

**BRAIN IMAGING STUDIES OF THE PITUITARY GLAND IN PEDIATRIC
MENTAL ILLNESS**

by

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Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
January 2007

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ISBN: 978-0-494-27166-7

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ISBN: 978-0-494-27166-7

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ABSTRACT

The currently used diagnostic system for psychiatric disorders is not based on etiology or pathophysiology, as these aspects of such illnesses are still poorly understood. Similarly, it remains very difficult to predict, with any accuracy, illness course or treatment response. This thesis aimed to investigate potential biomarkers for the course and response to treatment of several pediatric psychiatric illnesses. The pituitary gland was chosen as a viable candidate for such a biomarker, as it is a very plastic organ, responsive to changes in physiological function, and amenable for morphological study using *in vivo* imaging techniques such as magnetic resonance imaging (MRI). Childhood and adolescence are active periods of growth, and are often the time of onset for many major psychiatric illnesses. Moreover, sex differences in illness onset and prevalence are common during this period, and the pituitary gland displays marked sex-related morphological differences during this period of growth. Functionally, the pituitary gland plays a central role in the stress response, which is strongly implicated in both mood and anxiety disorders. The core hypothesis of this thesis is that childhood and adolescent psychiatric diseases involving the function of the pituitary gland will exhibit abnormal pituitary morphology that is detectable with MRI. Data on pituitary gland volumes were collected from patients with anxiety disorders (obsessive compulsive disorder or OCD), mood disorders (major depressive disorder or MDD and bipolar disorder) and behavioral disorders (attention deficit hyperactivity disorder or ADHD) as well as healthy control subjects. In mood disorders, the pituitary gland was found to be larger than those of healthy controls, while in anxiety disorders it was found to be smaller than healthy controls'. In behavioral disorders, no difference was noted in pituitary gland size. Pharmacological treatment, while effective at reducing symptoms, did not affect pituitary volume in any of these populations. In conclusion, pituitary gland volume appears to be differentially affected in these illnesses, but not relatable to the reduction of symptoms, *per se*, or to the effects of psychotherapeutic medication. Future studies of the pituitary gland in mental illness, conducted in concert with direct measures of endocrine function, will shed further light on the role of the gland in psychiatric disease.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ACTH	Adrenocorticotrophic Hormone
ADH	Antidiuretic Hormone (Vasopressin)
ADHD	Attention Deficit Hyperactivity Disorder
ANCOVA	Analysis of Covariance
AVP	Arginine Vasopressin
CDRS	Children's Depression Rating Scale
CGI	Clinical Global Improvement
CPRS	Connor's Parent Rating Scale
CRF	Corticotropin Releasing Factor
CRH	Corticotropin Releasing Hormone
CTRS	Connor's Teacher Rating Scale
CY-BOCS	Children's Yale Brown Obsessive-Compulsive Scale
DSM	Diagnostic and Statistical Manual
E2	Estradiol
ECT	Electroconvulsive Therapy
FLASH	Fast Low Angle Shot
FSH	Follicle-Stimulating Hormone
GC	Glucocorticoids
GH	Growth Hormone
GHRH	Growth Hormone Releasing Hormone
GR	Glucocorticoid Receptor
H	Hydrogen
HAMA	Hamilton Anxiety Scale
HAMD	Hamilton Depression Scale
HPA	Hypothalamic-Pituitary-Adrenal
ICV	Intracranial Volume
K-SADS	Kiddie-Schedule for Affective Disorders and Schizophrenia
LH	Luteinizing Hormone
MDD	Major Depressive Disorder
MPH	Methylphenidate

MR	Magnetic Resonance
MRc	Mineral Corticoid Receptor
MRI	Magnetic Resonance Imaging
mpPVN	Medial Parvicellular Region of the Paraventricular Nucleus
MSH	Melanocyte-Stimulating Hormone
OCD	Obsessive Compulsive Disorder
ODD	Oppositional Defiant Disorder
POMC	Pro-opio-melanocortin
PROG	Progesterone
PTSD	Post-Traumatic Stress Disorder
PVN	Paraventricular Nucleus
rf	Radiofrequency
SPGR	Spoiled Gradient Echo Pulse
SSRI	Selective Serotonin Reuptake Inhibitor
T	Tesla
T1	Spin-Lattice Relaxation
T2	Spin-Spin Relaxation
T3	Triiodothyronine
T4	L-thyroxine
TE	Echo Time
TH	Thyroid Hormone
TR	Repetition Time
TRH	Thyroid Releasing Hormone
TSH	Thyroid-Stimulating Hormone

ACKNOWLEDGEMENTS

I would like to thank Dr. Ronald Leslie for his infatigable support for my work and my career. Dr. Leslie has been an invaluable source of support and inspiration on this and many other scientific endeavors. He has become a valued mentor and collaborator. Not to mention, he has taught me more about writing than my mother (who was an English teacher no less!). Any clarity in my thought and prose is due in large part to his influence. I was lucky to have fantastic mentors, collaborators and support from the Dalhousie community (some of whom served as supervisors and members of the committee as well): Normand Carrey, MD, Matthias Schmidt, MD, Martin Alda, MD, Ben Rusak, PhD, William Baldrige, PhD, William Currie, PhD, Michael Wilkinson, PhD and Vivek Kusumakar, MD. I would also be remiss if I did not acknowledge the tremendous support that Drs Kazue Semba and John Connolly provided me back in 2000 at the start of my graduate portion of my career.

I would also like to thank my parents, in-laws, brothers and sisters and extended family for their patience and support throughout this process. All too often one hears about the badgered student (*"When are you going to finish?"*). All I heard was *"What do you need?"* and *"We're behind you"*. Needless to say, my wife, Jacqueline, has been especially critical to this effort. Being a husband and father is the most important aspect of my life, beyond being a scientist. As large as my work has been on this thesis, she has shouldered a larger burden at home. My children, Connor, Logan and Jack, have also borne the burden of a distracted father. I am fully aware of the large sacrifice of time my family has made. I also know that it was their patience and love that made this career possible for me. This work is dedicated to my wife and children.

