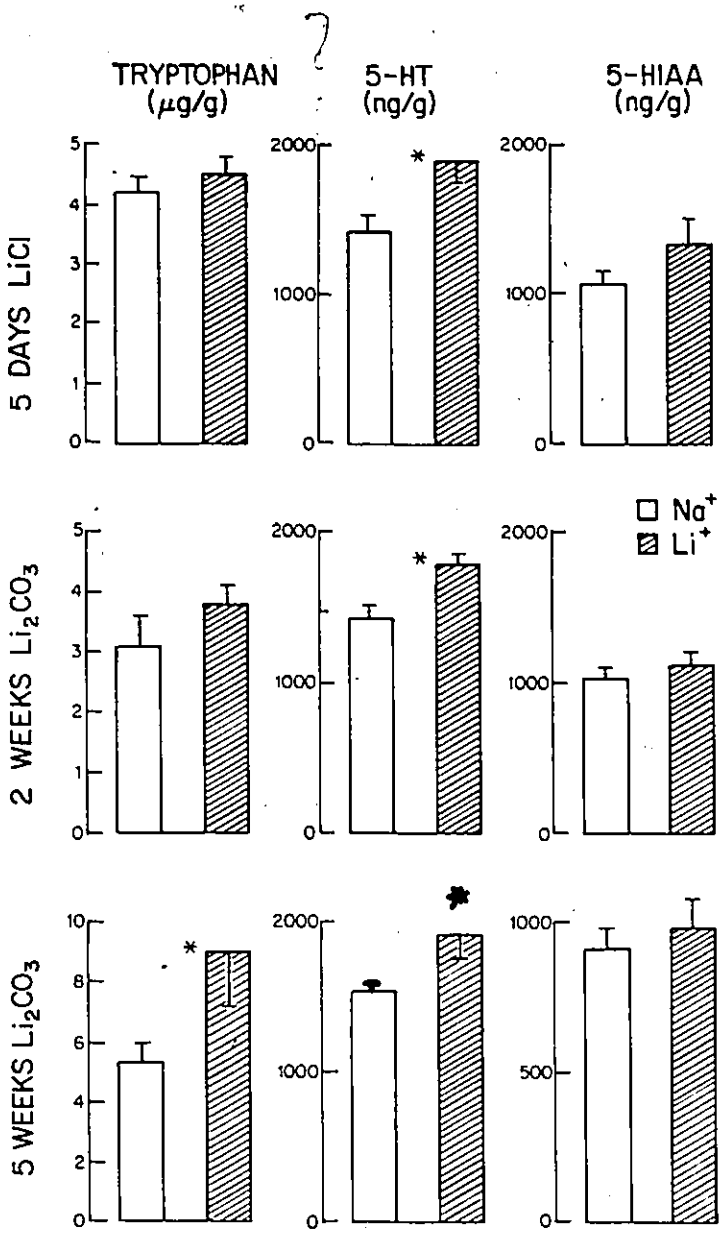

Fig. 8. Effect of three different lithium treatments on hypothalamic levels of tryptophan, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid. Each bar represents the mean ± S.E.M. of at least 6 animals. Rats were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks.

* Significantly different from control values at $p < 0.05$.

of lithium chloride with the greatest increases demonstrated in the hippocampus, pons, medulla and midbrain (40-65% above control values). Similarly, 2 weeks of oral lithium carbonate treatment was shown to produce significant elevations of 5-HIAA in the olfactory tubercle, bulb and tract, pons, midbrain and thalamus while no significant changes were observed in the amygdala, cerebellum, motor cortex, temporal cortex, hippocampus, hypothalamus, medulla and corpus striatum (Figs.6,8). Similarly 5 weeks of oral lithium carbonate treatment produced significant increases in the levels of the hydroxyindole in the cingulate gyrus, midbrain, pons-medulla, olfactory tubercle, bulb and tract, and thalamus but not in the amygdala, cerebellum, motor and temporal cortices, hippocampus, hypothalamus, and the corpus striatum (Figs. 7, 8).

In conclusion, lithium appears to cause an overall increase in tryptophan, 5-HT, and 5-HIAA in a number of brain regions; however no region appears to be markedly different from other regions. Moreover, the increases produced following the short term (5 days) lithium treatment appears to be greater than for 2 or 5 week experiments, effects which may be related to higher lithium content in brain regions (see Table 4.)

B. Effect of Lithium on 5-Hydroxytryptamine Turnover Rates in Discrete Brain Regions

Since lithium was shown to produce increased levels of hydroxyindoles in a number of brain regions, it was of interest to examine the serotonin turnover rate. It was decided to measure the turnover rate by the following two methods: a) by following the rate of increase of 5-HT and decrease in 5-HIAA following inhibition of monoamine oxidase (342) and b) by the rate of increase of 5-HIAA following inhibition of 5-HIAA transport with probenecid (343). Unfortunately, radioisotopic methods

involving injection of labelled precursor, L-tryptophan followed by isolation of labelled 5-HT have not proved to be sufficiently sensitive for use in small brain regions.

i. Effect of lithium treatment on pargyline-induced accumulation of 5-hydroxytryptamine

The rate of accumulation of 5-HT in discrete brain regions of ACI rats following pargyline hydrochloride (75 mg/Kg, i.p.) was linear for at least 60 minutes in control animals (Table 6, Fig. 9). Table 7 illustrates that the hypothalamus, midbrain and thalamus of lithium treated rats (5 days LiCl) showed significant increases in turnover rates by 62%, 33% and 44% respectively. The amygdala, corpus striatum, hippocampus, medulla, olfactory tubercle bulb and tract and the pons showed no significant alterations in the turnover rate.

Following two weeks of lithium carbonate treatment the rate of serotonin turnover appeared to be elevated in the pons (+ 24%) and midbrain (+ 31%) and reduced in the cerebellum (- 11%), corpus striatum (- 16%), hypothalamus (- 16%), motor cortex (- 17%), olfactory tubercle bulb and tract (- 39%) and the thalamus (- 21%) although the changes did not reach the $p < 0.05$ levels of significance in any brain region. Similarly, following five weeks of lithium treatment the turnover rate was not significantly altered in any brain region studied except the midbrain which showed a 27% increase (Table 7).

The only statistically significant effects of lithium on 5-HT turnover rates were a) significantly increased turnover in the hypothalamus, midbrain and thalamus following 5 days treatment, and b) increased turnover in the midbrain following 5 week treatment, perhaps since the 5-HT turnover data showed higher standard errors than

TABLE 6

THE EFFECT OF PARGYLINE HYDROCHLORIDE ON THE LEVELS OF 5-HYDROXYTRYPTAMINE IN SELECTED BRAIN REGIONS OF ACI RATS

Results are expressed as percentage of controls ± S.E.M. of at least 15 determinations. Rats were given a single injection of pargyline HCl (75 mg/Kg, i.p.) and groups of rats were killed at 0, 30, 60 and 90 minutes later.

Brain Region	5-HT levels (% control values)			
	Time after paragyline treatment (min)			
	0	30	60	90
Amygdala	100±6	165±12	224±13	244±16
Cerebellum	100±3	140±8	163±8	251±20*
Cortex-motor	100±5	151±7	208±12	214±11
-temporal	100±4	137±7	154±6	-
Hippocampus	100±6	144±7	172±7	263±24*
Hypothalamus	100±3	176±22	277±30	332±26
Medulla	100±4	166±12	228±6	-
Midbrain	100±3	151±6	229±16	447±8*
Olfactory tubercle bulb and tract	100±4	133±6	166±6	224±11*
Pons	100±4	141±6	157±5	-
Striatum	100±4	125±5	144±7	181±14
Thalamus	100±3	143±5	196±20	156±3

* Values are significantly different from the 60 minute data. All values including 30 and 90 minutes are significantly higher from 0 time ($p < 0.02$).

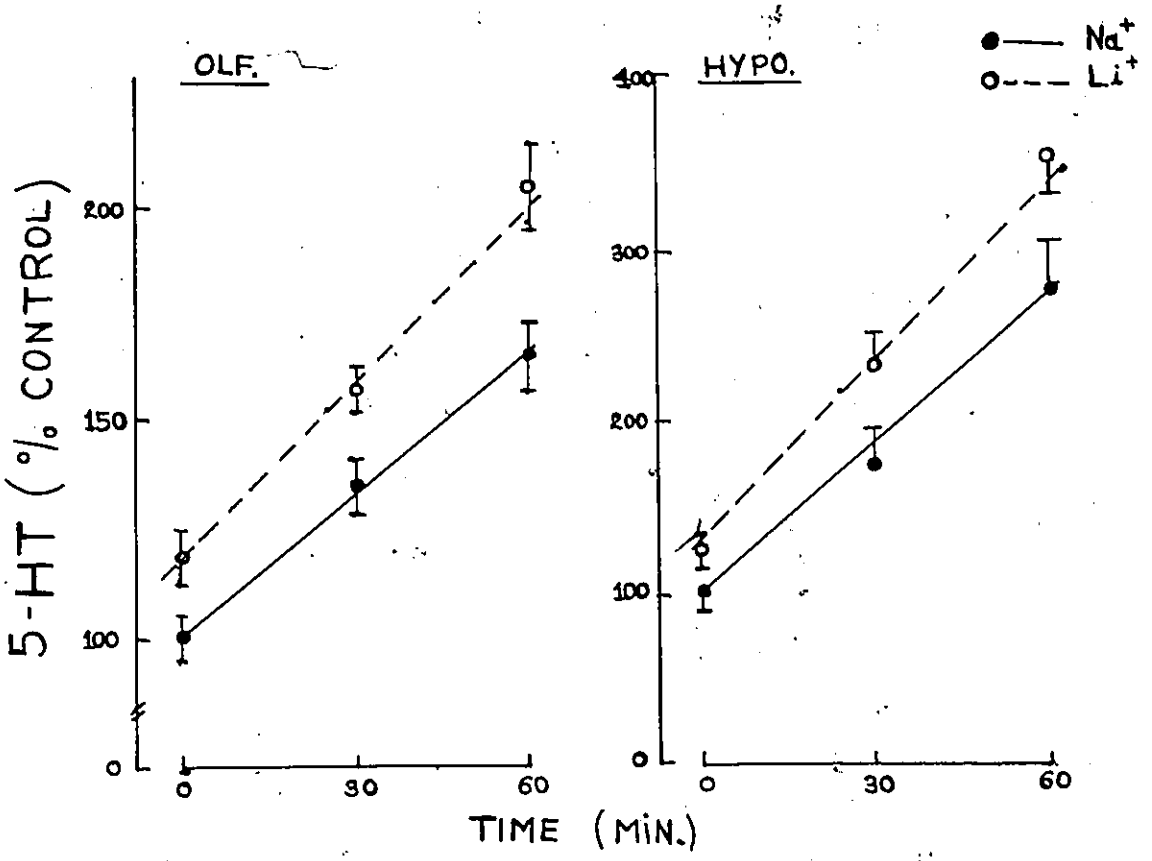

Fig. 9. Effect of lithium on pargyline-induced rise in 5-hydroxytryptamine levels in rat brain regions. Each point represents the mean ± S.E.M. of at least 5 determinations and are shown as percentage of control values. Rats were given Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 5 weeks. At the end of the treatment groups of rats were given a single injection of pargyline HCl (75 mg/Kg, i.p.) and killed 0, 30, 60 or 90 minutes later. Olfactory (olf), hypothalamus (hypo).

TABLE 7

THE EFFECT OF LITHIUM ON 5-HYDROXYTRYPTAMINE TURNOVER RATE AS MEASURED BY THE PARGYLINE-INDUCED RISE IN 5-HYDROXY-TRYPTAMINE LEVELS IN BRAIN REGIONS OF ACI RATS

Results are given as mean ± S.E.M. of at least 5 determinations. Rats were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks. At the end of the treatment, groups of 5 rats were given a single injection of pargyline HCL (75 mg/Kg, i.p.) and killed 0, 30, 60 or 90 minutes later. The figure in parenthesis is the percentage of control values.

Brain Region	Treatment	5-HT turnover rate (nmoles/g/hr) 5 days	2 weeks	5 weeks
Amygdala	Na+	2.989±0.328	-	2.672±0.294
	Li+	2.237±0.599 (75)	-	2.271±0.338 (85)
Cerebellum	Na+	-	0.480±0.085	0.678±0.034
	Li+	-	0.429±0.282 (89)	0.588±0.040 (87)
Cortex, motor	Na+	-	1.446±0.283	0.944±0.090
	Li+	-	1.198±0.322 (83)	0.763±0.158 (81)
Cortex, temporal	Na+	-	0.836±0.141	0.814±0.034
	Li+	-	0.859±0.220 (103)	0.695±0.130 (85)
Hippocampus	Na+	1.853±0.170	1.746±0.164	1.718±0.141
	Li+	1.458±0.469 (79)	1.655±0.367 (95)	2.040±0.203 (119)
Hypothalamus	Na+	6.316±1.203	6.356±1.260	6.836±1.300
	Li+	10.242±1.452 (162)*	5.305±1.017 (84)	8.011±0.870 (117)
Medulla	Na+	3.379±0.220	3.435±0.226	3.107±0.141
	Li+	3.475±0.633 (103)	3.396±0.237 (99)	3.288±0.170 (106)
Midbrain	Na+	3.797±0.509	3.910±0.758	4.220±0.582
	Li+	5.034±0.260 (133)*	5.118±0.746 (131)	5.362±0.350 (127)*
Olfactory tubercle bulb and tract	Na+	1.757±0.198	2.079±0.232	2.469±0.277
	Li+	1.300±0.367 (74)	1.260±0.379 (61)	2.887±0.328 (117)

TABLE 7
continued

Brain Region	Treatment	5-HT turnover rate (nmoles/g/hr) 5 days	2 weeks	5 weeks
Pons	Na+	2.040±0.260	1.785±0.226	-
	Li+	1.644±0.316 (81)	2.226±0.271 (124)	-
Striatum	Na+	1.605±0.249	1.695±0.260	2.181±0.300
	Li+	1.480±0.396 (92)	1.429±0.390 (84)	2.362±0.520 (138)
Thalamus	Na+	2.328±0.277	1.881±0.220	2.226±0.266
	Li+	3.350±0.339 (144)*	1.480±0.288 (79)	2.266±0.181 (102)

* $p < 0.05$ when compared with Na+-treated group of rats.

measurements of hydroxyindoles.

ii. Effect of lithium treatment on pargyline-induced reduction of 5-hydroxyindoleacetic acid

The rate of loss of 5-HIAA after pargyline did not appear to be significantly altered by 5 days of lithium treatment in the brain regions investigated, but there was a tendency of increased rate of loss in the amygdala (+ 61%), hippocampus (+ 89%), midbrain (+ 29) and olfactory tubercle, bulb and tract (+ 29%), and reduced rate of loss in the hypothalamus (- 16%), medulla (- 40%), striatum (- 28%) and the thalamus (- 10%) (Table 8).

Whereas 2 weeks of oral lithium treatment did not significantly alter the rate of loss of 5-HIAA following pargyline treatment, 5 weeks of lithium treatment resulted in an increased rate of loss of 5-HIAA in the midbrain (+ 49%) and the amygdala (+ 38%) (Table 8). Thus increases in the rate of loss of 5-HIAA were observed in the amygdala (and possibly the hippocampus, midbrain and olfactory tubercle) after 5 days and in the amygdala and midbrain after 5 weeks of lithium treatment.

iii. Effect of lithium treatment on probenecid-induced rise in 5-hydroxyindoleacetic acid

The regional rate of elevations of 5-HIAA following probenecid (200 mg/Kg, i.p.) to control animals was shown to be linear up to 180 minutes following drug administration (Fig. 10). After 5 days of lithium chloride treatment the rate of rise of 5-HIAA was essentially similar to that produced by control rats in most brain regions. The hypothalamus and thalamus exhibited moderate elevations in the rate of turnover by approximately 40% and 27% respectively (Table 9). When lithium was given in the diet for two weeks, the 5-HT turnover rate was significantly altered in only the thalamus (-43%) and the olfactory

TABLE 8

THE EFFECT OF LITHIUM ON 5-HYDROXYTRYPTAMINE TURNOVER RATE AS MEASURED BY PARGYLINE-INDUCED DECREASE IN 5-HYDROXYINDOLEACETIC ACID LEVELS IN BRAIN REGIONS OF ACI RATS

Results are expressed as mean ± S.E.M. of at least 5 determinations. Rats were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks. At the end of the treatment, groups of rats were given a single injection of pargyline HCl (75 mg/Kg, i.p.) and killed 0, 30, 60, or 90 minutes later. Data shown in parenthesis represent the result as percentage of control values.

Brain Region	Treatment	5-HT turnover rate (nmoles/g/hr) 5 days	2 weeks	5 weeks
Amygdala	Na^+	1.655±0.372	1.801±0.24	1.461±0.147
	Li^+	2.675±0.586 (161)	1.885±0.35 (105)	2.021±0.183 (138)*
Cortex, motor	Na^+	1.257±0.126	1.021±0.188	1.455±0.150
	Li^+	0.932±0.314 (74)	0.927±0.157 (91)	1.115±0.110 (77)
Hippocampus	Na^+	2.026±0.545	2.832±0.524	2.215±0.131
	Li^+	3.827±1.293 (189)	3.503±0.560 (124)	2.031±0.136 (92)
Hypothalamus	Na^+	5.382±0.890	6.283±0.246	4.932±0.508
	Li^+	4.524±1.487 (84)	5.513±0.408 (88)	3.880±0.497 (79)
Medulla	Na^+	2.147±0.649	3.440±0.958	
	Li^+	1.278±0.717 (60)	3.628±0.440 (106)	
Midbrain	Na^+	2.189±0.204	2.901±0.524	1.927±0.168
	Li^+	2.827±0.592 (129)	3.073±0.188 (106)	2.864±0.173 (149)*
Olfactory tubercle bulb and tract	Na^+	1.414±0.209	1.984±0.367	1.953±0.105
	Li^+	1.827±0.272 (129)	1.874±0.361 (95)	2.115±0.094 (108)
Pons	Na^+		3.780±0.461	
	Li^+		3.278±0.963 (87)	
Striatum	Na^+	1.199±0.236		1.246±0.068
	Li^+	0.859±0.278 (72)		1.340±0.105 (108)
Thalamus	Na^+	1.822±0.257	1.660±0.257	2.419±0.183
	Li^+	1.644±0.335 (90)	2.340±0.351 (141)	2.450±0.042 (101)

* $p < 0.05$ when compared with respective Na^+ group

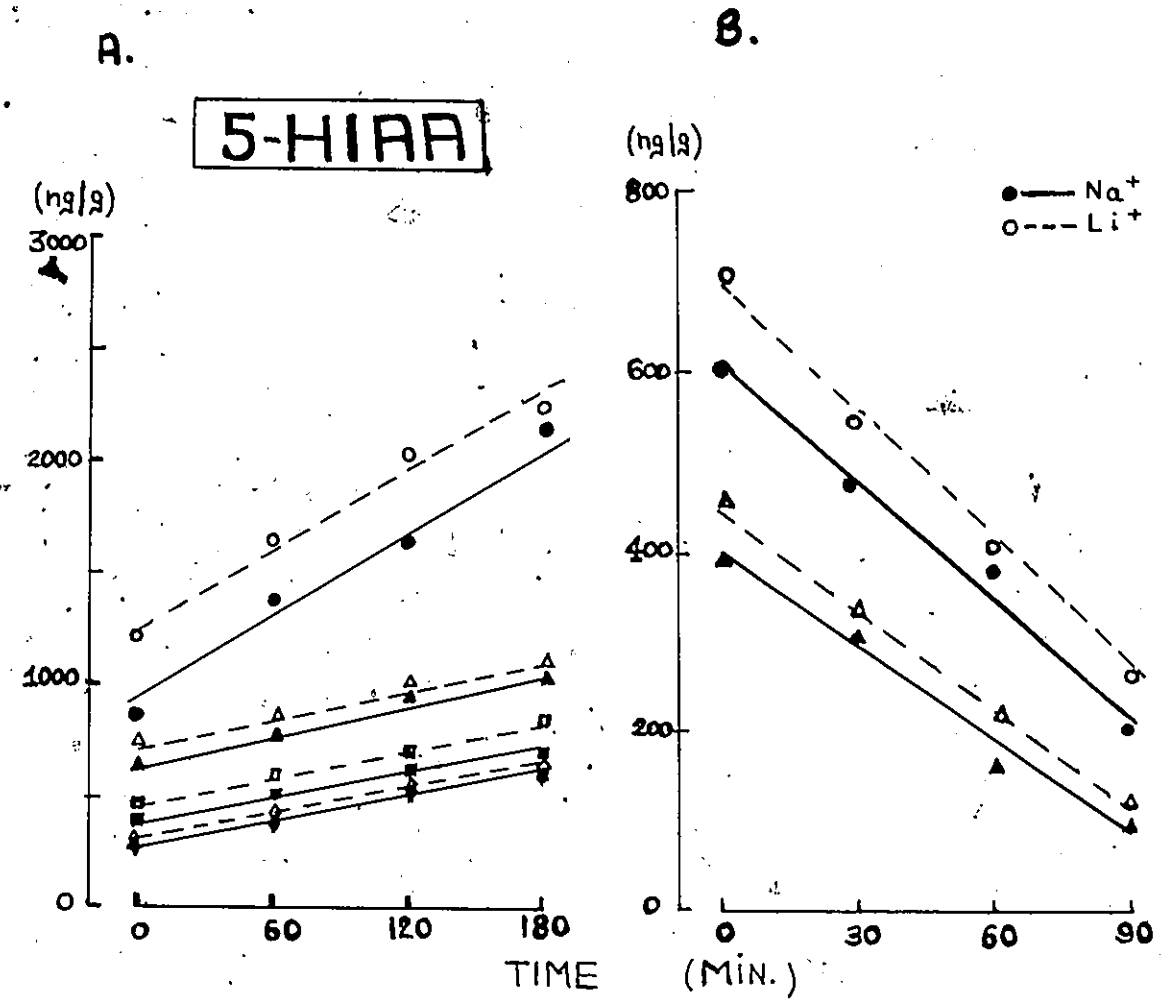

Fig. 10. Effect of lithium on 5-hydroxytryptamine turnover rate in rat brain regions. Each point represents the mean of at least 5 determinations. Rats were given Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 5 weeks. At the end of the treatment groups of rats were given either (A) a single injection of probenecid (200 mg/Kg, i.p.) and killed 0, 60, 120 or 180 minutes later. ●—, ○---pons; ▲—, △---thalamus; ■—, □---amygdala; ◆— ◇-- motor cortex; or (B) a single injection of pargyline HCl (75 mg/Kg, i.p.) and killed 0, 30, 60, 90 minutes later. ●— ○-- thalamus ▲— △--- olfactory tubercle.

TABLE 9

THE EFFECT OF LITHIUM ON 5-HYDROXYTRYPTAMINE TURNOVER RATE AS MEASURED BY THE PROBENECID-INDUCED ACCUMULATION OF 5-HYDROXYINDOLEACETIC ACID LEVELS IN BRAIN REGIONS OF ACI RATS

Results are given as mean ± S.E.M. of at least 6 determinations. Rats were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks. At the end of the treatment groups of 6 rats were given a single injection of probenecid (200 mg/Kg, i.p.) and killed 0, 60, 120 or 180 minutes later. (percentage of control values)

Brain Region	Treatment	5-HT turnover rate (nmoles/g/hr) 5 days	2 weeks	5 weeks
Amygdala	Na^+	0.853±0.094	0.445±0.021	0.387±0.010
	Li^+	0.780±0.105 (91)	0.476±0.063 (107)	0.503±0.031 (130)*
Cortex, motor	Na^+	0.529±0.031	0.497±0.031	0.387±0.016
	Li^+	0.529±0.063 (100)	0.435±0.016 (87)	0.414±0.026 (107)
Hippocampus	Na^+	1.576±0.126	1.131±0.089	0.733±0.021
	Li^+	1.618±0.147 (103)	0.864±0.079 (76)	0.712±0.037 (97)
Hypothalamus	Na^+	1.754±0.178	1.482±0.136	1.100±0.104
	Li^+	2.461±0.351 (140)*	1.346±0.089 (91)	1.691±0.255 (154)*
Medulla	Na^+	1.524±0.178	0.890±0.031	0.634±0.042
	Li^+	1.382±0.152 (91)	0.838±0.073 (94)	0.681±0.063 (107)
Midbrain	Na^+	1.047±0.021	0.764±0.042	0.885±0.110
	Li^+	1.021±0.115 (98)	0.712±0.052 (93)	0.890±0.058 (101)
Olfactory tubercle bulb and tract	Na^+	0.534±0.037	0.482±0.016	0.367±0.016
	Li^+	0.503±0.052 (94)	0.550±0.031 (114)*	0.461±0.021 (126)*
Pons	Na^+	1.853±0.094	1.251±0.094	1.293±0.026
	Li^+	2.084±0.267 (112)	1.168±0.099 (93)	1.011±0.068 (78)
Striatum	Na^+	0.675±0.026	0.707±0.079	0.665±0.068
	Li^+	0.812±0.147 (120)	0.476±0.03 (67)	0.833±0.068 (125)*
Thalamus	Na^+	0.743±0.042	0.681±0.016	0.539±0.031
	Li^+	0.948±0.042 (127)*	0.387±0.016 (57)*	0.529±0.016 (98)

* $p < 0.05$ when compared with respective Na controls

tubercle (+14%) when compared with control treated rats. Moreover, 5 weeks of lithium treatment did not change the rate at which 5-HIAA accumulated following probenecid treatment. Three brain regions, the corpus striatum and the olfactory tubercle, and amygdala showed slight elevations in the rate of 5-HIAA accumulation. When comparison is made between the effects of lithium on probenecid-induced elevations in 5-HIAA at each time period, only the 180 minute point was greater in the lithium-treated group than its respective controls.

