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BRAIN THYROID HORMONES IN MODELS OF DEPRESSION - AN INITIAL ASSESSMENT

by

Yael Friedman

A thesis submitted in conformity with the requirements for the degree of Master of Science Graduate Department of Pharmacology University of Toronto

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ABSTRACT

Brain Thyroid Hormones in Models of Depression - An Initial Assessment
Yael Friedman (1998)
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Department of Pharmacology, University of Toronto

Depression is among the most serious, costly and life-threatening of all mental disorders. Although specific alterations in brain thyroid hormone (TH) levels have been hypothesized as being causal factors in depressive illness, no information currently exists concerning the role of brain THs in depression. In this project, brain THs were evaluated for the first time in the learned helplessness (LH) model of depression and in experimental conditions related to depression. The first and second studies examined the effects of uncontrollable footshock stress on brain THs in adult rats. Although whole brain triiodothyronine (T₃) levels were found to change rapidly in response to acute stress, a clear relationship was not observed between whole brain TH levels and amount of shock received. In the third study, changes in THs in amygdala, hippocampus and frontal cortex were examined in helpless and non-helpless rats following the LH procedure. No significant differences in T₃ and thyroxine (T₄) concentrations were found among these two behaviourally distinct groups and shock-naive controls. The fourth study examined brain THs in rats selectively bred for helpless behaviour in the LH paradigm. Whole brain T₄ levels were elevated in congenitally helpless rats compared to LH-resistant controls. While the findings from the four studies do not provide definite answers with regards to the role of brain THs in depressive syndromes, the data suggest that complex relationships may exist between alterations in brain TH levels and depressive-like states.
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First and foremost, I would like to express my sincere gratitude to my supervisor Dr. J.N. Nobrega for his continued support, encouragement and guidance during this project. With your valuable insight, patience and sense of humor, you have made this project an extremely enjoyable and rewarding experience.

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LIST OF ABBREVIATIONS

3,3'-T₂ - 3,3'-diiodothyronine
5'D-I - type I deiodinase
5'D-II - type II deiodinase
5D-III - type III deiodinase
5-HT - serotonin
ANOVA - analysis of variance
BSA - bovine serum albumin
cAMP - cyclic adenosine monophosphate
cLH - congenitally helpless
cNLH - congenitally non-helpless
CNS - central nervous system
cpm - counts per minute
CSF - cerebrospinal fluid
DA - dopamine
DIT - diiodotyrosine
DTT - dithiothreitol
ECT - electroconvulsive therapy
EDTA - ethylenediaminetetraacetic acid
ES - escapable shock
G₅ - stimulatory G protein
HEPES - hydroxyethane piperazine sulphate
IS - inescapable shock
LH - learned helplessness/learned helpless
MAO - monoamine oxidase
MAOIs - monoamine oxidase inhibitors
MDE - major depressive episode
MIT - moniodotyrosine
NE - norepinephrine
NLH - non-helpless
NT - neurotransmitter
PTU - propylthiouracil
RIA - radioimmunoassay
rT₃ - 3,3',5'-triiodothyronine, reverse T₃
SEM - standard error of the mean
SSRIs - selective serotonin reuptake inhibitors
T₃ - 3,5,3'-triiodothyronine
T₄ - thyroxine
TCAs - tricyclic antidepressants
THR - thyroid hormone receptor
TREs - thyroid response elements
TRH - thyrotropin-releasing hormone
TSH - thyroid-stimulating hormone
1.0 INTRODUCTION

1.1 Structure and Properties of Thyroid Hormones

The thyroid gland is responsible for secreting two significant hormones: 3,5,3',5'-tetraiodo-L-thyronine (thyroxine, T₄) and 3,5,3'-triiodo-L-thyronine (T₃). The structure of T₄ was elucidated by Harington and Barger (1927). T₃ was identified 25 years later in plasma and thyroid extracts by Gross and Pitt-Rivers (1952) (see Figure 1 for structures of T₄ and T₃). T₄ is the principal secretory product of the thyroid gland (Puymirat, 1992). However, T₃ has 4-10 times the activity of T₄ and is present in much smaller quantities in the circulation. Both hormones are tightly bound to certain plasma proteins, although T₃ has a lower affinity for these binding proteins than T₄ (Samuels et al., 1988). Since the free hormone is metabolized quicker than the bound form, the half-life of T₃ in the circulation is shorter (2 days) than that of T₄ (7 days) (Schimmer and George, 1998). Due to the fact that T₃ binds to the nuclear receptor (see section 1.3) and is the more potent iodothyronine, it is considered to be the biologically active hormone, with T₄ functioning as a circulating prohormone (Larsen, 1972; Schwartz et al., 1971). Thus, the conversion of T₄ to T₃ represents the first step in thyroid hormone (TH) action.

Thyroid hormones (THs) are essential for the normal functioning of all body tissues. They are involved in development, growth and adaptation to environmental stress and have a profound effect of increasing the body’s basal metabolic rate. Although the thyroid gland is not essential to life, it maintains the level of metabolism in the tissues that is optimal for their normal function (Ganong, 1993).
1.2 Synthesis and Regulation of Thyroid Hormones

The formation of T₄ requires approximately 50 mg of iodine each year. Ingested iodine is converted to iodide and absorbed from the gastrointestinal tract into the blood. Iodide is first actively pumped from the extracellular fluid into the interior of the cell (iodide trapping) and is then oxidized to iodine by the enzyme thyroidal peroxidase. The iodine combines with tyrosine residues in the glycoprotein thyroglobulin resulting in monoiodinated (MIT) and diiodinated (DIT) L-tyrosine residues. These residues couple with each other to form T₄ (2 DIT molecules) and T₃ (1 MIT and 1 DIT). T₄ and T₃ are released from the thyroid gland into the circulation as a result of the hydrolysis of thyroglobulin to amino acids by lysosomal enzymes. The MIT and DIT residues that are not coupled are deiodinated by the enzyme deiodinase. This allows for recycling of iodine within the gland so that additional formation of THs can occur (Ganong, 1993).

In order to maintain normal levels of metabolic activity in the body, the amounts of THs must be tightly regulated at all times. Specific feedback mechanisms operate through the hypothalamus and the anterior pituitary to control the secretion of THs. Thyroid-stimulating hormone (TSH), also known as thyrotropin, is an anterior pituitary glycoprotein that acts to increase all of the known activities of thyroid glandular cells. TSH exerts its effects by binding to specific TSH receptors on the basal membrane surfaces of the cell. This binding results in an activation of adenylyl cyclase through Gₛ, which in turn activates intracellular cyclic adenosine monophosphate (cAMP). cAMP then activates protein kinases which causes multiple phosphorylations throughout the cell leading to an immediate increase in secretion of THs and prolonged growth of the thyroid glandular tissue.
Secretion of TSH from the anterior pituitary is controlled by the tripeptide L-pyroglutamyl-L-histidyl-L-prolinamide, known as thyrotropin-releasing hormone (TRH) which is secreted by nerve endings in the median eminence of the hypothalamus and is transported to the anterior pituitary. TRH binds to TRH receptors in the pituitary cell membrane which leads to an activation of phospholipase C and diacylglycerol which result in TSH release (Holsboer, 1995).

Increased levels of THs in the periphery cause a decrease in TSH secretion by directly affecting the anterior pituitary. This feedback regulation mechanism maintains an almost constant concentration of free THs in the circulation.

1.3 Actions of Thyroid Hormones

The effects of TH action on cellular function occurs, in large part, at the level of gene expression (Chin, 1994; Lazar and Chin, 1990). T₃ enters the cells and is transported to the cell nucleus where it binds to a nuclear TH receptor (THR), which has a 10-20 fold higher affinity for T₃ than T₄ (Escobar-Morreale, 1997; Larsen, 1997; Oppenheimer et al., 1987; Silva and Larsen, 1986). This receptor is a member of the superfamily of nuclear hormone receptor transcription factors that contains a DNA binding region and a T₃ binding region and is believed to be the site of initiation of TH physiological effects (Chin, 1994; Karin et al., 1993). Four different receptor subtypes have been identified in mature brain. These subtypes differ in their relative abundance and anatomical localization in brain (Bradley et al., 1989). The DNA binding targets of the THR complex are called TH response elements (TREs) and are located in the promoter region of specific genes (Chin, 1994). The binding of the THR
complex to the TREs causes an alteration in the transcription of certain genes, ultimately resulting in the increased synthesis of those proteins. It is believed that most of the actions of THs are the result of the synthesis of these new proteins.

1.3.1 Central Effects

1.3.1.1 In Development

It has long been recognized that THs are essential for normal development and maturation of the mammalian central nervous system (CNS) (Leonard et al., 1981; Pasquini and Adamo, 1994; Schwartz, 1983; Silva and Larsen, 1982). Absence of THs during critical periods of CNS maturation results in physiological, biochemical and morphological abnormalities. Neonatal hypothyroidism results in a deficiency in myelination, an impairment of proliferation and migration of nerve cells, retardation in synapse formation in several brain areas as well as a reduction in the number of arborizations of neurons in the cerebral cortex and cerebellum (Puymirat, 1992; Silva and Larsen, 1982). Hypothyroidism in the neonate results in a condition known as cretinism which is due to retardation of the growth, branching and myelination of neuronal cells (Leonard et al., 1981) and leads to mental retardation and dwarfism.

1.3.1.2 In Mature Brain

Despite the long-held notion that the adult brain is biochemically refractory to THs, mature brain processes have been shown to be affected by THs. These processes include expression of brain-specific genes, activity of enzymes involved in the degradation of myelin and neurotransmitters (Ahmed et al., 1993), mitochondrial respiration (Dembri et al., 1984)
and effects on monoaminergic neurotransmission at a number of sites (Henley and Koehnle, 1997). In addition, the adult brain contains significant amounts of intracellular THs and nuclear THRNs (Bradley et al., 1989; Leonard et al., 1992; Oppenheimer, 1979; Oppenheimer et al., 1987; Puymirat et al., 1991; Puymirat, 1992). In fact, only the liver contains more T₄ and T₃ per gram wet weight than the brain (Larsen et al., 1981; Leonard, 1992). Current evidence suggests that THs can affect mature brain function through nuclear T₃ receptors thereby affecting gene expression (Chin, 1994; Lazar and Chin, 1990; Lazar, 1993; Samuels et al., 1988; Tsai and Omalley, 1994) or through a non-nuclear mechanism that affects synaptic transmission in nerve terminals (Mason et al., 1987; Mason et al., 1993; Sarkar and Ray, 1994). Adults with severe hyperthyroidism show emotional lability, whereas those with chronic hypothyroidism develop memory impairment and cerebellar ataxia (Leonard et al., 1981). Additionally, clinical studies implicate THs in the regulation of mood and in the pathophysiology of depressive disorders as will be discussed in detail in section 1.7.

1.4 Thyroid Hormone Metabolism

The metabolism of THs is mediated by several enzymatic reactions, such as oxidative decarboxylation, deamination, conjugation and deiodination. Of these reactions, deiodination is predominant and represents a key step in TH regulation (Escobar-Morreale et al., 1997; Kaplan, 1986). Approximately 80% of the main secretory product of the thyroid gland, T₄, is deiodinated either to the biologically active T₃ or to the inactive iodothyronine reverse T₃ (rT₃) (Leonard and Visser, 1986). There are two distinct deiodination reactions, referred to as phenolic ring deiodination and tyrosyl ring deiodination. Phenolic ring (outer ring) deiodination is usually referred to as 5'-deiodination and is responsible for the conversion of
T₄ to T₃ and rT₃ to the inactive metabolite 3,3'-diiodothyronine (3,3'-T₂). Since T₄ is thought to have little or no intrinsic activity, this reaction is regarded as an activating pathway. Tyrosyl ring (inner ring) deiodination results in the formation of rT₃ from T₄ and 3,3'-T₂ from T₃. This process is referred to as 5-deiodination and is considered an inactivating pathway as the products formed are inactive. The sequential metabolism of T₄ is shown in Figure 1.

It is now established that there are at least three different iodothyronine deiodinases which are distinguished from each other by the selectivity of the reactions they catalyze, their localization, substrate specificities and enzyme kinetics (Leonard and Visser, 1986; Campos-Barros et al., 1994).

1.4.1 Deiodinase Enzymes

Type I deiodinase (5'D-I) is a non-selective enzyme, deiodinating both phenolic and tyrosyl rings of iodothyronines. Although its activity is highest in liver and kidney, there is high activity in the thyroid gland (Campos-Barros et al., 1994; Leonard and Visser, 1986). In the liver and kidney, this is the deiodinase that catalyzes the deiodination of all iodothyronines (Leonard and Visser, 1986). In brain, 5'D-I catalyzes only the degradation of rT₃ to 3,3'-T₂ (Campos-Barros et al., 1994; Leonard and Visser, 1986). It is present predominantly in glial cells and unlike in the liver and kidney, 5'D-I levels in the brain are unaffected by thyroid status (Leonard and Visser, 1986).

Type II deiodinase (5'D-II) is a selective outer-ring deiodinase that catalyzes the conversion of T₄ to T₃ and rT₃ to 3,3'-T₂. It has a more limited distribution in mammalian tissues than 5'D-I. It is located in the CNS, pituitary gland, brown adipose tissue and
Figure 1: Metabolism of $T_4$. Conversion of $T_4$ to $T_3$ and $rT_3$ to $3,3'T_2$ proceeds by outer ring deiodination, whereas conversion of $T_4$ to $rT_3$ and $T_3$ to $3,3'T_2$ proceeds by inner ring deiodination. Taken from Leonard and Visser (1986).
placenta (Campos-Barros et al., 1994; Leonard and Visser, 1986). This isoenzyme has a remarkable ability to increase or decrease rapidly in response to alterations in circulating levels of THs (Leonard and Visser, 1986). For instance, in the brains of thyroidectomized rats, 5'D-II levels rise 5-10 fold and decrease by >90% after TH replacement (Leonard et al., 1981). Unlike 5'D-I, T₄ is the preferred substrate and modulates 5'D-II activity (Leonard, 1992) which is discussed in section 1.5.1.