CHAPTER ONE: INTRODUCTION

THE QUESTION OF PSYCHIATRIC DIAGNOSIS

Mental illness remains one of the last great frontiers of modern medicine. Although these disorders are quite common, debilitating and costly to society, very little is known about them. The World Health Organization (www.who.int) offers startling statistics about mental illness: 1) almost one million people die from suicide each year worldwide, 2) one in four people visiting a health service suffer from a mental illness and 3) by the year 2020, major depression will be second only to ischemic heart disease in the amount of disability experienced by sufferers. Given the impact of mental illness on those afflicted and society, there is a pressing need for effective diagnosis and treatment.

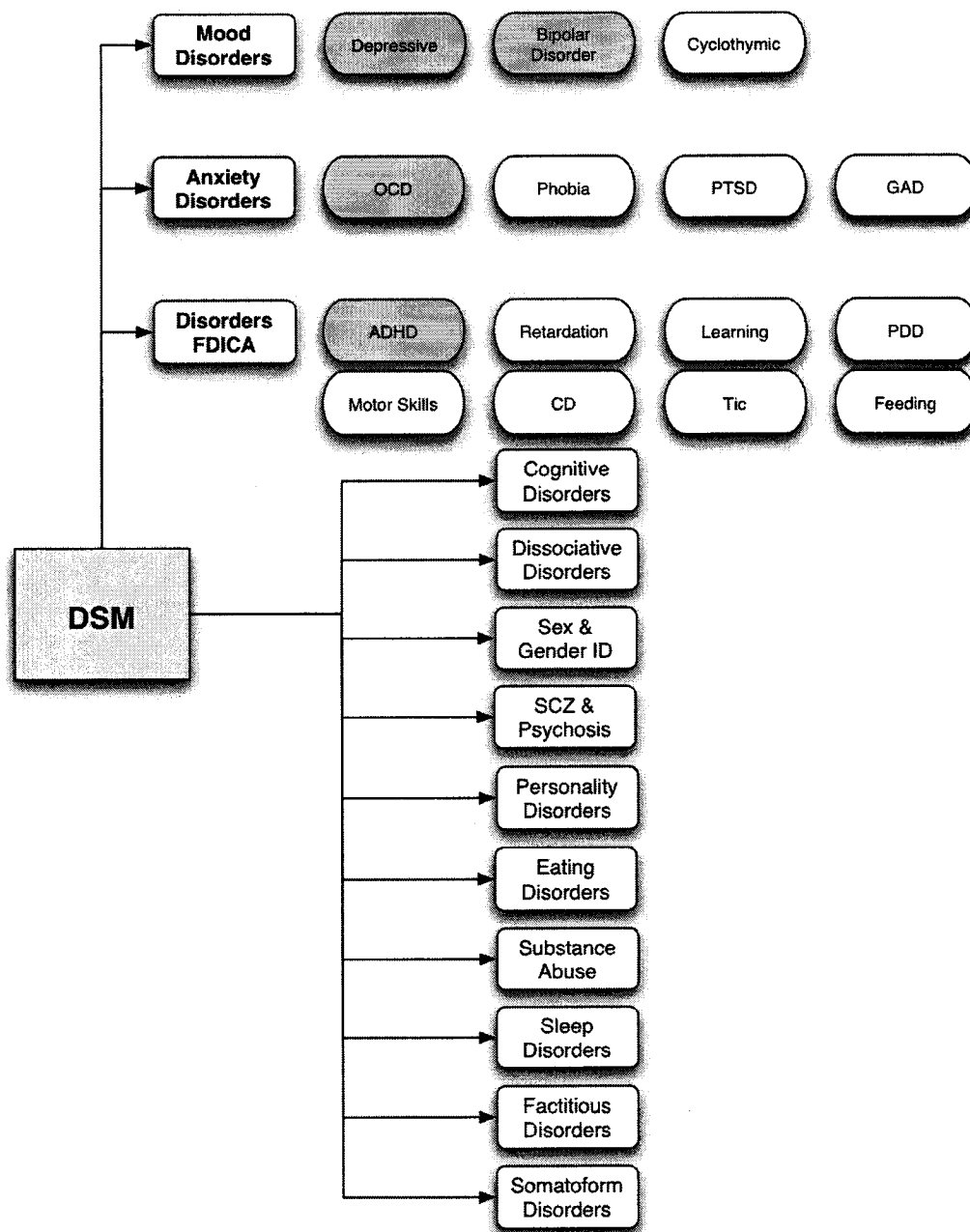
The diagnostic system (APA, 1994) is not based on etiology or pathophysiology. Nor does it predict illness course or treatment response. The intellectual framework from which these disorders are treated and investigated is based on behavioral evidence. The Diagnostic and Statistical Manual of Mental Disorders (commonly known as the DSM, APA, 1994) divides aberrant behaviors into various categories based on clusters of those behaviors (see figure 1). The divisions between these disorders are established via behavioral observation and self-report rather than by known biological markers. Given the commonly accepted division between the mind and brain and the stigma associated with mental illness, diagnostics development has been slow and suffered from many wrong turns (Boland and Keller, 1999).

HISTORICAL ASPECTS OF PSYCHIATRIC DIAGNOSIS

The theoretical basis of psychiatric disorders has oscillated wildly between various dogmas and conceptualizations. In the 18th century, Philippe Pinel (1798) provided one of the first comprehensive categorical systems for psychiatric illness (Boland and Keller, 1999). The emphasis was on phenomenology rather than pathophysiology as his perception of mental illness was based on “moral” difficulties. In the 19th century, Falret (1854) first described bipolar disorder (“la folie circulaire”) (Boland and Keller, 1999). In the early twentieth century, Kraepelin described psychiatric

Figure 1: Basic Structure of the Diagnostic and Statistical Manual of Mental Disorders (DSM).