The two methods of estimating 5-HT turnover rates did not always produce consistent results. At least part of the reason for the inconsistencies is that the inherent variability in the turnover measurements yielded a standard error of the mean of around 25%. Thus the methods appeared to be not sufficiently sensitive to measure the relatively small changes in turnover in small brain regions.

C. Effect of Lithium on Enzymes Associated with 5-Hydroxytryptamine Metabolism in Rat Brains

To examine whether short and long term lithium treatment given intraperitoneally or in the diet produces changes in enzymes associated with the 5-HT metabolism, the influence of lithium on tryptophan hydroxylase and monoamine oxidase activities was investigated in discrete brain regions.

i. Effect of lithium treatment on regional brain tryptophan hydroxylase activity

Limited studies were conducted on the possible effects of lithium on tryptophan hydroxylase activity. On the basis of previous studies it was predicted that the enzyme activity might be expected to increase following lithium treatment for short duration. However, five days LiCl treatment (2.67 mEq/Kg, i.p., twice daily) increased the activity

in the midbrain (+ 25% above controls) and medulla (+ 20%), only and decreased activity in the hippocampus (- 33%) and thalamus (Table 10).

Two weeks of lithium carbonate treatment did not produce significant changes in the enzyme activity in most brain regions. A significant increase was seen only in the thalamus (+ 23% above controls) (Table 10). It is interesting to note that the midbrain, a region known to contain tryptaminergic cell bodies, exhibited significant reductions in enzyme activity (- 15% below controls) following this dose schedule. This is in marked contrast to the changes in the activity shown for 5 days of lithium treatment. Moreover, hippocampal and thalamic enzyme activities while reduced after 5 days of lithium treatment were no longer significantly different from control rats following two weeks of lithium treatment.

ii. Effect of lithium treatment on regional monoamine oxidase activity

It was of interest to examine the regional monoamine oxidase enzyme activity following lithium treatment and exclude the possibility that lithium-induced changes in indole levels is a consequence of its enhanced activity.

Five days of lithium chloride treatment did not significantly change the activity in most regions investigated with the exception of a slight reduction in the corpus striatum (- 21% of control values Table 11). Similarly, 2 weeks of oral lithium carbonate treatment did not produce any changes in the enzyme activity in these regions. Moreover, when the lithium treatment was extended to five weeks no significant alterations in the enzyme activity was observed, however there was a tendency for increases in the septum (+ 27% above controls) and cingulate gyrus (+ 29% above controls) (Table 12). While lithium treatment increases the

TABLE 10

THE EFFECT OF LITHIUM TREATMENT ON TRYPTOPHAN HYDROXYLASE ACTIVITY IN SELECTED BRAIN REGIONS OF ACI RATS

Results are given as mean ± S.E.M. of at least 5 determinations and expressed as percentage of control values. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/kg food) for 2 weeks.

Brain Region	Tryptophan hydroxylase activity (% control)			
	5 days		2 weeks	
	Control	LiCl	Control	Li_2CO_3
Corpus striatum	100±7	89±6	100±8	91±4
Cortex, motor	100±9	107±14	100±4	113±11
Hippocampus	100±11	67±8*	100±9	104±4
Midbrain	100±3	125±8*	100±7	85±3*
Olfactory tubercle bulb and tract	100±4	92±5	100±11	125±14
Pons	100±10	102±13	100±5	98±6
Thalamus	100±10	83±11	100±8	123±8*

* $p < 0.05$ when compared with control values.

TABLE 11

THE EFFECT OF LITHIUM CHLORIDE TREATMENT ON MONOAMINE OXIDASE ACTIVITY IN DISCRETE BRAIN REGIONS OF ACI RATS

Results are given as mean ± S.E.M. for groups of at least 6 rats. Animals were given twice daily intraperitoneal injections of NaCl or LiCl (2.67 mEq/kg) for 5 days.

Brain Region	Monoamine Oxidase Activity (nmoles/g/hr)		
	Control	LiCl	% control
Amygdala	180±16	194±16	108
Cerebellum	64±4	64±4	100
Cingulate gyrus	194±10	175±7	90
Corpus striatum	211±14	166±12*	79
Cortex-motor	70±6	66±5	94
-temporal	70±6	69±8	100
Hypothalamus	261±21	299±12	115
Midbrain	165±13	157±15	95
Olfactory tubercle bulb and tract	155±15	152±14	98
Pons-medulla	159±15	134±10	84
Septum	243±22	270±12	111

* $p < 0.05$ when compared with controls.

TABLE 12

THE EFFECT OF ORAL LITHIUM CARBONATE TREATMENT ON MONOAMINE OXIDASE ACTIVITY IN DISCRETE BRAIN REGIONS OF ACI RATS

Results are given as mean ± S.E.M. of groups of at least 5 rats. Animals were given a Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/kg food) for 2 or 5 weeks.

Brain Region	Monoamine Oxidase Activity (nmoles/g/hr) 2 weeks			5 weeks		
	Control	Li_2CO_3	%**	Control	Li_2CO_3	%**
Amygdala	204±6	206±8	101	191±8	196±13	103
Corpus striatum	197±13	214±15	109	N.A.	N.A.	N.A.
Cortex-motor	76±3	74±6	98	68±6	70±3	103
Hippocampus	223±10	226±9	101	190±6	163±18	86
Hypothalamus	N.A.*	N.A.	N.A.	263±20	274±24	105
Midbrain	181±12	199±9	110	165±15	157±7	95
Olfactory tubercle bulb and tract	201±11	210±9	104	174±14	198±13	114
Pons-medulla	208±10	223±9	107	152±13	131±15	86
Septum	N.A.	N.A.	N.A.	239±24	309±44	127
Thalamus	186±13	180±10	97	N.A.	N.A.	N.A.

* N.A. indicates that the brain region was not assayed for MAO.

** % indicates value expressed as percentage of control values.

levels of 5-HIAA in a number of brain regions, it does not appear to significantly change monoamine oxidase activity.

D. Effect of Lithium on Brain Tryptophan Distribution

i. Effect of lithium on the relationship between plasma and brain tryptophan

Evidence has been presented (82 - 84) to suggest that the concentration of tryptophan may be the principal determinant of the rate of 5-HT synthesis, and that treatments which elevate plasma free tryptophan lead to corresponding increases of brain tryptophan. It was of interest therefore to examine whether the early changes in tryptophan levels are a consequence of changes in the plasma distribution of tryptophan following lithium treatment. Determination of total plasma tryptophan showed no significant difference between controls and lithium treated rats (5 days LiCl 2.67 mEq/Kg, i.p. twice daily; and 2 weeks lithium carbonate-containing diet, 30 mEq/Kg food) (Fig. 11). In contrast, free tryptophan in plasma of lithium treated rats was significantly elevated by 79% after 5 days and 58% after 2 weeks of lithium treatment (Table 15, Fig. 11).

In order to investigate the role of free and total plasma tryptophan levels in elevating brain tryptophan levels, analysis was performed comparing the plasma values to the specific brain region values. Figure 12 illustrates that plasma free tryptophan shows a positive correlation with the representative brain tryptophan values. Thus, whereas plasma total tryptophan values showed no significant correlation with regional tryptophan levels, plasma free tryptophan values were positively correlated with the regional tryptophan values for most regions studied (all data not shown for all other regions examined).

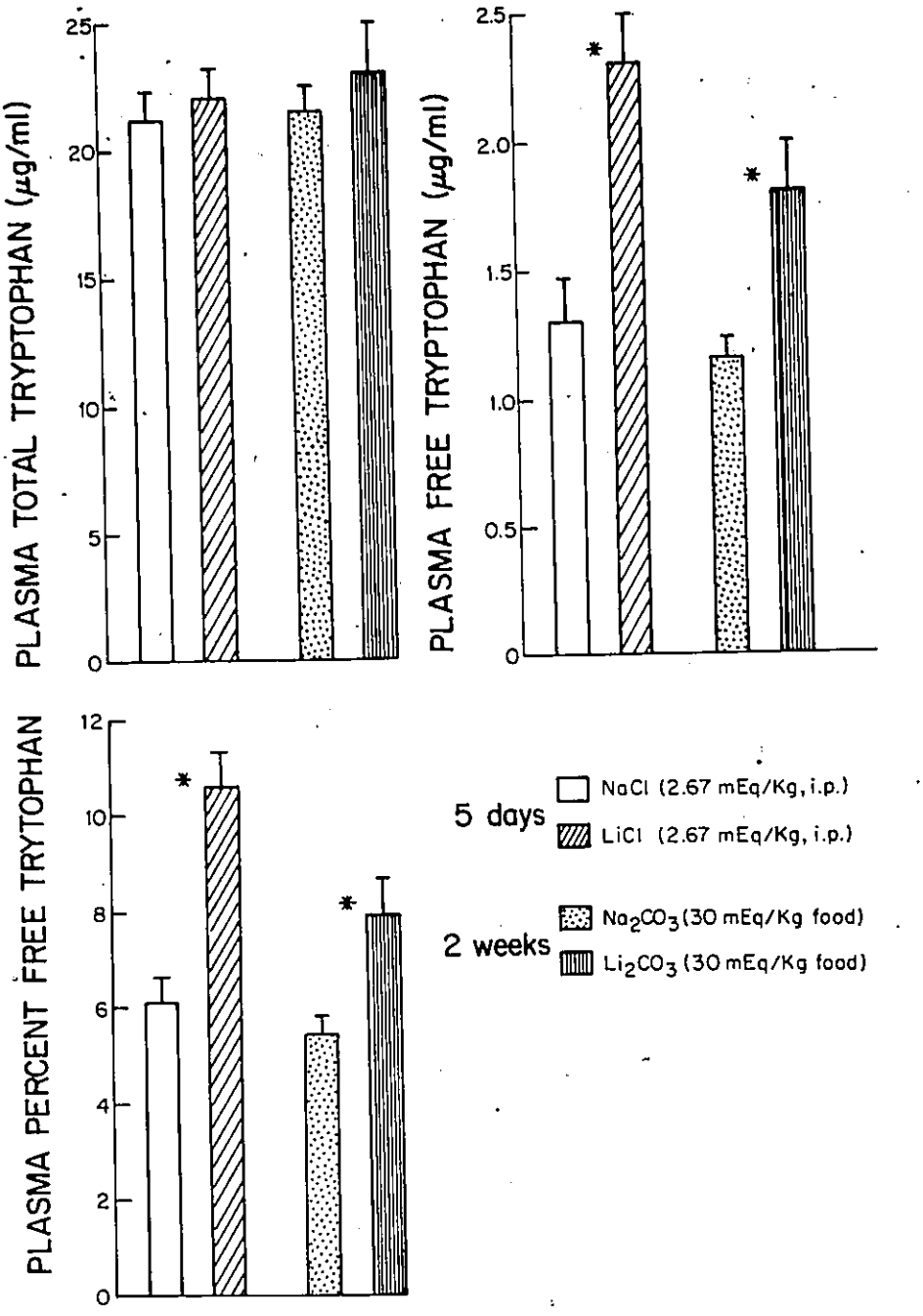

Fig. 11. Effect of lithium treatment on the distribution of tryptophan in blood. Each bar represents the mean ± S.E.M. of at least 5 determinations. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 weeks.

* $p < 0.05$ when compared with respective controls.

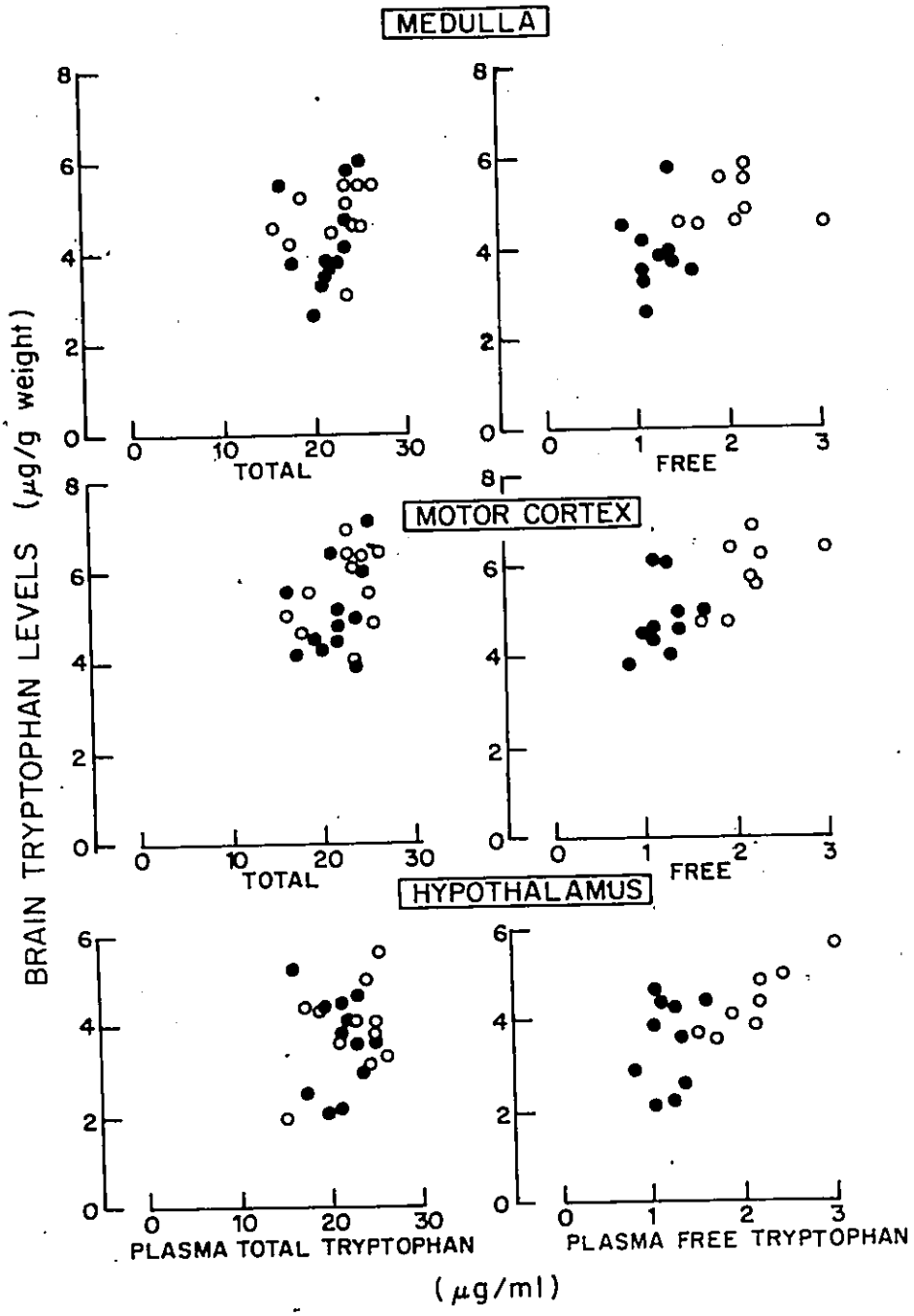

Fig. 12. Effect of lithium on the relationship between plasma and brain tryptophan levels. Each point represents one animal. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days and groups of rats killed 12 hours after the last injection.
● control, ◐ lithium-treated rat.

ii. Effect of lithium on subcellular distribution of tryptophan in brain

While plasma and uptake mechanisms may contribute to the elevated tryptophan levels in the CNS following lithium treatment, it remains a possibility that the lithium-induced changes in brain tryptophan do not simply result from a passive diffusion effect. Since it has been demonstrated that lithium stimulates the uptake of tryptophan into synaptosomal membranes (27), it remained a possibility that lithium may affect the endogenous pools of tryptophan in selected brain regions. It was therefore of interest to examine the subcellular distribution of tryptophan.

Five days of lithium treatment caused significant elevations in the levels of tryptophan of S_2 fractions of all brain regions (23-47%.above control values) except for the midbrain. Similarly, the tryptophan level appeared to be elevated in the P_2 fractions of most brain regions (30-57%) (Table 13), although the increases were statistically significant only in the cerebellum, motor cortex, midbrain and pons-medulla. Five weeks of lithium treatment produced a similar significant elevation in the S_2 fraction tryptophan levels (19-92%) in all regions. The P_2 tryptophan levels were significantly higher in the midbrain, pons-medulla, cerebellum, and motor cortex of lithium-treated rats when compared with control animals. Higher levels of tryptophan were observed in the P_2 fractions in the pons-medulla and the cerebellum than in the S_2 fraction, while the reverse was true for the cingulate gyrus, and the hippocampus after 5 weeks of lithium treatment. These preliminary data suggest that lithium may cause a shift in the subcellular tryptophan pools or to increase both fractions depending on the brain region under investigation.

TABLE 13

THE EFFECT OF LITHIUM TREATMENT ON SUBCELLULAR DISTRIBUTION OF TRYPTOPHAN IN SELECTED RAT BRAIN REGIONS

Values are given as mean ± S.E.M. of 5 determinations and expressed as percentage of control values. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 containing diet (30 mEq/Kg food) for 5 weeks. At the end of the treatment or 12 hours after the last injection groups of rats were killed and S_2 and P_2 fractions were obtained as described in Materials and Methods Section.

Brain Region	Treatment	Tryptophan Levels (% control) 5 days LiCl		5 weeks Li_2CO_3	
		S_2	P_2	S_2	P_2
Cerebellum	Na^+	100±8	100±11	100±4	100±11
	Li^+	142±12*	136±13*	119±6*	186±25*
Cortex-motor	Na^+	100±7	100±11	100±7	100±11
	Li^+	147±12*	130±4*	123±7*	129±8*
Cingulate gyrus	Na^+	100±13	100±8	100±10	100±8
	Li^+	128±10*	83±7	144±16*	75±5*
Hippocampus	Na^+	100±3	100±16	100±14	100±24
	Li^+	123±5*	122±14	192±27*	81±18
Midbrain	Na^+	100±8	100±14	100±8	100±16
	Li^+	103±7	157±19*	153±11*	156±20*
Pons-medulla	Na^+	100±5	100±17	100±10	100±15
	Li^+	132±18*	156±16*	124±10*	155±10*

* significantly different when compared with Na^+ controls ($p < 0.05$)

E. Effect of Lithium on Brain Tryptophan, 5-Hydroxytryptamine and 5- Hydroxyindoleacetic Acid Following a Tryptophan Load

i. Effect of lithium on tryptophan-induced alterations in the levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid

It was of interest to study the effects of tryptophan loading on the synthesis of 5-HT in lithium pretreated rats in order to establish whether following a tryptophan load, lithium might a) increase the uptake of tryptophan into brain cells alone or b) increase both the uptake of tryptophan into the brain and the maximum rate at which the brain tissue could synthesize 5-HT. In addition, it was of interest to examine the capacity of the brain for synthesizing 5-HT and the possible influence of lithium on this system.

Female ACI rats were given twice daily injections of either lithium chloride or sodium chloride for 5 days, and 12 hours after the last treatment a single injection of L-tryptophan (100 mg/Kg, i.p.) or its vehicle was given and the rats killed one hour later. Both lithium and tryptophan treatments alone significantly elevated 5-HT and 5-HIAA levels in most regions. However, in a number of regions there was a significantly greater rise in the 5-hydroxyindole levels in rats receiving both lithium and tryptophan than those given tryptophan alone. These preliminary results suggested that lithium and tryptophan exerted an additive effect, and was consistent with the "tryptophan uptake mechanism" which was postulated to be principally responsible for the enhanced indole levels in rat brain (27).

In subsequent experiments the dose of tryptophan was increased to determine if lithium increased the capacity of the brain tissue to synthesize 5-HT. Table 14 , and figures 13, 14 and 15 summarize the observed effects. In control animals, tryptophan-induced

TABLE 14

THE EFFECT OF L-TRYPTOPHAN LOADING ON THE LEVELS OF 5-HYDROXYINDOLEACETIC ACID (ng/g) IN LITHIUM AND SODIUM TREATED RAT BRAIN

Results are given as mean ± S.E.M. of at least 6 animals in a given group. Rats were given intraperitoneal injections of NaCl or LiCl for 5 days (2.67 mEq/Kg, twice daily). Approximately 12 hours after the last injection groups of rats were given a single i.p. injection of either vehicle or L-tryptophan at 50, 100, 200 or 400 mg/Kg dose and killed 60 minutes later.

Brain Region		L-tryptophan dose (mg/Kg)				
		Vehicle	50	100	200	400
Amygdala	NaCl	357±32	446±22	457±37	500±37	630±78
	LiCl	510±48	651±19	520±50	642±42	798±45
Cerebellum	NaCl	133±7	178±12	226±11	217±17	-
	LiCl	206±10	252±11	269±21	270±24	-
Corpus striatum	NaCl	720±23	942±38	1007±79	1009±51	998±61
	LiCl	1146±136	1329±60	1303±103	1398±58	1477±74
Cortex, motor	NaCl	366±16	476±21	476±19	512±30	522±26
	LiCl	549±44	697±42	607±28	679±29	709±26
Cortex, temporal	NaCl	351±12	465±21	487±47	543±20	-
	LiCl	562±20	702±33	730±50	729±24	-
Hippocampus	NaCl	823±51	1019±46	1234±52	1345±55	1241±116
	LiCl	1201±79	1559±61	1415±90	1556±114	1494±143
Medulla	NaCl	493±47	730±17	884±79	959±106	-
	LiCl	828±58	1099±54	1499±65	1355±45	-
Midbrain	NaCl	689±25	1147±65	1246±85	1471±99	1256±61
	LiCl	1057±83	1705±92	1498±119	1895±172	1684±21

TABLE 14 continued

Brain Region		L-tryptophan dose (mg/Kg)				
		Vehicle	50	100	200	400
Olfactory tubercle bulb and tract	NaCl	412±21	529±42	604±28	643±28	601±20
	LiCl	572±46	710±37	702±41	766±50	814±34
Pons	NaCl	897±30	1310±56	1645±114	1774±129	2183±266
	LiCl	1506±46	1856±106	2306±70	2289±145	2737±305
Thalamus	NaCl	630±24	769±30	874±83	903±40	941±69
	LiCl	919±37	1152±57	1367±33	1225±65	1191±38

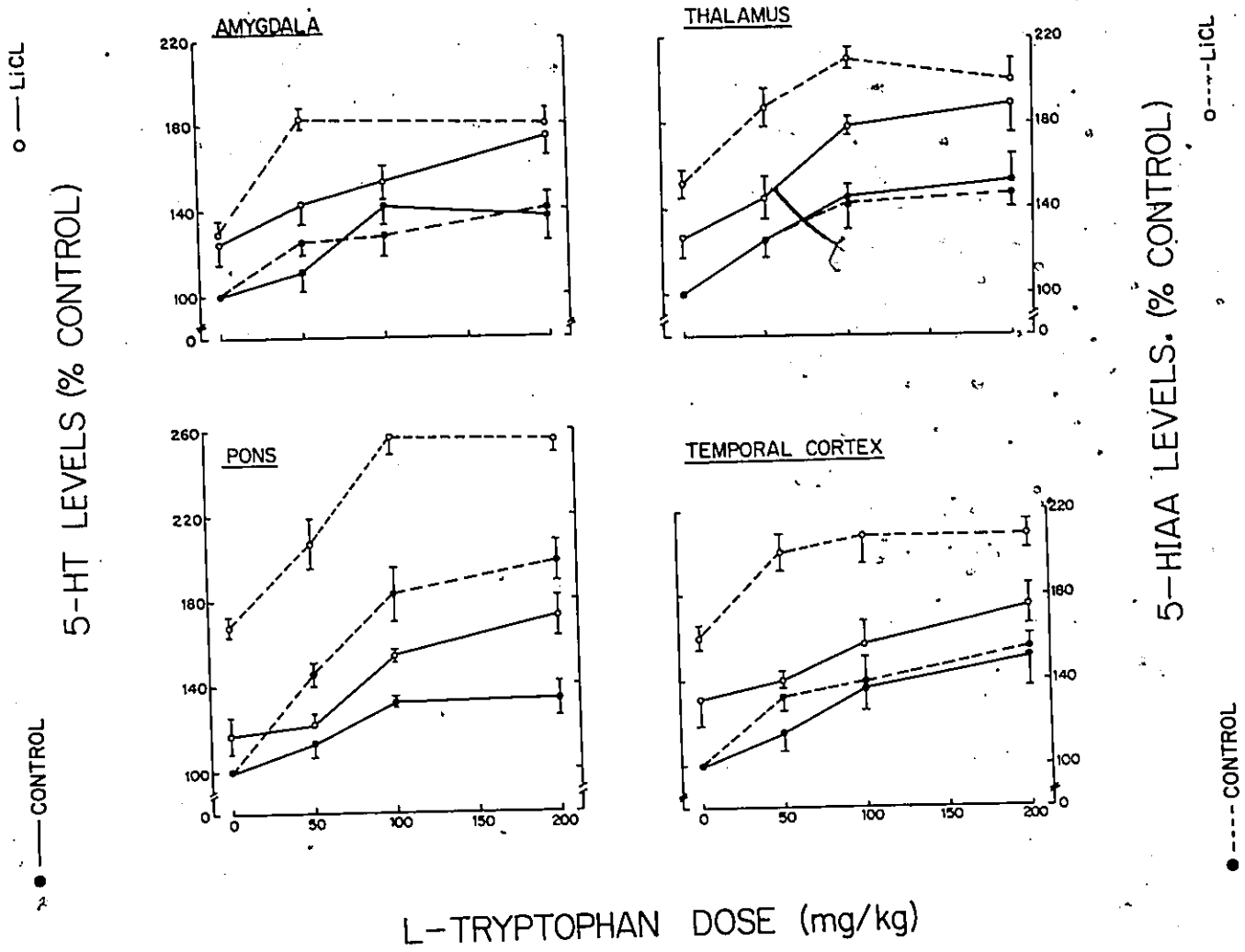

Fig. 13 Effect of precursor load on regional distribution of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in control and lithium-treated rats. Each point represents the mean ± S.E.M. of at least 6 determinations, and are expressed as percentage of control values. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days. Twelve hours later, groups of rats were given a single injection of L-tryptophan (50, 100, 200 mg/Kg, i.p.) or its vehicle and killed 60 minutes later. (Significance points are indicated in Fig. 14 and 15).