Type III deiodinase (5D-III), a selective inner-ring deiodinase, has been found in rat placenta, skin and is the most abundant deiodinase in the rat CNS (Kaplan and Yaskoski, 1981; Leonard and Visser, 1986). In the CNS, it is localized predominantly in glial cells (Cavalieri et al., 1986; Leonard and Larsen, 1985). It is a deactivating deiodinase responsible for converting T₄ to rT₃ and T₃ to 3,3'-T₂. Both T₄ and T₃ serve as alternate substrates for this enzyme (Leonard and Visser, 1986).

In summary, outer-ring deiodination, converting T₄ to T₃ and rT₃ to 3,3'-T₂ is catalyzed by both 5'D-I and 5'D-II, whereas inner-ring deiodination of T₄ and T₃ to the inactive metabolites 3,3'-T₂ and rT₃, respectively, is catalyzed by 5'D-I and 5D-III. Table 1 summarizes the major characteristics of the three deiodinases in the rat.

1.5 Regulation of Thyroid Hormones in the Central Nervous System

In the brain, T₄ metabolism is regulated by a different mechanism from that described in peripheral tissues, such as kidney and liver. In peripheral tissues, most of the T₃ is taken up from the blood directly (after peripheral monodeiodination of T₄), whereas intracerebral conversion of T₄ to T₃ by the enzyme 5'D-II contributes to 75-90% of T₃ bound to brain cell
Table 1: Characteristics of the Three Deiodinases in the Rat

<table>
<thead>
<tr>
<th>Deiodination Site</th>
<th>Type I (5'D-I)</th>
<th>Type II (5'D-II)</th>
<th>Type III (5'D-III)</th>
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<tr>
<td>Tissue Localization</td>
<td>Non-selective; inner and outer ring</td>
<td>Selective; outer ring</td>
<td>selective; inner ring</td>
</tr>
<tr>
<td>Reactions Catalyzed</td>
<td>high activity in peripheral tissues; low activity in CNS</td>
<td>CNS, BAT, pituitary</td>
<td>CNS, placenta, skin</td>
</tr>
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<td>Substrate Preference</td>
<td>all iodothyronines (periphery)</td>
<td>$T_4 \rightarrow T_3$ (activation)</td>
<td>$T_3 \rightarrow 3,3'T_2$ (deactivation)</td>
</tr>
<tr>
<td></td>
<td>$rT_3 \rightarrow 3,3'T_2$ (CNS)</td>
<td>$rT_3 \rightarrow 3,3'T_2$</td>
<td>$T_4 \rightarrow rT_3$</td>
</tr>
<tr>
<td></td>
<td>$rT_3$</td>
<td>$T_4$</td>
<td>$T_4 = T_3$</td>
</tr>
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*Adapted from Leonard and Visser (1986) and Campos-Barros et al. (1994)*
nuclei (the remaining 10-25% of intracerebral $T_3$ is obtained from the circulation) (Campos-Barros et al., 1994; Crantz and Larsen, 1980; Dratman et al., 1983; Leonard and Visser, 1986; Silva et al., 1982; Van Doorn et al., 1985). This implies that the main determinant of $T_3$ levels in the periphery are circulating levels of $T_3$, whereas the main determinant of $T_3$ levels in brain are circulating levels of $T_4$ and that the supply of $T_4$ is essential for $T_3$ function in the CNS. 5'D-II provides the CNS with the ability to regulate thyroid status within the CNS somewhat independently of the rest of the body (Dratman et al., 1983).

Once in the circulation, $T_4$ and $T_3$ are transferred to the extravascular space. Both iodothyronines enter the glial cell compartment, which is located between the capillaries and the neuronal compartment. In the glial cell compartment, $T_4$ and $T_3$ are metabolized to inactive iodothyronines. The r$T_3$ that is generated is further metabolized to 3,3'-T$_2$. Thus, the majority of $T_4$ and $T_3$ are degraded due to the high concentrations of 5'D-I and 5D-III. The remaining $T_4$ then diffuses to the neuronal cell compartment where it is converted to $T_3$ by 5'D-II (Leonard, 1992).

The anatomical separation of the three deiodinases in the brain ensures that there is minimal contribution of circulating levels of $T_3$ to the intracerebral pool. As well, r$T_3$, an inhibitor of 5'D-II activity (which could potentially interfere in the conversion of $T_4$ to $T_3$) is removed.

1.5.1 Regulation of Type II Deiodinase in Brain

In the brain, 5'D-II is localized predominantly to the plasma membrane of neuronal cell bodies, although brain regions that are rich in catecholaminergic fibre tracts have 5'D-II
in both neural and glial cells (Leonard, 1992). T4 is the most potent iodothyronine to
modulate 5'D-II activity (Dratman and Crutchfield, 1983) by promoting the formation of F-
actin stress fibres (part of the cytoskeleton) that are required for internalization of 5'D-II
(Leonard et al., 1984; Leonard, 1992; Siegrist-Kaiser et al., 1990). When 5'D-II becomes
associated with F-actin, it is inactivated. On the other hand, in the absence of F-actin stress
fibres, 5'D-II turnover is slow (Leonard, 1992).

1.6 Unipolar Depression

1.6.1 History and Symptoms

Unipolar depression, the most common mood disorder, was described in Hippocratic
writing in the fifth century BC. According to Hippocrates, moods were dependent upon the
balance of blood, phlegm, yellow and black bile. An excess of black bile was believed to be
the cause of depression (Kandel, 1991). Melancholia, the ancient Greek term for depression,
actually means black bile.

It was extremely difficult to update this Hippocratic view because of an inability to
classify affective disorders precisely. Only in the last two decades have precise criteria for
classifying affective (mood) disorders emerged.

The core symptoms of a major depressive episode (MDE) include a duration lasting at
least two weeks in which there is a dysphoric mood or a generalized loss of interest or
pleasure (anhedonia) in almost all activities that were previously pleasurable. These
symptoms are present most of the day and persist nearly every day. The individual is often
described as being sad, discouraged, and has feelings of worthlessness, excessive or

In addition to the core symptoms, the individual must experience at least four of the following incidental symptoms: 1) sleep disturbances, 2) reduced or increased appetite, 3) fatigue and loss of energy, 4) psychomotor agitation or retardation and 5) diminished ability to think or concentrate (American Psychiatric Association, 1994; Willner, 1991).

The most common sleep disturbance is insomnia in which individuals will wake up during the night and have difficulty returning to sleep (middle insomnia) or terminal insomnia whereby the individual wakes up early and cannot return to sleep. In most individuals, appetite is reduced and if severe, may lead to a significant weight loss, although other individuals may have an increased appetite. Psychomotor changes include agitation or retardation that is observable by others. Individuals have less energy and fatigue easily without physical exertion. Many people report that they have an impaired ability to concentrate, think or make decisions as well as being easily distracted and having impaired memory. In general, there is an interference in social, occupational and other important areas of daily functioning (American Psychiatric Association, 1994).

In addition to the inclusion criteria outlined above, there are exclusion criteria. For instance, a MDE can not be caused by physiological or side effects of medications or drugs of abuse or by a general medical condition. Bereavement, schizophrenia or other neurological diseases must be excluded (American Psychiatric Association, 1994).
1.6.2 Course and Incidence

A MDE may begin at any age, although the average age of onset is in the mid-20s (Kandel, 1991). The course of the disorder is variable in that some people experience isolated episodes whereas others have clusters of episodes. The number of prior episodes predicts the likelihood of developing subsequent episodes (American Psychiatric Association, 1994). For instance, 50-60% of individuals who have had a single episode will have a second episode; individuals who have had two episodes have a 70% chance of developing another episode and this increases to 90% for those who have had three episodes (American Psychiatric Association, 1994). In two-thirds of cases, there is complete remission of symptoms, whereas in 5-10% of individuals, full criteria of a MDE continue to be met for more than two years following the initial MDE (American Psychiatric Association, 1994).

Depression affects 5% of the world’s population and it is estimated that at least 1 in 10 adults will experience a MDE in their life time. Depression is among the most serious, costly and life-threatening of all mental disorders and is the leading cause of suicide. In 1990, international health experts identified depression as the fourth-ranked cause of disability and premature death worldwide (Judd, 1997).

In prepubertal children, girls and boys are equally affected, although adolescent and adult women are affected 2-3 times more often than men (Willner, 1991). There is also a strong genetic predisposition for depression, so that a MDE is 1.5-3 times more common in first-degree biological relatives of a person with depression than among the general population (Barchas et al., 1994).
1.6.3 Etiology

Despite research on various fronts, brain substrates of depression have not been elucidated. Research on antidepressant drugs has led to several hypotheses which have implicated alterations in neurotransmitter (NT) function. Since drugs that are clinically effective in treating these disorders act on serotonergic and noradrenergic systems of the brain, it was hypothesized that depressive symptoms were a result of disturbances in the neurotransmission mediated by serotonin (5-HT) and catecholamines, such as norepinephrine (NE) (Kirkegaard and Faber, 1998).

As a result of studies on antidepressants, two generalized monoamine-based hypotheses emerged relative to the etiology of depression: the catecholamine hypothesis and the serotonin hypothesis (Henley and Koehnle, 1997; Maes and Meltzer, 1995). It was hypothesized that there was a decreased availability in 5-HT or NE or both amines in depression. These hypotheses derived from an initial study in which it was observed that reserpine, an anti-hypertensive agent, precipitated depressive-like symptoms in 15% of patients as well as in animals. It was found that reserpine caused a depletion of brain 5-HT, NE and other amines by causing the transmitter-containing vesicles to release NTs into the cytoplasm, where they are subject to degradation by the enzyme monoamine oxidase (MAO) prior to exocytosis (Barchas et al., 1994).

These hypotheses are not consistent with all of the available clinical evidence. For instance, it has been observed that the onset of depression in some patients is actually associated with an increase in NE levels in cerebrospinal fluid (CSF) (Kandel, 1991). Thus,
it is now generally accepted that depression is most likely a disorder with several underlying pathologies and that transmitter systems are not independent of each other.

1.6.4 Antidepressant Treatments

There are two basic types of effective antidepressant treatments for depressive illness: electroconvulsive therapy (ECT) and antidepressant drugs. Antidepressant drugs can be further classified into the following groups: monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs). Approximately 70-80% of depressed patients respond to antidepressant drugs, whereas the remaining 20-30% are resistant to such medication (Aronson et al., 1996; Henley and Koelnle, 1997; Howland, 1993; Vandoolaege et al., 1997).

Among antidepressant treatments, ECT has been used for the longest period of time. It involves the passing of an electric current to cause a brief seizure. It is very effective in drug-resistant patients and in those who experience severe and disabling symptoms (Barchas et al., 1994; Tremont and Stern, 1997). Despite this, it is often reserved as a final intervention because of the possibility of cognitive effects (Tremont and Stern, 1997).

The MAOIs are known as the first generation antidepressants. Following inhibition of the enzyme MAO, there is a significant increase in dopamine (DA), 5-HT and NE concentrations in brain. The increased availability of 5-HT in the brain leads to an increase in serotonergic transmission. Since MAO is widely distributed throughout the body, side effects do occur and are due to excessive stimulation of the CNS (Warsh and Khanna, 1998).
The TCAs, known as second generation antidepressants, are usually first-line treatments for primary affective illness (Howland, 1993). They function as potent reuptake blockers of 5-HT and NE. However, the exact mechanism of action has yet to be elucidated since inhibition of reuptake occurs rapidly, whereas their clinical effects develop slowly over a period of 2-4 weeks. Since TCAs have potent effects on the cardiovascular system, side effects such as hypertension and arrhythmias are observed (Warsh and Khanna, 1998).

Consistent with the serotonin hypothesis, compounds that act selectively on this NT were produced. All the SSRIs are potent inhibitors of 5-HT reuptake causing little inhibition on DA or NE reuptake. Although there is a significant reduction in the adverse effects produced with SSRIs compared with the conventional TCAs, the SSRIs produce anxiety and headaches, which may be related to increased stimulation of 5-HT receptor subtypes (Warsh and Khanna, 1998).

1.7 Thyroid Hormones and Depression

The literature on hyper- and hypothyroid disorders clearly indicate that THs are important in maintaining cognitive and affective homeostasis (Henley and Koehnle, 1997). Moreover, a significant body of evidence implicates THs in the pathophysiology of depressive disorders and in the mechanisms of action of antidepressant treatments.

1.7.1 Clinical and Subclinical Thyroid Disorders

Clinical studies have documented the occurrence of psychiatric symptomatology in patients with both hyper- and hypothyroidism. Emotional lability and anxiety have been reported in patients with clinical hyperthyroidism (Bauer and Whybrow, 1988; Joffe and
Sokolov, 1994; Nomura, 1994). On the other hand, clinical hypothyroidism is associated with features of depression, especially lethargy, fatigue and cognitive impairment (Joffe and Sokolov, 1994). In fact, virtually 100% of patients with severe hypothyroidism suffer from depression (Haggerty and Prange, 1995; Henley and Koehnle, 1997). Even in patients with subclinical thyroid disease, where abnormalities of the hypothalamic-pituitary-thyroid axis are subtle, there is an association between alterations in TH levels and mood and cognitive disturbances (Joffe and Sokolov, 1994; Nomura, 1994). Furthermore, it appears that subclinical hypothyroidism may be more prevalent in refractory depression that in other types of depression (Howland, 1993; 1995; Joffe and Levitt, 1992).