The DSM groups behaviors into clusters and categories based on similarity of symptoms of those afflicted. For example, mood disorders are composed of depressive, bipolar and cyclothymic disorders. All have a mood (e.g. sad or euphoric) element at their core. Anxiety disorders have feelings of anxiety at their core, but the expression may vary widely from obsessive-compulsive disorder (OCD), phobias, generalized anxiety disorder (G AD) or post-traumatic stress disorder (PTSD). Disorders usually first diagnosed in infancy, childhood or adolescence (FDICA) have their timeframe of onset in common and include attention deficit hyperactivity disorder (ADHD), learning disabilities, pervasive developmental disorder (PDD), conduct disorder (CD), tic disorders, etc. The focus of this thesis is on those broad categories (anxiety, mood and behavioral disorders). Other disorders include schizophrenia (SCZ), sex and gender identity (ID) disorders, and eating disorders. Each of these categories is based on behavioral or demographic relationships, not known biology. The hope is to incorporate biological knowledge into later editions of the DSM (Kupfer et al., 2002).



disorders based on their symptoms while trying to avoid theories and etiological considerations (Boland and Keller, 1999). Psychosocial models developed around World War II, melding Adolph Meyer's social perspective with Sigmund Freud's focus on early life experiences. Walter Menninger proffered that all psychiatric illness arose from the failure of the person to adapt to their environment (Wilson, 1993). This was a dramatic swing away from Kraepelin's classification system. The initial Diagnostic and Statistical Manual (DSM, APA, 1952) reflected this psychosocial model with diagnoses classed as reactions to environmental stimuli. This broad classification approach was not conducive to research, and a more systematic method of classification was needed (Wilson, 1993).

PHENOMENOLOGY MEETS PATHOPHYSIOLOGY

Despite these difficulties, evidence for a biological basis for mental illnesses, like depression, began to develop. Electroconvulsive therapy (ECT) was shown to be effective for treating depression (Bennett and Wilbur, 1944). Psychotropic agents were more widely introduced in the 1950s, with the monoamine oxidase inhibitor Iproniazid (Crane, 1957) and the tricyclic antidepressant Imipramine (Kuhn, 1958) providing a new tool for treating depression. By the 1960s, the efficacy of the antidepressants (e.g., tricyclics) was firmly established (Klerman and Cole, 1965). In 1964, reserpine, which binds to the storage vesicles of, and causes release of dopamine, norepinephrine, and serotonin, was found to induce depressive symptoms (Sulser et al., 1964). Papez (1937) had theorized a so-called limbic circuit involving the hippocampus, amygdala and limbic lobe that were paramount in emotion. Lithium for treating mania became more widely used. These chemical agents gave rise to a new biological perspective for investigating psychiatric disorders. Their true impact on psychiatry etiology and treatment was slow in North America compared to Europe (Boland and Keller, 1999).

The description of depressive disorders evolved from organic/reactive to psychosis and neurosis with DSM-II (APA, 1968). The psychotic mood disorders were considered treatable with somatic treatments (i.e., medications, ECT). On the other

hand, depressive neurosis was still considered an excessive reaction to internal or external stimuli and therefore thought to be more readily addressed by psychotherapy. Hence, the advent of somatic therapies for mental illness altered the perception of the phenomenology of the disorders. The more intractable, severe forms became candidates for medications while less severe problems remained under the realm of 'talk therapy'. The classification system became influenced by treatment choices/possibilities.

During the last half of the twentieth century, research into the mechanism of action of antidepressants evolved from simple ideas about brain chemical concentrations to considerations of neurotransmitter balance and receptor/intracellular effects. For example, serotonin changed from simple concentration considerations to its actions on various receptors (Sjoerdsma and Palfreyman, 1990). The desire to improve therapeutic decision-making (pharmacotherapy versus psychotherapy) necessitated a more standardized system of disease classification (Boland and Keller, 1999). The efforts to develop better-targeted agents also helped inspire this move (Boland and Keller, 1999). Epidemiological research was also hampered by the vague systems used to date. Cooper et al., (1972) even noted that the differences in incidence of bipolar disorder and schizophrenia in the United States as compared to the United Kingdom was actually due to biases in diagnosis. In the United States, the Presidential Commission on Mental Health (1978) was forced to conclude that improvements in treatment could not be made without first improving the diagnosis.

In an effort to address this problem, the Research Diagnostic Criteria (RDC) was developed (Spitzer et al., 1978). A standardized interview approach was implemented as part of this approach. This provided a way for psychiatrists to reliably establish a diagnosis among different examiners and sites. The RDC laid the groundwork necessary for the development of the DSM-III (APA, 1980). The DSM-III was a radical departure from the DSM-II as the new version attempted to 're-medicalize' mental illness (APA, 1980). For example, mood disorders were no longer considered part of the continuum of human behavior (APA, 1968), but a distinct pathological

state (APA, 1980). The DSM-III also marks the first time that mood disorders were divided into bipolar and unipolar disorders. Based on the classification based medical model approach, the differences between the two disorders with regard to inheritance, symptomatology and course, could be accounted for (APA, 1980). The increase in the amount of research conducted into psychiatric illness based on the criteria as described in the DSM-III has led to an even more refined medical model for DSM-IV (APA, 1994).

However, the criteria used in the DSM-IV still reflected phenomenology rather than pathophysiology. The criteria were developed from clinical observation, rather than biological study. Starting with the categorical system of diagnosing mental illness with the DSM-III, structured interviews were constructed. This in turn led to a more standardized type of diagnosis, as standardized criteria were available (APA, 1980, 1994). Psychiatrists could feel confident that their diagnosis was reliable; but the question of validity still remains (Boland and Keller, 1999). In order to begin to address this question of validity, one needs to take a step back from the phenomenology and try to uncover the disorder's biological roots. A diagnostic system based on biology, rather than clinical observation may look quite different from the DSM series and conceptualizations of the disorders may be changed dramatically (Kupfer et al., 2002). Rather than discrete behavioral entities as described in the DSM-IV (APA, 1994), some symptom overlap exists between disorders (Kupfer et al., 2002). Indeed, the neurobiology may overlap between disorders (non-disorder specific) with other brain regions of interest being disorder-specific. These potential points of convergence and divergence of the various types of disorders must be addressed.

There are many large categories of mental illness (APA, 1994). Three will be discussed in this thesis; mood, anxiety and behavioral disorders. These were selected as they reflect emotional and behavioral symptoms: 1) emotion (major depression and bipolar disorder), 2) behavior and emotion (obsessive-compulsive disorder) and 3) behavior (attention deficit hyperactivity disorder). Each disorder has set clusters of

behaviors and epidemiological characteristics. These will be discussed briefly in the following sections.