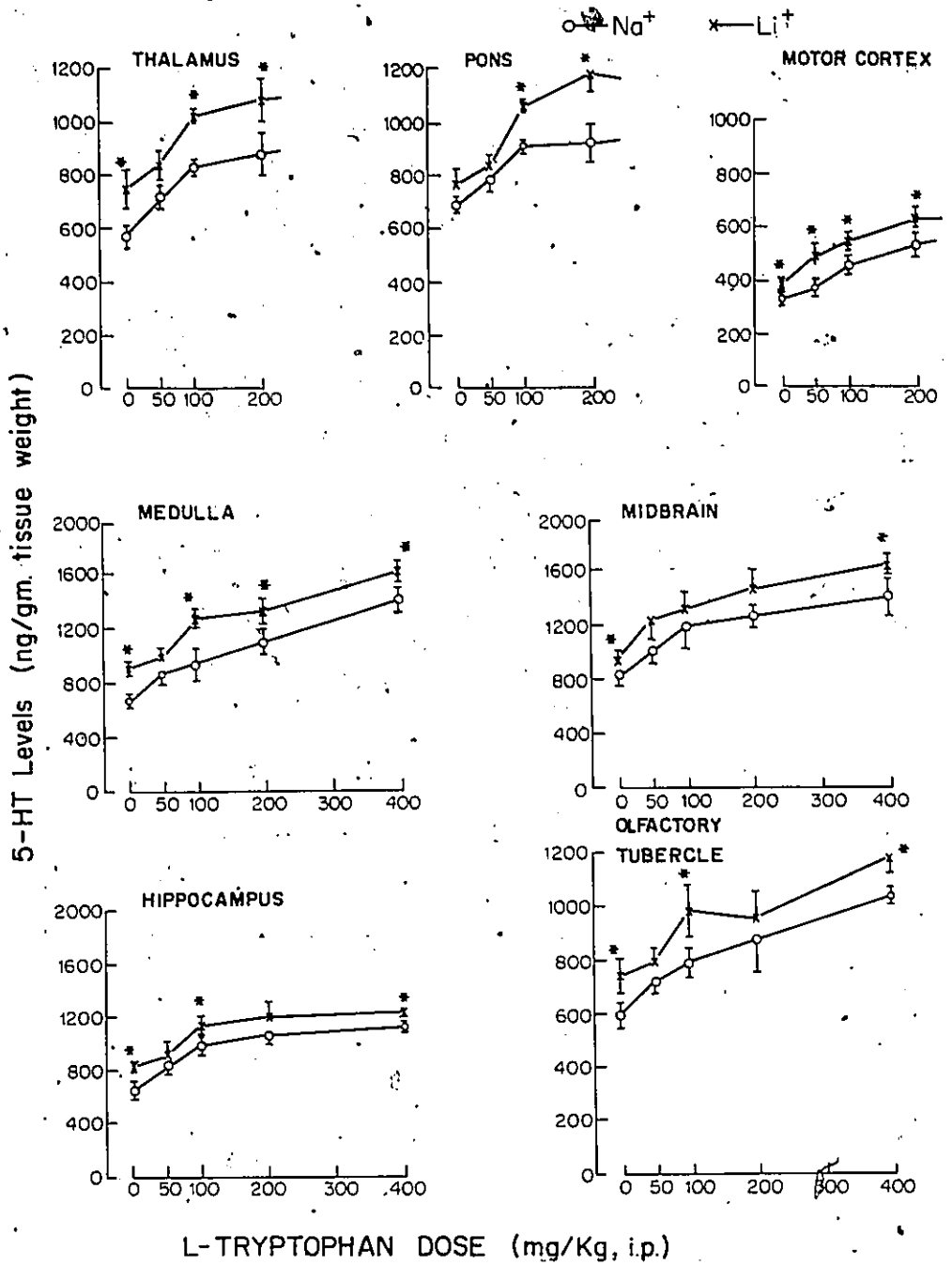

Fig. 14 Effect of precursor load on regional levels of 5-hydroxytryptamine in control and lithium-treated rats. Each point represents mean ± S.E.M. of at least 6 determinations. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days. Twelve hour later groups of rats were given a single injection of L-tryptophan (50, 100, 200 or 400 mg/Kg, i.p.) or its vehicle and killed 60 minutes later.

* $p < 0.05$ when compared with Na^+ control group.

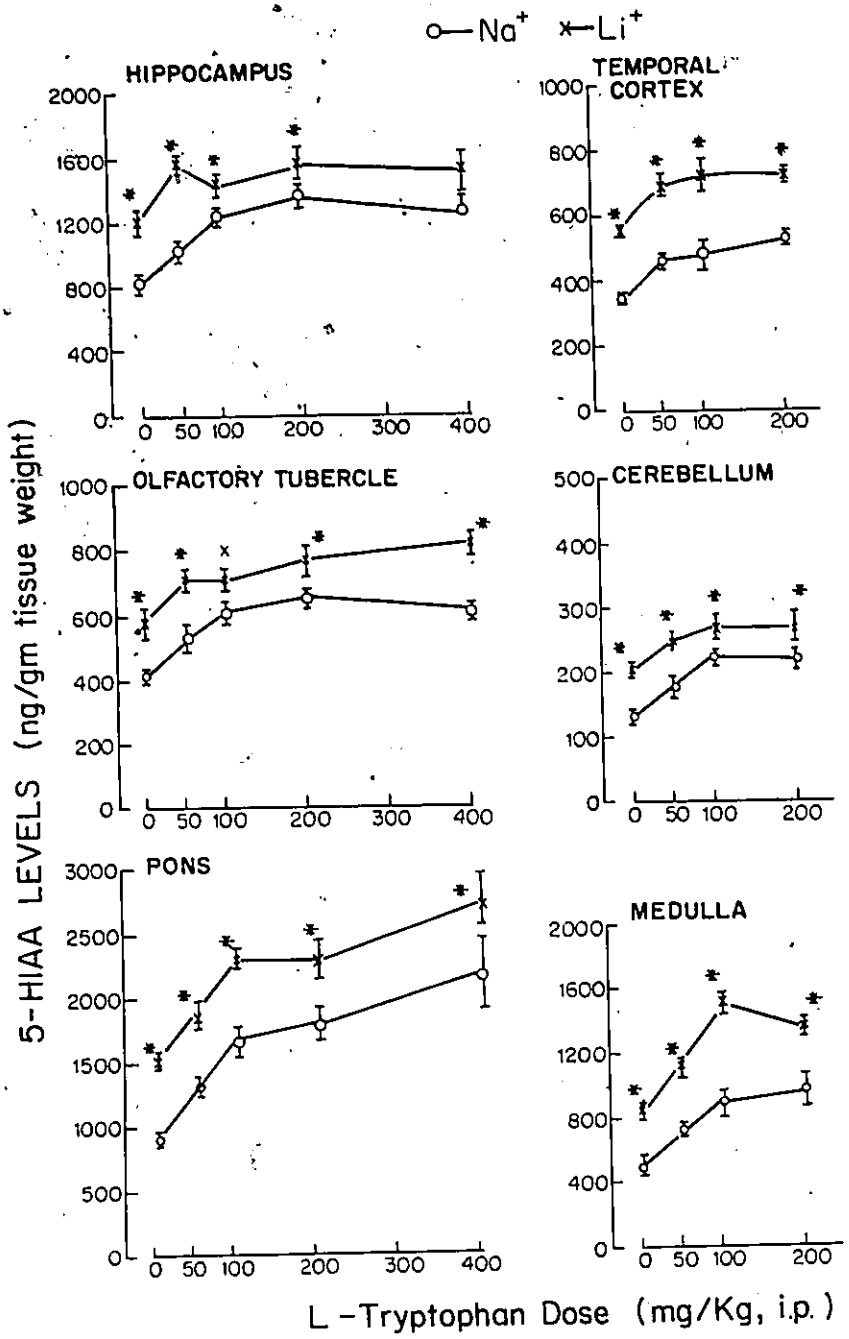

Fig. 15 Effect of precursor load on regional levels of 5-hydroxyindoleacetic acid in control and lithium treated rats. Each point represents mean ± S.E.M. of at least 6 determinations. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days. Twelve hour later groups of rats were given a single injection of L-tryptophan (50, 100, 200 or 400 mg/Kg, i.p.) or its vehicle and killed 60 minutes later.

* $p < 0.05$ when compared with Na^+ control group.

enhancement of 5-HT and 5-HIAA appears to be dose related in all the brain regions, and a plateau effect was achieved at doses of tryptophan between 100-200 mg/Kg. In lithium pretreated rats, the tryptophan-induced increase of 5-HT and 5-HIAA appeared to be significantly higher than in control animals. In addition, it was possible to achieve higher plateau levels in most brain regions following lithium treatment than in control animals.

Based on this data it is suggested that the effect of lithium is not simply to increase the availability of the substrate, but seems to involve a mechanism which allows for increased capacity of the tissue to convert tryptophan to 5-hydroxyindoles. The data is not consistent with the idea that the ability of lithium to increase brain 5-HT synthesis involves an increased transport ALONE into the brain, since lithium pretreatment produced a much enhanced effect on 5-hydroxyindoles in animals which had an excess of tryptophan in its system.

ii. Effect of precursor load on regional distribution of tryptophan in control and lithium-treated rats

Lithium was shown to produce significant elevations in both tryptophan levels and 5-hydroxyindoles in discrete regions of rat brain. It was therefore of interest to investigate whether the effects of lithium on the 5-HT-containing neurons could be explained by enhanced uptake into the brain from peripheral sources as well as explore the possibility that lithium may alter the endogenous pools of tryptophan, as was suggested by preliminary studies.

The results shown in Table 15 suggest that a tryptophan load of 100 mg/Kg causes significant elevations in the total plasma and plasma free tryptophan when given to rats treated with lithium for either

TABLE 15

THE EFFECT OF TRYPTOPHAN-LOAD ON PLASMA TRYPTOPHAN LEVELS OF LITHIUM TREATED RATS

Results are expressed as mean ± S.E.M. of at least 6 determinations. Rats were given twice daily NaCl or LiCl (2.67 mEq/Kg, i.p.) injections for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for two weeks. At end of treatment or 12 hours after the last injection groups of rats were given a single injection of L-tryptophan (100 mg/Kg, i.p.) and killed 60 minutes later. Figure shown in parenthesis represent the results as percentage of control values.

Lithium Treatment	Control + Vehicle	Control + L-Tryptophan	Lithium + Vehicle	Lithium + L-Tryptophan
Total plasma tryptophan (µg/ml)				
5 days	21.2±1.3 (100)	76.75±4* (362)	21.85±1.3 (103)	70.4±7.2* (332)
2 weeks	21.4±1.0 (100)	62.75±3* (294)	22.85±2.0 (108)	74.6±6.0* (349)
Plasma free tryptophan (µg/ml)				
5 days	1.29±0.13 (100)	20.90±1.5* (1625)	2.31±0.2* (179)	20.6±0.3* (1605)
2 weeks	1.14±0.08 (100)	11.13±1.1* (974)	1.80±0.21* (158)	11.1±0.6* (973)
Plasma % free tryptophan (%)				
5 days	6.06±0.6 (100)	27.23±3.0* (449)	10.55±0.7* (174)	28.7±1.3* (474)
2 weeks	5.35±0.4 (100)	17.74±2.0* (332)	7.90±0.8* (148)	14.9±1.6* (279)

* significantly different from control+vehicle groups ($p < 0.02$)

5 days or for 2 weeks, but this effect was not significantly greater than that produced by the precursor load given to control animals. The lithium-induced elevations in plasma free and total tryptophan produced prior to loading treatment was no longer observable when a high dose of tryptophan was given.

The effect of precursor loading on the brain levels of tryptophan in control and lithium pretreated rats is illustrated in Table 16, and figures 16 and 17. Whereas several brain regions demonstrated an additive effect of lithium treatment on tryptophan levels (cerebellum, pons, medulla, motor and temporal cortices, amygdala, hippocampus and thalamus following 5 days of lithium treatment; and cerebellum, pons, medulla and thalamus following 2 weeks of lithium treatment), a number of regions demonstrated effects which appear to be significantly greater than that produced by control rats receiving a tryptophan treatment (hypothalamus, olfactory tubercle bulb and tract, striatum, amygdala and midbrain). The latter effect appears to be more pronounced following 2 weeks of lithium treatment in a larger number of regions, viz- amygdala, motor and temporal cortices, hippocampus, hypothalamus, mid- brain, olfactory tubercle bulb and tract, pons and striatum. These data suggest that lithium alters the compartmentation of tryptophan in several brain regions in a time dependent manner since the observed changes were more pronounced following prolonged treatment with lithium.

TABLE 16

THE EFFECT OF PRECURSOR LOAD ON THE LEVELS OF TRYPTOPHAN IN DISCRETE BRAIN REGIONS OF LITHIUM TREATED RATS

Results are expressed as percentage of control values ± S.E.M. of at least 6 determinations. Rats were given intraperitoneal injections of NaCl or LiCl for 5 days (2.67 mEq/Kg, twice daily). Approximately 12 hours after the last injection, groups of 6 rats were given a single i.p. injection of L-tryptophan (100 mg/Kg) or its vehicle and killed 60 minutes later.

Brain Region	Control Rats			Lithium Treated Rats		
	Na^+ + Vehicle	Na^+ + L-tryptophan	% increase	Li^+ + Vehicle	Li^+ + L-tryptophan	% increase
Amygdala	100±8	569±44*	+469	118±5*	707±31*	+499
Cerebellum	100±16	590±19*	+490	121±11	650±64*	+437
Cortex-motor	100±4	520±25*	+420	118±6*	606±38*	+414
-temporal	100±8	496±37*	+396	150±9*	669±51*	+346
Hippocampus	100±6	660±41*	+560	131±9*	741±48*	+466
Hypothalamus	100±6	460±26*	+360	107±7	581±47*	+443
Medulla	100±12	439±16*	+339	99±4	489±52*	+393
Midbrain	100±14	581±34*	+481	105±6	708±49*	+574
Olfactory tubercle bulb and tract	100±8	438±18*	+338	104±6	593±20*	+470
Pons	100±8	495±24*	+395	129±14*	637±33*	+394
Striatum	100±7	475±24*	+375	104±6	575±27*	+453
Thalamus	100±6	489±28*	+389	117±6*	578±33*	+343

* $p < 0.025$ compared with Na^+ -vehicle treated rats

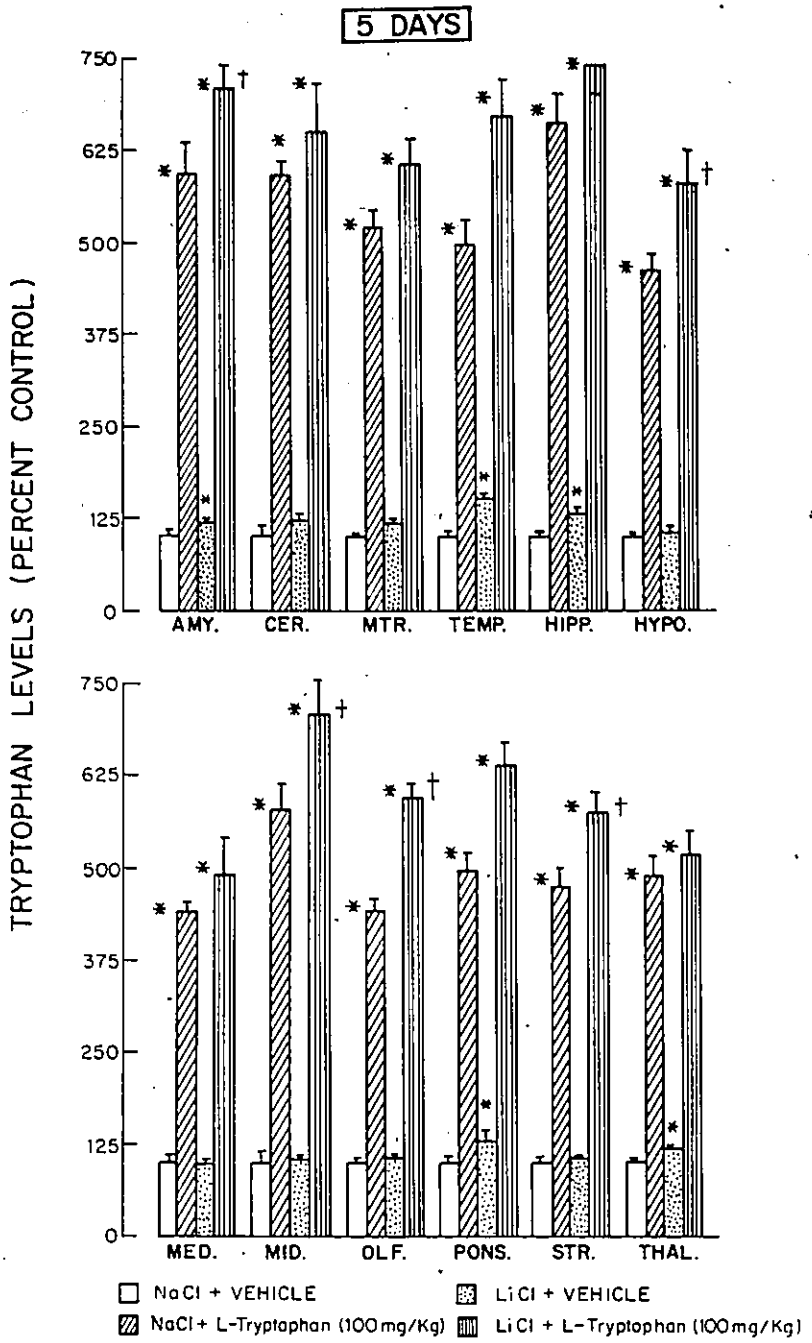

Fig. 16. Effect of precursor load on regional distribution of tryptophan in control and lithium-treated rats. Each bar represents the mean ± S.E.M. of at least 5 determinations, and are expressed as percentage of control values. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days. Twelve hours later, groups of rats were given a single injection of L-tryptophan (100 mg/Kg, i.p.) or it vehicle and killed 60 minutes later. Abbreviations as in Fig. 5.

* $p < 0.05$ when compared with NaCl + vehicle group
\+ $p < 0.05$ when compared with NaCl + L-tryptophan group

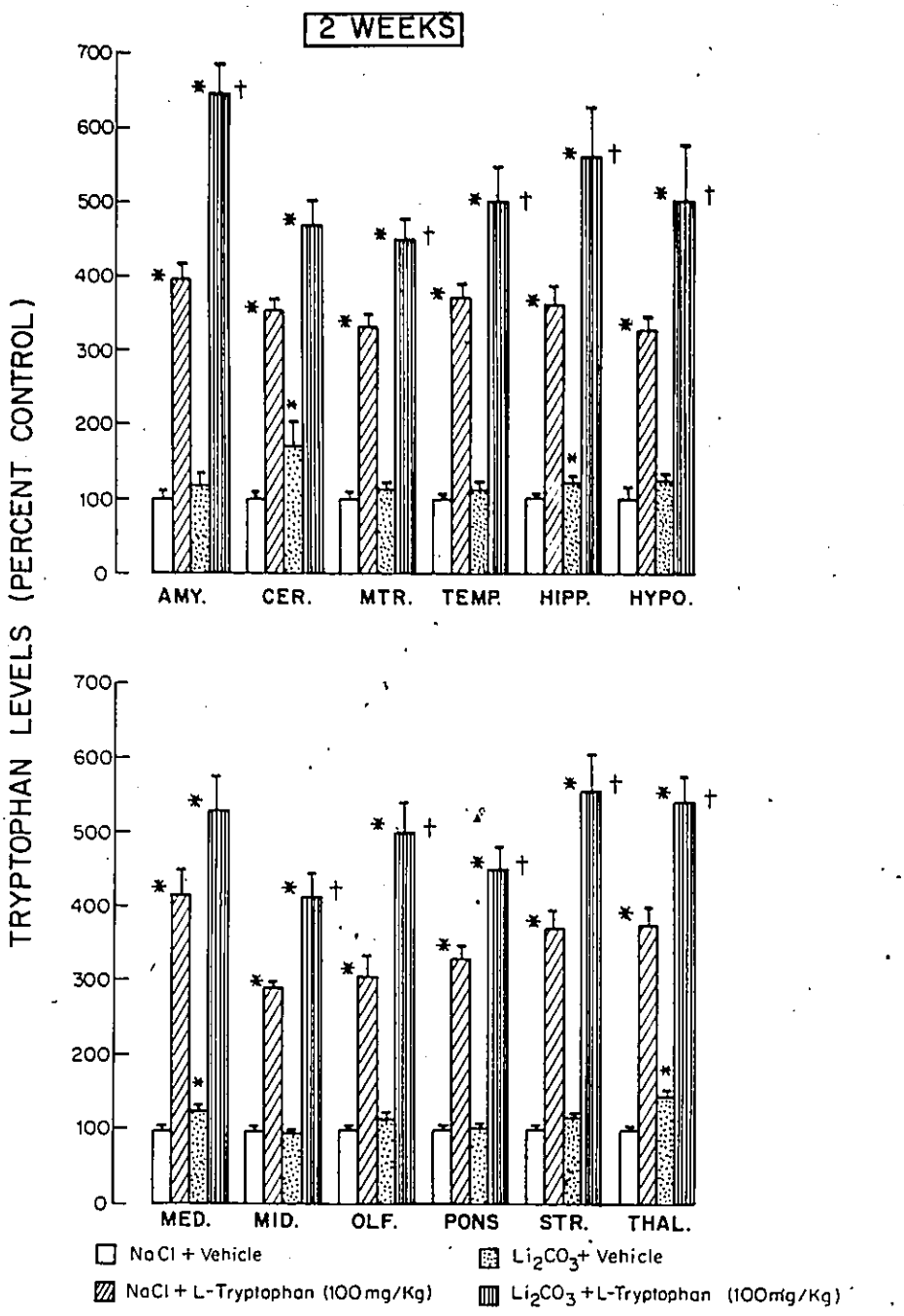

Fig. 17. Effect of precursor load on regional distribution of tryptophan in control and lithium-treated rats. Each bar represents the mean ± S.E.M. of at least 5 determinations, and are expressed as percentage of control values. Animals were given Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 weeks. Groups of rats were given a single injection of L- tryptophan (100 mg/Kg, i.p.) or its vehicle and killed 60 minutes later.

* $p < 0.05$ when compared with NaCl + vehicle group
\+ $p < 0.05$ when compared with NaCl + L-tryptophan group.

III. EFFECT OF LITHIUM ON CATECHOLAMINE-CONTAINING NEURONS

A. Effect of lithium on Regional Levels of Tyrosine, Norepinephrine and Dopamine

To examine whether short and long term lithium administration produces changes in catecholaminergic neurons, the influence of lithium on the levels of tyrosine, NE and DA and associated enzyme, tyrosine hydroxylase (TH) was investigated. While lithium treatment for 5 days did not produce significant changes in the levels of tyrosine and NE in most regions, significant increases were observed in the corpus striatum (32% above controls), olfactory tubercle bulb and tract (+ 15%) and the medulla (+ 19%) (Fig. 18 , Fig. 19). In contrast, no changes in the levels of DA were observed in the corpus striatum although a significant 23% elevation was found in the olfactory tubercle bulb and tract (Table 17).

Two weeks of oral lithium treatment (30 mEq/Kg food) did not modify regional tyrosine levels and significantly elevated the NE content in the pons only (20% above controls). There was a marked increase in the DA level in the olfactory tubercle bulb and tract, however no such changes were produced in the corpus striatum (Table 17). Similarly, following 5 weeks of oral lithium treatment the levels of tyrosine, NE and DA were not markedly altered in most brain regions, with the exception of an increase in the pontine NE and olfactory tubercle bulb and tract DA levels (Table 17, Figs. 18 and 19).