1.7.2 Peripheral Thyroid Hormone Levels in Depressed Patients

The majority of depressed patients have TH levels within the normal or euthyroid range (Gold et al., 1981; Joffe and Sokolov, 1994; Nomura, 1994). Only 5-10% have evidence of clinical hypothyroidism (Barsano et al., 1994; Gold et al., 1981; Joffe and Levitt, 1992). The studies which have examined circulating levels of THs in depressed patients have produced inconsistent results. Many studies have reported slight elevations in T₄ levels in depressed individuals compared to healthy controls (Bauer and Whybrow, 1988; Joffe and Sokolov, 1994). The results are even less consistent with regards to T₃. In general, it has been reported than no changes in circulating T₃ levels were found (Bauer and Whybrow, 1988; Joffe and Sokolov, 1994), although rT₃ levels may be increased in depressed patients (Kirkegaard and Faber, 1998). One of the most widely documented abnormality of thyroid function is that 25-30% of depressed patients have a blunted TSH response to TRH (Loosen, 1985; Loosen and Prange, 1982; Prange et al., 1987). However, an abnormal TSH response
to TRH is not specific to depression (Loosen, 1985; Nomura, 1994) and has been observed in alcoholic patients and those suffering from anorexia nervosa (Devlin and Walsh, 1988). There also appears to be a blunting of the circadian variation of TSH and an increase in TRH levels in the CSF of depressed patients (Kirkegaard and Faber, 1981).

1.7.3 Effect of Antidepressants on Peripheral Thyroid Hormone Levels

Unlike the findings of circulating TH levels in depressed patients, the evaluation of the effects of antidepressants on circulating TH levels has produced relatively consistent results. Most (Baumgartner et al., 1988; Brady and Anton, 1989; Hoflich et al., 1992; Joffe and Singer, 1990b; Kirkegaard and Faber, 1986; Roy-Byrne et al., 1984; Whybrow et al., 1972), but not all (Leichter et al., 1979; Nordgren and Scheele, 1981) studies have demonstrated that treatment with a variety of antidepressants including cognitive therapy (Joffe et al., 1996) is associated with significant decreases in T4 levels (Bauer and Whybrow, 1988; Joffe and Sokolov, 1994). Moreover, in two of these studies, responders to TCAs had greater reductions in circulating T4 levels than nonresponders (Baumgartner et al., 1988; Joffe and Singer, 1990a). Reductions in circulating T4 levels following chronic treatment with the tricyclic desipramine have also been reported in animal studies (Campos-Barros and Baumgartner, 1994; Joffe et al., 1993).

1.7.4 Thyroid Hormone Augmentation of Tricyclic Antidepressants

THs have been used to treat patients with depressive illness in the absence of thyroid disease (Banki, 1975; Joffe and Singer, 1990a; Joffe and Levitt, 1994). Most studies have shown that the addition of small amounts (25-50 μg) of T3 will convert 67% of tricyclic
nonresponders to responders within a few days to 2 to 3 weeks (Henley and Koehnle, 1997; Joffe and Singer, 1990a). Thus, T₃ potentiation provides a rapid and effective way to treat refractory depression. This potentiation was not affected by the type of antidepressant used, sex or baseline thyroid status of the patient (Joffe and Sokolov, 1994). Recently, it has been suggested that patients who respond to T₃ augmentation may have elevated levels of THs prior to any antidepressant treatment compared to those who do not respond to such augmentation (Sokolov et al., 1997). The mechanism of action of T₃ is unknown and it does not appear to increase plasma levels of the antidepressants (Brochet et al., 1987; Joffe and Sokolov, 1994).

Since T₃ is derived from the peripheral monodeiodination of T₄, it was assumed that T₄ would have a similar potentiating effect. The potentiating effect of T₄ and T₃ were compared in a randomized, double-blind study by Joffe and Singer (1990a) in which they found that T₃ was significantly more effective than T₄ in converting tricyclic nonresponders to responders. However, no placebo group was included in this study, so it is uncertain whether T₄ treatment was superior to placebo.

1.7.5 Hypotheses about the Role of Thyroid Hormones in Depression

Although the clinical findings implicate TH alterations in depressive illness and in the mechanism of action of antidepressant treatments, the nature of this involvement is not clear. In fact, these clinical observations have led to the development of two conflicting hypotheses that are supported by contrasting interpretations of the same data.
1.7.5.1 Thyroid Hypofunction Hypothesis

The first hypothesis was suggested by Whybrow and Prange (1981) whereby it was hypothesized that depression is a state of thyroid hypofunction and that increases in TH levels are required for antidepressant response. This hypothesis is consistent with the association between clinical hypothyroidism and depressive symptoms. As well, T₃ augmentation of TCAs in refractory depression partially supports this hypothesis. The relative increases in peripheral T₄ levels in depressed patients are seen as a compensatory response to the depressed state (Joffe and Sokolov, 1994). Thus, the elevations in TH levels are seen as a response to the illness, rather than a component of it. However, this hypothesis is not consistent with the findings that T₃ is more effective in potentiating the efficacy of TCAs in nonresponders than T₄ and that a variety of antidepressants cause reductions in circulating T₄ levels.

1.7.5.2 Thyroid Hyperfunction Hypothesis

An alternative hypothesis has been proposed by Joffe (Joffe and Sokolov, 1994). They hypothesize that depression is a state of excess THs in brain. Thus, the relative increases in circulating T₄ levels are seen as a pathological finding rather than a compensatory response. Consistent with this hypothesis are the findings that decreases in T₄ are observed with antidepressant treatments (Bauer and Whybrow, 1988; Joffe and Sokolov, 1994) as well as the blunted circadian variation in TSH levels, reduced TSH response to TRH (Prange et al., 1987) and elevated TRH levels in the CSF of depressed patients (Kirkegaard and Faber, 1981). This hypothesis is also supported by the finding that T₃ is more effective than T₄ in augmenting TCA response in refractory depression and is based on the differential regulation
of THs in peripheral organs and the brain. \(T_3\) administration may not lead to enhanced brain TH function as hypothesized by Bauer and Whybrow (1988) since exogenous \(T_3\) administration would result in elevations in plasma \(T_3\) levels. This would ultimately lead to a decrease in plasma \(T_4\) levels due to negative feedback regulation. As circulating levels of \(T_4\) are determinants of intracerebral \(T_3\), this would paradoxically make less \(T_3\) available in the brain. On the other hand, exogenous \(T_4\) administration would lead to elevations in plasma \(T_4\) levels and consequently make more THs available to the brain.

Neither the TH deficiency or TH excess hypotheses are fully consistent with all of the available data. In order to understand and delineate the role of THs in depression, brain measurements of THs are needed. However, obtaining measurements from post-mortem brains from depressed patients have potential confounds in that there is lack of control over post-mortem freezing time (which would affect the deiodinase enzymes) and medication history. In addition, there are currently no suitable in vivo imaging techniques available to study brain THs in depressed individuals (Bauer and Whybrow, 1988). Animal models are able to circumvent these problems and may provide important information on a number of key issues regarding the role of brain THs in depressive-like states.

1.8 Animal Models

There are numerous animal models of depression including the forced swim test ("behavioural despair"), olfactory bulbectomy, restraint stress, chronic mild stress and the learned helplessness (LH) paradigm. However, few are able to reproduce core features or conditions that prevail in human depression. Uncontrollable stress models are among the few
models that approach reasonable standards in terms of fulfilling the criteria for face, predictive and construct validity (see section 1.8.2) (Anisman et al., 1991; Healy, 1987; Katz, 1981; Maier and Seligman, 1976; Willner, 1986).

1.8.1 Learned Helplessness Model of Depression

Exposure to uncontrollable stress is well documented to induce deficits in avoiding subsequent stressors. In a typical procedure, animals are initially exposed to inescapable footshock and are then tested in an escapable shock session whereby animals can terminate shock delivery. This escape deficit effect was first reported by McCulloch and Bruner (1939). Since then, the phenomenon has been extensively observed across the animal kingdom (Eisenstein and Carlson, 1997) and has been referred to as “learned helplessness” following the conceptualization of Overmeir and Seligman (1967). Overmeir and Seligman proposed that it is the subject's lack of control over a stressor that produces the “helpless” behaviour. This paradigm resembles what is observed in depressed individuals, in which they believe that they are powerless and helpless in the face of adversity. However, as is discussed later, there are questions with regards to the cause(s) of the “helplessness”.

1.8.2 Validity of the Learned Helplessness Model of Depression

In evaluating animal models of psychiatric disorders, three sets of criteria must be met before the model can be utilized for neurobiological research (Willner, 1986).

1.8.2.1 Face Validity

In order for the criteria for face validity to be met, the model should resemble the condition being modeled in symptomatology. Exposure to uncontrollable stress is known to
induce weight loss (Dishman, 1997; Eisenstein and Carlson, 1997; Willner, 1986), anhedonia (Dishman, 1997; Willner, 1986), deficits in motoric function (Anisman et al., 1978), sleep and sexual behaviour (Dishman, 1997; Healy, 1987; Willner, 1986) as well as changes in brain neurochemical systems (Anisman et al., 1980; Dishman, 1997; Overmier and Hellhammer, 1991; Sherman et al., 1982). Thus, exposure to uncontrollable stress is known to induce a variety of behavioural and physiological changes that are analogous to what is observed in human depression (see section 1.6.1).

1.8.2.2 Predictive Validity

A model has predictive validity if it can successfully differentiate between effective and ineffective therapeutic treatments as observed in the actual disorder. The LH model has good predictive validity in that TCAs, MAOIs and ECT prevent and reverse escape deficits (Overmier and Hellhammer, 1991; Sherman et al., 1979; 1982; Sherman and Petty, 1980; Thiebot et al., 1992; Willner, 1986) whereas psychoactive drugs that are ineffective in treating human depression (such as anxiolytics and neuroleptics) do not reverse the escape deficits (Eisenstein and Carlson, 1997; Overmier and Hellhammer, 1991). Furthermore, low doses of T₃ reverse LH (Martin et al., 1985; 1987; Massol et al., 1990), potentiate the effectiveness of antidepressants in reversing the escape deficits (Martin et al., 1987) and make antidepressants effective in rats that were previously resistant to such treatment in the LH model (Martin et al., 1987).
1.8.2.3 Construct Validity

Unlike face validity, in which only the demonstration of similarity between the symptoms in the model and the disorder is required, construct validity requires that the model be consistent with some sound theoretical rationale (Willner, 1986). This means that the cause of the condition that is being modeled should be the same as the cause of the actual disorder. Part of the difficulty in establishing the construct validity of the LH model lies in the fact that while feelings of “helplessness” are a common feature in human depression, it is not clear that such feelings are a result of experiences with inescapable stress. However, the essential difficulty lies in the fact that the primary cause of depression in humans remains unknown. For this reason, it is not possible at present to be certain about the construct validity of this or any other model of depression. Nevertheless, it is instructive to consider the nature of the behavioural deficits seen in the LH model.

1.8.2.4 The Nature of the Behavioural Deficits

Although the behavioural consequences of exposure to uncontrollable stress have been well documented, there are differing views of the specific cause(s) of these escape deficits. These include reinforcement of competing motor responses, adaptation to shock and learned helplessness (Overmier and Seligman, 1967; Seligman and Maier, 1967). The LH hypothesis is based on the assumption that inescapable stress makes subjects “learn” that responding does not terminate the stress, so that they become helpless in avoiding new and subsequent stressors. Nevertheless, others have questioned whether any learning is involved and attribute the behavioural deficits to the uncontrollability of the stressor (Anisman et al., 1991; Anisman and Zacharko, 1990; Overmier and Hellhammer, 1991) due to the fact that
much of the symptomatology that occur in the LH model is short-lived (lasting between 48-72 hours after shock exposure) whereas learned responses are normally long-lasting and persist for months (Weiss et al., 1981). As a result of this discrepancy, a second group of explanations argues that the deficits observed following inescapable shock are a result of depletions in brain catecholamines which can lead to impairments in motor function (motor activation deficit hypothesis) and interfere with responding (Weiss et al., 1981).

Direct evidence in favour of the LH interpretation of the behavioural deficits comes from the finding that performance deficits after inescapable shock were also observed when animals were required to reduce responding (passive avoidance) in order to escape subsequent stressors (Jackson et al., 1978). Although these observations cannot be attributed to the motor inactivation explanation, they do not indicate that the behavioural deficits are only a result of cognitive changes ("helplessness"). In fact, it is clear that inescapable shock exposure produces both a learning and a motor deficit (Maier and Jackson, 1979).

1.8.2.5 Assumptions involved in the choice of the LH model

Our choice of the LH model is not predicated on a cognitive theory of depression, or on whether the behavioural deficits are cognitive (vs. motoric) in nature. Rather, we regard the escape deficits as a marker of a broader physiological state that arises in some animals as a result of the inescapable shock experience. In this context, the use of the term "learned helplessness" for the behavioural effect to be studied follows common usage but does not imply specific assumptions about the nature of the response.
Our choice of this particular stress model has also been dictated by one additional characteristic, namely the fact that the behavioural deficits are seen in only a percentage of animals (20-30%) previously exposed to inescapable stress (Lachman et al., 1993). This suggests a role for genetic variables, as is otherwise the case in human depression. In the particular paradigm we have chosen, the behavioural response is seen as a marker of predisposition to depressive type responses to uncontrollable stress. Stated differently, stress does not “create” a depressive state, but may serve to precipitate the condition in susceptible individuals.

1.9 Measurements of Thyroid Hormones in Brain

The two hypotheses concerning the nature of TH abnormalities in depressive disorders claim that depressive illness and antidepressant treatments are associated with TH alterations in brain. Although it is relatively easy to obtain serum measurements of the hormones, these are limited indices of brain THs due to the differential regulation of THs in brain and the differences in rates of formation of $T_3$ and $T_4$ in brain as compared to peripheral organs (Crantz and Larsen, 1980; Leonard et al., 1981). Since brain THs are maintained within very narrow limits by specific homeostatic mechanisms (Dratman et al., 1983; Leonard et al., 1980; Morreale de Escobar et al., 1985), small fluctuations in brain THs may lead to significant functional effect that may not necessarily cause significant changes in peripheral TH activity.