MOOD DISORDERS

Mood disorders have at their core, obviously, a disturbance in mood. There are many types of mood disorders, each with differing characteristics and prevalence, but all with affect-related symptoms at their center (APA, 1994). These disorders include, based on DSM-IV classifications, major depressive disorder (or MDD), bipolar disorder (types I and II), dysthymic disorder, cyclothymic disorder, substance induced mood disorder, mood disorder due to a general medical condition, and finally, major depression and bipolar disorder not otherwise specified. These disorders are characterized by their episodic nature (periods of wellness followed by periods of illness). The exemplars of mood disorders are major depressive disorder and bipolar disorder.

MAJOR DEPRESSION

Major depressive disorder (MDD) is characterized by a pervasive change in mood, lasting over two weeks, that manifests as a depressed or irritable mood and/or loss of interest in pleasure (table 1). Other core clinical characteristics include changes in appetite, weight, sleep, activity, concentration, energy level, self-esteem, and motivation (APA, 1994). In children, MDD has more anxiety and somatic (affecting the body) complaints associated with it (Chambers et al., 1982; Mitchell et al., 1988; Ryan et al., 1987). Adolescents tend to exhibit stronger changes in sleep and appetite than younger children.

MDD was once thought to be rare in pediatric populations. Its prevalence is 2% in children, with a 1:1 sex ratio and between 4 – 8% in adolescents with a 1:2 sex ratio biased towards females (Fleming and Offord, 1990; Kashani et al., 1987a, b; Lewinsohn et al., 1994). By the age of 18, the cumulative incidence for MDD is 20% (Lewinsohn et al., 1993). Interestingly, since 1940, the risk for developing MDD has

Table 1: Diagnostic Criteria for Major Depressive Disorder (MDD)

A diagnosis is made when at least 2 of the following symptoms are reported for a period longer than 2 weeks (American Psychiatric Association, 1994)

Depressed mood

Irritability

Low self esteem

Feelings of hopelessness, worthlessness

Guilt

Impaired concentration and thinking

Weight loss or gain

Sleep disturbance

Fatigue, low energy, agitation

Decreased interest in pleasurable stimulus

Thoughts of death and suicide

Table 2: Diagnostic Criteria for Bipolar Disorder

A diagnosis is made when the following symptoms are reported (American Psychiatric Association, 1994)

- Bipolar I: mania and/or depression
 - Bipolar II: hypomania and/or depression
 - Bipolar Mixed States: combination of manic and depressed features
 - Not better accounted for by another disorder
-

risen with each successive generation and has also been identified in steadily younger populations (Kovacs and Gatsonis, 1994; Ryan et al., 1992).

BIPOLAR DISORDER

There are two core types of bipolar disorder. Bipolar I disorder is characterized by periods of mania or mixed episodes coupled with one or more major depressive episodes (table 2). The hallmarks of bipolar II disorder include episodes of hypomania coupled with major depressive episodes. Mania, and to a lesser extent, hypomania is characterized by erratic behaviors, increased energy levels, with significant impairment on functioning. This “high” may not necessarily be ego-dystonic (in conflict with one’s self-image or ego) to the patient. Bipolar disorder is also commonly mistaken for attention deficit hyperactivity disorder or other behavioral disorders in children. Bipolar depression is similar to MDD, but it may also include symptoms of psychosis and psychomotor retardation. A family history of bipolar disorder is also a strong indicator of bipolar disorder in a depressed youth (Geller et al., 1994; Strober and Carlson, 1982; Strober et al., 1993). Young people also tend to have increased occurrences of rapid cycling, which makes the disorder harder to treat, and increases the risk for suicide (Brent et al., 1988, 1993; Geller and Luby, 1997).

The prevalence of bipolar disorder is between 0.2 – 0.4% prepubertally and 1.0% in adolescents (Lewinsohn et al., 2003). There has been no sex difference identified with regard to prevalence. The age of onset for bipolar disorder tends to be earlier (17 to 29 years) than MDD (24 to 30 years), with over 60% of patients with bipolar disorder reporting onset of a mood disorder with depressive symptoms in adolescence and young adulthood (Bland, 1997).

ANXIETY DISORDERS

Anxiety disorders have at their core anxiety-related symptoms such as nervousness, agitation, worry and apprehension. The expression and manifestation of those symptoms can vary widely. Anxiety disorders, as defined by DSM-IV criteria,

include panic attacks, various phobias, obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), generalized anxiety disorder (GAD), anxiety due to a medical condition or substance induced, and finally anxiety disorder not otherwise specified (does not fit other categories). OCD is the exemplar of this class of disorders as it is chronic with clear diagnostic criteria.

Obsessive-Compulsive Disorder

Obsessions are persistent, recurring thoughts, images or impulses that are ego-dystonic (table 3). That means they are considered intrusive, inappropriate and distressing to the person afflicted. They are not excessive worries over realistic problems. Compulsions are repetitive behaviors or mental acts that the person feels driven to perform in order to reduce distress from a dreaded outcome. These acts, behavioral or mental, must be performed in accordance with a rigid set of standards. In order to reach clinical relevance, these obsessions and compulsions must cause marked distress and impairment, be time consuming (greater than 1 hour per day) and negatively impact one's daily routine, social and academic functioning (APA, 1994).

In pediatric samples, the prevalence of OCD is estimated between 1.0 – 3.6% (Flament et al., 1988; Valleni-Basile et al., 1994). The age of onset is believed to be prior to age 15 years in one third to half of OCD cases (Pauls et al., 1995). Males tend to have a prepubertal onset and females a pubertal or adolescent onset. Early on, the ratio of males to females with OCD is 3:2 with it becoming closer to equal during adolescence (Swedo et al., 1989).