B. Effect of Lithium on Brain Tyrosine Hydroxylase Activity

Tyrosine hydroxylase exists in both soluble and particulate forms in the brain and it has been suggested that the particulate enzyme might be the more functionally active form (61). It was of interest therefore to study the effect of lithium on both the particulate and soluble

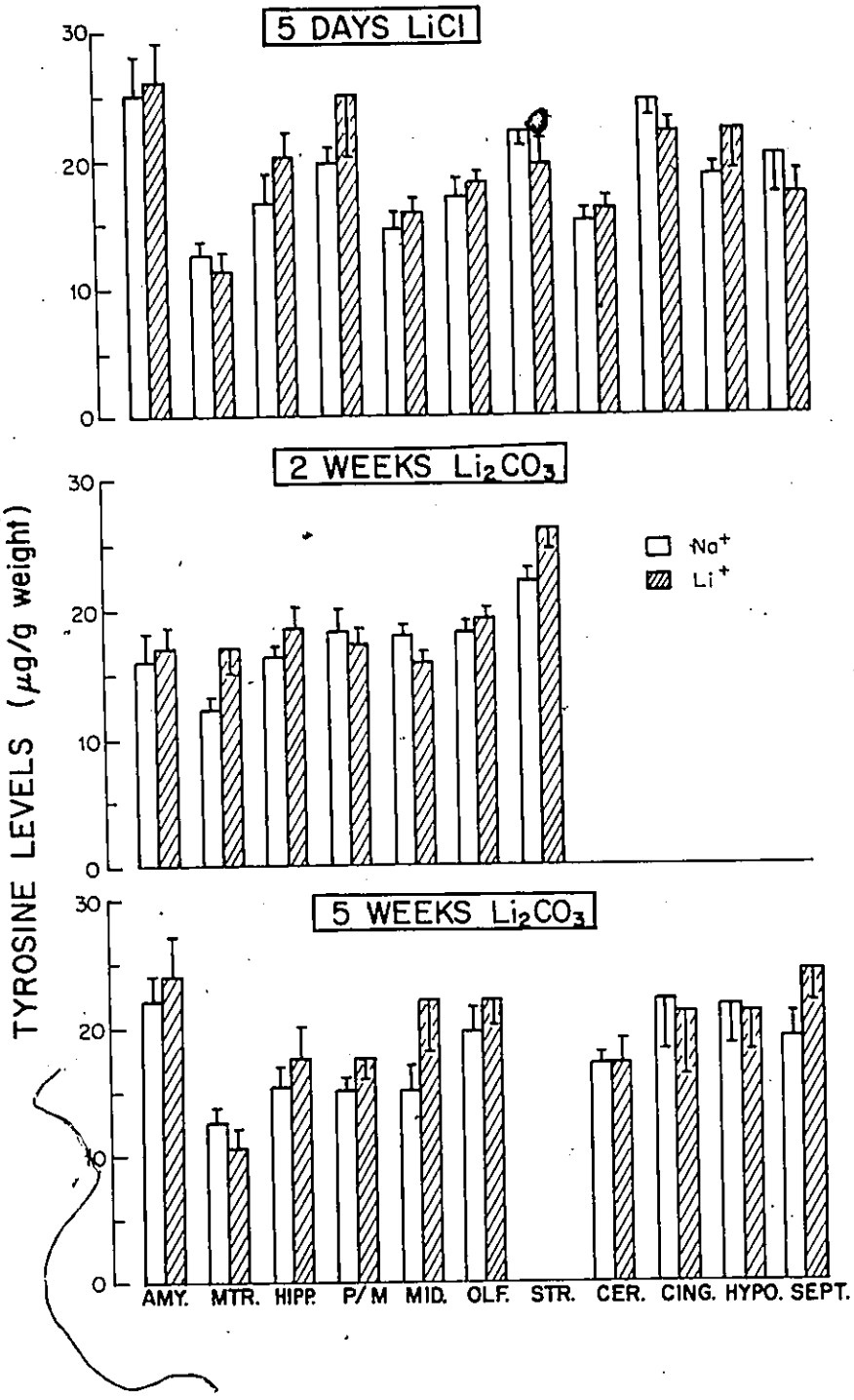

Fig. 18. Effect of lithium treatment on regional levels of tyrosine. Each bar represents the mean ± S.E.M. of 5-6 determinations. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks. Abbreviations as in Fig. 5.

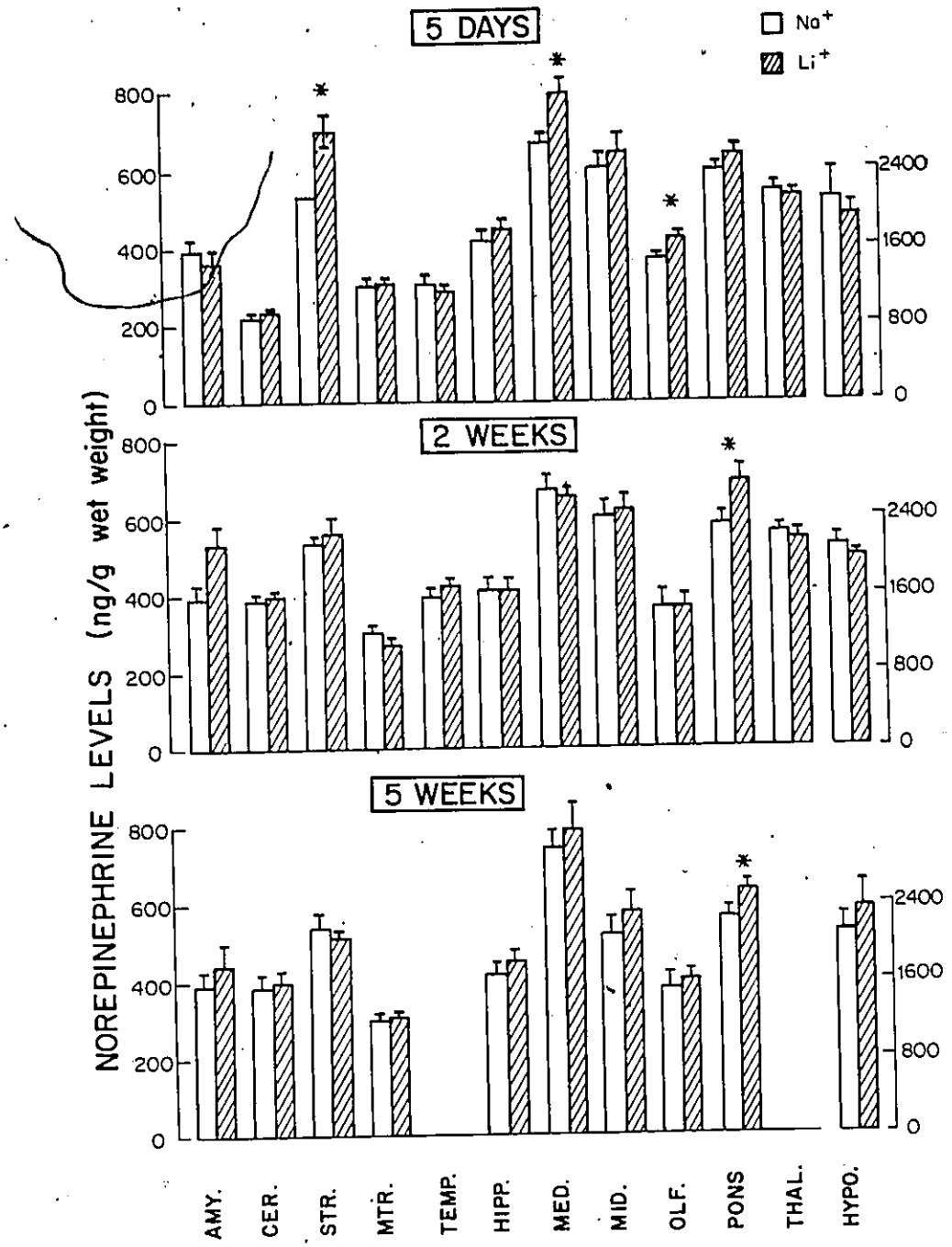

Fig. 19. Effect of lithium treatment on regional levels of norepinephrine. Each bar represents the mean ± S.E.M. of at least 6 determinations. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/Kg, i.p.) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks. Abbreviations as in Fig. 5.

* $p < 0.05$ when compared with respective control.

TABLE 17

LEVELS OF DOPAMINE AND NOREPINEPHRINE IN SELECTED BRAIN REGIONS FOLLOWING SHORT AND LONG TERM LITHIUM ADMINISTRATION

Results are represented as mean ± S.E.M. of 6-10 determinations. Rats were given either intraperitoneal injections of NaCl or LiCl (2.67 mEq/kg, twice daily) for 5 days or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/kg food) for 2 or 5 weeks.

Brain Regions	Treatment	DOPAMINE (ng/g)			NOREPINEPHRINE (ng/g)		
		Na^+	Li^+	%	Na^+	Li^+	%
Olfactory tubercle bulb and tract							
	5 days	3266±165	4030±505*	123	340±7	393±20*	116
	2 weeks	3155±316	4153±370*	132	364±45	367±33	101
	5 weeks	4038±356	5171±515*	128	378±37	403±28	107
Corpus striatum							
	5 days	6084±380	6428±426	106	533±36	703±45*	132
	2 weeks	5391±789	4584±542	85	533±14	555±43	104
	5 weeks	5523±226	6551±435	119	533±38	512±20	96

* significantly different from respective Na^+ control group of rats at $p < 0.05$

forms of the enzyme in a number of brain regions. Following 5 days of lithium treatment, the tyrosine hydroxylase activity measured in the intact sucrose homogenate was significantly increased in the hypothalamus (+ 23% above controls), but decreased in the cingulate gyrus (- 25%) (Table 18). The tyrosine hydroxylase activity of solubilized homogenate was not significantly different from control rats. However, there was a tendency of increased activity in the cerebellum (+ 65%), hypothalamus (+ 25%), cingulate gyrus (+ 56%) and decreased in the pons-medulla (- 25%).

Two weeks of lithium treatment did not modify the regional enzyme activity when measured in both the solubilized and intact sucrose homogenates with the exception of a significant 25% rise in activity of the intact fraction derived from the olfactory tubercle, bulb and tract (Table 19).

IV EFFECT OF NEONATAL ADMINISTRATION OF CENTRAL NEUROTOXINS ON BIOGENIC AMINES IN RAT BRAIN REGIONS

A. Time Course of Norepinephrine Changes in Brain Regions Following 6-Hydroxydopamine

The alterations in brain NE levels following a 100 µg/g dose of 6-OHDA injected subcutaneously on days one and two after birth is illustrated in Figure 20. The content of NE was significantly below control levels (Table 20) in the hypothalamus, hippocampus, olfactory tubercle bulb and tract, spinal cord and motor and temporal cortices at the age of 20 days. In contrast, at this age the NE content in the midbrain, pons, medulla and cerebellum was significantly elevated above

TABLE 18

THE EFFECT OF 5 DAYS LITHIUM CHLORIDE TREATMENT ON THE TYROSINE HYDROXYLASE ACTIVITY OF BRAIN REGIONS OF ACI RATS

Results are given as mean ± S.E.M. of 4-8 determinations. Animals were given twice daily injections of NaCl or LiCl (2.67 mEq/kg, i.p.) for 5 days. Approximately 12 hours after the last injection, groups of animals were killed.

Brain Region	Tyrosine Hydroxylase Activity (nmoles/g/hr)					
	Sucrose			Total		
	Control	LiCl	% Control	Control	LiCl	% Control
Amygdala	13.5±0.8	11.9±2.4	88	27.6±4.2	30.1±3.4	109
Cerebellum	3.8±0.2	3.3±0.4	88	3.9±0.7	6.5±1.2	165
Cingulate gyrus	14.9±1.0	11.1±1.0*	75	12.4±1.8	19.4±3.8	156
Corpus striatum	192±29	193±16	101	195±10	189±11	93
Cortex-motor	3.1±0.3	2.6±0.2	84	7.5±0.6	7.3±0.4	96
Hippocampus	7.7±1.2	10.1±0.9	130	20.3±4.8	15.7±1.4	77
Hypothalamus	24.1±1.2	29.7±2.2*	123	63.3±13.5	79.1±12.7	125
Midbrain	10.9±0.8	10.7±1.8	98	18.8±3.0	20.3±2.5	108
Olfactory tubercle bulb and tract	35.0±3.7	29.7±4.1	85	69.2±1.8	65.5±1.1	95
Pons-medulla	6.6±0.8	6.8±0.6	103	14.0±1.5	10.5±1.4*	75
Septum	57.1±6.8	51.4±7.8	90	60.8±5.5	57.7±4.3	95

* $p < 0.05$ when compared with respective controls

TABLE 19

THE EFFECT OF 2 WEEKS LITHIUM CARBONATE TREATMENT ON THE TYROSINE HYDROXYLASE ACTIVITY OF BRAIN REGIONS OF ACI RATS

Results are given as mean ± S.E.M. of 6 determinations. Animals were given sodium carbonate or lithium carbonate containing diet (30 mEq/kg food) for 2 weeks. At the end of the treatment groups of animals were killed.

Brain Region	Tyrosine Hydroxylase Activity (nmoles/g/hr)					
	SUCROSE			TOTAL		
	Control	Li_2CO_3	% Control	Control	Li_2CO_3	% Control
Amygdala	13.5±2.5	16.2±1.8	120	27.7±2.3	32.0±3.4	116
Corpus striatum	182±6	176±9	97	254±6	255±13	100
Cortex-motor	4.8±0.2	4.7±0.3	98	9.9±1.1	9.1±0.9	92
Hippocampus	10.1±1.3	11.1±0.7	110	21.7±4.7	27.2±2.1	125
Medulla	5.2±0.1	5.8±0.4	112	12.0±0.5	13.2±0.5	110
Midbrain	5.8±0.6	5.4±0.4	93	22.0±2.2	19.0±1.0	86
Olfactory tubercle bulb and tract	17.8±1.3	22.2±1.2*	125	67.4±4.8	69.8±2.5	104
Pons	5.8±0.3	5.6±0.5	97	16.6±0.5	15.1±2.7	91
Thalamus	6.1±0.2	5.6±0.3	92	30.2±1.2	29.3±1.3	97

* $p < 0.05$ when compared with respective controls

TABLE 20

NOREPINEPHRINE (NE) LEVELS IN RAT BRAIN REGIONS
AT 20, 40 and 60DAYS OF AGE

Results are given as mean ± S.E.M. for groups of at least 12 rats. All animals received once daily subcutaneous injections of saline on days 1-6 after birth*

Brain Region	Norepinephrine (ng/g) 20 days	40 days	60 days
Amygdala	465±23	434±63	432±15
Cerebellum	213±8	194±9	234±12
Corpus striatum	767±31	960±161	861±44
Cortex-motor	222±10	209±24	210±11
-temporal	243±10	209±19	226±7
Hippocampus	516±23	456±95	408±31
Hypothalamus	1944±102	1914±136	1921±123
Medulla	660±35	717±68	735±29
Midbrain	583±20	547±93	550±22
Olfactory tubercle, bulb and tract	520±16	593±58	576±28
Pons	817±18	805±94	836±23
Spinal cord	444±39	238±44	213±27
Thalamus	559±15	516±29	511±17

*Vehicle control injections on days 1 and 2 were not significantly different from saline-treated (1-6 days) group of rats.

control values. After this time both the increases and decreases in NE content continued to remain significant or more pronounced in several brain regions. In addition, the thalamus and the corpus striatum which at 20 days of age did not demonstrate significant changes, at later time periods (40 and 60 days) showed a significant elevation and reduction respectively.

B. Time Course of 5-Hydroxytryptamine and 5-Hydroxyindoleacetic Acid Changes in Brain Regions Following 6-Hydroxydopamine

The effect of 6-OHDA given neonatally on the levels of 5-hydroxyindoles was minimal for 20, 40 and 60 days of age. Out of some 13 brain regions only the spinal cord, thalamus, and the pons demonstrated significant differences in the levels of 5-HT and/or 5-HIAA (Table 21) at 60 days. The spinal cord demonstrated elevated 5-HT (+ 29% above controls) and 5-HIAA (+ 42%) content at approximately 58 days after the last injection. Similarly, the pons and the thalamus exhibited significant increases in the levels of 5-HIAA only after 60 days. Therefore, 6-OHDA did not produce profound disruption of 5-HT-containing neurons and appears to have selectively interrupted catcholamine-containing neurons.

C. Time Course of 5-hydroxyindoles Changes in Brain Regions Following 5,7-Dihydroxytryptamine

The administration of two subcutaenous injections of 5,7-DHT to 1 and 2 day old rat pups at a daily dose of 100 µg/g was shown to produce changes in the levels of both 5-HT and 5-HIAA in a regional-specific manner when measured at later time periods up to maturity (Figure 21). Two major patterns emerge: a) those brain regions which demonstrate a temporally-dependent increase in the level of both indoles (e.g. pons, medulla, thalamus, spinal cord, striatum,

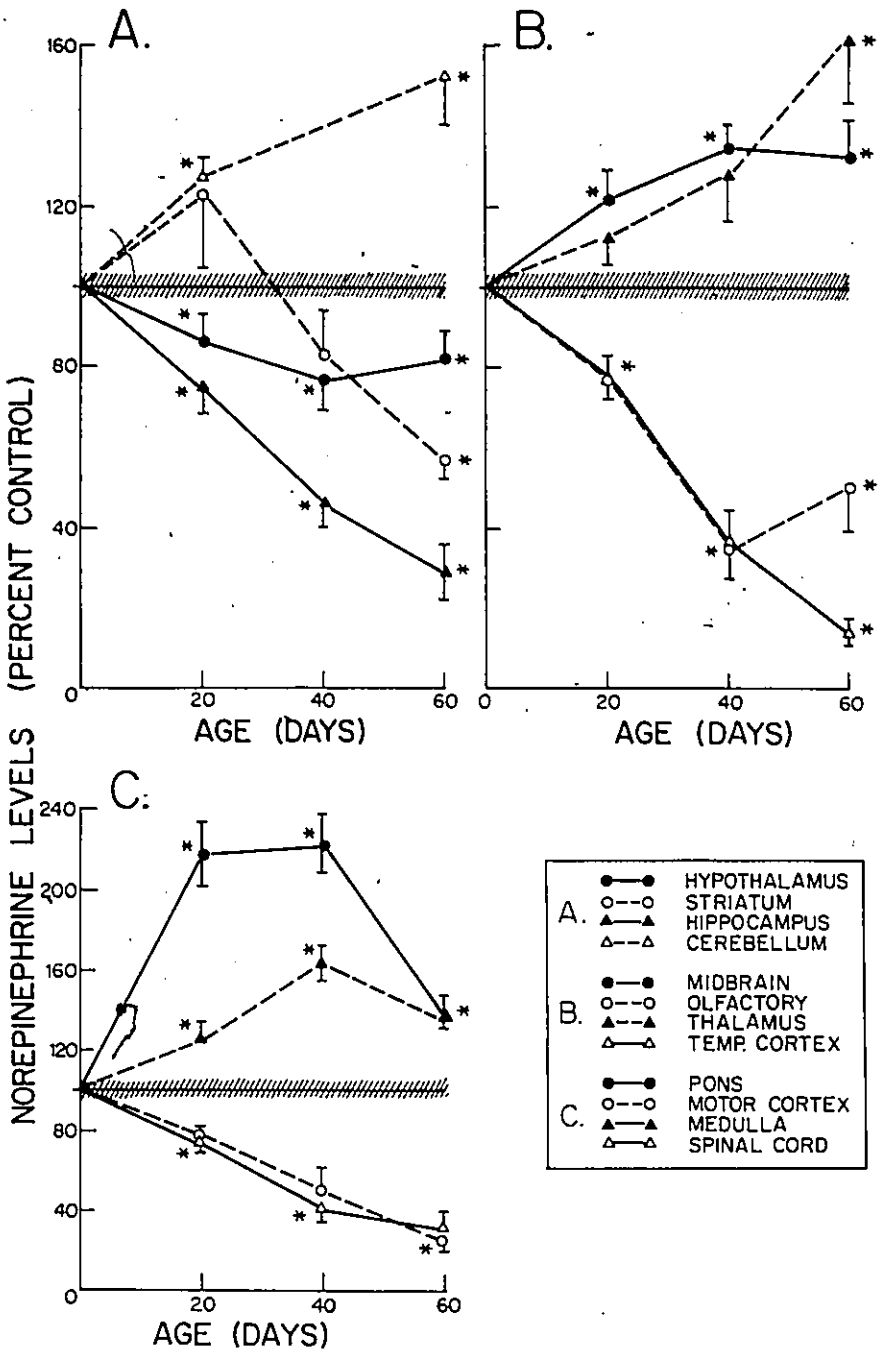

Fig. 20. Time course norepinephrine changes in brain regions following neonatal 6-hydroxydopamine. Each point represents the mean ± S.E.M. of at least 5 determinations and are expressed as percentage of control values (shown in shaded area). Pups received a single daily subcutaneous injections of 6-OHDA (100 µg/g) or its vehicle on days 1 and 2 after birth. Rats were killed at 20 days and the remainder weaned at 21 days and killed at 40 and 60 days of age.

* $p < 0.05$ when compared with respective control values.

TABLE 21

THE EFFECT OF NEONATAL 6-HYDROXYDOPAMINE (6-OHDA) ON 5-HYDROXYTRYPTAMINE METABOLISM IN BRAIN REGIONS OF 20, 40 AND 60 DAY OLD RATS

Results are given as mean ± S.E.M. of groups of at least 5 rats and expressed as percentage of control values. One and two day old pups received a daily subcutaneous injection of 100 µg/g 6-hydroxydopamine or its vehicle in a volume of 1 µl/g. Rats were weaned at 21 days and groups of animals were killed at 20, 40 or 60 days of age.

Brain Region	Age (days)	5-HT		5-HIAA	
		Control	6-OHDA	Control	6-OHDA
		(% controls)			
Pons	20	100±10	92±5	100±9	100±8
	40	100±6	96±4	100±7	115±10
	60	100±12	78±8	100±4	119±7*
Spinal cord	20	100±10	114±6	100±13	102±8
	40	100±8	100±14	100±10	104±8
	60	100±11	129±10*	100±7	142±10*
Thalamus	20	100±7	98±3	100±5	99±4
	40	100±9	117±11	-	-
	60	100±6	101±8	100±12	124±4*

* $p < 0.05$ when compared with respective controls

cerebellum); and b) those regions which show a temporally-related decrease in the levels of both indoles (e.g. amygdala, motor and temporal cortices, hippocampus, and the olfactory tubercle, bulb and tract). Invariably, robust changes in the levels of 5-HT and 5-HIAA were consistently observed by 60 days of age. However, two regions, the hypothalamus and the midbrain appeared to be resistant to 5,7-DHT treatment. It appears that 5,7-DHT was effective in depleting the levels of the indoles in the majority of the terminal regions, while elevating the levels in some regions containing 5-HT cell bodies.

D. Effect of 5,7-Dihydroxytryptamine on Brain Levels of Norepinephrine and Dopamine

Neonatal treatment with 5,7-DHT did not significantly alter the endogenous levels of NE in the majority of the brain regions when examined at later time periods (Table 22). However, several brain regions demonstrated reduced levels (corpus striatum, spinal cord and the olfactory tubercle bulb and tract) while the midbrain demonstrated increased levels at either 40 and/or 60 days of age. Therefore, 5,7-DHT treatment in the neonatal period did not produce profound disruptions of catecholaminergic neurons and appear to have more or less selectively interfered with the 5-HT-containing neurons.

E. Effect of Neonatal 4-Chloroamphetamine on Brain Regional Monoamine Levels

While doses of 10 and 15 µg/g 4-chloroamphetamine when given neonatally were found to be highly toxic and fatal to rat pups, a dose of 5 µg/g was found to be less toxic, and most of the animals survived this treatment. When 4-chloroamphetamine was injected subcutaneously on days 1-6 after birth it did not produce its characteristic depleting effect observed by comparable dose given to adult rodents. At 20 days

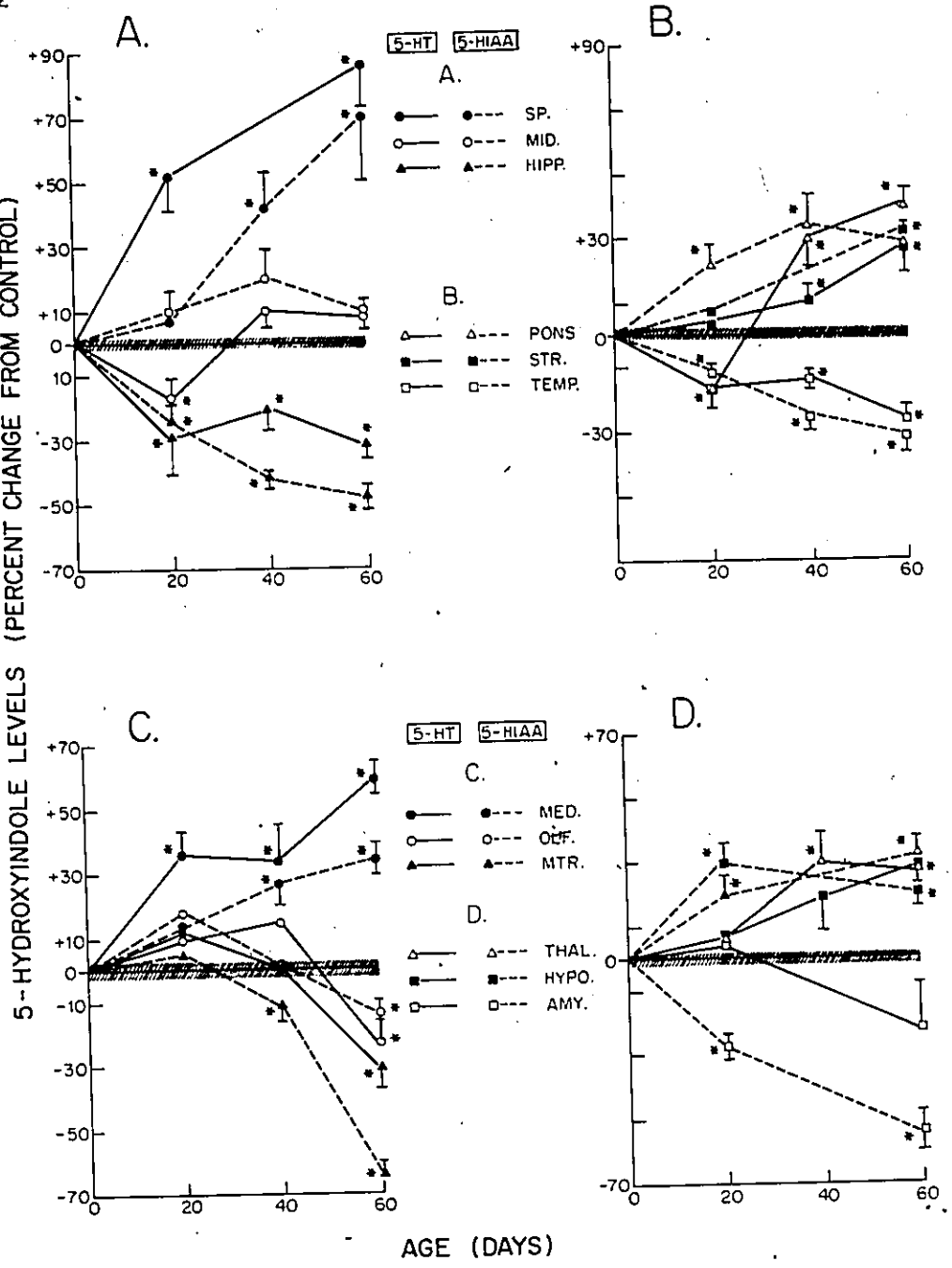

Fig. 21. Time course 5-hydroxyindoles changes in brain regions following neonatal 5,7-dihydroxytryptamine. Each point represents the mean ± S.E.M. of at least 5 determinations and are expressed as percentage of control values (shown in shaded area). Pups received a single daily subcutaneous injections of 5,7-DHT (100 µg/g) or its vehicle on days 1 and 2 after birth. Group of rats were killed at 20 days and the remainder weaned at 21 days and killed at 40 and 60 days of age.