Measuring THs in post-mortem brain presents a number of technical difficulties. A very sensitive radioimmunoassay (RIA) method was first described by Morreale de Escobar et al (1985) for fetal brain tissue. However, the assay requirements dictate using relatively
large amounts of brain tissue. Baumgartner et al (1994) and Campos-Barros et al (1994) have improved the methodology thereby reducing the assay tissue requirements, although these authors still used proprietary T₃ and T₄ antibodies. Recently, Mason and Prange (1993) and Sarkar and Ray (1994) have adapted the method described by Morreale de Escobar et al (1985) to measure synaptosomal and whole brain TH levels by using a commercially available RIA kit. The work described in this thesis followed a similar approach. From a methodological point of view, one objective of the present project was to develop assay conditions suitable for detection of THs in brain tissue using a commercially available RIA kit.
1.10 Summary, Rationale and Objectives

As noted in the introduction, there is substantial evidence for the involvement of THs in depressive illness. However, the mechanisms underlying this involvement are currently unknown. Specific hypotheses have been proposed, but have been difficult to test in the clinic. While clinical studies have provided insight regarding the association between depression and circulating levels of THs, no information currently exists concerning the role of brain THs in depressive illness.

It is difficult to test the two hypotheses regarding the role of brain THs in depressive illness in the clinic since there are currently no suitable in vivo imaging techniques available and measurements in post-mortem brains from human depressed patients can be confounded. For these reasons, we have chosen to use an animal model of depression for this initial assessment of possible changes in brain THs. The learned helplessness model was selected because it appears to reproduce key aspects of human depression.

Since the LH animal model of depression is an uncontrollable stress model, it was initially important to investigate the effects of acute stress on brain THs. These were the objectives of the first and second studies. The third study evaluated brain THs in rats that were behaviourally screened following the LH paradigm. The fourth study evaluated brain THs in selectively bred rats with an increased vulnerability to developing escape deficits in the LH paradigm.
The goals of this thesis can be summarized as follows:

1) To determine whether there are changes in brain THs in rats following acute footshock stress.

2) To determine whether a "dose-response" relationship exists between changes in brain THs and amount of shock received by the rats.

3) To use the learned helplessness model of depression to examine brain THs in rats showing escape deficits after uncontrollable stress exposure.

4) To examine brain THs in a genetic model of depression whereby rats are selectively bred for increased susceptibility to escape deficits in the learned helplessness paradigm.
2.0 GENERAL METHODS

2.1 Animals

Subjects were male and female Sprague-Dawley rats (Charles River Laboratories, Montreal, Quebec) arriving at weights between 200-225 g.

2.2 Housing, Maintenance and Grouping

Upon arrival, the rats were housed individually in hanging wire mesh cages or in groups of 3 in plastic cages with wire mesh tops. The room was maintained at constant temperature (21° ± 1°C) with a 12 hour light cycle (lights on at 07:00 h). Food (Purina rat chow) and water were given ad libitum. Rats were given a seven day acclimation period to the housing conditions and to daily handling by the experimenter. The rats were weighed daily prior to experimentation. All testing was conducted during the light phase and all experimental procedures conformed to the guidelines laid down by the Canadian Council on Animal Care and were approved by the Animal Care Committees at the University of Toronto and the Clarke Institute of Psychiatry. On the day of the experiment, the rats were divided into three groups matched for body weight using the mean of the last three days of body weight recordings.

2.3 Shock Procedures

Rats were placed in sound-attenuated operant boxes measuring 24 cm long, 30 cm wide, and 21 cm high (Model ENV-008CT, Med Associates Inc., St. Albans, VT). Each chamber contained two retractable response levers, 4.5 cm wide and 7 cm above the floor of the chamber as well as a food pellet dispenser. The rats were tested three at a time in the
operant boxes. The shock stimulus consisted of scrambled pulsed 0.8 mA shock delivered to the metal bar floor of the operant chamber by a software-controlled shock generator (Med Associates Inc., St. Albans, VT). Rats in the Escapable Shock (ES) group could terminate shock delivery by pressing a retractable lever. Rats in the Inescapable Shock (IS) group had no control over shock termination. Rats in the control group received no shock. 15 trials of footshock were given. Each trial began with the onset of the shock stimulus accompanied by a red light. The trial ended either when an ES rat pressed the lever or when 60 seconds elapsed. Intertrial intervals were randomly varied from 1.5 and 24 seconds. Response latencies (seconds) for each subject were recorded automatically on a 386-SX IBM computer.

2.4 Sacrifice

Following shock exposure, rats were sacrificed by decapitation. Their brains were rapidly removed, rinsed in 0.9% ice-cold saline and cut along the midline. The half-brains were placed on aluminum foil and immediately frozen over dry ice. The brain samples were then stored at -80°C until assayed.

During the sacrificing, trunk blood was obtained and collected in evacuated blood collecting tubes (Terumo Europe Inc, Belgium) with 0.2 mL heparin. The tubes were centrifuged (Medifuge centrifuge) at 2600 x g for 10 minutes. Plasma was then collected by pipetting the clear supernatant and aliquoting into eppendorfs which were frozen over dry ice immediately. The plasma samples were stored at -80°C until assayed.
2.5 Brain Thyroid Hormone Assay

2.5.1 Resin Preparation

Resin (AG 1-X2, 200-400 mesh chloride form, Bio Rad Laboratories, Hercules, CA) was weighed out and placed in a volume of 70% glacial acetic acid five times the weight of the resin. The resin was left for 2 days in a 4°C refrigerator. Then, a vacuum filtration apparatus was used to filter the resin. Filtration consisted of pouring the resin in 70% acetic acid through the apparatus followed by washing the resin with 1-2 L of dH2O and 1-2 L of sodium acetate buffer (pH 7.0). The resin was then stored in the sodium acetate buffer (pH 7.0) in a 4°C refrigerator.

2.5.2 Extraction

Extraction of the iodothyronines followed modifications of the procedure described by Morreale de Escobar et al. (1985). Frozen half-brains were weighed and placed in centrifuge tubes. 4 mL of 80% reagent-grade methanol, 20% 0.02N NaOH, 3 μM iopanoic acid (deiodinase inhibitor) were added to each tube and the samples were homogenized on ice for 1-2 minutes with a Brinkmann polytron (Switzerland) set at level 7. After each sample was homogenized, the polytron probe was rinsed with an additional 1 mL of the methanol solution. Then, 100 μL of [125I] L-triiodothyronine (T3) tracer in phosphate buffer with sodium salicylate (ICN, Montreal, Quebec) and [125I]L-thyroxine (T4) tracer in pH 7.9 buffer (ICN, Montreal, Quebec) were added to the homogenates (using the following arbitrary convention: [125I]T3 was added to the odd-numbered samples and [125I]T4 added to even-numbered samples). 10 glass beads (Fisher Scientific, Pittsburgh, PA) were added to
each centrifuge tube and the tubes were centrifuged (Medifuge centrifuge) at a speed of 2600 x g for 10 minutes. The first supernatants were pipetted into new centrifuge tubes and the remaining pellets were resuspended in 4 mL of ice-cold 100% reagent-grade methanol and 3 μM iopanoic acid solution. Resuspension included vortexing the pellets vigorously for 1 minute. The procedure was repeated three times until the volume of supernatant collected from the 3 spins was 13 mL. The pellets were kept at room temperature and left to dry overnight. 17 mL of chloroform (hydrophobic solvent) and 6.5 mL of 0.05% calcium chloride (CaCl₂) (hydrophilic solvent) were added to the second set of centrifuge tubes for a final volume of 36.5 mL. The tubes were vortexed vigorously and centrifuged (Silencer, H-103NA series) at 4°C for 2 minutes at 700 x g. The upper layer was collected into a third set of centrifuge tubes. Once again, this procedure was repeated three times. After the initial spin, a chloroform:methanol:CaCl₂ solution (3:49:48) was used to maintain the volume at 36.5 mL. After 3 spins (Silencer, H-103NA series) at 700 x g, the upper layers were kept in a 4°C refrigerator overnight until purification by column chromatography.

2.5.3 Purification of Extracts

The following day, polyprep columns (Mandel Scientific Company, Guelph, Ontario) were filled with resin to a height of 2.5 cm. Contents of the third centrifuge tube were pipetted and allowed to run through the columns. This was followed by washing of the columns with the following solutions in the following order: 2 mL sodium acetate buffer (pH 7.0), 2 mL ethanol and 3 μM iopanoic acid, 4 mL sodium acetate buffer (pH 7.0), 2 mL ethanol and iopanoic acid solution, 2 mL sodium acetate (pH 7.0), 2 mL sodium acetate buffer (pH 4.0), 2 mL acetate buffer (pH 3.0), 2 mL 1% glacial acetic acid and 2 mL 35%
glacial acetic acid. Finally, the iodothyronines were eluted with 70% glacial acetic acid. Two fractions; one with a volume of 500 μL and one with a volume of 2 mL 70% glacial acetic acid were collected separately in culture tubes and the radioactivity was counted in a gamma (γ) counter (Beckman 5500B) for one minute/sample. % recoveries were calculated by comparing the cpm from the 2 mL 70% acetic acid fraction with 100 μL of the [125I]T₃ or [125I]T₄ tracers. The efficiency of the extraction was 70-80% for T₃ and 50-60% for T₄. The 2 mL fractions were aliquoted into 2 mL eppendorfs and placed in a speed vacuum (Savant Instruments Inc.) for overnight drying. The following day, the samples were removed from the speed vacuum and frozen at -80°C until RIA was carried out. T₃ and T₄ were measured using commercially available reagent kits (ICN, Montreal, Quebec) and procedures followed modifications of the RIA method described by Mason and Prange (1993) and Sarkar and Ray (1994).

2.5.4 Triiodothyronine Determination

On the day of the assay, the dried-down samples were reconstituted in 300 μL of stripped-down human serum (ICN, Montreal, Quebec). For T₃ determination, a solid phase component system RIA kit (ICN, Montreal, Quebec) was used. 50 μL of the reconstituted samples and the serum standards (ICN, Montreal, Quebec) containing T₃ in human serum were added to polypropylene tubes coated with T₃ rabbit antiserum (ICN, Montreal, Quebec) with high specificity to T₃ (% cross-reactivity to T₄, 3,5-diiodothyronine, 3,3′,5′-L-triiodothyronine (rT₃) was 0.18%, 0.44% and 0.01% respectively). The standard curve ranged from 0-800 ng/dL. This was followed by addition of 1 mL [125I]T₃ tracer. The tubes were vortexed gently and incubated in a 37° ± 1°C water bath for 60 minutes. At the end of
the incubation period, the liquid in the tubes was decanted and the rims of the tubes were blotted dry. Then, the tubes were rinsed with 4 mL dH2O and blotted dry again. The radioactivity in the tubes was counted in a γ counter. Sensitivity of the assay was 0.067 pg T3/μL.

2.5.5 Thyroxine Determination

T4 levels were determined by using a monoclonal solid phase RIA kit (ICN, Montreal, Quebec). The serum standards (ICN, Montreal, Quebec) containing L-thyroxine in human serum were diluted 1:10 with stripped-down human serum (ICN Montreal, Quebec). The diluted standard curve ranged from 0-1000 ng/dL. The dried-down samples were reconstituted with 300 μL stripped-down serum and 50 μL of samples and standards were added to free T4 plastic tubes coated with mouse T4 antiserum (ICN, Montreal, Quebec) with high specificity to T4 (% cross-reactivity to D-thyroxine, T3 and 3,5-diiodo-L-thyronine was 30.9%, 1.0% and <0.002 respectively). This was followed by the addition of 1 mL [125I]T4 tracer to each tube and a gentle vortexing. The rest of the procedure was identical to that described for T3. Sensitivity of the assay was 7.6 pg T4/μL.

2.6 Thyroid Hormone Determination in Plasma

2.6.1 Triiodothyronine Determination

On the day of the assay, the frozen plasma samples were thawed at room temperature. T3 levels were determined using a solid phase component system RIA kit (ICN, Montreal, Quebec). After thawing, 100 μL of each sample were added to the corresponding antibody-coated tubes. This was followed by addition of 1 mL [125I]T3 tracer and a gentle vortexing
of the tubes. Then, the tubes were incubated in a 37° ± 1° C water bath for 60 minutes. At the end of the incubation period, the liquid in the tubes was decanted and the rims of the tubes were blotted dry. The tubes were rinsed with 1 mL dH2O. The standard curve was prepared using the serum standards ranging from 0-800 ng/dL. The radioactivity in all tubes was counted with a γ counter. Sensitivity of the assay was 6.7 ng T3/dL.

2.6.2 Thyroxine Determination

For T4 determination, a monoclonal solid phase RIA kit (ICN, Montreal, Quebec) was used. T4 levels were determined by adding 25 μL of each sample to total T4-antibody coated tubes which was followed by the addition of 1 mL of [125I]T4 tracer to each tube. The tubes were then incubated at room temperature for 60 minutes. At the end of the incubation period, the liquid in the tubes was decanted and the tubes were allowed to drain for one minute in an inverted position and the rims were blotted dry. A standard curve was prepared using serum standards, ranging from 0-20 μg/dL. The radioactivity in the tubes was counted using a γ counter. Sensitivity of the assay was 0.76 μg T4/dL.

2.7 Corticosteroid Determination in Plasma

On the day of the assay, plasma samples were allowed to thaw at room temperature. Plasma corticosteroid levels were determined using an Immunchem™ double antibody 125I RIA kit (ICN, Montreal, Quebec). Plasma samples were diluted 1:200 with steroid diluent (ICN, Montreal, Quebec). 100 μL of the diluted samples and standards ranging from 25-1000 ng/mL were pipetted into eppendorf tubes. This was followed by addition of 200 μL of [125I] corticosterone and 200 μL anti-corticosterone to all tubes. Then, the samples were
vortexed and left to incubate at room temperature for 2 hours. After incubation, 500 μL of precipitant solution were added to the tubes. Once again, the assay tubes were vortexed thoroughly. The tubes were centrifuged in a refrigerated Beckman Microfuge™ 12 at 500 x g for 15 minutes. Following centrifugation, the supernatants were decanted and the rim of the tubes were blotted dry. The precipitates were counted in a γ counter.