BEHAVIORAL DISORDERS

Attention deficit hyperactivity disorder (ADHD) is classed by the DSM –IV under disorders first diagnosed in infancy, childhood or adolescence. This class also includes mental retardation, learning disorders, motor skills disorders, communication disorders, pervasive developmental disorders, feeding and eating disorders of infancy or early childhood, tic disorders, and elimination disorders. The reason ADHD is included in this category is that age of onset must occur prior to age 7 years in order

Table 3: Diagnostic Criteria for Obsessive-Compulsive Disorder (OCD)

A diagnosis is made when the following symptoms are reported (American Psychiatric Association, 1994)

- Obsessions: recurrent/persistent thoughts, impulses or images that are intrusive, inappropriate and cause marked anxiety or distress
 - Compulsions: repetitive behaviors or mental acts that a person feels driven to perform in response to an obsession (to prevent or reduce anxiety)
 - Obsessions or compulsions recognized as excessive or unreasonable (does not apply in children)
 - Cause marked distress and time consuming (> 1 hour/day) or significantly interferes with the person's life
 - Not the result of another DSM or medical diagnosis
-

Table 4: Diagnostic Criteria for Attention Deficit Hyperactivity Disorder (ADHD)

A diagnosis is made when the following symptoms are reported (American Psychiatric Association, 1994)

- Inattention (6 or more): fails to give close attention to details, difficulty sustaining attention, does not seem to listen, poor follow through, difficulty organizing, avoids or dislikes tasks that require sustained attention, often loses things, easily distracted by extraneous stimuli, forgetful
 - Hyperactivity (6 or more): fidgets, leaves seat in class, runs or climbs excessively, difficulty playing quietly, often “on the go” or “driven by a motor”, talks to excess
 - Impulsivity: blurts out answers to questions, difficulty awaiting turn, interrupts or intrudes on others
 - Some symptoms prior to age 7
 - Impairment in 2 or more settings (e.g. school and at home)
 - Causes significant impairment
 - Not the result of another disorder
-

to establish the diagnosis. When looking at the symptoms though, as described by the DSM (APA, 1994), ADHD is more truly akin to the other behavioral disorders such as conduct disorder (CD), oppositional defiant disorder (ODD) and disruptive behavior disorder not otherwise specified. Basically, these disorders have at their core an inability to inhibit behaviors that are clearly not appropriate to a given situation.

Attention Deficit Hyperactivity Disorder

In ADHD, there are two core groups of symptoms: inattention and hyperactivity-impulsivity (table 4). Inattention includes the following: failure to attend to details or make careless mistakes, difficulty sustaining attention, not listening, not following through, difficulty organizing, avoidance or dislike of sustained mental effort, losing things, being easily distracted and forgetfulness. Hyperactivity includes: fidgeting, being out of seat (e.g., in a school class), running or climbing excessively, difficulty playing quietly, being “on the go” and talking excessively. The impulsivity symptoms include: blurting out answers, difficulty awaiting their turn, and often interrupting or intruding on others (APA, 1994). ADHD is a serious public health problem that affects between 3 – 6% of children and accounts for as many as 30% to 50% of child referrals to mental health services . The disorder is also much more common in males than females (ranging from 4:1 to 9:1; APA, 1994).

PREMISE FOR THESIS STATEMENT

The neurobiology of psychiatric illness, if not responsible for the etiology of the disorders (a matter of considerable debate), is at least responsible for mediating the expression of the disorders, as emotions and behaviors are processed in the brain. The disorders themselves, by their biological processes, may in turn be responsible for pathology in the brain. For example, stress (including psychological stress) adversely affects hippocampal volume (McEwen, 2000; Sapolsky, 2000). So while not necessarily causing the stress, the hippocampus is affected by the stress process (McEwen, 2000; Sapolsky, 2000). As stated above, all psychiatric diseases are understood and investigated from the vantage given by the expressed behaviors of the disorder. The disorders, while behaviorally very complex overall converge on a key

anatomical 'nexus point', the pituitary gland (see below; Boyer, 2000; Arborelius et al., 1999). This gland, which is also available for investigation *in vivo*, is the anatomical region of study for this thesis.

THE PITUITARY GLAND

WHY FOCUS ON THE PITUITARY?

There are three reasons for choosing the pituitary gland (or 'pituitary') as the subject of this thesis. First, regardless of the cortical and subcortical alterations noted in the above conditions, one commonality among pediatric mental illnesses is the behavioral and biological interaction of the disease state with the pituitary gland, via stress-related pathways (e.g., Boyer, 2000; Arborelius et al., 1999; Lucassen et al., 2006; Kluge et al., 2006). Second, changes in neuroendocrine activity are reflected in alterations in pituitary gland morphology. During pregnancy, for example, the pituitary gland increases in volume; this was suggested to involve lactotrophic hyperplasia (increase in secretory cell number; Gonzalez et al., 1988; Dinc et al., 1998). In children with shunted hydrocephalus, an enlarged pituitary and a reduction in plasma growth hormone have been reported (Lopponen et al., 1997). Levels of follicle stimulating hormone and luteinizing hormone (FSH and LH) have also been correlated with pituitary size in the shunted hydrocephalus patients (Lopponen et al., 1997). Furthermore, hypothyroid individuals have enlarged pituitary glands (Shimono et al., 1999). In dwarfs with a growth hormone releasing hormone receptor gene mutation, smaller than normal pituitaries were found (Murray et al., 2000). Smaller gland size was also noted in isolated growth hormone deficiency and in multiple pituitary hormone deficiency compared with healthy controls (Arslanoglu et al., 2001). The third reason is that the pituitary gland is a viable candidate for *in vivo* study using magnetic resonance imaging (see chapter 2). There is currently no post-mortem literature regarding the pituitary gland (or any other brain region for that matter) in pediatric mental illness. Hence, without *in vivo* imaging, there would be no way to directly assess pituitary volume.

HISTORICAL ASPECTS

The term hypophysis is the Latin term for the pituitary gland (American Heritage Dictionary of the English Language, 2000). The term Hypophysis was coined by Samuel Thomas Soemmerring (1755-1830) (Moog and Karenberg, 2004). It is derived from the Greek word hupuphysis meaning attachment underneath (American Heritage Dictionary of the English Language, 2000). Aristotle (384 – 322BC) believed that the brain was a cooling mechanism for the human body and that waste products (like phlegm) were excreted through the nose via the pituitary gland (Davy, 1987). Indeed, 'pituitary' is derived from the Latin pituitarius, meaning 'phlegm' (Online Etymology Dictionary. Douglas Harper, Historian. 27 March, 2007). Galen (130 – 200AD) kept the idea of waste production, but had the waste arising from the process of distilling animal spirits from the vital spirits in the blood (Davy, 1987). Galen's precepts were held until the 14th century (Davy, 1987).