* $p < 0.05$ when compared with respective control values.

TABLE 22

EFFECT OF NEONATAL 5,7-DIHYDROXYTRYPTAMINE ON NOREPINEPHRINE LEVELS IN RAT BRAINS AT 40 AND 60 DAYS OF AGE

Data is presented as mean ± S.E.M. of at least 5 animals in a given group. One and two day old rat pups were given a single daily injection of either 5,7-DHT (100 µg/g) or its vehicle s.c. in a volume of 1 µl/g. Rats were allowed to mature and were weaned at 21 days of age, and groups of animals were killed at 40 or 60 days of age. Values are given as percentages of control values.

Brain Region	NE (% controls) 40 days	60 days
Corpus striatum	58±6*	71±12*
Cortex-motor	119±6	91±4
-temporal	95±6	93±4
Hippocampus	114±17	93±10
Hypothalamus	103±7	124±13
Medulla	110±10	103±3
Midbrain	132±11*	98±6
Olfactory tubercle bulb and tract	89±13	103±4
Pons	123±12	91±4
Spinal cord	83±8*	98±12
Thalamus	108±15	96±9

* Significantly different when compared with respective control values at $p < 0.05$.

of age, 4-chloroamphetamine was shown to produce significant increases in striatal and medullary 5-HT and/or 5-HIAA and significant decreases in the temporal cortex (Table 23). Reduced levels of indoles were shown to persist only in the temporal cortex when measured at 60 days, with a tendency for lowered levels ($p > 0.05$) in the pons, medulla, hippocampus and the spinal cord. This neurotoxin did not significantly change the levels of NE in any of the regions except for the midbrain which demonstrated a small yet significant rise at 20 and 60 days. Based on these observations it is concluded that neonatal 4-chloroamphetamine is not effective in disrupting 5-HT and catecholamine-containing neurons during periods of early development.

V EFFECT OF NEONATAL ADMINISTRATION OF PSYCHOACTIVE DRUGS ON BIOGENIC AMINE LEVELS AT DIFFERENT PERIODS OF POSTNATAL DEVELOPMENT

A. Chlorpromazine (CPZ)

i. Effect of neonatal chlorpromazine on body and brain weight

Both 3 and 10 µg/g injections of chlorpromazine on days 1 through 6 resulted in prolonged decrease in body weight in male and female SD rats (Table 24). In contrast, the brain and cerebellar weights showed only minor decreases of up to 10% of control values when measured at 20 or 60 days of age.

ii. Effect of neonatal chlorpromazine on brain levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid

Table 26 shows the levels of 5-hydroxyindoles, calculated as percentage of control values (Table 25) in brain regions of rats receiving CPZ (3 or 10 µg/g) on days 1-6 after birth. In animals treated with 3 µg/g dose of CPZ the 5-HT levels were significantly elevated in 4 of 14 brain regions (corpus striatum, hypothalamus, medulla, spinal cord) when studied at 20 days of age, whereas when 10 µg/g dose of CPZ

TABLE 23

EFFECT OF NEONATAL 4-CHLOROAMPHETAMINE ON BRAIN LEVELS OF 5-HYDROXYTRYPTAMINE, 5-HYDROXYINDOLEACETIC ACID AND NOREPINEPHRINE AT 20 AND 60 DAYS OF AGE

Data is presented as mean ± S.E.M. of at least 10 determinations. Single daily subcutaneous injections of 4-chloroamphetamine (5 µg/g) or saline were given to pups on the first 6 days after birth, in a volume of 1 µl/g. Groups of rats were killed at 20 days and the remaining groups were weaned at 21 days of age and killed at 60 days. Values are given as percentage of control values.

Brain Region	20 days			60 days		
	5-HT	5-HIAA	NE	5-HT	5-HIAA	NE
Amygdala	94±13	97±9	126±8	-	-	-
Cerebellum	106±7	96±5	-	-	-	-
Cortex-motor	98±3	95±3	104±4	97±9	95±6	96±8
-temporal	87±10	82±4*	97±3	75±5*	81±6*	94±8
Hippocampus	101±4	94±5	112±7	80±5*	105±7	98±8
Hypothalamus	94±4	97±4	85±8	108±4	-	112±13
Medulla	112±7	126±10*	108±4	89±7	87±9	101±4
Midbrain	109±6	96±3	113±3	87±14	116±10	119±5*
Olfactory tubercle bulb and tract	95±3	89±4	95±6	112±6	-	121±8*
Pons	98±3	98±4	109±9	75±2*	84±15	89±9
Spinal cord	93±8	91±5	88±7	88±9	74±17	103±11
Striatum	112±6	121±4*	107±7	94±11	-	-
Thalamus	100±6	95±5	111±4	109±11	-	99±12

* $p < 0.05$ when compared with corresponding control values.

TABLE 24

THE EFFECT OF REPEATED CHLORPROMAZINE (CPZ) INJECTIONS TO NEONATAL RATS ON BODY AND BRAIN WEIGHTS AT 20 AND 60 DAYS OF AGE

Results are given as mean ± S.E.M. for groups of at least 12 rats. CPZ was given subcutaneously once daily on days 1-6 after birth at a dose level of either 3 or 10 µg/g. Controls received the saline vehicle alone. The figure in parenthesis is the percentage of the control value.

Treatment (dose µg/g)	Body weight (g)		Brain weight (mg)			
			Whole brain		Cerebellum	
	20 Days	60 Days	20 Days	60 Days	20 Days	60 Days
Male rats						
Saline	45.0±0.8	314±8	1582±23	2078±23	189±2	277±6
CPZ (3)	35.9±1.0* (80)	264±10* (84)	1487±23* (94)	2082±34 (100)	178±3 (94)	270±5 (97)
CPZ (10)	39.4±1.6* (88)	237±17* (75)	1479±44* (93)	2037±71 (98)	179±4 (95)	258±12 (93)
Female rats						
Saline	45.0±0.8	210±4	1557±33	2038±31	182±3	264±4
CPZ (3)	41.8±1.5 (93)	191±7* (91)	1483±27 (95)	1928±52 (95)	178±3 (98)	242±7* (92)
CPZ (10)	38.4±4.0* (85)	170±8* (81)	1450±32* (93)	1881±55* (92)	179±4 (98)	238±10* (90)

* $p < 0.05$ when compared to control values.

TABLE 25

5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) LEVELS IN RAT BRAIN AT 20, 40 AND 60 DAYS

Results are given as mean ± S.E.M. for groups of at least 12 rats consisting of equal number of males and females. All rats received once daily subcutaneous injection of saline on days 1-6 after birth.

Brain Region	5-HT (ng/g)			5-HIAA (ng/g)		
	20 Days	40 Days	60 Days	20 Days	40 Days	60 Days
Amygdala	687±79		813±47	347±21		498±21*
Cerebellum	278±20	-	334±29	158±5	-	203±10
Corpus striatum	751±36	893±48	826±97	514±34	596±36	613±28*
Cortex-motor	293±11	298±4	382±17*	176±4	260±8	252±7*
-temporal	337±27	330±4	317±10	165±9	260±7	276±8
Hippocampus	478±37	576±24	629±46*	473±16	506±22	664±22*
Hypothalamus	1198±55	1373±178	1322±87	827±47	-	886±54
Medulla	840±78	740±36	1189±54*	650±38	709±12	670±33
Midbrain	826±78	710±51	865±31	706±34	720±25	676±42
Olfactory tubercle, bulb and tract	466±54	433±84	540±31	469±34	-	445±32
Pons	923±30	752±40	850±18	837±50	874±64	862±38
Septum	2041±101	-	1813±138	1408±66	-	1322±117
Spinal cord	740±38	1004±64*	983±101*	398±28	342±15	423±27
Thalamus	726±42	706±66	868±52*	503±23	-	576±20

* $p < 0.05$ when compared with 20 day old animals

TABLE 26

THE EFFECT OF REPEATED CHLORPROMAZINE (CPZ) INJECTIONS TO NEONATAL RATS ON 5-HT AND 5-HIAA LEVELS IN RAT BRAIN REGIONS AT 20 AND 60 DAYS OF AGE

Results are given as mean ± S.E.M. for groups of at least 12 rats and are expressed as percentage of control values (given in Table 25). The groups consisted of equal numbers of male and female rats. CPZ was given subcutaneously once daily on days 1-6 after birth at a dose level of either 3 or 10 µg/g.

Brain Region	Dose of CPZ (µg/g)	5-HT (% control) 20 days	5-HT (% control) 60 days	5-HIAA (% control) 20 days	5-HIAA (% control) 60 days
Amygdala	3	111±7	135±6*	109±14	118±6*
	10	136±15*	150±6*	99±6	110±6
Cerebellum	3	115±10	110±4	96±6	105±7
	10	108±7	118±9	102±5	91±9
Corpus striatum	3	126±16*	98±5	104±8	88±4*
	10	138±14*	104±5	115±8	95±7
Cortex-motor	3	107±5	113±5	110±5	98±9
	10	80±4*	111±5	102±5	84±6*
-temporal	3	90±7	103±3	87±9	114±6
	10	128±11*	102±5	91±6	105±5
Hippocampus	3	100±6	114±4	105±5	88±8
	10	84±9	109±5	100±8	90±5
Hypothalamus	3	155±16*	100±7	101±8	116±8
	10	155±17*	153±16*	116±10	128±10*
Medulla	3	129±7*	102±4	91±5	104±6
	10	108±7	106±5	98±8	86±5*
Midbrain	3	110±9	104±4	105±3	100±8
	10	142±13*	97±4	101±7	99±9
Olfactory tubercle	3	106±8	125±6*	91±4	111±5
bulb and tract	10	129±10*	112±6	99±4	93±6
Pons	3	113±5	113±5	110±3	103±4
	10	117±7*	101±4	111±6	96±7
Septum	3	86±11	127±13*	90±11	110±9
	10	152±24*	109±11	112±15	85±10
Spinal cord	3	142±12*	115±9	124±14	100±6
	10	113±19	107±5	96±9	78±10*
Thalamus	3	102±5	103±4	92±5	97±4
	10	124±10*	104±4	98±7	86±4*

* $p < 0.05$ when compared to control animals

was used the 5-HT levels were significantly elevated in 9 brain regions and reduced in one (motor cortex) (Table 26). The extent of the CPZ-induced changes were much reduced by 60 days of age at which time the 5-HT levels showed significant increases only in the amygdala, hypothalamus, olfactory tubercle bulb and tract and septum (3 and/or 10 µg/g).

In contrast, 5-HIAA levels were not significantly different from control values at 20 days of age but showed significant differences at 60 days. With 3 µg/g dose of CPZ the 5-HIAA level was elevated in the amygdala and reduced in the corpus striatum, whereas in the group treated with the 10 µg/g dose of CPZ the 5-HIAA level was elevated in the hypothalamus and reduced in the motor cortex, medulla, spinal cord and thalamus.

iii. Effect of neonatal chlorpromazine on brain tryptophan levels

Of the ten brain regions examined none was shown to produce any significant change in the levels of endogenous tryptophan content of 20-day old CPZ pretreated rats (Table 27). Thus the changes in 5-hydroxyindoles induced by CPZ cannot be simply accounted for by changes in the levels of its precursor, L-tryptophan.

iv. Effect of neonatal chlorpromazine on brain monoamine oxidase activity

Data shown in Table 28 demonstrates that subcutaneous injections of CPZ (3 or 10 µg/g) on days 1-6 after birth causes only minor changes in the monoamine oxidase activity at 20 days of age. Following a dose of 3 µg/g of CPZ small but significant decreases in the activity was demonstrated in the amygdala, hippocampus and thalamus, while following a 10 µg/g dose of CPZ the enzyme activity was shown to decrease by up to 18% of control values in the amygdala, thalamus and the septum.

TABLE 27

THE EFFECT OF NEONATAL CHLORPROMAZINE (CPZ) ADMINISTRATION ON TRYPTOPHAN LEVELS IN RAT BRAIN REGIONS AT 20 DAYS OF AGE

Results are given as mean ± S.E.M. for groups of 6 rats. CPZ was given subcutaneously once daily on days 1-6 after birth at a dose level of either 3 or 10 µg/g. Controls received the saline vehicle alone.

Brain Region	Tryptophan		
	Control (µg/g)	CPZ (3) (% control)	CPZ (10)
Cerebellum	9.67±0.78	95±6	94±4
Corpus striatum	13.53±0.88	105±5	92±4
Cortex-motor	6.47±0.26	117±7	111±6
-temporal	6.73±0.27	102±6	102±6
Hippocampus	11.70±1.47	90±7	106±13
Medulla	11.73±0.56	98±4	96±7
Midbrain	11.32±0.53	104±5	105±5
Olfactory tubercle bulb and tract	9.63±0.31	103±10	106±6
Pons	11.56±1.31	112±9	104±8
Thalamus	12.37±0.22	92±3	105±8

TABLE 28

THE EFFECT OF NEONATAL CHLORPROMAZINE (CPZ) ADMINISTRATION ON THE MONOAMINE OXIDASE ACTIVITY IN BRAIN REGIONS OF RATS AT 20 DAYS OF AGE

Results are expressed as mean ± S.E.M. of 5-6 determinations. CPZ was given subcutaneously once daily on days 1-6 after birth at the dose of 3 or 10 µg/g. Control rats received the saline vehicle alone.

Brain region (dose µg/g)	Monoamine oxidase activity (nmoles/g/hr)		
	Control	CPZ (3)	CPZ (10)
Amygdala	150±8	131±7*	130±7*
Cerebellum	74±5	72±3	73±4
Corpus striatum	174±3	173±8	170±5
Cortex-motor	61±3	64±4	66±4
-temporal	61±4	55±4	57±4
Hippocampus	179±7	162±5*	166±6
Hypothalamus	277±10	306±22	291±12
Medulla	189±10	201±4	181±7
Midbrain	155±5	156±8	162±13
Olfactory tubercle bulb and tract	118±8	120±8	112±4
Pons	185±12	200±7	177±5
Septum	245±10	233±11	214±8*
Spinal cord	157±12	188±15	171±14
Thalamus	148±4	137±2*	121±3*

* $p < 0.05$ when compared with respective control values.

v. Effect of neonatal chlorpromazine on the brain levels of norepinephrine, tyrosine and tyrosine hydroxylase activity

Both 3 and 10 µg/g injections of CPZ on days 1-6 after birth failed to change the levels of NE in any of the brain regions when studied at 20 days of age (Table 29). The lower dose of CPZ did not produce a significant change in the tyrosine content in any of the regions except the hippocampus which demonstrated a decrease and the pons a significant increase when compared with controls. Similarly, the higher dose of CPZ caused a significant elevation in the levels of tyrosine in both the pons and the corpus striatum (Table 29). Tyrosine hydroxylase activity measured in solubilized sucrose homogenates, failed to change in 8 of 10 brain regions. Two brain regions, the hippocampus and the midbrain exhibited significant reductions of up to 25% of controls at 3 and/or 10 µg/g dose. It appears that neonatal CPZ causes only minor changes in catecholaminergic neurons when examined at 20 days of age.

B. Haloperidol

i. Effect of neonatal haloperidol on body and brain weight

Both 0.3 and 1.0 µg/g injections of haloperidol on days 1-6 resulted in no significant effect on rat body or brain weights at 20 days of age (Table 30).

ii. Effect of neonatal haloperidol injection on the brain levels of 5-hydroxyindoles

The effect of subcutaneous injections of haloperidol at doses of 0.1, 0.3 or 1.0 µg/g given to rats on days 1-6 after birth on the levels of 5-HT and 5-HIAA in selected brain regions is shown in Figure 22. There was a statistically significant dose-related increase in the levels of 5-HT in the cerebellum, pons and thalamus (to 116-149% of controls) whereas significant increases in the levels

TABLE 29

THE EFFECT OF REPEATED CHLORPROMAZINE (CPZ) INJECTIONS TO NEONATAL RATS ON THE LEVELS OF TYROSINE, NOREPINEPHRINE AND TYROSINE HYDROXYLASE (TH) ACTIVITY AT 20 DAYS OF AGE

Results are given as mean ± S.E.M. of groups of 6 rats and expressed as percentage of control values. CPZ was given subcutaneously once daily on days 1-6 after birth at a dose levels of either 3 or 10 µg/g. Controls received the saline vehicle alone.

Brain Region	CPZ dose (µg/g)	Tyrosine	NE	TH Activity
		(% controls)		
Cerebellum	3	102±3	97±8	107±5
	10	112±7	91±8	111±7
Corpus striatum	3	105±11	113±12	96±8
	10	157±15*	110±10	91±5
Cortex-motor	3	118±14	114±12	96±6
	10	113±18	105±10	96±12
-temporal	3	97±9	107±11	90±10
	10	103±9	115±13	102±15
Hippocampus	3	79±6*	95±14	81±4*
	10	89±3	98±9	83±3*
Medulla	3	105±4	102±12	90±9
	10	87±13	113±11	87±2
Midbrain	3	97±7	99±8	74±6*
	10	117±6	107±10	91±14
Olfactory tubercle bulb and tract	3	109±9	118±10	106±7
	10	95±9	103±9	100±6
Pons	3	136±8*	103±9	94±5
	10	128±8*	106±10	98±4
Thalamus	3	100±7	108±6	100±7
	10	100±9	104±6	121±12

* $p < 0.05$ when compared with control values

TABLE 30

THE EFFECT OF REPEATED HALOPERIDOL INJECTIONS TO NEONATAL RATS ON BODY AND BRAIN WEIGHTS AT 20 DAYS OF AGE

Results are given as mean ± S.E.M. for groups of at least 4 male and 4 female pups. Animals were given daily subcutaneous injections of haloperidol (0.3 or 1.0 µg/g) on days 1-6 after birth and were killed at 20 days of age. The figure in parenthesis is the percentage of the control value.

Treatment (Dose, µg/g)	Body weight (g)		Brain weight (g)	
	Male	Female	Male	Female
Saline	35.8±1.0	33.5±0.7	1508±25	1425±9
	(100)	(100)	(100)	(100)
Haloperidol (0.3)	35.0±1.4	33.3±1.2	1540±19	1462±17
	(98)	(99)	(102)	(103)
Haloperidol (1.0)	33.7±0.8	32.3±3.2	1493±15	1482±29
	(94)	(96)	(99)	(104)

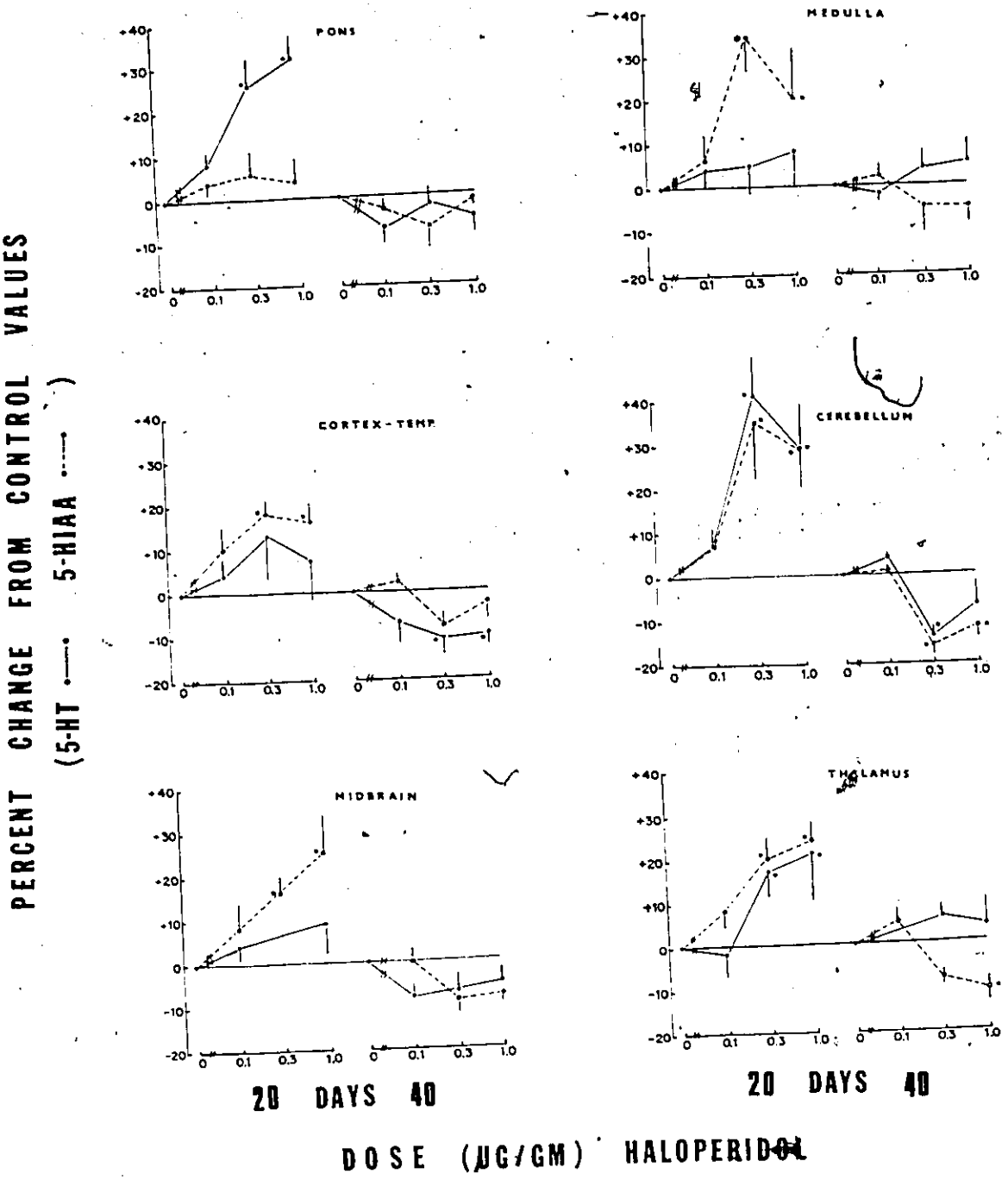

Fig. 22. Effect of repeated haloperidol injection to neonatal rats on the levels of 5-hydroxyindoles at 20 and 40 days of age. Each point represents the mean ± S.E.M. of 8-10 rats and expressed as percent change from control values. Rat pups were given daily subcutaneous injections of haloperidol (0.1, 0.3 and 1.0 µg/g) on days 1 through 6 after birth and were killed at 20 or 40 days of age.

* $p \leq 0.05$ when compared with respective control values (saline-treated).

of 5-HIAA (to 117-134% of controls) were observed in the medulla, midbrain, temporal cortex and thalamus in haloperidol pretreated rats. Neither 5-hydroxyindole was significantly affected in the amygdala, corpus striatum, hippocampus, hypothalamus, motor cortex, olfactory tubercle bulb and tract, or spinal cord when measured at 20 days of age. In contrast, at the age of 40 days only two brain regions, the cerebellum and temporal cortex, demonstrated significant reductions in the levels of 5-HT at 0.3 and/or 1.0 µg/g dose of haloperidol. In addition, the cerebellum exhibited significant reduction in the levels of 5-HIAA at 0.3 and 1.0 µg/g dose of haloperidol and the thalamus at 1 µg/g dose.

iii. Effect of neonatal haloperidol injections on the brain levels of catecholamines

Table 31 shows the NE and DA levels in two brain regions at 20 days of age following subcutaneous injections of haloperidol at the doses of 0.3 and 1.0 µg/g given on days 1-6 after birth. The hippocampus showed small but statistically significant increases in the NE level at 0.3 and 1.0 µg/g dose and an increased DA level at the highest dose level only. The NE level was significantly reduced in the hypothalamus in rats pretreated with the highest dose of haloperidol. There were no significant changes in either NE or DA in any of the remaining 11 brain regions. The haloperidol-induced changes at 20 days were not shown to persist to age of 40 days, since there was no longer any significant difference in the level of NE or DA in any of the brain regions (data not shown).