2.8 Brain Type II Deiodinase Activity Assay

2.8.1 Purification of Radioiodide

A 50% slurry was made using Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) and dH₂O. 400 μL of this slurry was loaded onto polyprep columns (Bio Rad Laboratories, Hercules, CA). The columns were activated with 2 mL 0.1 M HCl. Next, 200,000 cpm of the 3,3',5'-[¹²⁵I]triiodothyronine (rT₃) substrate (specific activity: 791 μCi/μg, NEN Life Sciences Products) was diluted 1:10 with 0.1 M HCl and loaded onto the columns. This was followed by washing of the column with 4 mL 0.1 M HCl and 2 mL dH₂O. The purified iodothyronine rT₃ was eluted with 800 μL of ethanol: NH₄OH (99:1) and this eluant was collected and dried under a stream of compressed nitrogen gas for 30 minutes.

2.8.2 Type II Deiodinase Assay Procedure

The measurement of 5'D-II activity was based on the release of radioiodide from the purified [¹²⁵I]rT₃ substrate. Frozen half-brains were homogenized on ice in 5-6 v/w homogenization buffer consisting of 320 mM sucrose, 10 mM dithiothreitol (DTT), 10 mM HEPES buffer (pH 7.0) and 1 mM EDTA for 1 minute using a polytron set at level 7. The reaction mixture consisting of 0.1 μM rT₃, 200 mM DTT in 0.1 M KH₂PO₄ at pH 6.5, 1 M
KH₂PO₄/10 mM EDTA and 10 mM propylthiouracil (PTU) was added to the purified dried-down culture tube. Each tube contained 29.5 µL of the reaction mixture, 40.5 µL dH₂O and the reaction in each tube was started by the addition of 30 µL of the corresponding tissue homogenate, so that the final volume in each tube was 100 µL. Control tubes (representing non-enzymatic deiodination) were included with every assay and contained 30 µL of the homogenization buffer instead of tissue homogenate. The tubes were incubated at 37° ± 1°C for 60 minutes. Following the incubation period, the reaction in each tube was stopped with the addition of 50 µL ice-cold 10 mM PTU:8% bovine serum albumin (BSA) followed by 350 µL ice-cold trichloroacetic acid. The tubes were centrifuged (Silencer, H-103NA series) at 4°C for 30 minutes at 1500 x g. 400 µL of the supernatant containing the free ¹²⁵I were further purified by column chromatography (polyprep columns) using 1.6 mL of resin (Dowex 50W-X2 strongly acidic cationic exchanger, Sigma, St. Louis, MO). The free ¹²⁵I was eluted with 2 mL 10% glacial acetic acid into culture tubes and the tubes were counted in a γ counter. Specific enzymatic activity was expressed as femtomoles of ¹²⁵I released/mg protein/hour and was determined by subtracting the ¹²⁵I produced in the samples from the ¹²⁵I produced from the tissue-free controls. Because the [¹²⁵I]rT₃ substrate was randomly labeled with ¹²⁵I in the equivalent 3' or 5' positions of the phenolic ring, the labeled iodide release was half that of the degraded iodothyronines. This was accounted for in the analysis of the data. Each experimental point was determined in triplicate. The remaining tissue homogenates were aliquoted into eppendorfs and immediately frozen at -80°C for subsequent protein analysis.
2.8.3 Protein Determination

Quantitation of protein content in the homogenates was performed using the method of Bradford (1976). On the day of the assay, frozen homogenates were thawed at room temperature. BSA at a concentration of 1 mg/mL was diluted 1:10 with dH₂O. A standard curve using the diluted BSA was created ranging from 0-25 μg BSA. Tissue homogenates were diluted 1:1000 with dH₂O to ensure that the absorbance readings would lie in the 0 to 50 μg range. 2 mL of each standard and sample were used in the assay. 500 μL of dye reagent (Bio Rad Laboratories, Hercules, CA) were added to each culture tube for a final volume of 2.5 mL followed by a thorough vortexing of each tube. Absorbance was measured at 595 nm in polystyrene cuvettes using a Shimadzu UV-160 spectrophotometer, with the first standard (0 μg protein) as a calibration blank.

2.9 Data Analysis

T₃ and T₄ brain and plasma levels, plasma corticosteroid levels and protein concentrations were determined using Graphpad Prism™, version 2.0. Data are expressed as means ± standard error of the mean (SEM) throughout and were analyzed by one-way analysis of variance (ANOVAs) followed, where appropriate, by independent two-tailed t-tests. ANOVAs, independent t-tests, means and SEM were performed using Excel, version 5.0. Correlation statistics were also performed using Excel (version 5.0) and utilized the Pearson product moment coefficient of correlation, r. Statistical significance was defined by p values of 0.05 or less.
3.0 INDIVIDUAL STUDIES
STUDY 1: THE EFFECTS OF STRESS CONTROLLABILITY ON BRAIN THYROID HORMONES*

[* Portions of the data are included in Friedman Y et al., Acute Stress Increases Thyroid Hormones in Rat Brain, Biological Psychiatry, in press]
In experimental animals, acute exposure to *uncontrollable* footshock stress is well documented to induce a variety of behavioural and neurochemical changes that are not observed if the animals are exposed to escapable shock (Anisman and Zacharko, 1992; Thiebot et al., 1992). For instance, feeding and sleep patterns are disturbed (Zacharko and Anisman, 1989), locomotor activity is disrupted (Weiss et al., 1981) and there is suppression of responding for different types of reward, including intracranial electrical self stimulation (Zacharko and Anisman, 1991). Due to the similarities between the effects of uncontrollable stress and symptoms seen in human depression (Hellriegel and D’Mello, 1997), brain THs were examined in animals exposed to uncontrollable footshock stress. In addition, since female rats may differ from males in response to stress, gender differences in brain TH levels in response to stress were also examined. The objective of this study was to examine *group* effects in male and female rats as the behavioural deficits observed following uncontrollable stress exposure show considerable within-group variability (Anisman and Zacharko, 1982).

3.1 Procedures

Upon arrival, Sprague-Dawley rats were housed in groups of 3 in plastic cages with wire-mesh tops. In separate experiments, male or female Sprague-Dawley rats were allocated into three different groups. Rats in the first group (n=8) were exposed to 15 trials of escapable shock (ES) whereby shock delivery could be terminated by a lever press. Rats in the second group (n=8) were “yoked” to the first group, which meant that these rats were exposed to the same amount of shock for the same duration as the ES rats, but had no control over shock termination. Thus, rats in the second group were exposed to inescapable shock (IS). Control rats were placed in the operant boxes but did not receive any footshock. Three
hours after shock exposure, all rats were sacrificed and their brains were retrieved as described in section 2.4.

3.2 Results

3.2.1 Whole Brain Levels of $T_4$ and $T_3$ in Male Rats

Whole brain levels of $T_4$ and $T_3$ in male rats are shown in Figure 2. ANOVA indicated no significant differences in whole brain $T_4$ levels between controls, ES and IS groups ($F_{2,19} = 0.45, p = 0.63$). In contrast, whole brain $T_3$ levels were significantly elevated ($F_{2,19} = 4.04, p < 0.03$). The IS rats had a 21% increase in $T_3$ levels compared to controls ($t = 2.78, p < 0.012$). Although the ES group in the male rats did not have significantly different whole brain $T_3$ levels than controls, there was an 11% increase in whole brain $T_3$ levels.

3.2.2 Whole Brain Levels of $T_4$ and $T_3$ in Female Rats

Whole brain levels of $T_4$ and $T_3$ in female rats are shown in Figure 2. There were no significant differences in whole brain $T_4$ levels between the control, ES and IS rats ($F_{2,21} = 0.16, p = 0.84$) as indicated by ANOVA. Similar to what was observed in male rats, whole brain $T_3$ levels were significantly elevated in the three groups ($F_{2,21} = 3.5, p < 0.05$). Whole brain $T_3$ levels were significantly increased by 19% in the ES rats compared to the control group ($t = 2.49, p < 0.026$). The IS rats had a 9% increase in $T_3$ levels compared to controls, although this elevation was not statistically significant.

Since each ES rat could terminate shock delivery for itself and its yoked partner during the 15 trials, different rats received different amounts of shock. The range was 186-
Figure 2: Whole brain levels of T₃ and T₄ (expressed as pg/g tissue) in male and female rats sacrificed 3 hours after a shock session. Each bar is a mean of 8 observations and error bars represent SEM. ***p<0.012, **p<0.026 compared to control group (2-tailed t-tests).
878 seconds for the males rats and 137-851 seconds for the female rats. Correlations as shown in Table 2 were done between the total amount of shock received and brain T₃ levels and these were -0.59 for males (p<0.025) and 0.32 for females (NS). Correlations between T₄ levels and total amount of shock were -0.11 (NS) for male rats and -0.36 (NS) for female rats.

3.2.3 Plasma Levels of T₃, T₄ and Corticosteroids

Plasma levels of the hormones as shown in Table 3 were also measured. Separate analyses of variance indicated that there were no significant differences in plasma T₃ levels for males (F₂,23 = 0.09, p= 0.92) and females (F₂,23 = 0.93, p = 0.42) as well as in plasma T₄ levels for male (F₂,23 = 0.90, p = 0.42) and female rats (F₂,23 = 0.32, p = 0.73).

Since the rats were exposed to shock, plasma corticosteroids (see Table 3) were measured as an indicator of the stress response. No significant differences were found among the three groups for male (F₂,21 = 1.4, p = 0.27) and female rats (F₂,21 = 0.33, p = 0.72). However, in males, corticosteroid levels were elevated by 44% and 91% in the ES and IS groups, respectively, when compared to controls whereas no observable increases in corticosteroids were found in the ES and IS female rats when compared to female controls. Although the ES and IS female rats did not have increased plasma corticosteroid levels compared to shock-naive controls, they had higher baseline corticosteroid levels (4-10 fold) than the male rats.

No significant correlations (Table 2) were found between brain T₃ levels and plasma corticosteroid levels in males (r = -0.32) and in females (r = 0.16). Similarly, there were no
Table 2: Correlations between Total Shock, Plasma Corticosteroids and Brain $T_3$ and $T_4$\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Shock</td>
<td>Cort.</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Shock</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cort.</td>
<td>0.057</td>
<td>0.26</td>
</tr>
<tr>
<td>Brain $T_3$</td>
<td>-0.59$^*$</td>
<td>-0.32</td>
</tr>
<tr>
<td>Brain $T_4$</td>
<td>-0.11</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>-0.36</td>
<td>0.31</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values are Pearson product-moment correlations, $^p < 0.025$. 
Table 3: Plasma Levels of T₃, T₄ and Corticosteroids in Male and Female Rats After a Single Shock Session

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₃</td>
<td>T₄</td>
<td>bCort.</td>
<td>T₃</td>
</tr>
<tr>
<td>Control</td>
<td>43.27</td>
<td>4.44</td>
<td>27.9</td>
<td>94.7</td>
</tr>
<tr>
<td></td>
<td>± 4.28</td>
<td>± 0.18</td>
<td>± 8.3</td>
<td>± 4.5</td>
</tr>
<tr>
<td>Escapable Shock</td>
<td>45.32</td>
<td>4.61</td>
<td>53.2</td>
<td>86.3</td>
</tr>
<tr>
<td></td>
<td>± 4.13</td>
<td>± 0.23</td>
<td>± 13.8</td>
<td>± 8.2</td>
</tr>
<tr>
<td>Inescapable Shock</td>
<td>42.95</td>
<td>4.76</td>
<td>40.2</td>
<td>100.2</td>
</tr>
<tr>
<td></td>
<td>± 4.72</td>
<td>± 0.23</td>
<td>± 9.3</td>
<td>± 8.4</td>
</tr>
</tbody>
</table>

Values are means ± SEM in ng/dL (T₃), µg/dL (T₄) and ng/mL (b corticosteroids) in male and female rats. N = 8 per group.
significant correlations (Table 2) found between brain T₄ levels and plasma corticosteroids in either male rats (r = 0.33) or female rats (r = 0.31) as well as between plasma corticosteroid levels and total amount of shock received in male (r = 0.06) and female rats (r = 0.26).

### 3.3 Discussion

Exposure to acute stress produced significant increases in whole brain T₃ levels in both male and female rats. To our knowledge, this is the first demonstration of environmentally-induced changes in whole brain TH levels in mature organisms. These elevations in whole brain T₃ levels occurred in the absence of parallel increases in whole brain T₄ levels and in circulating levels of T₃ and T₄ in males and females. These observations suggest that the effects of acute stress are specific to brain tissue, and perhaps specific to T₃.

One possibility is that stress may have increased the activity of the 5'D-II enzyme, which is responsible for maintaining the intracerebral supply of T₃. An enhancement of 5'D-II activity would increase the deiodination of T₄, thereby leading to a decrease in the tissue concentrations of T₄ accompanied by increases in T₃ levels. This possibility is consistent with the observation that in male rats, the group exposed to inescapable shock had the highest levels of T₃ and the lowest levels of T₄. However, in female rats, such trends in whole brain T₃ and T₄ levels were not observed since the rats exposed to escapable shock had the highest levels of T₃, although T₄ levels in the ES and IS groups were similar to control values. Thus, the possibility of changes in the activity of 5'D-II may not account for the increased T₃ levels observed in female rats.
Although THs are not generally considered to be stress hormones, marked effects of very different types of acute stress on TH metabolism and concentrations in mature rat brain have also been reported in one study presented in abstract form (Pinna et al., 1997). In this study, all forms of acute stress (ranging from sleep deprivation to motoric stress) were associated with increased 5'D-II activity in frontal cortex and amygdala, region-specific elevations in \( T_3 \) concentrations and decreases in regional \( T_4 \) concentrations in rats sacrificed two hours post stress. These observations are consistent with the findings in the present study whereby elevations in whole brain \( T_3 \) levels were observed in male and female rats sacrificed three hours following acute footshock stress.