Andreas Vesalius (1514-1564) provided the first clearly illustrated anatomy of the pituitary gland (Vesalius, 1534 as cited by Davy, 1987). He posited that the pituitary acted as a structure that distilled fluid from the ventricles and excreted the waste products into the nose and pharynx. In essence, while Vesalius added greatly to the anatomical understanding of the pituitary, he only modernized Galen's functional precepts. Schneider and Lower finally discredited the phlegm theory of pituitary gland function. Schneider (1660 as cited by Davy, 1987) described the mucous membranes of the nose and their role in the production of phlegm. Richard Lower, in 1680 also excluded the brain as the origin of mucus (Davy, 1987). It was also Richard Lower, in 1672, who suggested that substances were removed from the cerebrospinal fluid (CSF) and deposited directly into the bloodstream (Davy, 1987). This laid down one of the fundamental concepts that later came to describe the endocrine system.

It was not until the 20th century that the first microscopic description of the pituitary gland appeared (Benda, 1900; Erdheim, 1903 as cited by Davy, 1987). The pituitary was divided into three cell types, based on the staining techniques available at the time, into acidophilic, basophilic and chromophobic. It was also during this period

that the pituitary was first linked with pathology. Mohr (1840) described obesity concurrent with a tumor in the pituitary gland (as cited by Davy, 1987). Marie (1886) linked the pituitary with acromegaly. By excising it from animals, it was also found that the pituitary was necessary for life (Marinesco, 1892; Vassale and Secchi, 1892; as cited by Davy, 1987)). Paulesco (1908) refined this further by noting that the anterior lobe was necessary, but an animal could survive without the posterior lobe (Davy, 1987). The true function of the gland remained elusive until Cushing's tome "Is the Pituitary Gland Essential to the Maintenance of Life" (1909). This paper marked the founding of modern endocrinology. Cushing's (1912) work was finally encapsulated in his book "The Pituitary Body and Its Disorders", considered by some to be a true milestone in medicine. These past efforts have laid the groundwork for our current understanding of the anatomy and physiology of the pituitary gland as outlined in the following section.

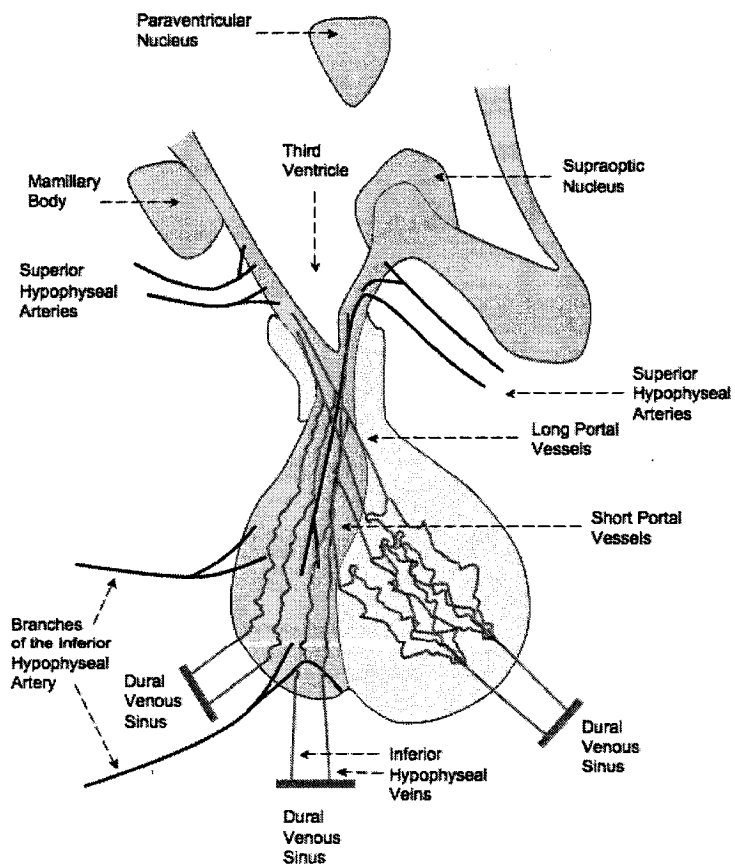
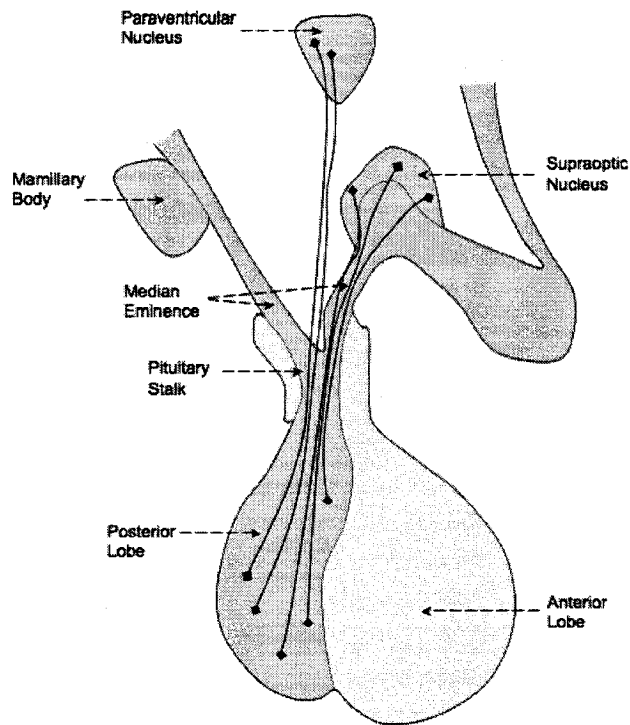
ANATOMY AND PHYSIOLOGY

The hypothalamus lies superior to the pituitary gland, connected to it by the pituitary stalk (or infundibulum) (Gray, 1918). The sella turcica forms a bony socket surrounding the pituitary gland inferiorly. The bone of the posterior wall of the sphenoid air sinus bounds the anterior aspect of the sella turcica. The posterior aspect of the sella turcica is bounded by the clivus (Gray, 1918). The lateral aspects of the sella turcica are made up of a layer of dura and then the cavernous sinus (Gray, 1918). The sella turcica is a depression in the sphenoid bone, including the planum sphenoidale, the tuberculum sellae, anterior clinoid processes, sella floor and the dorsum of the sellae (see figure 2 for the basic anatomy) (Gray, 1918). There is a wide range in the normal size (50 mm^2 to 129 mm^2) of the sella turcica (Israel, 1970). The pituitary gland fills approximately three fourths of the sellar cavity. The superior aspect is covered by the diaphragma sellae, with a foramen allowing the infundibulum to pass through (Sabshin, 1987).