C. Lithium

The administration of lithium chloride at dose levels higher than 2 µEq/g to rat pups on days 1-6 after birth proved to be highly toxic and fatal for all the animals. However, following lower doses of lithium

TABLE 31

THE EFFECT OF REPEATED HALOPERIDOL INJECTIONS TO NEONATAL RATS ON THE LEVELS OF NOREPINEPHRINE (NE) AND DOPAMINE (DA) at 20 DAYS OF AGE

Results are given as mean ± S.E.M. of groups of at least 8 rats. Animals were given daily subcutaneous injections of haloperidol (0.3 or 1.0 µg/g) on days 1 through 6 after birth and were killed at 20 days of age. The figure in parenthesis represents percentage of saline control values.

Treatment (dose, µg/g)	NE (ng/g)		DA (ng/g)	
	Hippocampus	Hypothalamus	Hippocampus	Hypothalamus
Saline	249±11 (100)	1834±78 (100)	344±23 (100)	1035±59 (100)
Haloperidol (0.3)	292±13* (117)	1718±75 (94)	369±23 (107)	1087±95 (105)
Haloperidol (1.0)	289±17* (116)	1470±75* (80)	415±14* (121)	1161±91 (112)

* $p < 0.05$ when compared with saline controls.

TABLE 32

THE EFFECT OF REPEATED LITHIUM INJECTION TO NEONATAL RATS ON BODY AND BRAIN WEIGHTS AT 20 AND 60 DAYS OF AGE

Results are given as mean ± S.E.M. of at least 12 rats. Lithium was given subcutaneously once daily on days 1-6 after birth at a dose level of 1 or 2 μEq/g. Controls received the saline vehicle alone. The figure in parenthesis is the percentage of the control value.

Treatment (dose μEq/g)	Body weight (g)		Brain weight (mg)			
			whole brain		cerebellum	
	20 days	60 days	20 days	60 days	20 days	60 days
Male rats						
Saline	45.0±0.8	314±8	1582±23	2078±23	189±2	277±6
Lithium (1)	48.4±1.3 (108)	295±13 (94)	1589±15 (100)	2143±34 (103)	192±3 (102)	282±6 (102)
Lithium (2)	46.7±1.1 (104)	-	1500±18 (95)	-	181±7 (96)	-
Female rats						
Saline	45.0±0.8	210±4	1557±33	2038±31	182±3	264±4
Lithium (1)	48.4±1.3 (108)	200±6 (95)	1589±15 (102)	1983±42 (97)	192±3 (106)	255±6 (97)
Lithium (2)	46.7±1.1 (104)	-	1500±18 (96)	-	181±7 (100)	-

(1 and 2 μEq/g) all the injected pups survived the treatment. Both injections resulted in no significant effect on rat body and brain weights at 20 or 60 days of age (Table 32).

i. Effect of neonatal lithium injection on norepinephrine levels in rat brain

A dose of 1 μEq/g LiCl given once daily on days 1-6 after birth produced significant elevation in the levels of NE in the corpus striatum and hippocampus, and significant reduction in the pons , hypothalamus and thalamus when measured at 20 days of age (Table 33). A dose of 2 μEq/g LiCl produced significant elevations in the hippocampus, olfactory tubercle, bulb and tract and the thalamus, and significant reduction in the hypothalamus and temporal cortex. The lithium induced changes did not persist since at the age of 60 days, lithium no longer produce significant alterations in any of the brain regions (data not shown).

ii. Effect of neonatal lithium on tyrosine hydroxylase activity in rat brain

A subcutaneous injection of LiCl at a dose of 1 μEq/g produced significant increases in the enzyme activity in the olfactory tubercle, bulb and tract (+ 30% above controls) and the corpus striatum (+ 19%) and significant decreases in the amygdala (- 21%) and the pons (- 13%) when measured at 20 days of age (Table 34). At this dose, lithium did not produce significant changes in the activity in the remaining nine brain regions.

Lithium-induced changes in the levels of NE in the olfactory tubercle bulb and tract, corpus striatum and the pons may be related to similar changes observed in the tyrosine hydroxylase activity. However, no such a relationship appears to exist for the amygdala, hippocampus and the thalamus.

TABLE 33

THE EFFECT OF NEONATAL LITHIUM CHLORIDE ADMINISTRATION ON THE LEVELS OF NOREPINEPHRINE IN BRAIN REGIONS OF 20-DAY OLD RATS

Results are given as mean ± S.E.M. for groups of at least 8 rats and expressed as percentage of control values. Lithium was given subcutaneously once daily on days 1-6 after birth at a dose level of 1 or 2 μEq/g. Control pups received the saline vehicle.

Brain region (dose, μEq/g)	NE (% Controls)		
	Control	Lithium (1)	Lithium (2)
Amygdala	100±10	107±9	120±9
Cerebellum	100±7	94±2	100±6
Corpus striatum	100±8	134±11*	-
Cortex-motor	100±4	102±4	92±7
-temporal	100±6	102±5	82±8*
Hippocampus	100±4	118±5*	124±5*
Hypothalamus	100±6	81±4*	72±5*
Medulla	100±7	90±7	95±8
Midbrain	100±4	108±5	103±3
Olfactory tubercle bulb and tract	100±6	89±3	121±10*
Pons	100±7	84±5*	102±4
Spinal cord	100±9	115±12	120±24
Thalamus	100±3	85±3*	133±17*

* $p < 0.05$ when compared with saline controls

TABLE 34

THE EFFECT OF REPEATED LITHIUM CHLORIDE INJECTIONS TO NEONATAL RATS ON TYROSINE HYDROXYLASE (TH) ACTIVITY IN RAT BRAIN REGIONS AT 20 DAYS OF AGE

Results are given as mean ± S.E.M. for groups of 6-12 rats. Lithium was given subcutaneously once daily to pups on days 1-6 after birth at a dose level of 1 μEq/g and animals were killed at 20 days of age.

Brain Region	Tyrosine Hydroxylase Activity (nmoles/g/h)		
	Control	Lithium	% Control
Amygdala	16.5±2.0	13.1±0.5*	79
Cerebellum	6.4±0.3	6.5±0.7	102
Cingulate gyrus	30.4±3.4	28.2±2.8	93
Corpus striatum	155.0±7.0	184.4±8.8*	119
Cortex-motor	7.1±0.5	7.1±0.4	100
-temporal	5.1±0.3	5.2±0.8	102
Hippocampus	20.8±1.0	18.6±2.0	89
Hypothalamus	67.4±2.6	69.0±3.0	102
Medulla	24.1±1.4	22.4±1.0	93
Midbrain	29.9±2.6	35.2±5.6	118
Olfactory tubercle bulb and tract	35.1±1.1	45.6±0.8*	130
Pons	29.9±0.8	25.9±1.1*	87
Septum	90.3±5.2	101.9±10.6	113
Spinal Cord	30.2±1.8	26.6±2.8	88
Thalamus	41.2±3.0	39.0±5.5	95

* $p < 0.05$ when compared with control values.

iii. Effect of neonatal lithium on brain 5-hydroxytryptamine metabolism

The levels of 5-HT and 5-HIAA in discrete brain regions of 20 days old rats which had been pretreated with lithium on days 1-6 after birth is shown in Table 35 . In a number of regions significant changes in the 5-hydroxyindoles were noted. The levels of 5-HT following neonatal administration of LiCl at a dose of 1 μEq/g were significantly elevated in 4 brain regions (hippocampus, midbrain, temporal cortex and the thalamus), while the 5-HIAA levels were significantly increased in the amygdala and reduced in the hippocampus, hypothalamus, medulla, olfactory tubercle bulb and tract, and the thalamus. The extent of the lithium-induced changes was reduced by 60 days of age when significant increases in 5-HT levels were observed in the amygdala and hypothalamus and decreases in 5-HT and/or 5-HIAA in the hippocampus, medulla, olfactory tubercle, bulb and tract, pons, and the thalamus (Table 36).

A dose of 2 μEq/g LiCl caused significant increases in the levels of 5-HT at 20 days of age in the corpus striatum, temporal cortex, hippocampus, midbrain and thalamus. 5-HIAA levels were significantly reduced in three brain regions (corpus striatum, hypothalamus, and olfactory tubercle, bulb and tract) and increased in 4 brain regions (amygdala, motor cortex, midbrain and pons) (Table 35).

iv. Effect of neonatal lithium on monoamine oxidase activity in discrete regions of rat brain

Neonatal lithium treatment (1 μEq/g, s.c., on days 1-6 after birth) failed to modify the MAO activity in all brain regions except for the hypothalamus which showed significant increases (to 149% of controls), and the pons and hippocampus which showed significant decreases (to 80% of controls) at 20 days of age (Table 37).

TABLE 35

THE EFFECT OF REPEATED LITHIUM CHLORIDE INJECTIONS TO NEONATAL RATS ON THE LEVELS OF 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN BRAIN REGIONS OF 20-DAY OLD RATS

Results are given as mean ± S.E.M. of groups of at least 12 rats and expressed as percentage of control values. Lithium was given subcutaneously once daily to pups on days 1-6 after birth at a dose level of 1 or 2 μEq/g. Groups of animals were killed at 20 days of age.

Brain region (dose, μEq/g)	5-HT (% Control)		5-HIAA (% Control)	
	Lithium (1)	Lithium (2)	Lithium (1)	Lithium (2)
Amygdala	105±5	102±8	122±10*	117±8*
Cerebellum	94±4	96±5	99±3	104±4
Corpus striatum	103±3	126±8*	104±4	88±5*
Cortex-motor	107±6	84±12	98±4	118±5*
-temporal	121±9*	117±3*	106±5	100±5
Hippocampus	112±3*	128±8*	81±3*	-
Hypothalamus	108±4	99±10	78±7*	83±7*
Medulla	101±3	101±10	73±4*	-
Midbrain	120±6*	116±4*	103±4	122±3*
Olfactory tubercle bulb and tract	93±4	100±6	74±6*	87±4*
Pons	103±3	105±2	112±4	117±7*
Spinal cord	102±7	78±13	75±12	75±19
Thalamus	129±8*	137±10*	84±5*	100±3

* $p < 0.05$ when compared with saline controls.

TABLE 36

THE EFFECT OF REPEATED LITHIUM CHLORIDE INJECTIONS TO NEONATAL RATS ON THE 5-HYDROXYINDOLE LEVELS IN BRAIN REGIONS OF 60-DAY OLD RATS

Results are given as mean ± S.E.M. of groups of at least 12 rats and expressed as percentage of control values. Lithium was given subcutaneously once daily to pups on days 1-6 after birth at a dose level of 1 μEq/g. Animals were weaned at 21 days of age and were killed at 60 days of age.

Brain region	5-HT (% Control)	5-HIAA (% Control)
Amygdala	114±6*	95±7
Cerebellum	90±8	88±10
Corpus striatum	99±4	87±8
Cortex-motor	106±9	89±8
-temporal	93±4	114±6
Hippocampus	81±6*	77±6*
Hypothalamus	117±9*	94±8
Medulla	94±4	79±4*
Midbrain	102±5	109±7
Olfactory tubercle bulb and tract	91±5	69±6*
Pons	98±5	87±6*
Spinal cord	96±1	86±8
Thalamus	109±6	80±4*

* $p < 0.05$ when compared with saline controls.

TABLE 37

THE EFFECT OF REPEATED LITHIUM CHLORIDE INJECTIONS TO NEONATAL RATS ON MONOAMINE OXIDASE (MAO) ACTIVITY IN RAT BRAIN REGIONS AT 20 DAYS OF AGE

Results are given as mean ± S.E.M. for groups of 6-12 rats. Lithium was given subcutaneously once daily to pups on days 1-6 after birth at a dose level of 1 μEq/g. Animals were killed at 20 days of age.

Brain Region	Monoamine oxidase activity (nmoles/g/hr)		
	Control	Lithium	% Control
Amygdala	158±14	158±3	100
Cerebellum	85±3	85±6	100
Cingulate gyrus	206±5	226±16	110
Corpus striatum	189±19	181±20	96
Cortex-motor	92±6	87±5	95
-temporal	71±2	62±6	87
Hippocampus	201±4	173±2*	86
Hypothalamus	240±32	358±23*	149
Medulla	204±3	198±9	97
Midbrain	166±8	169±10	102
Olfactory tubercle bulb and tract	150±4	136±8	91
Pons	239±7	191±3*	80
Septum	225±12	239±12	106
Spinal cord	197±5	187±11	95
Thalamus	149±8	144±8	97

* $p < 0.05$ when compared with control values.

5. DISCUSSION

I. EFFECT OF NEUROTOXINS ON THE DEVELOPMENT OF BRAIN BIOGENIC AMINES

Since it was shown that the administration of 6-OHDA to rats in the immediate postnatal period produces long lasting effects on the catecholamine-containing neurons, it was of interest to examine the time course of this effect in a number of brain regions. Our results show that subcutaneous injections of 6-OHDA on days one and two after birth produce long term decreases in the NE levels of spinal cord, and in selected brain regions of the rat brain (e.g. hypothalamus, hippocampus, olfactory tubercle with bulb and tract, motor and temporal cortices) which persisted until at least 60 days of age. Unlike intraventricular injections (288) the striatal and hypothalamic NE levels were more resistant to damage following peripheral administration. This may result from an inability of the drug to reach these sites in high enough concentration to destroy the NE terminals. Our results confirm and extend earlier work in this area (35, 36, 37, 282, 284-288) in which either peripheral or intracranial routes of administrations were used.

In contrast to the major depletions of NE in forebrain regions, NE levels in brain stem regions and the cerebellum were dramatically elevated at 20 days of age and remained significantly elevated until at least 60 days of age. In cerebellum and thalamus the increases were more pronounced at 60 days than at 20 days whereas in the pons the reverse was true. While the mechanisms responsible for the elevated NE levels is not clearly established, there are a number of theories which were proposed to account for the observed changes. It has been

suggested that the elevations may be due to the accumulation of both enzymes and storage granules in the cell bodies (283), following destruction of terminals and perhaps axons. Alternatively, it has been suggested that destruction of forebrain terminals of axons arising from the NE cell body, the locus coeruleus results in anomalous sprouting in the regions containing the cell bodies or collateral growth from adjacent neurons (282, 283). In addition, it remains a possibility that the damage produced to certain catecholamine-containing neurons may also result in a reduced feedback inhibition at the level of the cell bodies, leading to enhanced firing rate and a compensatory increase in NE synthesis in regions containing the cell bodies (37, 294). The existence of such a feedback system acting on the locus coeruleus has been proposed to explain the actions of a number of drugs on the firing of the locus coeruleus neurons (294).

The effect of neonatal 6-OHDA on 5-HT containing neurons was shown to be minimal with reports of no change in 5-HT (284, 293), or the ^{3}H-5-HT uptake into cerebral cortex (282), although the 5-HIAA content and tryptophan hydroxylase activity were shown to be significantly increased in both the hypothalamus and the brain stem regions (35, 293). Our study is in general agreement with these reports. Out of 13 brain regions only the spinal cord, thalamus and the pons demonstrated significant increases in the levels of 5-HIAA. Thus neonatal 6-OHDA injections did not produce profound disruption of 5-HT containing neurons and appear to selectively interrupt catecholamine-containing neurons. The limited changes in 5-HIAA levels may be the result of compensatory changes secondary to the dramatic effects on NE neurons.

In the light of recent biochemical and morphological evidence that dihydroxytryptamines and 4-chloroamphetamine may be selective neurotoxins acting on the serotonergic neurons (30-32, 49), an attempt was made to demonstrate biochemically terminal or axonal degeneration similar to that shown for 6-OHDA. Our results demonstrate that subcutaneous injection of 5,7-DHT to one- and two-day old rats only produces long term decreases in the levels of 5-HT and/or 5-HIAA in selected brain regions (e.g. amygdala, motor and temporal cortices and the hippocampus) for at least 60 days. In contrast, the levels of 5-hydroxyindoles were significantly elevated in a number of brain regions (pons, medulla, thalamus, spinal cord, striatum and the cerebellum) with more pronounced increases observed at 60 days of age. However the midbrain appeared to be resistant to 5,7-DHT treatment.

Our results indicate both similarities and differences between the effects of intracisternal and peripheral 5,7-DHT administration in the neonatal rat. Unlike the intracisternal procedure (38) peripheral injections substantially elevated spinal cord 5-HT and 5-HIAA levels and did not significantly alter the indole levels in the midbrain and hypothalamus at the age of 60 days. The differences may be attributed to the differences in the amount of drug that reaches the tissue after intraventricular injection, and the time of biochemical estimation. It is possible that the 5,7-DHT-induced changes following intraventricular injections may be due to more nonspecific damage to regions lying in close proximity to the site of injection.

Lytle et al. (38) reported that following intracisternal injections of 5,7-DHT in doses up to 50 µg, whole brain and spinal cord 5-HT

and 5-HIAA levels were markedly reduced for as long as 240 days. It is of interest that the 5-HT reduction following 5,7-DHT was more pronounced following neonatal treatment (38, 39) than that produced in the adult rat. Sachs and co workers (29, 300) showed that systemic administration of newborn rats with 5,7-DHT at a dose similar to that used in the present study caused a 50% reduction of ^{3}H-5-HT uptake into cerebral cortex, or a 50% elevations in the ^{3}H-5-HT uptake into the pons-medulla at 7 days of age. Our observation of elevated levels of 5-hydroxyindoles in the pons and medulla and the reduced levels in the motor and temporal cortices is consistent with the uptake studies of Sachs (29, 300). The reduced levels of 5-hydroxyindoles in a number of terminal regions is consistent with the idea that the nerve terminals of 5-HT containing neurons are most sensitive to this drug.

The increases in 5-hydroxyindoles level in regions known to contain 5-HT cell bodies (e.g. pons, medulla) may be explained by the sprouting of drug-damaged 5-HT axons, and a possible collateral sprouting from adjacent neurons. Support for this notion is derived from studies involving adult rats. It was reported that the newly formed terminals can normalize the function of 5-HT synapses (302-304). Morphological and biochemical evidence suggest that more active and extensive regeneration occurs in the lower brain stem regions (29, 300, 304, 305). A number of likely explanations for the increased levels of the 5-hydroxyindoles can be offered. It is possible that 5-HT terminals arising from the 5-HT containing cell bodies degenerate to cause intensive sprouting in the region of the cell body (31). Alternatively, the increases may reflect increased synthesis in the

surviving terminals (38). It is conceivable that in addition to sprouting, damage to certain 5-HT containing terminals may result in a reduced feedback inhibition at the level of the raphe cell bodies, leading to enhanced firing rate and compensatory increase in 5-HT synthesis in regions containing the cell bodies. The existence of such a feedback inhibition system acting on the raphe system has been proposed to explain the effect of certain drugs on the firing of the raphe nuclei (91, 111).

In our study the thalamus was shown to be exceptionally sensitive to the actions of 5,7-DHT, while the hypothalamus was sensitive at later time periods. Since these regions were shown to contain one nucleus densely innervated with serotonergic terminals (ventrolateral geniculate nucleus and the suprachiasmatic nucleus respectively) (59, 344), it is possible that either enhanced synthesis of 5-HT or terminal regeneration during development may be responsible for the observed effects. However, confirmation of this awaits future research. The spinal cord, a region rich in both axons and axon terminals, was shown to be exceptionally sensitive to the actions of 5,7-DHT. It is conceivable that the elevated levels of 5-HT may reflect the combination of two effects: 1. terminal degeneration in the axon terminal portion of the cord, and 2. a subsequent compensatory sprouting in the axon rich aspect of the cord. However, more research is needed to explore this possibility.

Neonatal treatment with 5,7-DHT did not significantly alter the levels of NE in most brain regions examined with the exception of the corpus striatum and the spinal cord which showed decreases and the midbrain which showed increases. Our data on NE changes agree with

those of Lytle et al. (38) and Breese and Cooper (39) who showed only small decreases in the brain amine following intracranial administration, and of Jonsson (300) who demonstrated only slight decreases in the ^{3}H-NE uptake into the cerebral cortex and the corpus striatum following peripheral administration.

Available evidence suggests that there is a critical amount of 6-OHDA, 5,7-DHT or similar neurotoxins which a nerve terminal must concentrate before degeneration of the terminal occurs. The amount of the neurotoxin in the vicinity of the nerve terminal and hence the amount available for uptake depends on many factors such as dose of drug, route of administration, blood supply to the particular region and perhaps the proximity to the ventricular system. In neonatal studies additional factors are involved such as the maturity of the amine active uptake system in specific terminals and the local development of the blood-brain barrier.

Massive destruction of monoamine terminals appear to occur in certain brain regions as anticipated. Thus the hippocampus, temporal and motor cortices and the olfactory tubercle bulb and tract show marked depletion of NE following neonatal 6-OHDA. It is interesting that the same brain regions also show similar marked decreases in 5-hydroxyindole levels following neonatal 5,7-DHT treatment. All of these regions contain relatively high monoamine terminals.

However, other regions with numerous monoamine terminals such as the hypothalamus show little or no reduction in monoamine levels.

It is interesting that regions which show no decrease in monoamine content, and in fact tend to show highly significant increases are almost without exception fairly close to the regions containing the monoamine cell bodies. Thus medulla, pons, midbrain and thalamus usually show significant elevation in monoamine levels. It appears that terminals in these regions, since they are close to the cell bodies may mature earlier than those remote from the cell bodies such as in the cerebral cortex. Several authors speculated (29,282) that critical periods of maturation exist during which the terminals are particularly sensitive. For example, this could conceivably occur at a time after an effective active uptake system has formed but before there was a sufficiently rapid synthesis of the endogenous monoamine to dilute the neurotoxic analog (i.e. 6-OHDA or 5,7-DHT) in the terminal stores. If there is indeed a critical period during which the terminals are particularly sensitive it is possible that terminals in the hindbrain may have already passed this critical period before birth and thus be spared of massive destruction.

One of the puzzling features of this work is the marked increase in monoamine levels following neonatal administration of 6-OHDA or 5,7-DHT. This effect has been known for a number of years but our study showed the increases to be much more widespread than previously realized. Several theories have been advanced to explain this increase. An early suggestion was that the increases in the hindbrain were due to

a buildup of the transmitter in the cell bodies and axons following degeneration (283). However, the very long-lasting nature of the effect seems to argue against this possibility. Most of the current speculation centers around the possibility that following destruction of terminals, there follows regenerative or collateral "sprouting" in the vicinity of the cell bodies (282). Our results are generally consistent with this hypothesis.

It may be relevent that following treatment of adult rats with p-chloroamphetamine (4-CA), a drug with actions postulated to be similar to 5,7-DHT, there was an increased 5-HT level in parts of the spinal cord containing 5-HT axons (white matter) but a decrease in portions containing 5-HT terminals (grey matter) (314). Thus even in the adult, damage to terminals seems to be accompanied by an increased 5-hydroxyindole content in regions containing axons. It is interesting therefore that in our study, regions containing high 5-HT axons or dendrites (e.g. pons, medulla) usually show increases in monoamines.

One of the few regions in which the effect of 6-OHDA on NE level differs markedly from the effect of 5,7-DHT on 5-hydroxyindole levels was the spinal cord. 5,7-DHT produced a marked increase in spinal cord 5-hydroxyindole levels whereas 6-OHDA produced a marked depletion of NE. However, spinal cord was unusual in that it was one of only three brain regions that showed a significant change in 5-hydroxyindole levels following 6-OHDA. Interestingly, 6-OHDA produced a significant <u>increase</u> in 5-Hydroxyindoles in spinal cord similar to that produced after 5,7-DHT. This result may be relevant to a further hypothesis

regarding the ability of the neurotoxic analogs of the monoamines to produce long-lasting increases in monoamine levels in hindbrain regions. Since increases in monoamine levels can be produced without evidence of destruction of terminals containing that particular monoamine (i.e. increased 5-HT levels or turnover or increased tryptophan hydroxylase activity following 6-OHDA)(35, 293) a trans-synaptic mechanism may be involved. Interference with the functioning of certain neurons during development may result in a modified development of associated neurons, whether it involves altered number of processes or terminals or simply a modified activity of existing terminals. This may involve an alteration in the normal transsynaptic feedback inhibition control system.

There is substantial evidence to suggest that 4-CA exerts cytotoxic effects on 5-HT containing neurons (32, 49, 50, 306-313) but there is no general agreement as to the primary site of this effect (32, 309, 310, 313, 314). Since the bulk of the evidence was conducted on mature rodents, it was of interest to examine whether similar cytotoxic effects could be demonstrated to occur in the developing brain.

4-CA administration in neonatal rats has been shown to have no significant effect on the 5-HT content in the pontine raphe nuclei, area B9 and the forebrain (312). Our results confirm that regional 5-HT levels are practically unchanged by 4-CA treatment of neonatal rats, although the 5-HIAA levels are markedly increased in both medulla and striatum and decreased in the temporal cortex when measured at 20 days of age. However, significant reductions in the levels of 5-HT are observed in the pons, hippocampus and temporal cortex at 60 days of age. These results suggest that perhaps the depleting effect of

4-CA may become more pronounced at later periods of brain development. The lack of consistent changes produced by 4-CA on the 5-HT containing neurons suggest that it is not useful tool in studies involved in the elucidation of the function of these neurons in the developing brain. One cannot exclude the possibility that the dose used was not adequately high enough to be able to demonstrate persistent neurotoxic effects. Alternatively, it is conceivable that the active metabolite of 4-CA which might be responsible for the cytotoxic effects in the adult cannot be produced in adequate amounts in the neonate.