The choice of the sacrifice point (three hours after shock administration) for this initial study took into account the turnover of \( T_3 \) and \( T_4 \) in rat circulation (six hours for \( T_3 \) and ten hours for \( T_4 \)). It is possible that there is a faster turnover of the iodothyronines in brain (Silva et al., 1984; Silva and Matthews, 1984). This does not imply that the alterations in brain \( T_3 \) levels which were observed in males and females after shock administration represent peak effects. Thus, it would be important to characterize the full time course of shock effects on brain THs to assess whether peak effects were captured. Regardless of whether peak effects were captured or not, the data indicate that whole brain TH levels, particularly \( T_3 \), can change rapidly in response to acute stress in both male and female rats.

Although increases in whole brain \( T_3 \) levels were detected in both males and females, gender differences were observed as well. In males, the rats exposed to inescapable shock had significantly higher brain \( T_3 \) levels than shock-naive controls, whereas in females, the rats exposed to escapable shock had a significant elevation in \( T_3 \) levels compared to controls.
This raises the possibility that real gender differences in response to different types of stress may exist and that stress controllability effects on brain T₃ may be different for males and females. There are known gender differences in the behavioural and neurochemical response to stress (Blanchard et al., 1995). For instance, Kennett et al (1986) reported that female rats failed to adapt to repeated restraint stress and became progressively less active when tested in a novel environment compared to similarly-stressed male rats. This inability to adapt to stress is associated with defective serotonin function and elevated glucocorticoid levels in females. This is in agreement with our findings in that female rats had 4-10 times higher basal corticosteroid levels compared to the male rats. Such elevations in corticosteroids are associated not only with abnormal adaptation to stressors, but with precipitation of depressive symptoms in predisposed subjects (Kennett et al., 1986). Naturally, one would expect the shocked rats to have higher corticosteroids than shock-naive controls. In male rats, this was the case, whereas in female rats, the control group had elevations in plasma corticosteroid levels compared to the shocked groups.

It is also possible that stress controllability may not be a critical factor for either males or females with regards to brain T₃ levels, since all shocked groups had elevations in these levels compared to controls. This possibility suggests that there may be a clear association between shock and brain T₃ levels. In fact, there was a significant and unexpected negative correlation between the amount of shock received and brain T₃ levels in male rats. However, no such correlation was found in female rats. Correlations could be spurious (caused by 1-2 rats) and investigation of shock effects on brain THs at various time points should help clarify these findings.
In summary, brain T3 levels, but not T4, were found to increase in response to acute stress exposure in male and female rats. This response seemed to be differentially affected by stress controllability in males and females. This study raised important questions regarding the effects of different amounts of shock on brain THs, particularly on brain T3. The next study attempted to address the effects of different amounts of stress on brain THs.
STUDY 2: EFFECTS OF DIFFERENT AMOUNTS OF FOOTSHOCK STRESS ON BRAIN THYROID HORMONES
The findings from the first experiment suggested that acute footshock stress had marked effects on whole brain T₃ levels in male and female rats. An unanticipated significant negative correlation between T₃ levels in whole brain and amount of shock received in male rats was found. It was important to assess the effects of different amounts of shock on TH-related brain parameters. We concentrated on male rats, since there was no significant correlation found between whole brain T₃ levels and amount of shock received in female rats and the fact that inescapable shock had clear effects on T₃ levels in male rats. Accordingly, the objective of this study was to examine the effects of different amounts of stress on whole brain TH concentrations in a controlled manner in order to determine whether a “dose-response” relationship existed between brain T₃ and T₄ levels and amount of shock. Additionally, the effects of varying amounts of footshock stress on 5’D-II enzyme activity were measured as an indicator of central TH metabolism and regulation of intracerebral T₃ production.

3.1 Procedures

In this experiment, male Sprague-Dawley rats were divided into the following three groups: rats in the first group (n=8) were exposed to a single session of 20 minutes of inescapable shock; rats in the second group (n=8) were exposed to a 40 minute IS session whereas rats in the control group (n =8) received no footshocks. All rats were sacrificed twenty-four hours following shock exposure and their brains were retrieved as outlined in section 2.4.
3.2 Results

3.2.1 Whole Brain Levels of T3 and T4 and 5’D-II Activity

Whole brain T3, T4 levels and 5’D-II activity are shown in Figure 3. ANOVA indicated no significant differences in whole brain levels of T3 (F2,22 = 0.08, p= 0.92), T4 (F2,23 = 0.86, p= 0.44) and 5’D-II activity among the three groups (F2,23 = 0.62, p= 0.55).

3.2.2 Plasma Levels of T3, T4 and Corticosteroids in Male Rats

Plasma levels of T3 and T4 as shown in Table 4 were measured and analysis of variance indicated no significant differences in plasma levels of either T3 (F2,23 = 0.016, p= 0.99) or T4 (F2,23 = 1.12, p= 0.35) in the three groups. Despite the lack of statistical significance in plasma T4 levels, there was an increase in these levels in the 20 minutes IS and 40 minutes IS groups by 5% and 15%, respectively, compared to controls.

Plasma corticosteroids were also measured as shown in Table 4 and no significant differences were observed between the groups (F2,23 = 0.89, p= 0.43), although the 20 minutes IS and 40 minutes IS groups had pronounced elevations in corticosteroid levels compared to controls (97% increase for 20 minutes IS rats and 62% increase for 40 minutes IS rats).

3.3 Discussion

Exposure to different amounts of inescapable footshock did not appear to affect whole brain T3 and T4 levels as well as the activity of the 5’D-II enzyme measured twenty-four hours later. In addition, there were no changes observed in circulating levels of the hormones. In contrast, plasma corticosteroids were elevated in the shocked groups compared
Figure 3: Whole brain levels of $T_3$ and $T_4$ (top panel) and Type II deiodinase activity (expressed as fmol/μg protein/hour) (bottom panel) in male rats sacrificed 24 hours after an inescapable shock (IS) session. Each bar is a mean of 8 observations and error bars represent SEM. One-way ANOVA indicated no significant differences between the groups.
Table 4: Plasma Levels of $T_3$, $T_4$ and Corticosteroids in Male Rats After an Inescapable Shock Session

<table>
<thead>
<tr>
<th></th>
<th>$T_3$</th>
<th>$T_4$</th>
<th>Corticosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>$81.96 \pm 8.16$</td>
<td>$4.48 \pm 0.27$</td>
<td>$18.43 \pm 6.67$</td>
</tr>
<tr>
<td><strong>20 min Inescapable Shock</strong></td>
<td>$80.48 \pm 3.26$</td>
<td>$4.71 \pm 0.41$</td>
<td>$36.34 \pm 12.22$</td>
</tr>
<tr>
<td><strong>40 min Inescapable Shock</strong></td>
<td>$81.43 \pm 5.59$</td>
<td>$5.12 \pm 0.24$</td>
<td>$29.79 \pm 8.55$</td>
</tr>
</tbody>
</table>

*Values are means $\pm$ SEM in ng/dL ($T_3$), $\mu$g/dL ($T_4$) and ng/mL (corticosteroids) in male rats. $N = 8$ per group.
to shock-naive controls, although this did not achieve statistical significance. This indicates that shock exposure was a stressful experience, activating the hypothalamic-pituitary-adrenal axis and leading to increased corticosteroid release, which could be detected even twenty-four hours following shock administration.

While the findings from this experiment suggest that a “dose-response” relationship does not exist between the amount of shock the animals received and TH-related brain parameters, the sacrifice time should be considered. It is possible that alterations in brain THs and 5′D-II activity could have occurred shortly following shock exposure (as was observed in study 1) given the rapid turnover of the iodothyronines in rat brain. After twenty-four hours, a compensatory mechanism could have been activated thereby restoring TH levels and maintaining TH homeostasis in the brain. As in the first study, it would be important to characterize the time course of the shock effect on brain TH-related parameters.

This study also served as a control experiment for the LH paradigm (see study 3) whereby animals are initially exposed to an inescapable shock session and are then tested in a shock escape paradigm whereby shock delivery can be terminated. The shock escape session, which is used to gauge behavioural depression, results in the helpless animals being exposed to a greater amount of shock than other animals. Hence, if any brain changes were to be observed in these animals, it would be unclear whether these changes were a consequence of the shock exposure or an effect associated with the depressed state. Therefore, it is important to separate brain effects due to shock from the effects associated with behavioural helplessness as seen in the LH paradigm. The findings from this study indicate that shock is not a confounding factor in the brain analyses.
STUDY 3: BRAIN THYROID HORMONES IN THE LEARNED HELPNESS PARADIGM
The LH paradigm is an uncontrollable stress model of depression. As discussed in section 1.8.2, it is one of the few animal models that approaches reasonable standards in terms of fulfilling the criteria for face, predictive and construct validity and as such, it is a useful animal model of human depression. The objective of this study was to determine for the first time whether there are changes in brain THs in rats showing escape deficits after acute uncontrollable stress. As discussed in the introduction, in this paradigm, not every animal that is exposed to inescapable stress will be behaviourally impaired. This was an important element in our choice of this paradigm as an animal model of depression. This phenomenon is consistent with the notion that exposure to uncontrollable shock does not create a depressive state, but may serve to precipitate such a condition in susceptible subjects. Our interest lay primarily in identifying animals that displayed such susceptibility. Thus, individual, rather than group effects, were examined.

3.1 Procedures

Male Sprague-Dawley rats that were housed individually were divided into three groups matched for body weight. Rats in the IS group (n=34) underwent an initial training session consisting of the delivery of a series of scrambled pulsed footshocks over a 40 minute period. Twenty-four hours after the initial training session, each rat in the IS and ES group (n=32) was tested in an escape situation whereby 15 trials of footshock were administered and could be terminated if the rat pressed a lever or after a maximum of 60 seconds per trial. Latencies to escape were automatically recorded and a failure to escape was defined as any latency over 20 seconds in each of the 15 escape trials. We chose to determine the behavioural deficits (escape deficits) based on a definition that has been described previously
(Edwards et al., 1986; 1991; Sherman et al., 1982) and has been successfully used to study several neurochemical systems (Edwards et al., 1991; Sherman and Petty, 1982). We have also chosen to employ this definition of helplessness since a genetic line from the laboratory of Dr. Edwards was available for study (see study 4). The following criteria were used: rats scoring 0-5 failures were considered non-helpless (NLH), those with 11-15 failures were considered learned helpless (LH) and those scoring 6-10 failures were not used in the analysis (see Table 5). Control rats were placed in the apparatus but received no electric shock during the two-day shock session. The ES rats were only tested in the shock escape paradigm and thus provided us with baseline responding indices (see Figure 4a). The ES rats were not used in the TH brain analyses. Twenty-four hours following the shock escape session, all rats were sacrificed as described in section 2.4.

3.1.1 Modified Extraction Method

Preliminary work using a modified extraction method described by Pinna et al. (in press) indicated that tissue requirements for the brain TH assay could be reduced. In this study, methodology became available for the measurement of T₃ and T₄ in specific brain regions.

The frozen half brains were removed from the -80°C freezer and dissected on a thermoelectric cold plate into the following regions: olfactory bulbs, hypothalamus, thalamus, amygdala, frontal cortex, parietal cortex, caudate-putamen, pons/medulla, occipital cortex, hippocampus, cerebellum and the 'rest' of the brain. For regional TH analysis, the following limbic regions were used: amygdala, frontal cortex and hippocampus. These brain regions were chosen because they are known to be involved in stress responses and in LH (Edwards
Table 5: Number of Rats Showing Escape Deficits in the Learned Helplessness Paradigm

<table>
<thead>
<tr>
<th></th>
<th>Total number of failures in 15 trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5</td>
</tr>
<tr>
<td>Escapable Shock</td>
<td>18/32</td>
</tr>
<tr>
<td>Inescapable Shock</td>
<td>13/34</td>
</tr>
</tbody>
</table>

*Escapable Shock refers to those rats tested only in the shock escape session consisting of 15 escape trials without prior exposure to inescapable shock. Inescapable Shock refers to those rats exposed to a 40 minute inescapable shock session 24 hours prior to testing in the shock escape session.
et al., 1991). All of the dissected brain regions were placed in pre-weighed eppendorfs, weighed immediately to obtain the tissue weight and frozen over dry ice. At the end of the dissections, the eppendorfs were placed in a -80°C freezer until assayed further.

The day before the extraction, polyprep columns (Bio Rad Laboratories, Hercules, CA) were set up and activated. This process included loading the columns with 1.4 mL resin (AG 1-X2, 200-400 mesh chloride form, Bio Rad Laboratories, Hercules, CA) stored in 70% glacial acetic acid. The columns were washed with 9 mL 70% glacial acetic acid, followed by washing with 20-25 mL dH2O and activation with 10 mL sodium acetate buffer (pH 7.0). The columns were capped so as to prevent dripping and stored in the sodium acetate buffer (pH 7.0) in a 4°C cold room overnight.

The following day, the tissue samples were placed in centrifuge tubes containing 3 mL of 100% reagent-grade methanol and 100 mM PTU solution. Then, the samples were homogenized on ice with a Brinkmann polytron set at level 7 for 1-2 minutes. Next, 100 μL of [125I]T3 and [125I]T4 tracers were added to the homogenates using the arbitrary convention described in section 2.5.2. The homogenates were centrifuged (Medifuge centrifuge) at a speed of 1200 x g for 15 minutes in a 4°C cold room. The first supernatants were pipetted into new centrifuge tubes and the pellets were resuspended (as described in section 2.5.2) in 3 mL of ice-cold 100% methanol and 100 mM PTU solution. This procedure was repeated three times, so that the volume of the supernatants that was collected reached 9 mL. After three spins (Medifuge centrifuge) at 1200 x g, the extracts were purified by column chromatography in a manner identical to that outlined in section 2.5.3. T3 and T4 levels were determined by RIA as described in sections 2.5.4 and 2.5.5.
3.2 Results

3.2.1 Performance in Shock Escape Session

Rats exposed to inescapable stress twenty-four hours prior to testing in the shock escape session had significantly higher mean latencies ($t = 3.8$, $p < 0.001$) for the 15 escape trials ($34.7 \pm 0.99$ sec) than those of rats exposed only to the shock escape session ($28.9 \pm 1.14$ sec) as shown in Figure 4a. Furthermore, consistent with findings in previous studies (Edwards et al., 1986; 1991), the rats exposed to uncontrollable stress prior to testing in the shock escape session showed widely different performances in the shock escape paradigm. According to the adopted definition, LH rats had mean escape latency scores greater than 20 seconds ($53.8 \pm 1.29$ sec) and these were significantly higher ($t = 20.4$, $p < 0.0001$) than those of the NLH rats ($14.04 \pm 1.37$ sec) as shown in Figure 4b.