The typical dimensions of the adult human pituitary gland are 10 mm in diameter in the anterior to posterior direction, 15mm in diameter in lateral dimensions and 5 mm

Figure 2: Diagrammatic Representation of the Anatomy of the Pituitary Gland

The top of the figure shows the basic breakdown of the pituitary gland. It is composed of two lobes, the anterior lobe, or adenohypophysis (comprised of the pars anterior and pars intermedia) and the posterior lobe, or neurohypophysis. The posterior lobe receives neuronal input (represented by diamond headed lines) from the paraventricular nucleus and the supraoptic nucleus. The bottom half of the figure demonstrates the blood supply of the pituitary gland. The blood supply is derived from the superior and inferior hypophyseal arteries. Venous drainage goes into the dural sinuses (inferior petrosal and cavernous sinuses). The inferior hypophyseal artery flows into the posterior lobe of the pituitary gland to form a capillary plexus that drains into the venous sinuses. The superior hypophyseal artery forms two or more branches above the diaphragm that go on to form a circumfundibular plexus around the pituitary stalk, hypothalamus and median eminence (Sabshin, 1987). This then drains into the hypophyseal portal system, then into the long hypophyseal portal veins, down into the pituitary forming a secondary plexus that drains ultimately into the dural sinuses. The bilateral trabecular arteries have direct branches from the superior hypophyseal artery into the anterior lobe that drain into the short hypophyseal veins. (Figure adapted from Gray (1918)).



in diameter in the rostral-to-caudal plane (Sabshin, 1987). The weight of an adult pituitary gland is between 500 and 700 mg (Sabshin, 1987). The pituitary gland is 20% larger in adult females compared to males and increases by another 10% during pregnancy (Randall et al., 1982). At birth the pituitary gland is one fifth the size and weight of the adult pituitary (Kaplan et al., 1976).

Functionally and embryologically (see next section), the human pituitary is divided into two separate lobes: (1) the anterior lobe, or adenohypophysis (pars anterior and pars intermedia; Gray, 1918), and (2) the posterior lobe, or neurohypophysis. The anterior lobe contains at least six cell types, including cells that produce growth hormone (GH or somatotropin), prolactin, adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH or thyrotropin), follicle-stimulating hormone (FSH) and luteinizing hormone (LH; both FSH and LH are produced by the same cell type) and melanocyte-stimulating hormone (MSH). Please see the following sections for details on each of these functions of the pituitary. The posterior lobe is responsible for the production of vasopressin (or arginine-vasopressin, AVP, or antidiuretic hormone, ADH) and oxytocin (Sabshin, 1987).

The blood supply is derived from paired superior and inferior hypophyseal arteries (Gray, 1918). The superior hypophyseal artery arises from the ophthalmic segment and/or the clinoid segment of the internal carotid artery (Bouthillier et al., 1996). The inferior hypophyseal artery arises from the cavernous segment of the internal carotid (Bouthillier et al., 1996). Venous drainage goes into the dural sinuses (inferior petrosal and cavernous sinuses) then into the internal jugular veins (Gray, 1918). The inferior hypophyseal artery flows into the posterior lobe of the pituitary gland to form a capillary plexus that drains into the venous sinuses (Gray, 1918). The superior hypophyseal artery forms two or more branches above the diaphragm that go on to form a circumfundibular plexus around the pituitary stalk, hypothalamus and median eminence (Sabshin, 1987). This then drains into the hypophyseal portal system, then into the long hypophyseal portal veins, down into the pituitary forming a secondary plexus that drains ultimately into the dural sinuses (Gray, 1918). The bilateral

trabecular arteries have direct branches from the superior hypophyseal artery into the anterior lobe that drain into the short hypophyseal veins (Sabshin, 1987). There are also some connections from the inferior hypophyseal arteries to the anterior lobe that have allowed for the survival of the anterior lobe during surgical stalk resection (Sabshin, 1987). This complex relationship between the blood supply and the pituitary gland has important implications for the function of the gland, for it is via the circulatory system that many of the pituitary gland's functions are carried out (Sabshin, 1987).

EMBRYOLOGY AND DEVELOPMENT

Maternal hormones do not cross the placenta. Hence, the fetus is responsible for forming its own pituitary hormones (Decherney and Naftolin, 1980). For most bodily structures, development and differentiation occur during the first trimester, with the remaining time spent developing the function of those structures. This is not true for the neuroendocrine system. It develops early and plays a critical role in the development of the rest of the body (Decherney and Naftolin, 1980; Swaab et al., 1978; Winter, 1982), becoming functional by mid-gestation.

The development of the pituitary gland reflects the growth of the two main pituitary lobes, the adenohypophysis and neurohypophysis. The posterior lobe, or neurohypophysis, arises from the termination of unmyelinated axons from the supraoptic and paraventricular subnuclei of the hypothalamus (Sabshin, 1987). By 6 to 8 weeks of gestation, both oxytocin and vasopressin are expressed in these subnuclei (Sabshin, 1987). The anterior lobe, or adenohypophysis, arises from the oral epithelium in an area termed Rathke's pouch around the third gestational week (Sabshin, 1987). At 6 weeks gestation, it separates from the roof of the mouth. Capillary connections are established by the 12th-week of gestation. By the 7th month, it is close to the adult anterior pituitary in terms of anatomy and function (Sabshin, 1987).