II. THE EFFECT OF NEONATAL CHLORPROMAZINE HALOPERIDOL AND LITHIUM ON THE DEVELOPMENT OF CATECHOLAMINE AND 5-HYDROXYTRYPTAMINE CONTAINING NEURONS

The perinatal period appears to be a vulnerable one in that certain drugs given during this period have been shown to produce persistent alterations in behavior and/or brain monoamine levels suggesting alterations in the development of monoamine-containing neurons. The mechanism by which such a change could occur remains unclear. It is possible that a drug-induced interference in synaptic transmission during the period of rapid terminal proliferation can produce changes in the structure or number of synapses formed leading to an altered steady-state level of the associated neurotransmitter. A drug action such as an extended period of DA-receptor blockade may also have effects on the development of post-synaptic neurons of other transmitters.

The present study demonstrates that whereas neonatal chlorpromazine resulted in only minor changes in the catecholamine-containing neurons when examined at 20 days of age using the levels of tyrosine, NE and TH

activity as a measure of the functioning of the neuronal system, CPZ was shown to cause significant changes in the 5-HT containing neurons. In fact, significant dose-related increases in the levels of 5-HT occurred in the amygdala, corpus striatum, temporal cortex, hypothalamus, midbrain, olfactory tubercle with the bulb and tract, the pons, septum and thalamus at 20 days-effects which cannot be explained by changes in the levels of tryptophan, or the monoamine oxidase activity. It is of interest, that in a number of regions known to contain high local concentration of 5-HT terminals (e.g. the amygdala, hypothalamus, olfactory tubercle, bulb and tract, and the septum), CPZ was shown to produce persistant increases in the levels of 5-HT up to at least 60 days. In contrast, the levels of 5-HIAA in a number of regions which at 20 days did not reveal any changes, by 60 days was shown to be significantly reduced (e.g. corpus striatum, motor cortex, medulla, spinal cord, and the thalamus). Assuming these changes to be a manifestation of an altered activity in specific 5-HT pathways, this data appears to be consistent with the notion that CPZ alters the development of a specific groups of 5-HT-containing neurons.

Studies involving the pre and neonatal exposure to CPZ on brain monoamines (41, 324, 325) showed inconsistent results. Tonge (324) reports a significantly increased 5-HT level in almost all the brain regions studied with little or no change in 5-HIAA levels. In contrast Nair (41) found reduced 5-HT and elevated 5-HIAA levels in whole brain following CPZ treatment, the effects being maximal when the drug was given either throughout the pregnancy or during days 18-21 of gestation. Evidently the effects of CPZ depends on the age at which

the drugs are given and/or whether the CPZ is given to the mother or directly to the offspring.

In addition, the present study demonstrates that normal development of neonatal rats following injections of CPA in the immediate postnatal week is disrupted. We observed a significant body and brain weight deficit which persisted to at least 60 days of age when CPZ was administered directly to the offspring on days 1-6 after birth. CPZ injections to pregnant female rats and mice have been shown to reduce body weight gain in the offspring when given at a daily dose of 10 mg/Kg or more (41, 322), although lower doses (1-8 mg/Kg) have usually been reported to have no effect on body weight (46, 319, 320, 323). Nair (41) reported that the suppression of body weight gain was maximal in animals receiving CPZ (10 mg/Kg, i.p.) during the last few days of pregnancy. Our data suggest that the reduction in body weight gain following prenatal exposure to CPZ is at least partly the result of a direct effect of the drug on the fetus.

The effect of CPZ on the serotonergic neurons could be a direct one, or it may be an indirect one acting through a secondary factor such as release of a hormone or neonatal malnutrition. It is therefore interesting that CPZ has been reported to block release of growth hormone (345, 346). Moreover, Stern et al. (348) have shown that if lactating female rats are given a low protein diet the offsprings show significantly higher brain 5-HT and 5-HIAA levels at 21 days of age, and when the offspring are fed a similar low protein diet after weaning, they continued to show high 5-HT and 5-HIAA levels at most

time periods studied until at least 300 days of age. However, it appears unlikely that our results could be explained solely in terms of a malnutrition effect. In the malnutrition experiments (348) the body weights were profoundly reduced being only 24% of control at 60 days of age. Similarly the brain weights were reduced by approximately 20% in the period up to 60 days. Moreover, a prominent effect of neonatal malnutrition was the increase in 5-HIAA which tended to be greater than the increase in 5-HT (347, 348). This is in marked contrast to our data on the effect of neonatal CPZ treatment. We attempted to produce animals which had their body weights reduced by the same amount as following neonatal CPZ by using larger than usual litters (16-18 pups per litter). The body weight gain in the pups was decreased to a similar amount as in our CPZ study (by approximately 10%) but there was no detectable change in either 5-HT or 5-HIAA levels in these animals (Taub and Peters, unpublished data). It was therefore concluded that the CPZ-induced changes in 5-HT and 5-HIAA changes in 5-HT and 5-HIAA levels are probably not a consequence of reduced food intake.

Our data is in agreement with the hypothesis that CPZ given in early life produces long-lasting alterations in 5-HT and 5-HIAA levels in certain regions of rat brain suggesting an altered development of 5-HT-containing neurons. It is conceivable that this effect may be related to the behavioral deficits produced by perinatal CPZ treatment (40). Although the present study may seem to suggest that synthesis and perhaps utilization of 5-HT is increased in several brain regions of CPZ treated rats, no conclusions can be drawn since it is not clear whether the increased 5-HT synthesis is secondary to

increased release from serotonergic neurons or to reduced intraneuronal deamination of 5-HT. However, from the available data it seems probable that the latter possibility is unlikely since MAO activity remained unchanged in most brain regions of neonatally CPZ-pretreated rats.

Treatment of newborn rats with haloperidol was shown to result in a significantly lowered maze performance in later life (321) Similar behavioral abnormalities were shown to occur following the administration of a pharmacologically similar drug, penfluridol. Biochemical evidence suggests that the offsprings exposed to penfluridol had reduced levels of monoamines and reduced rate of tyrosine hydroxylation at 28 days in the limbic system and brain stem areas (42, 48). Engel and Lundborg (42) suggested that the persistent changes after penfluridol is the consequence of treatment with the DA-receptor antagonist during the vulnerable period for the functional maturation of the central dopamine system. It was therefore decided to study whether treatment of newborn rats with haloperidol can also produce persistent changes in brain monoamine levels. It was found that rats treated with one of 3 doses of haloperidol showed a significantly increased level of 5-HT and/or 5-HIAA in 6 of 13 brain regions studied (pons, medulla, cerebellum, temporal cortex, midbrain and thalamus) whereas the catecholamine levels were virtually unaffected when studied at 20 days of age. However, the changes in 5-hydroxyindoles were transient since no effect was found at 40 days of age. It is of interest that the brain stem regions which contain 5-HT cell bodies showed significant increases in the levels of 5-HT and/or 5-HIAA following neonatal haloperidol treatment. However, the

haloperidol induced changes were not restricted to regions with high 5-HT cell body density since significant changes were also found in the cerebellum and cortex, regions with low levels of 5-HT. It is conceivable that the effects of neonatal treatment with haloperidol on 5-hydroxyindole levels in various brain regions are the result of abnormal development of specific groups of 5-HT containing neurons. The mechanism by which these changes occur remain unclear but it may be an indirect one involving an initial action on post-synaptic DA receptors. However, a direct effect cannot be ruled out, although a single 10 mg/Kg injection of haloperidol was reported to have no effect on brain 5-hydroxyindole levels in the adult rat (196).

In contrast, Grabowska (349) provided evidence to suggest that apomorphine, one of whose actions is as a central dopamine receptor stimulating agent (70a) increases the 5-HT turnover rate secondary to the stimulation of central dopamine receptors. In fact she provided evidence to suggest that apomorphine does not alter the levels of 5-HT in the adult brain but does cause an elevation in the levels of 5-HIAA. This observation was also confirmed by Scheel-Kruger and Hasselager (351). In addition it was shown that the apomorphine-induced elevations in 5-hydroxyindoles were prevented by prior treatment with haloperidol (350).

The apparent increased activity of 5-HT-containing neurons in several brain regions at least at 20 days is not consistent with the findings of Engel et al. (48) who studied penfluridol and reported that tryptophan hydroxylation was decreased in the limbic system (regions which they included to be contained within, olfactory tubercle, nucleus accumbens, septum and amygdala) and the brain stem of these rats.

It was of interest to explore the possibility that lithium, a drug with structural and pharmacological properties different from that of chlorpromazine and haloperidol, could produce long lasting alteration in the development of the monoamine-containing neurons. We find that in contrast to the effects produced by neonatal CPZ and haloperidol, lithium treatment produced significant changes in the levels of NE at 20 days of age. However these changes were transient since no effect was found at 60 days of age. Both increases and decreases in the levels of NE were shown to occur in a dose-related manner. Whereas the hypothalamus and the temporal cortex were shown to have reduced levels of NE, the corpus striatum, hippocampus, thalamus and olfactory tubercle with bulb and tract were shown to have increased levels of NE. Consistent with the increased NE levels in the olfactory tubercle and the corpus striatum, tyrosine hydroxylase activity was also shown to be increased in these regions. The data may suggest that catecholamine-containing neurons of the mesolimbic and nigrostriatal dopaminergic pathways may be activated by lithium treatment. However, it is also possible that lithium exerts an inhibitory influence on the dorsal noradrenaline-containing neurons, since pontine, cortical, hypothalamic, and amygdaloid NE levels and/or tyrosine hydroxylase activity were shown to be significantly reduced. However, the hippocampus which also belongs to this system showed a significantly increased NE level. The changes in NE levels were not restricted to regions containing the cell bodies of NE and DA, since terminal regions also demonstrated significant increases or decreases.

Treatment of newborn rats with lithium was shown to significantly increase the levels of 5-HT in a dose-related manner in 5 out of 13 brain regions (corpus striatum, temporal cortex, hippocampus, midbrain, and thalamus), whereas the levels of 5-HIAA were increased in the amygdala, motor cortex, pons, and midbrain, and reduced in the corpus striatum, hypothalamus, and olfactory tubercle when examined at 20 days of age. These changes were transient since many of the changes were no longer observable at 60 days. It appears that lithium produces an early stimulation of the 5-HT containing neurons, whereas by 60 days of age the net effect was a reduced activity as assessed biochemically with decreased 5-hydroxyindole levels found both in regions containing terminals (hippocampus, olfactory tubercle, and hypothalamus) and in regions containing the 5-HT cell bodies (e.g. pons and medulla).

The mechanism by which lithium produces these effects remain unclear, but studies on the development of non-mammalian species hints at the possible underlying mechanism (110). Indeed, Flickinger et al. (352), working with frog embryos, provided evidence that lithium produces teratogenic effects by inhibition of DNA synthesis, and reduction in cell numbers undergoing cell division. In addition, it was reported that early exposure of neonates to lithium seems to disorganize the development of CNS structures (138). Studies with mammalian species suggested that lithium may produce teratogenic effects (140-145), which may be related, at least in part, to altered development

of the CNS. It is therefore conceivable that lithium when given to the newborn rats may produce changes in the development of the monoamine-containing neurons. However, in our study no significant changes in the whole brain weight or body weights were observed in periods up to 60 days of age.

In conclusion, it was demonstrated that structurally dissimilar psychoactive drugs are capable of altering the development of certain groups of monoamine containing neurons. Our data provides additional biochemical evidence to support the suggestion that in fact the neurotransmitters may play a role in the development of the monoamine-containing neurons during critical stages of development. More consistent changes in the actions of these agents have been demonstrated on the 5-HT than catecholamine-containing neurons.

Since lithium, a metal cation with similar properties to functionally important cations such as Na^{+}, K^{+}, Mg^{++} and Ca^{++}, is capable of producing marked changes in the development of the brain monoamine-containing neurons, it may be speculated that some of the observed changes produced by CPZ and haloperidol may also be related at least in part to a possible influence on electrolytes in critical brain regions. It is not clear at the present what the common factor is, if indeed there is a common mechanism by which these drugs of widely differing chemical structure alter the development of the monoamine containing neurons. The suggestion has been made by several authors that the monoamines may play an important role in cell development in addition to their better known role as neurotransmitters (44) Since all the drugs studied appear to interact with monoamine receptors, either directly or indirectly, it is conceivable that the common

factor involves their interactions with 5-HT, NE or DA receptors. Other mechanisms, such as one involving interference with the actions of endocrine factors (e.g. corticosterone, thyroid hormones, sex hormones) on neuronal cell development cannot at present be excluded.

Indeed there is considerable evidence linking the central neurotransmitters with neuroendocrine regulation. Involvement of hypothalamic catecholamines in the modulation of release of hormones such as growth hormone, prolactin, ACTH and the gonadotrophins has been reported in many studies (345). Drugs which act on biogenic amine receptors may affect the release of these hormones. For example, in the mature rat apomorphine, a potent DA receptor stimulant, releases growth hormone (355) while chlorpromazine, one of whose action is to block dopamine receptors blocks growth hormone release (346), and secretion of gonadotrophins. Thus in the fetus and neonate, many drugs have the potential of altering the release of hormones essential for normal growth and development.

III. DISTRIBUTION OF LITHIUM IN THE CENTRAL NERVOUS SYSTEM

An important problem which complicates the study of the neurochemistry of lithium as related to its therapeutic efficacy in the treatment of mania, or manic-depressive psychosis in man is the fact that the distribution of the ion in the brain following its acute and chronic administrations has not been clearly established. It is desirable to investigate the distribution of lithium in discrete brain regions following long-term treatment at doses which are clinically appropriate and animal studies should demonstrate plasma lithium levels in the same relative range as human patients undergoing lithium therapy.

In our studies, treatment of two different strains of rats with a diet containing 30 mEq/Kg of lithium carbonate for 2 or 5 weeks resulted in plasma lithium levels in the human therapeutic range. Similarly, in studies involving repeated injections of lithium chloride over a period of 5 days, resulted in plasma lithium levels in the subtoxic range but significantly higher than those produced in studies involving chronic oral administrations.

Several authors reported a non-uniform distribution of lithium following its acute (110, 161, 166-168) and chronic (14-16, 114, 164) administration. Only those studies which are clinically relevent can be compared. Bond et al. (14) studied lithium levels in several brain regions of Wistar rats given a diet containing 30 mEq/Kg LiCl. After two weeks of treatment the lithium levels showed only small inter-regional variation, the hypothalamus having one of the lowest levels, and the hippocampus, cortex, and corpus striatum with relatively higher levels. In contrast, Edelfors (114) reported that both 3 and 5 weeks treatment of Wistar rats with the same dietary lithium content, produced a hypothalamic levels higher than all other brain regions studied with the exception of a sample of white matter. We find high levels of lithium in the septum and cingulate gyrus in both Sprague-Dawley and ACI (Micro) rats following 2 and 5 weeks of lithium carbonate containing diet (30 mEq/Kg food). Other regions also showed high lithium levels such as the hypothalamus, olfactory tubercle with bulb and tract and the amygdala. In contrast, lithium content in the cerebellum, pons, medulla and midbrain and cortical lobes showed significantly lower levels following this treatment schedule. It is interesting that several regions which are known to subserve emotionality (169) (septum, cingulate gyrus, hippocampus,

olfactory cortex, amygdala) tended to concentrate or retain lithium in greater amounts than in non-limbic regions. A similar non-uniform ion distribution was also demonstrated following 5 days of repeated LiCl treatment. It is tempting to suggest that the greater localization of lithium in brain structures associated with the limbic system may be related to its therapeutic efficacy in modulating emotional behavior (16). These results appear to agree with those of Delgato and DeFeudis (250), who were one of the first to suggest a limbic site of action for lithium. However other reports of greater concentrations of lithium in such regions as the pituitary (15, 157), corpus striatum (15, 164) and the posterior hemisphere (159) do not lend support to this suggestion. Support for our earlier suggestion (160) was provided by the recent study of Sprites (164) on the distribution of lithium in 32 brain regions of primates. They showed that following 3-6 weeks of oral lithium carbonate treatment (13 mg/Kg/day), lithium was found to be higher in the anterior thalamus, caudate nucleus, cingulum, posterior hippocampus and the olfactory bulb than the remaining 27 regions analyzed. Furthermore, of these regions the cerebellum, pons, midbrain and the temporal and frontal lobes exhibited the lowest levels of lithium.

It has therefore been suggested that the higher lithium concentrations in these regions may be related to the behavioral, functional, and biochemical effects of lithium. The results of the present study which attempted to link the biochemical effects of lithium, at least as far as the catecholamine and serotonergic systems are concerned, to the regional distribution of the ion do not offer a simple relationship. However, the possibility that lithium may interact with other substrates within these regions cannot be excluded.

IV. EFFECT OF LITHIUM ON CATECHOLAMINE CONTAINING NEURONS

A number of recent studies indicate that catecholamine metabolism in brain is affected by acute and possibly chronic exposure to lithium. However there is disagreement as to the direction and extent of the change in the metabolism of both NE and DA. Most of these studies are difficult to compare due to the diversity of experimental approaches which were used. In the case of NE metabolism, lithium generally seems to cause increased uptake of NE following short term treatment and increased intraneuronal metabolism (17, 181-185). Less work has been done on DA metabolism and the results have not been consistent (19, 20, 22, 176, 179, 195).

In the present study NE and DA metabolism was studied in a large number of brain regions of animals treated with lithium salts for 5 days, 2 weeks or 5 weeks. Our results demonstrate increased levels of NE in the corpus striatum, olfactory tubercle bulb and tract and the medulla following 5 days of lithium treatment whereas following 2 and 5 weeks of lithium treatment the only significant change in NE level was an increase in the pons. DA levels were significantly increased only in the corpus striatum and the olfactory tubercle bulb and tract, after 5 days of lithium treatment. The increase persisted in the olfactory tubercle up to 5 weeks of treatment.

Changes in the levels of NE and DA might be caused by altered rates of synthesis, metabolism, release or reuptake of the transmitter. It was therefore of interest to investigate whether lithium caused changes in the tyrosine hydroxylase activity, or the rate of tyrosine

hydroxylation in these regions which might explain the changes in the levels of NE and DA. We find that tyrosine hydroxylase activity was not significantly different from control values in any of the brain regions examined following 5 days or 2 weeks of lithium exposure.

As was discussed in Materials and Methods section, we considered that the ability of sucrose homogenates to hydroxylate ^{14}C-tyrosine to ^{14}C-dopa might be a better measure of an altered ability of nerve terminals to synthesize catecholamines than measurements of the tyrosine hydroxylase activity. We found that the tyrosine hydroxylating capacity of the brain tissue, measured in the sucrose homogenate, was significantly increased in the hypothalamus (and possibly the hippocampus) after 5 days, and in the olfactory tubercle bulb and tract (and possibly the amygdala) following 2 weeks of lithium treatment. In the remaining brain regions, no changes in the ability of the tissue to hydroxylate tyrosine was observed. Our results agree with the report of Segal et al. (178) that tyrosine hydroxylase activity was not changed in regions which contain the dorsal NE pathway. However, they also reported significantly increased tyrosine hydroxylase activity in the substantia nigra and the caudate nucleus following either acute or 8 days of lithium treatment. Based on these observations they concluded that lithium modified the dopaminergic nigrostriatal pathway. The study of Stefanini et al. (185) may be relevent since they reported increased DA uptake into caudate synaptosomal preparation isolated from rats chronically treated with lithium for 20 days. However, Friedman and Gershon (20) showed decreased DA synthesis following 2 weeks of lithium treatment in striatal slices; whereas Hesketh et al. (354) reported no changes in

the striatal tyrosine hydroxylase activity following lithium treatment for 1, 2 or 3 weeks.

The increase in tyrosine hydroxylating ability in the olfactory tubercle of rats treated with lithium for 2 weeks was consistent with the increase in the DA levels. Thus at least as far as the olfactory tubercle is concerned, our data suggest that increased hydroxylating ability in vitro is better correlated to the levels of catechoalamines in vivo than is the tyrosine hydroxylase activity.

The mechanism by which lithium affects the NE or DA is not clear, but there is evidence that in several brain regions increased NE and/or DA levels may be accounted for by increased turnover rates and/or increased tyrosine hydroxylase activity. It is not clear whether the actions of lithium are secondary to the decreased levels at post-synaptic receptor sites which may conceivably cause increased synthesis and turnover in NE and DA by a postulated feedback mechanisms. Such adaptive changes in the biosynthetic capacity was suggested by Segal et al. (27, 178) to be responsible for changes in the steady state levels of NE or DA. It is suggested that lithium may produce its effects on the mesolimbic system, since increased levels and/or enzyme activity was more pronounced in the olfactory tubercle bulb and tract. It is interesting that the hypothalamus, a region shown to contain high levels of lithium, also showed an increased ability to hydroxylate ^{14}C-tyrosine to ^{14}C-dopa after 5 days of lithium treatment. In contrast, two brain regions also shown to have exceptionally high lithium levels, the septum and cingulate gyrus, did not show changes in the levels of NE. While the TH activity was shown to be

unaltered in the septum, the TH activity in the cingulate gyrus was reduced. Therefore a clear relationship between the levels of lithium in these regions and the changes in DA and/or NE metabolism cannot be demonstrated.

V. EFFECT OF LITHIUM ON 5-HYDROXYTRYPTAMINE-CONTAINING NEURONS

In the present study lithium treatment increased the level of tryptophan, 5-HT and 5-HIAA in a number of brain regions without evidence of marked regional specificity. The increases following 5 days of lithium chloride treatment appeared to be greater than those produced after 2 and 5 weeks of lithium carbonate treatment. These differences between treatments may be related to higher lithium content in the brain regions of rats pretreated with LiCl for 5 days. The present results confirmed the biochemical findings of earlier reports suggesting a generalized activation of 5-HT mechanisms in the brain (23-25, 27, 194, 196, 206).

Although this effect has been known for a number of years our study showed the increases to be much more widespread than previously realized, and the effect could be demonstrated at clinically relevent doses. It is interesting that the profound changes which occurred following 5 days of treatment were no longer observable in a number of brain regions when the treatment was extended for periods up to 5 weeks. These observations are in general agreement with a number of earlier chronic studies (18, 19, 27, 179, 198). Lithium-induced elevations in 5-hydroxyindoles persisted in the olfactory tubercle, pons medulla, midbrain and the thalamus for at least 5 weeks, although the lithium-induced elevations in the cerebellum, motor and temporal cortices,

striatum, hippocampus and the amygdala were no longer observable after 2 or 5 weeks of treatment.

It is possible that the early changes were restored by mechanisms which tend to re-establish steady state conditions. It was therefore of interest to examine whether the increased levels of 5-hydroxyindoles could be explained by altered rates of 5-HT turnover. When the 5-HT turnover rate was estimated using the pargyline-induced rise in 5-HT three brain regions, the hypothalamus, midbrain and thalamus demonstrated a statistically significant increase in turnover rate (5 days). Moreover, when the lithium treatment was extended to 2 or 5 weeks, and given in the diet, the midbrain, amygdala and olfactory tubercle showed significant increases in turnover. Using the probenecid method, 5-HT turnover rates appeared to be increased in the thalamus and hypothalamus after 5 days of lithium treatment, and in the hypothalamus, striatum, amygdala and olfactory tubercle after 5 weeks of treatment. It should be pointed out that the two methods used to estimate the 5-HT turnovers did not always produce consistent results, therefore one must interpret the turnover results with caution. A possible explanation for this inconsistency is that the methods are not sufficiently sensitive to measure the relatively small changes in turnover rates in small brain regions. Although it was not possible to state definitely that the 5-HT was increased in all brain regions the results were consistent with a general increase in 5-HT turnover of about 15-20%.

Since it was not possible to clearly determine whether the increased levels of 5-HIAA following lithium treatment reflect changes in turnover rates, we decided to examine whether altered

rates of enzyme activities associated with 5-HT metabolism may be responsible for the elevated 5-hydroxyindoles in selected brain regions. It has been suggested by Knapp and Mandel (27, 198) that two of the factors which are involved in lithium's action are the substrate availability and the regulation of the rate-limiting enzyme, tryptophan hydroxylase. They provided evidence to suggest that lithium initially increases but later decreases the enzyme activity with a resulting reduced synthesis and the establishment of steady state levels.

The results of our study demonstrate that 5 days of lithium treatment produced an increased tryptophan hydroxylase activity in the midbrain and decreased activity in the hippocampus. However, whereas hippocampal enzyme activity after two weeks of lithium treatment was no longer different from control rats, midbrain tryptophan hydroxylase activity was significantly reduced. These results are consistent with the report of Knapp and Mandel (27, 198).

Changes in the 5-HIAA levels following lithium treatment were not associated with changes in the activity of MAO, since its activity did not change in any brain region following 5 days to 5 weeks of lithium treatment. It is therefore interesting that Murphy and Weiss (174) reported lower MAO activity in platelets obtained from depressed bipolar patients, whereas Bockar et al. (175) showed an increase in the platelet MAO activity during lithium therapy. However, in vitro, lithium had no significant effect on MAO activity in either rat brain (172) or human platelets (195).

There is growing body of evidence to suggest that the synthesis, storage and degradation of 5-HT is influenced by the brain tryptophan concentrations and this in turn by the plasma free tryptophan level.