3.2.2 Brain $T_4$ and $T_3$ Levels and Whole Brain 5'D-II Activity

Analysis of variance indicated that there were no significant differences in $T_4$ levels in amygdala ($F_{2,17} = 0.25$, $p = 0.78$), hippocampus ($F_{2,21} = 1.01$, $p = 0.38$) and frontal cortex ($F_{2,26} = 0.55$, $p = 0.59$) in the NLH, LH and control groups as shown in Figure 5. Similarly, no significant differences were found in $T_3$ levels in amygdala ($F_{2,33} = 1.87$, $p = 0.17$), hippocampus ($F_{2,29} = 0.85$, $p = 0.44$) and frontal cortex ($F_{2,31} = 0.19$, $p = 0.83$) in the three experimental groups (see Figure 6).
Figure 4: Performance in Shock Escape Session. (a) Escape response in rats exposed only to escapable shock (lower line) compared to those exposed to an inescapable shock training session 24 hours prior to testing in the shock escape session (upper line). (b) Differentiation of two distinct groups of rats (NLH and LH) exposed to inescapable shock according to their response latencies in the shock escape session. Error bars are omitted for clarity.
Figure 5: Levels of T₄ in different brain regions in male rats following the learned helplessness paradigm. Error bars represent SEM. One-way ANOVA indicated no significant differences in brain T₄ levels between the groups.
Brain T₃ Levels

![Graph showing levels of T₃ in different brain regions in male rats following the learned helplessness paradigm. Error bars represent SEM. One-way ANOVA indicated no significant differences in brain T₃ levels between the groups.]

Figure 6: Levels of T₃ in different brain regions in male rats following the learned helplessness paradigm. Error bars represent SEM. One-way ANOVA indicated no significant differences in brain T₃ levels between the groups.
Despite the lack of statistically significant differences in T₄ and T₃ levels in the brain areas examined, certain trends did emerge (see Figure 7). For instance, T₄ amygdala levels appeared to be elevated in NLH and LH rats by 26% and 3%, respectively, compared to controls, whereas hippocampus T₄ levels were reduced by 23% in the NLH group and by 19% in the LH group compared to the control group. Similarly, amygdala T₃ levels were increased in NLH rats (21% increase compared to controls) and LH rats (17% increase compared to controls) whereas hippocampus T₃ levels decreased by 15% and 19% in NLH and LH rats respectively. Frontal cortex T₄ levels were reduced by 18% in the NLH group and increased by 3% in the LH group compared to controls. Unlike T₄, frontal cortex T₃ values were reduced in both NLH and LH rats by 11% and 2% respectively.

The above considerations suggested that there might be effects associated with shock exposure in general. When NLH and LH rats were combined for the three regions and compared with controls, the combined NLH and LH group had significantly higher T₃ levels in amygdala (19% increase compared to shock-naive controls, t = 2.08, p< 0.047). When this type of analysis was done for the other brain regions for both T₃ and T₄, no significant differences were found.

Since NLH and LH rats differ in their behavioural responses when tested in the shock escape session, rats in the two groups were exposed to different amounts of shock during the fifteen trials. Correlations between the total amount of shock received over the two day session (range: 2454-2670 seconds for the NLH group and 3106-3300 seconds for the LH group) and brain T₄ and T₃ levels were done as shown in Table 6 in order to assess whether the amount of shock had an effect on the levels of iodothyronines in the three brain regions
Figure 7: Changes (represented as % change from control) in levels of T₄ (upper graph) and T₃ (lower graph) in amygdala (amyg), hippocampus (hippo) and frontal cortex (frcx) in male rats following the learned helplessness paradigm.
examined. No significant correlations were found between T4 levels in amygdala (r = 0.20), hippocampus (r = 0.016) and frontal cortex (r = 0.29) as well as T3 levels in amygdala (r = 0.08), hippocampus (r = 0.05) and frontal cortex (r = 0.14) and amount of shock that the rats received.

In addition, 5'D-II enzyme activity in whole brain was measured in several of the rats and analysis of variance indicated no significant differences between 5'D-II activity in controls (n=12), NLH (n=6) and LH (n=6) groups (F2,23 = 1.59, p = 0.23), although enzyme activity was reduced by 47% (23.2 ± 10.1 vs. 44.0 ± 7.8 fmol Γ/mg protein) and 37% (27.7 ± 10.1 vs. 44.0 ± 7.8 fmol Γ/mg protein) in the NLH and LH groups, respectively, compared to controls. There was no significant correlation found between the total amount of shock received over the two day session and whole brain 5'D-II activity (r = 0.13).

3.2.3 Plasma Levels of T3 and T4

Analysis of variance indicated that there were no significant differences among the three groups in plasma T3 (F2,34 = 0.59, p = 0.56) and T4 levels (F2,23 = 1.95, p = 0.16) as shown in Table 7.

3.3 Discussion

In this experiment, brain THs were examined in amygdala, hippocampus and frontal cortex of NLH and LH rats and shock-naive controls. Although no significant changes between NLH and LH rats were found in T3 and T4 levels in the three brain areas, there were some interesting trends. Both T3 and T4 levels in amygdala appeared to be elevated in NLH and LH groups compared to controls, whereas the trend was for levels of both hormones in
Table 6: Correlations between Total Shock and Regional $T_3$ and $T_4$ Levels in the Learned Helplessness Paradigm$^a$

<table>
<thead>
<tr>
<th></th>
<th>$T_3$</th>
<th></th>
<th>$T_4$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amyg</td>
<td>Hip</td>
<td>Frcx</td>
<td>Amyg</td>
</tr>
<tr>
<td>Total Shock</td>
<td>0.08</td>
<td>0.05</td>
<td>0.14</td>
<td>0.20</td>
</tr>
</tbody>
</table>

$^a$Values are Pearson product-moment correlations. Abbreviations used: amyg = amygdala; hip = hippocampus; frcx = frontal cortex.
Table 7: Plasma Levels of $T_3$ and $T_4$ in Male Rats Following Learned Helplessness Paradigm

<table>
<thead>
<tr>
<th></th>
<th>$T_3$</th>
<th>$T_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=13)</td>
<td>86.37 ± 3.19</td>
<td>5.77 ± 0.22</td>
</tr>
<tr>
<td>$^b$NLH (n=11)</td>
<td>81.56 ± 4.29</td>
<td>5.51 ± 0.16</td>
</tr>
<tr>
<td>$^c$LH (n=11)</td>
<td>81.06 ± 4.40</td>
<td>6.20 ± 0.32</td>
</tr>
</tbody>
</table>

$^a$Values are means ± SEM in ng/dL ($T_3$) and µg/dL ($T_4$) in male rats following the learned helplessness paradigm. $^b$NLH refers to non-helpless rats and $^c$LH refers to learned helpless rats.
the hippocampus to be reduced in these groups compared to controls. No such trends were observed in T\textsubscript{3} and T\textsubscript{4} levels in the frontal cortex of NLH and LH rats. The data suggest that there are differences in T\textsubscript{3} and T\textsubscript{4} levels in amygdala and hippocampus in animals exposed to uncontrollable shock compared to controls -- whether or not these animals showed escape deficits. This was confirmed in the case of amygdala T\textsubscript{3} concentrations, since NLH and LH rats combined had significant elevations in T\textsubscript{3} levels compared to controls.

Changes in brain catecholamines have been documented to occur following a single session of uncontrollable footshock. For instance, animals exposed to uncontrollable stress had reductions in NE levels in hippocampus, hypothalamus and frontal cortex (Dishman, 1997; Weiss et al., 1981) with less reliable decreases in brain 5-HT and DA levels (Dishman, 1997; Martin et al., 1990).

Additionally, there appeared to be regional brain differences in T\textsubscript{3} and T\textsubscript{4} values in NLH and LH rats when compared to the control group. In amygdala, T\textsubscript{3} and T\textsubscript{4} levels were elevated in these two groups; in hippocampus, the levels of the hormones were reduced, and in frontal cortex, T\textsubscript{3} and T\textsubscript{4} values were reduced with the exception of T\textsubscript{4} values in the LH group. These findings suggest that significant complexity may exist in regional TH alterations in depressive states. Although all of these brain areas are limbic regions which are often thought to be involved in stress responses and in LH, the data suggest that uncontrollable shock may have distinct effects on THs within this neuroanatomical system.

In a different context, Campos-Barros and Baumgartner (1994) reported elevated T\textsubscript{3} concentrations in hypothalamus and limbic forebrain during treatment with desipramine (5 mg/kg). In addition, T\textsubscript{4} concentrations were reduced in hypothalamus, midbrain and limbic
forebrain following desipramine (50 mg/kg) treatment. These results suggest that the effects of the tricyclic desipramine (and perhaps other antidepressants) on brain THs are dependent on the drug dose used as well as on the brain area examined. The findings from this study suggest that changes in brain THs in depressive states may also depend on the specific brain region investigated.

Although no significant differences in T3 and T4 levels among the NLH, LH and control rats were found, the changes in hormone levels observed were quite pronounced (ranging from 3-26% for T4 and 2-21% for T3) (see Figure 7). The lack of statistical significance can be attributed to the large variance in the levels of the iodothyronines. It is possible that methodological aspects may have contributed to this large variation. The experiment needs to be repeated in order to increase the sample sizes. As can be noted from the sample sizes in Figures 5 and 6, our attempts to measure THs in small brain areas resulted in a loss of samples, particularly for T4. These aspects are discussed in greater detail in the General Discussion.

Interestingly, the changes in T3 and T4 levels that were observed in NLH and LH rats compared to controls were similar in amygdala and hippocampus since levels of both hormones were increased in amygdala and reduced in hippocampus. This suggests that 5'D-II enzyme activity was not altered in these specific brain regions. If the changes in T3 and T4 levels were caused by changes in enzyme activity, it would be expected that T3 and T4 levels would be affected differentially in different brain regions, since this enzyme is responsible for converting T4 to T3 in brain. Consequently, increased enzyme activity would result in increased levels of T3 and reductions in brain T4 levels and this would be reversed if enzyme
activity was found to be reduced. However, this was not the case, since both T₃ and T₄ levels were affected similarly in amygdala and hippocampus. 5'D-II activity was measured in several of the LH and NLH rats in whole brain (which was partly dictated by assay tissue requirements), and it was observed that the activity of this enzyme was reduced in NLH and LH rats when compared to controls. Since enzyme activity was measured in whole brain, it is unclear how these measurements relate to the T₃ and T₄ measurements obtained in amygdala, hippocampus and frontal cortex in NLH and LH rats. Thus, in future work, it will be important to obtain measurements of 5'D-II activity in different brain areas in order to examine which regions contribute to the overall reductions in whole brain enzyme activity in NLH and LH rats.

It is likely that a different pattern of changes in brain TH levels might have been obtained if the definition of helplessness that was employed was less conservative. Following exposure to uncontrollable footshock stress, rats were screened based on their performance in the shock escape session. This behavioural screening procedure included placing the animals into three different groups according to the number of failures in 15 escape trials. This approach was conservative in that those animals whose scores were intermediate (6-10 failures) were not included in the brain TH analyses. It is possible that if these rats were included in the study by employing, for example, a median split design, different trends in brain TH levels would have been observed.

In summary, the results from this study suggest that THs are altered as a result of uncontrollable shock and that the nature of these changes is dependent on the brain area examined. However, this initial study provided no evidence that THs are differentially
altered in the two behaviourally distinct groups of rats, since the regional changes in $T_3$ and $T_4$ levels were similar in magnitude in NLH and LH rats.

The trends observed suggest that inescapable shock exposure alone is sufficient to cause TH alterations regardless or whether the animals were helpless or not. However, these findings are contradicted by study 2, whereby it was observed that rats sacrificed twenty-four hours following either a 20 or 40 minute inescapable session did not have alterations in whole brain $T_3$ or $T_4$ levels or 5'D-II activity compared to shock-naive controls.

It is possible that there really are no differences in brain TH alterations in NLH and LH rats although their behaviours are clearly different.

The next study examined brain THs and related brain parameters in animals that were selectively bred for helplessness in the LH paradigm. As a result, brains of these rats can be examined in the absence of any experience of shock.
STUDY 4: BRAIN THYROID HORMONES IN A GENETIC MODEL OF DEPRESSION
In humans, depressive disorders are well known to have a genetic contribution. Similar to what is seen in human depression, vulnerability in developing helpless behaviour is governed by a genetic component. Following an inescapable shock session, approximately 20% of Sprague-Dawley rats will display helpless behaviour (Lachman et al., 1993). By selective inbreeding, there is a progressive increase in the number of escape deficient rats in successive generations. By the 26th generation, 95% of these animals meet the criteria for helplessness (Lachman et al., 1993). These congenitally helpless (cLH) rats show other behavioural deficits (such as disturbances in sleep and sexual behaviour) similar to what is observed in human depression (Edwards, personal communication). Examination of brains from these animals provided us with an opportunity to determine whether there are changes in TH-related brain parameters in animals with a high predisposition to this particular type of depressive behaviour in the absence of any shock stimulation.

3.1 Procedures

The shock procedures, breeding and sacrificing of the rats were carried out in the laboratory of Dr. Emmeline Edwards (University of Maryland) in a manner identical to that described previously (Edwards et al., 1986; 1991). Sprague-Dawley rats were tested in the LH paradigm and differentiated (according to their performance in the shock escape session) into NLH and LH groups. Following this, a selective breeding strategy was employed where susceptibility to LH behaviour has been markedly enhanced so that after 30 or so generations of inbreeding, 100% of these animals are either spontaneously helpless (cLH) or resistant to developing helpless behaviour (designated as congenitally non-helpless, cNLH) and do not
require uncontrollable shock stimulation to display the appropriate behaviour. The animals used in this experiment were 42nd generation cLH and cNLH rats.