The enzyme, tryptophan hydroxylase appears to be unsaturated with respect to substrate, and the indices of 5-HT synthesis in the brain appear to change in the same direction as tryptophan levels (80-84). Since we have shown in the present study elevated levels of both 5-hydroxyindoles and tryptophan in the brain, it was of interest to examine whether the early increases are a consequence of changes in the distribution of tryptophan in the blood and the brain compartments. We demonstrated increased plasma free-tryptophan levels in the blood after 5 days or 2 weeks of lithium treatment which correlated with the increases in the brain regional tryptophan levels.

It is known that lithium stimulates the uptake of tryptophan into synaptosomes (27), and it remained a possibility that lithium might affect the endogenous pools of tryptophan. Our preliminary results suggest that lithium may indeed cause increases in either soluble or bound tryptophan or increases in both. This suggestion is in agreement with the studies of Shaw et al. (290) and Knapp and Mandel (81) who demonstrated increased subcellular tryptophan levels following lithium treatment. Results from our experiments are consistent with the hypothesis that lithium stimulates the synthesis of brain 5-HT by increasing brain tryptophan levels, and by increasing its uptake into nerve endings. The ability of enhanced tryptophan uptake to increase 5-HT synthesis is believed to be due to the fact that under normal conditions tryptophan levels are insufficient to saturate tryptophan hydroxylase, i.e. increasing the substrate concentrations results in marked increases in 5-HT synthesis (80-84, 88-92). An important point was whether the actions

of lithium on 5-HT synthesis could be explained solely in terms of altered availability of tryptophan or whether an altered ability of the tissue to synthesize 5-HT was also involved.

Two possibilities could be visualized for the actions of lithium. Firstly, a lithium treatment might increase only the uptake of tryptophan into brain cells, or secondly, lithium might increase both uptake of tryptophan into the CNS and the maximum rate at which the brain tissue could synthesize 5-HT. The latter effect could be achieved by activation or induction of the rate limiting enzyme tryptophan hydroxylase (94-97). If the first possibility is correct, then in the presence of a tryptophan load sufficiently large enough to saturate the brain tryptophan hydroxylase with substrate there should be no difference between the ability of lithium-treated and control rat brains to synthesize 5-HT. However, if the second postulate is correct then even after tryptophan loading the lithium treated rat brains should be capable of synthesizing 5-HT at a rate greater than that produced by control rat brain tissues. We therefore, studied the effects of tryptophan loading on the synthesis of 5-HT in lithium pretreated rats to test these possibilities. It was considered that if we could increase tryptophan levels in brain to the point that the rate of synthesis was no longer dependent on the substrate concentration, then it might be possible to unmask changes in the ability of the tissue to synthesize 5-HT by mechanisms other than changes in available substrate.

ACI rats were given NaCl or LiCl injections for 5 days (2.67 mEq/Kg, i.p.) and loading doses of tryptophan were subsequently given. Our results demonstrated a dose related enhancement in the levels of 5-HT and 5-HIAA in all brain regions examined. However, in the lithium treated rats, the tryptophan-induced increases of 5-hydroxyindoles were significantly higher than in control rats. In addition it was possible to achieve higher plateau levels in most brain regions following lithium treatment than in control rats. Based on this data it is suggested that lithium does not simply increase the availability of the substrate, but seems to involve a mechanism which allows for increased capacity of the tissue to convert tryptophan to 5-hydroxy-indoles.

Our results suggest that lithium produces several effects on the distribution of tryptophan. Firstly, lithium appears to alter the distribution of plasma tryptophan such that it promotes its entry into the CNS - by a mechanism which may involve liberation from its blood storage pool. Secondly, lithium also causes a shift in the endogenous pools of tryptophan within a number of brain regions. However, it is evident that lithium also increases maximum rate at which the brain tissue can synthesize 5-HT in the presence of excess substrate. It is therefore apparent that the effect of lithium on brain 5-HT synthesis cannot be explained by a single mechanism, but that several stages in the formation of 5-HT from circulating tryptophan are simultaneously affected.

6. SUMMARY

Neonatal administration of 6-hydroxydopamine (100 µg/g, s.c.) on days one and two after birth produced long term changes in norepinephrine levels (NE) with marked decreases in the spinal cord, hypothalamus, hippocampus, olfactory tubercle bulb and tract, motor cortex and temporal cortex which persisted until at least 60 days of age. Marked increases in NE levels were observed in the midbrain, pons, medulla, and the cerebellum. The only evidence of interference with the development of 5-HT containing neurons was significant increases in the levels of 5-hydroxyindoleacetic acid (5-HIAA) in the spinal cord, thalamus and the pons. Treatment of neonatal rats with 5,7-dihydroxytryptamine (100 µg/gm s.c., on days 1 and 2 after birth only) produced profound and widespread changes in the levels of brain 5-hydroxyindoles for periods up to 60 days of age. Marked decreases in the levels of 5-hydroxyindoles were shown to occur in the amygdala, motor and temporal cortices and the hippocampus, and increases in the pons medulla, thalamus, spinal cord, corpus striatum and the cerebellum. Similar treatment of newborn rats with 4-chloroamphetamine (5 µg/g. s.c. on days 1-6 after birth) had little or no effect on the levels of the biogenic amines in the brain at later life.

Daily treatment of neonatal rats with chlorpromazine (3 or 10 µg/g, s.c., on days 1-6 after birth) resulted in a significant body and brain weight deficit which persisted to at least 60 days of age. Neonatal chlorpromazine had little or no effect on the catecholamine-containing neurons when examined at 20 days of age as shown by a lack of changes in tyrosine or NE levels or tyrosine hydroxylase activity in any of the brain regions studies. In contrast, CPZ produced

significant increases in the levels of 5-hydroxytryptamine (5-HT) in most brain regions at 20 days of age but in only 4 brain regions at 60 days of age. 5-HIAA levels were either significantly increased or decreases at 60 days of age. Treatment of newborn rats with haloperidol (0.1, 0.3, or 1.0 µg/g, s.c. on days 1-6 after birth) showed transient increases in the levels of 5-HT and/or 5-HIAA in the pons, medulla, cerebellum, temporal cortex, midbrain and thalamus only, whereas catecholamine levels were virtually unaffected at 20 days of age. Treatment of newborn rats with lithium (1 or 2 µEq/g, s.c., on days 1-6 after birth) produced transient increases in 5-HT in the corpus striatum, temporal cortex, hippocampus, midbrain, and thalamus, whereas the 5-HIAA levels were increased in the amygdala, motor cortex, pons, midbrain, and reduce in the corpus striatum, hypothalamus, and olfactory tubercle when examined at 20 days of age. Significantly reduced levels of 5-HT and/or 5-HIAA were noted in a number of brain regions at 60 days of age. Moreover, neonatal lithium produced significant increases in NE and/or tyrosine hydroxylase activity in the corpus striatum and olfactory tubercle bulb and tract. However pontine, cortical, hypothalamic, and amygdaloid NE levels or tyrosine hydroxylase activity were significantly reduced. It is suggested that a number of psychoactive drugs are capable of altering the development of certain groups of monoamine containing neurons, with more consistent changes demonstrated on the 5-HT containing neurons.

Treatment of adult ACI rats with lithium chloride (2.67 mEq/Kg, i.p.) for 5 days or lithium carbonate containing diet (30 mEq/Kg food) for 2 or 5 weeks achieved plasma lithium levels within the human therapeutic range, and the lithium distribution was shown to be non-

uniform. Several brain regions (septum, cingulate gyrus, olfactory tubercle, hypothalamus, and the amygdala) were shown to have higher lithium levels than other brain regions (midbrain, thalamus, pons, medulla, cerebellum, and temporal cortex).

Increased levels of norepinephrine were observed in the corpus striatum, olfactory tubercle bulb and tract following 5 days of lithium treatment, whereas following 2 or 5 weeks of lithium NE levels were increased in only the pons. Dopamine levels were increased in the corpus striatum and olfactory tubercle bulb and tract following five days, and in the olfactory tubercle following 2 and 5 weeks of lithium treatment. While the tyrosine hydroxylase activity was not changed in any brain region, the ability of the brain to synthesize catecholamines, measured by the rate of tyrosine hydroxylation in brain homogenates was significantly increased in the hypothalamus after 5 days of lithium treatment and the olfactory tubercle bulb and tract after 2 weeks.

The administration of lithium salts for 5 days or 2 weeks or 5 weeks caused significant increases in the levels of tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid levels in a number of brain regions without evidence of marked regional specificity. The increases were shown to be widespread following 5 days of lithium chloride treatment, and appeared to be greater than those produced by 2 and 5 weeks of lithium carbonate treatment. Lithium induced elevations of 5-hydroxyindoles persisted in the olfactory tubercle bulb and tract, pons medulla, midbrain and the thalamus for at least 5 weeks, although the lithium-induced elevations in the cerebellum, motor and temporal cortices, corpus striatum, hippocampus and the amygdala were no longer observable after 2 and 5 weeks of lithium treatment.

5-HT turnover rates were increased in the hypothalamus, midbrain and thalamus after 5 days, and in the midbrain, amygdala, and olfactory tubercle bulb and tract after 2 and/or 5 weeks of lithium treatment as estimated by the pargyline-induced rise in 5-HT levels. Using the probenecid method, 5-HT turnover rates were shown to be increased in the thalamus, and hypothalamus after 5 days and hypothalamus, striatum, amygdala and olfactory tubercle after 5 weeks of lithium treatment.

Five days of lithium treatment produced an increased tryptophan hydroxylase activity in the midbrain and decreased activity in the hippocampus, while following two weeks of treatment, midbrain enzyme was reduced. Lithium treatment did not modify the monoamine oxidase activity in any of the brain regions examined. Increased plasma free tryptophan levels in the blood after 5 days or 2 weeks of lithium treatment correlated with the increase in the tryptophan levels in brain regions, and the increases in subcellular levels of tryptophan in a number of brain regions. Tryptophan loading study (50, 100, 200 or 400 mg/Kg, i.p.) suggested that tryptophan-induced increases in the levels of 5-hydroxyindole in lithium pretreated rats were significantly higher than respective controls, and was demonstrable in virtually all brain regions examined.

Thus the present study demonstrates that lithium produces its stimulatory effect on 5-HT metabolism in adult rats by at least two processes operating simultaneously — increased availability of substrate and a mechanism which allows for increased capacity of the tissue to convert tryptophan to 5-hydroxyindoles.

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8. APPENDIX

TABLE 38

EFFECT OF SHORT AND PROLONGED LITHIUM TREATMENT ON REGIONAL 5-HYDROXYTRYPTAMINE TURNOVER AS MEASURED BY PARGYLINE-INDUCED ACCUMULATION OF 5-HYDROXYTRYPTAMINE

Results are given as mean ± S.E.M. of at least 5 determinations. Rats were given either intraperitoneal injections of NaCl or LiCl for 5 days (2.67 mEq/Kg, twice daily) or Na_2CO_3 or Li_2CO_3 containing diet (30 mEq/Kg food) for 2 or 5 weeks. At the end of the treatment or 12 hours after the last injection groups of rats were given a single i.p. injection of pargyline HCl (75 mg/Kg) and killed 0, 30, 60 or 90 minutes later. Fractional rate constants, K (h^{-1}), slopes of regression lines and turnover rates were estimated as described in Materials and Methods section.

Brain Region	Lithium Treatment	Slope	Fractional rate constant, K, (h^{-1})	5-HT turnover rate (ng/g/hr)
Amygdala	Control	0.364±0.04	0.838	529±58
	5 days	0.244±0.07	0.561	396±106
	5 weeks	0.329±0.05	0.757	402±60
Cerebellum	Control	0.213±0.03	0.490	85±15
	2 weeks	0.149±0.09	0.340	76±50
	5 weeks	0.232±0.03	0.534	105±14
Cortex-motor	Control	0.314±0.03	0.723	167±16
	2 weeks	0.329±0.09	0.757	212±57
	5 weeks	0.249±0.05	0.573	135±28
Cortex-temporal	Control	0.196±0.03	0.451	123±21
	2 weeks	0.214±0.06	0.492	152±39
	5 weeks	0.139±0.04	0.320	87±27
Hippocampus	Control	0.217±0.02	0.499	328±30
	5 days	0.134±0.05	0.308	258±83
	2 weeks	0.189±0.04	0.435	293±65
	5 weeks	0.272±0.03	0.626	361±36
Hypothalamus	Control	0.342±0.07	0.787	1125±223
	5 days	0.419±0.06	0.960	1813±257*
	2 weeks	0.123±0.04	0.526	939±180
	5 weeks	0.321±0.04	0.739	1418±154
Medulla	Control	0.364±0.02	0.838	598±39
	5 days	0.275±0.05	0.633	615±112
	2 weeks	0.362±0.07	0.833	601±42
	5 weeks	0.309±0.09	0.711	582±30

TABLE 38 continued

Brain Region	Lithium Treatment	Slope	Fractional rate constant, K, (h^{-1})	5-HT turnover rate (ng/g/hr)
Midbrain	Control	0.357±0.05	0.820	710±95
	5 days	0.422±0.02	0.970	891±46*
	2 weeks	0.352±0.05	0.809	930±135
	5 weeks	0.399±0.03	0.920	949±62*
Olfactory tubercle +	Control	0.225±0.03	0.518	372±42
	5 days	0.149±0.04	0.343	230±65
	2 weeks	0.124±0.04	0.285	223±67
	5 weeks	0.224±0.03	0.515	435±49
Pons	Control	0.199±0.03	0.458	340±36
	5 days	0.152±0.03	0.350	291±56
	2 weeks	0.227±0.03	0.522	394±48
Striatum	Control	0.163±0.03	0.375	300±46
	5 days	0.144±0.08	0.331	262±70
	2 weeks	0.129±0.04	0.297	256±69
	5 weeks	0.183±0.04	0.422	334±74
Thalamus	Control	0.279±0.03	0.643	380±44
	5 days	0.303±0.03	0.698	547±55*
	2 weeks	0.209±0.04	0.481	299±58
	5 weeks	0.243±0.02	0.559	401±32

* Statistically significant when compared with values of control animals ($p < 0.05$).

TABLE 39

THE EFFECT OF 5 DAYS LITHIUM TREATMENT ON 5-HYDROXYTRYPTAMINE TURNOVER AS MEASURED BY PARGYLINE-INDUCED REDUCTION OF 5-HYDROXYINDOLEACETIC ACID IN DISCRETE RAT BRAIN REGIONS

Results are given as mean ± S.E.M. of at least 6 animals in each group. Rats were given intraperitoneal injections of NaCl or LiCl for 5 days (2.67 mEq/Kg, twice daily). Approximately 12 hours after the last injection groups of animals were given a single i.p. injection of pargyline HCl (75 mg/Kg) and killed 0, 30, 60 or 90 minutes later. Fractional rate constants, $K(h^{-1})$, and slopes of regression lines, and turnover rates were calculated as described in Materials and Methods Section.

Brain Region	Treatment	Slope	Fractional rate constant $K(h^{-1})$	5-HT Turnover rate (ng/g/hr)
Amygdala				
	NaCl	-0.386±0.09	0.89	318±71
	LiCl	-0.395±0.09	0.91	511±112
Cortex-motor				
	NaCl	-0.370±0.04	0.85	240±24
	LiCl	-0.188±0.06	0.43	178±60
Hippocampus				
	NaCl	-0.178±0.04	0.47	387±104
	LiCl	-0.343±0.06	0.58	731±247
Hypothalamus				
	NaCl	-0.420±0.07	0.97	1028±170
	LiCl	-0.293±0.09	0.67	864±284
Medulla				
	NaCl	-0.364±0.11	0.83	410±124
	LiCl	-0.146±0.08	0.34	244±137
Midbrain				
	NaCl	-0.286±0.03	0.66	418±39
	LiCl	-0.236±0.05	0.54	540±113
Olfactory tubercle bulb and tract				
	NaCl	-0.353±0.05	0.81	270±40
	LiCl	-0.321±0.05	0.74	349±52
Striatum				
	NaCl	-0.166±0.03	0.38	229±45
	LiCl	-0.072±0.08	0.17	164±53
Thalamus				
	NaCl	-0.239±0.03	0.55	348±49
	LiCl	-0.149±0.03	0.34	314±64

TABLE 40

THE EFFECT OF 5 DAYS LITHIUM TREATMENT ON 5-HYDROXYTRYPTAMINE TURNOVER RATE AS MEASURED BY THE PROBENECID-INDUCED ELEVATION OF 5-HIAA IN DISCRETE BRAIN REGIONS OF ACI RAT

Results are given as mean ± S.E.M. of at least 5 animals in each group. Rats were given intraperitoneal injections of NaCl or LiCl for 5 days (2.67 mEq/Kg, twice daily). Approximately 12 hours after the last injection groups of animals were given a single i.p. injection of probenecid (200 mg/Kg) and killed 0, 60, 120 or 180 minutes later. Fractional rate constants, $K(h^{-1})$ and slopes of regression lines, and turnover rates were calculated as described in Materials and Methods Section.

Brain Region	Treatment	Steady State 5-HIAA Levels (ng/gm)	Slope	Fractional rate constant $K(h^{-1})$	5-HT Turnover Rate†
Amygdala					
	NaCl	358±53	0.198±0.032	0.456	163±18
	LiCl	561±74*	0.115±0.029	0.265	149±20
Corpus striatum					
	NaCl	483±18	0.116±0.017	0.268	129±5
	LiCl	776±138	0.087±0.030	0.200	155±28
Cortex, motor					
	NaCl	282±17	0.155±0.018	0.357	101±6
	LiCl	413±49*	0.106±0.020	0.244	101±1
Hippocampus					
	NaCl	823±65	0.159±0.026	0.366	301±24
	LiCl	1261±99*	0.102±0.029	0.234	295±28
Hypothalamus					
	NaCl	1060±106	0.137±0.031	0.316	335±34
	LiCl	1290±185	0.158±0.029	0.364	470±67
Medulla					
	NaCl	494±58	0.256±0.048	0.589	291±34
	LiCl	718±78*	0.160±0.062	0.368	264±29
Midbrain					
	NaCl	579±11	0.151±0.015	0.346	200±4
	LiCl	943±106*	0.090±0.027	0.207	195±22
Olfactory tubercle bulb and tract					
	NaCl	254±17	0.174±0.024	0.400	102±7
	LiCl	366±38*	0.114±0.024	0.263	96±10
Pons					
	NaCl	824±41	0.187±0.040	0.430	354±74
	LiCl	1416±110*	0.083±0.027	0.191	270±88
Thalamus					
	NaCl	632±34	0.098±0.018	0.225	142±8
	LiCl	924±40*	0.085±0.016	0.196	181±8*

* $p < 0.05$ when compared with respective controls

† (ng/g/hr)

CURRICULUM VITAE

Name: Hillel TAUB (Mr.)

Date of Birth: August 16, 1946

Degrees:

1. B.Sc., Sir George Williams University 1968 (Biochemistry)
2. M.Sc., Queen's University 1973 (Pharmacology)

Awards:

1. 1975-1976, Ontario Mental Health Foundation Pre-Doctoral Research Studentship
2. 1976-1977, Ontario Mental Health Foundation Pre-Doctoral Research Studentship
3. 1977-1978, University of Ottawa Research Fellowship

Publications:

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ABSTRACT

Neonatal administration of 6-hydroxydopamine (100 µg/g, s.c.) on days one and two after birth produced long term changes in norepinephrine levels (NE) with marked decreases in the spinal cord, hypothalamus, hippocampus, olfactory tubercle bulb and tract, motor cortex and temporal cortex which persisted until at least 60 days of age. Marked increases in NE levels were observed in the midbrain, pons, medulla, and the cerebellum. The only evidence of interference with the development of 5-HT containing neurons was significant increases in the levels of 5-hydroxyindoleacetic acid (5-HIAA) in the spinal cord, thalamus and the pons. Treatment of neonatal rats with 5,7-dihydroxytryptamine (100 µg/gm s.c., on days 1 and 2 after birth only) produced profound and widespread changes in the levels of brain 5-hydroxyindoles for periods up to 60 days of age. Marked decreases in the levels of 5-hydroxyindoles were shown to occur in the amygdala, motor and temporal cortices and the hippocampus, and increases in the pons medulla, thalamus, spinal cord, corpus striatum and the cerebellum. Similar treatment of newborn rats with 4-chloroamphetamine (5 µg/g, s.c. on days 1-6 after birth) had little or no effect on the levels of the biogenic amines in the brain at later life.

Daily treatment of neonatal rats with chlorpromazine (3 or 10 µg/g, s.c., on days 1-6 after birth) resulted in a significant body and brain weight deficit which persisted to at least 60 days of age. Neonatal chlorpromazine had little or no effect on the catecholamine-containing neurons when examined at 20 days of age as shown by a lack of changes in tyrosine or NE levels or tyrosine hydroxylase activity in any of the brain regions studies. In contrast, CPZ produced

significant increases in the levels of 5-hydroxytryptamine (5-HT) in most brain regions at 20 days of age but in only 4 brain regions at 60 days of age. 5-HIAA levels were either significantly increased or decreases at 60 days of age. Treatment of newborn rats with haloperidol (0.1, 0.3 or 1.0 μg/g, s.c. on days 1-6 after birth) showed transient increases in the levels of 5-HT and/or 5-HIAA in the pons, medulla, cerebellum, temporal cortex, midbrain and thalamus only, whereas catecholamine levels were virtually unaffected at 20 days of age. Treatment of newborn rats with lithium (1 or 2 μEq/g, s.c., on days 1-6 after birth) produced transient increases in 5-HT in the corpus striatum, temporal cortex, hippocampus, midbrain, and thalamus, whereas the 5-HIAA levels were increased in the amygdala, motor cortex, pons, midbrain, and reduce in the corpus striatum, hypothalamus, and olfactory tubercle when examined at 20 days of age. Significantly reduced levels of 5-HT and/or 5-HIAA were noted in a number of brain regions at 60 days of age. Moreover, neonatal lithium produced significant increases in NE and/or tyrosine hydroxylase activity in the corpus striatum and olfactory tubercle bulb and tract. However pontine, cortical, hypothalamic, and amygdaloid NE levels or tyrosine hydroxylase activity were significantly reduced. It is suggested that a number of psychoactive drugs are capable of altering the development of certain groups of monoamine containing neurons, with more consistent changes demonstrated on the 5-HT containing neurons.

Treatment of adult ACI rats with lithium chloride (2.67 mEq/Kg, i.p.) for 5 days or lithium carbonate containing diet (30 mEq/Kg food) for 2 or 5 weeks achieved plasma lithium levels within the human therapeutic range, and the lithium distribution was shown to be non-

uniform. Several brain regions (septum, cingulate gyrus, olfactory tubercle, hypothalamus, and the amygdala) were shown to have higher lithium levels than other brain regions (midbrain, thalamus, pons, medulla, cerebellum, and temporal cortex).

Increased levels of norepinephrine were observed in the corpus striatum, olfactory tubercle bulb and tract following 5 days of lithium treatment, whereas following 2 or 5 weeks of lithium NE levels were increased in only the pons. Dopamine levels were increased in the corpus striatum and olfactory tubercle bulb and tract following five days, and in the olfactory tubercle following 2 and 5 weeks of lithium treatment. While the tyrosine hydroxylase activity was not changed in any brain region, the ability of the brain to synthesize catecholamines, measured by the rate of tyrosine hydroxylation in brain homogenates was significantly increased in the hypothalamus after 5 days of lithium treatment and the olfactory tubercle bulb and tract after 2 weeks.

The administration of lithium salts for 5 days or 2 weeks or 5 weeks caused significant increases in the levels of tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid levels in a number of brain regions without evidence of marked regional specificity. The increases were shown to be widespread following 5 days of lithium chloride treatment, and appeared to be greater than those produced by 2 and 5 weeks of lithium carbonate treatment. Lithium induced elevations of 5-hydroxyindoles persisted in the olfactory tubercle bulb and tract, pons medulla, midbrain and the thalamus for at least 5 weeks, although the lithium-induced elevations in the cerebellum, motor and temporal cortices, corpus striatum, hippocampus and the amygdala were no longer observable after 2 and 5 weeks of lithium treatment.

5-HT turnover rates were increased in the hypothalamus, midbrain and thalamus after 5 days, and in the midbrain, amygdala, and olfactory tubercle bulb and tract after 2 and/or 5 weeks of lithium treatment as estimated by the pargyline-induced rise in 5-HT levels. Using the probenecid method, 5-HT turnover rates were shown to be increased in the thalamus, and hypothalamus after 5 days and hypothalamus, striatum, amygdala and olfactory tubercle after 5 weeks of lithium treatment.

Five days of lithium treatment produced an increased tryptophan hydroxylase activity in the midbrain and decreased activity in the hippocampus, while following two weeks of treatment, midbrain enzyme was reduced. Lithium treatment did not modify the monoamine oxidase activity in any of the brain regions examined. Increased plasma free tryptophan levels in the blood after 5 days or 2 weeks of lithium treatment correlated with the increase in the tryptophan levels in brain regions, and the increases in subcellular levels of tryptophan in a number of brain regions. Tryptophan loading study (50, 100, 200 or 400 mg/Kg, i.p.) suggested that tryptophan-induced increases in the levels of 5-hydroxyindole in lithium pretreated rats were significantly higher than respective controls, and was demonstrable in virtually all brain regions examined.

Thus the present study demonstrates that lithium produces its stimulatory effect on 5-HT metabolism in adult rats by at least two processes operating simultaneously - increased availability of substrate and a mechanism which allows for increased capacity of the tissue to convert tryptophan to 5-hydroxyindoles.