Brains from 16 female Sprague Dawley rats were shipped from the University of Maryland to our laboratory over dry ice. 8 of the brains were from cLH rats and the remaining 8 from the control rats (cNLH). The half-brains were assayed as outlined in section 2.5. The remaining half-brains were analyzed to determine 5'D-II activity as described in section 2.8.

Due to the sample losses in regional TH analysis (study 3), the decision was made to obtain an initial assessment of THs in cLH and cNLH rats using whole brain.

3.2 Results

3.2.1 Whole Brain levels of T₃, T₄ and 5'D-II Activity

Whole brain levels of T₄, T₃ and 5'D-II in cLH and cNLH rats are shown in Figure 8. Whole brain T₃ levels were not significantly different in the cLH and cNLH rats, although T₃ levels were elevated by 32% in cLH rats compared to cNLH controls (t = 1.77, p = 0.10). In contrast, whole brain T₄ levels were significantly elevated by 12% in cLH rats compared to the cNLH rats (t = 2.35, p<0.04). 5'D-II activity was not significantly different between the two groups (t = 0.34, p= 0.74), with cLH rats having a 5% increase in enzyme activity compared to the cNLH rats.
Figure 8: Whole brain levels of $T_3$ and $T_4$ (top panel) in congenitally helpless (cLH) and non-helpless (cNLH) rats and brain type II deiodinase activity (bottom panel). Each bar is a mean of 8 observations and error bars represent SEM. *$p<0.04$ compared to cNLH controls. No significant differences were found in whole brain $T_3$ levels and type II deiodinase activity between cLH and cNLH controls (2-tailed $t$-tests).
3.3 Discussion

In this experiment, whole brain levels of both T₃ and T₄ levels were increased in cLH rats when compared to cNLH controls. Although the elevation in T₃ levels in cLH rats compared to cNLH rats was not statistically significant, it was quite a pronounced increase (32%). The lack of statistical significance in whole brain T₃ levels between the two groups may have been due to the large within-group variability in T₃ levels compared to T₄ levels. The elevations in whole brain T₃ and T₄ levels in cLH rats are of functional importance since brain THs are normally regulated within a very narrow range mainly through the actions of activating and deactivating deiodinase (Dratman et al., 1983; Leonard et al., 1981; Morreale de Escobar et al., 1985).

5'D-I1 activity was not significantly different between cLH and cNLH rats. This indicates that the observed elevations in T₃ and T₄ are most likely not a result of changes in central TH metabolism. Theoretically, this is consistent with what is expected since increased enzyme activity should result in elevated tissue concentrations of T₃ accompanied by reductions in tissue levels of T₄. However, this was not observed, since both hormones were increased in the brains of cLH rats.

One limitation of this study is that the breeding of these rats was not conducted in our laboratory and no normal shock-naive female controls were included in the study. This meant that comparisons could only be made between the congenitally helpless and LH-resistant strains. Even though our interest was to identify whether brain THs were altered in these two subpopulations, it would be important to compare these brain parameters to those
of animals that have not received any shock in order to assess whether brain THs in cLH and cNLH rats differ from baseline hormone values.

Notwithstanding the lack of normal controls, the findings suggest that whole brain THs are elevated in animals that are predisposed to developing behavioural depression. It would be worthwhile to identify which brain regions contribute to the overall elevations in whole brain T₃ and T₄ levels in cLH rats. In addition, it would be important to determine the effects of uncontrollable stress on brain THs in these animals that are vulnerable to developing depressive illness since it has been suggested that stress can interact with putative genetic factors and act as a precipitating factor in depressive syndromes.
4.0 GENERAL DISCUSSION

Although a vast body of clinical literature indicates that there is an involvement of THs in the biological basis of primary depressive illness, the nature of such an involvement is unclear. The aim of this research project was to examine brain THs, the activity of the 5′D-II enzyme in brain and circulating levels of THs in four experimental conditions related to depression. This is the first time that brain THs and related parameters have been investigated in depressive states.

4.1 Summary of Findings

Study 1 demonstrated that whole brain TH levels, particularly T3, are extremely sensitive to acute stress in male and female rats. Study 2 demonstrated that changes in brain THs and 5′D-II activity do not persist twenty-four hours following uncontrollable stress exposure and that a clear relationship does not seem to exist between TH-related brain parameters and amount of shock received at this time point. In study 3, no significant alterations in TH levels in amygdala, hippocampus and frontal cortex were found between NLH and LH rats following the LH paradigm. In study 4, whole brain T4 levels were significantly elevated in cLH rats compared to cNLH rats.

4.2 Other Relevant Animal Studies

Work in this area has focused on the effects of antidepressant treatments on brain THs and central TH metabolism. Both the clinical and animal studies suggest that all effective antidepressant therapies, however different their biochemical mechanisms of
actions, affect serum TH levels. With regards to the effects on brain THs, the animal studies have demonstrated that treatment with several antidepressants, such as desipramine and fluoxetine, enhance the activity of the 5’D-II enzyme in various brain regions including limbic forebrain and hippocampus and reduce circulating T₄ levels (Campos-Barros and Baumgartner, 1994; Joffe et al., 1993). Campos-Barros and Baumgartner (1994) have found that chronic treatment with desipramine also resulted in a reduction in brain T₄ levels, whereas Joffe et al (1993) did not observe such brain effects. These apparently contradictory findings may be accounted for due to the different doses of desipramine used since Campos-Barros and Baumgartner (1994) used doses of 5 mg/kg and 20 mg/kg desipramine, whereas Joffe et al (1993) used a dosage of 10 mg/kg of desipramine. Although both of these studies provide support for the hypothesis advanced by Joffe (Joffe and Sokolov, 1994) in which it has been suggested that reductions in serum T₄ levels are required for antidepressant response, it is important to note that these animal studies were conducted in healthy rats that do not reflect the clinically depressed state. This project was an initial attempt to directly measure and evaluate brain THs in depression and experimental conditions related to depression.

4.3 Congenitally Helpless vs. Helpless Rats

One intriguing finding from the four studies was that both brain T₃ and T₄ were elevated in congenitally helpless rats compared to the control line that are LH-resistant. This effect was observed in a relatively small sample size (n=8 per group) so that the trends are very suggestive of the notion that brain THs are altered in depressive-like states. This genetic model of depression is useful for the examination of THs in depressive states, since the
“helpless” phenotype has been selected for by employing a breeding strategy. As a result, these animals are spontaneously helpless or resistant, so that they do not require shock stimulation to display the particular type of behaviour.

The observation that brain THs are elevated in cLH rats is consistent with the hypothesis offered by Joffe et al (see section 1.7.5.2) that depression is a state of thyroid excess in brain and that reductions in T₄ levels are required for antidepressant response. Nonetheless, this tendency was not consistent with what was observed in study 3, since THs were not significantly different between the NLH and LH rats in amygdala, hippocampus and frontal cortex. A number of aspects should be considered in connection with the differences observed between cLH and LH rats.

4.3.1 Whole Brain vs. Regional Thyroid Hormone Measurements

It is important to consider that the elevations in T₃ and T₄ brain levels in cLH rats were found in whole brain and the measurements from the LH paradigm were conducted in specific brain regions. It is likely that other brain regions in NLH and LH rats will indicate whether there are changes in THs between these two groups of rats. Thus, key brain areas that will show the relevant differences still need to be analyzed in NLH and LH rats. Regional TH analysis should also be performed on cNLH and cLH rats in order to assess which specific brain areas are responsible for the overall elevations observed in whole brain T₃ and T₄ levels in cLH rats.
4.3.2 Genetic Factors

A second significant difference between the rats from the LH paradigm and those in the genetic model of depression is that the NLH and LH rats were first generation and the cNLH and cLH rats were rats from the 42\textsuperscript{nd} generation of breeding of rats originally screened following the LH paradigm. Just like the behavioural deficits between NLH and LH rats have been enhanced by selective breeding, it is conceivable that any neurochemical differences that may exist between cNLH and cLH rats have been genetically “amplified” as a result of selective breeding.

4.3.3 Gender Differences

Yet another difference between the rats employed in studies 3 and 4 is that the cNLH and cLH rats were females, whereas the NLH and LH rats were males. There are known gender differences that exist in the incidence of stress-related and depressive disorders in humans (Blanchard et al., 1995). It is unclear whether these differences contribute to neurochemical differences. The contribution of female steroids and possible differences in corticosteroid levels (both basal and in response to uncontrollable and controllable stress) should be examined. Furthermore, the findings from study 1 suggested that gender differences in the effects of stress controllability on brain T\textsubscript{3} may exist. However, it is unclear how the controllability effects relate to the differences observed between studies 3 and 4.
4.3.4 Methodological Considerations

Another possibility for the differences between cLH and LH rats is the methodology that was used to measure THs in brain tissue. After the iodothyronines were extracted from the brains or specific brain regions and purified by column chromatography, RIA was used as a quantitative tool to measure the levels of the hormones. This procedure was dependent on using a commercially available RIA kit. Our first attempts to measure T3 and T4 levels in whole brain using this commercial RIA kit were quite successful. We then attempted to measure THs in small brain regions and were not as satisfied with these measurements as with the whole brain measurements. For regional TH analysis, it was observed that some of the samples had low levels of the hormones that were very close to background levels. After the samples were dried-down, they were reconstituted in stripped-down human serum. Although the majority of the iodothyronines were removed from this serum, trace levels of the iodothyronines could still be detected. When RIA was carried out on the brain regions, it was observed that some of the samples had T3 and T4 levels very close to those detected in the stripped-down serum. This suggests that the assay may not have been sensitive enough to discern very small differences between background levels and those present in the small brain areas, although this did not pose a problem when the hormones were measured in whole brain. The issue of assay sensitivity manifested itself by introducing a large inter-assay variability. This meant that levels of T3 and T4 in brain could only be compared within a certain run and not between different runs. This was dealt with by ensuring that all of the samples from a given experiment were run together so that group comparisons could be made appropriately.
A possible way of dealing with this problem in future work would be to reconstitute the dried-down samples in another medium that is known not to contain any iodothyronines. Another way to increase assay sensitivity might be to obtain purified T₃ and T₄ monoclonal antibodies and use a precipitant RIA assay rather than the current RIA assay that employs antibody-coated tubes.
4.4 Conclusions

1) Whole brain T$_3$ levels were elevated in response to acute footshock stress in male and female rats.

2) A “dose-response” relationship was not found between whole brain TH levels and 5’D-II enzyme activity and amount of shock the rats received twenty-four hours earlier.

3) T$_3$ and T$_4$ levels in amygdala, hippocampus and frontal cortex were not significantly altered in helpless and non-helpless male rats following the learned helplessness paradigm.

4) Whole brain T$_4$ levels were elevated in congenitally helpless female rats in a genetic model of depression.

5) 5’D-II enzyme activity in whole brain was not significantly different in helpless and non-helpless male rats in the learned helplessness paradigm as well as in congenitally helpless and non-helpless female rats in a genetic model of depression.

6) Circulating levels of T$_3$ and T$_4$ were not altered in response to acute controllable and uncontrollable footshock stress and in the learned helplessness paradigm.

In conclusion, the findings from these four studies indicate that brain THs change rapidly in response to acute stress and are altered in congenitally helpless rats. Although the results from the learned helplessness paradigm (study 3) are inconclusive, they demonstrate that specific brain areas may contribute differently to the changes in brain TH levels. While
the functional significance of the changes in brain THs observed in the four studies is not known, the findings raise the possibility that abnormalities in brain TH responses to stress and behavioural depression may contribute to the pathophysiology of depressive disorders.
5.0 RECOMMENDATIONS

A number of studies should be pursued in order to delineate the role of brain THs in stress-induced behavioural syndromes as well as in depressive illness. The following are recommendations for future studies:

1) The time course of shock effects on TH-related brain parameters needs to be fully characterized to determine when peak effects occur.

2) It would be important to continue efforts to increase the sensitivity of the TH brain assay in order to further reduce inter-assay variability.

3) The contribution of different brain regions (both limbic and non-limbic) to the changes observed in brain THs should be ascertained in the learned helplessness paradigm and in the genetic model of depression.

4) Gender differences in the learned helplessness paradigm and in the genetic model of depression should be examined as females appear more prone than males to a number of stress-related and depressive disorders.

5) Since it has been suggested that stress can precipitate depressive illness in predisposed subjects, it would be useful to examine brain THs and related brain parameters in animals that are predisposed to developing escape deficits and have been exposed to a single inescapable shock session.
6) In order to further establish a role for THs in this model of depression, it would be important to investigate if the administration of antidepressants would reverse the brain TH alterations observed in the learned helplessness paradigm.

These studies should be done as the data to be obtained would make a significant contribution to the current understanding of the involvement of brain THs in the biological basis of depressive illness.


Bauer, MS and Whybrow, PC. Thyroid hormones and the central nervous system in affective illness: interactions that may have clinical significance. *Integrative Psychiatry*, 6: 75-100 (1988).


Friedman, Y., Bacchus, R., Raymond, R., Joffe, RT. and Nobrega, JN. Acute stress increases thyroid hormone levels in rat brain. *Biological Psychiatry* (in press).


Joffe, RT. and Sokolov, STH. Thyroid hormones, the brain, and affective disorders. *Critical Reviews in Neurobiology*, 8: 45-63 (1994).


Morreale de Escobar, G., Pastor, R., Obregan, MJ. and Escobar del Rey, F. Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues, before and after onset of fetal thyroid function. *Endocrinology, 117*: 1890-1900 (1985).


Sokolov, STH., Levitt, AJ. and Joffe, RT. Thyroid hormone levels before unsuccessful antidepressant therapy are associated with later response to T₃ augmentation. *Psychiatry Research*, 69: 203-206 (1997).


Whybrow, PC., Coppen, A., Prange, AJ., Noguera, AR. and Bailey, JE. Thyroid function and the response to liothyronine in depression. *Archives of General Psychiatry*, 26: 242-245 (1972).