NORMAL ENDOCRINE FUNCTION OF THE PITUITARY GLAND

The pituitary is part of a multi-level system that regulates the endocrine system via negative and positive feedback mechanisms. Pituitary function is best understood as groups of hypothalamic-pituitary-target organ interactions (see figure 3 for a summary). Neural and hormonal feedback regulates the activity via releasing hormones and inhibitory hormones released into a complex vasculature (Goodrich and Lee, 1987). Certain axon terminals from neurons in the hypothalamus related to these terminate in the median eminence at the base of the hypothalamus. The median eminence consists of three zones: (1) the ependymal zone, (2) the internal zone and (3) the external zone (Caldwell and Kayne, 1987). The ependymal zone surrounds the infundibular recess of the third ventricle (Gray, 1918). The internal zone contains axons transporting vasopressin and oxytocin (from supraoptic and paraventricular nuclei) that terminate in the posterior pituitary. The external zone of the median eminence contains axon terminals that interface with the vessels of the portal plexus.

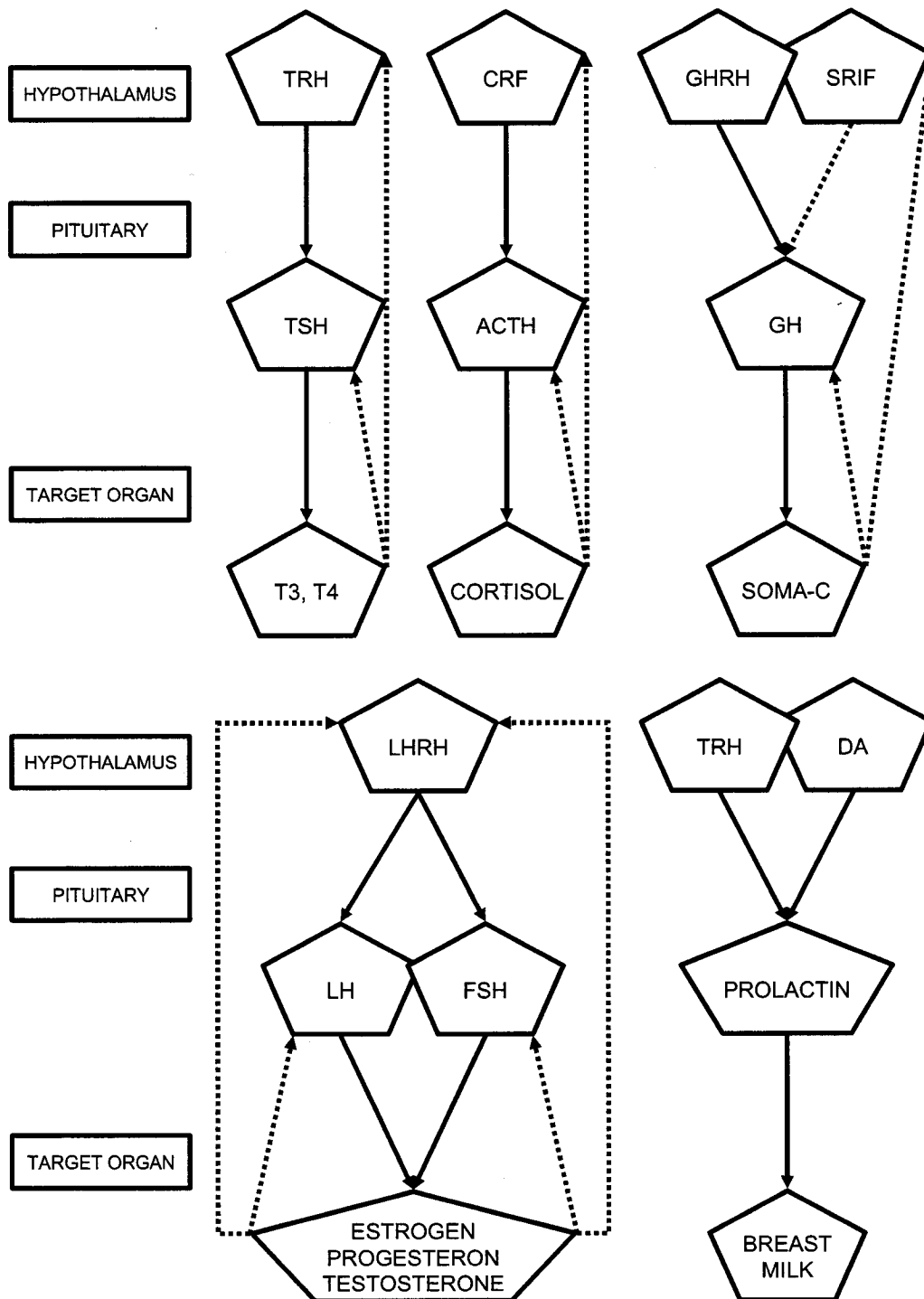
In the anterior pituitary gland, the cells are specialized to respond to and produce specific hormones (Caldwell and Kayne, 1987). For example, thyrotropes respond to thyroid releasing hormone (TRH) by releasing thyroid stimulating hormone (TSH). Somatotrophs are regulated by both releasing (growth hormone releasing hormone or GHRH) and inhibitory (somatostatin) mechanisms that control the release of growth hormone. These adenohypophyseal hormones enter into the systemic circulation to reach the receptors on the target organs. These target organ endocrine cells release a further hormone that then has its effect on various bodily systems.

Hormone secretion from the hypothalamus and anterior pituitary is pulsatile in nature (Caldwell and Kayne, 1987). Secretion occurs in ultradian pulses about every 60 – 180 minutes with the pulses from the hypothalamus driving the pulsatile release in the pituitary (Akil et al., 1999). Disruption of this pulsatile secretion can result in poor regulation of the hormonal system in question. There is also a circadian regulatory pattern to hormonal secretion. Some of these rhythms are truly circadian while others are dependant on sleep-wake cycles. The hypothalamic-pituitary adrenal (HPA) axis

Figure 3: Pituitary-Target Organ Interactions

There are five main hypothalamus-pituitary-organ axes: TOP (1) Thyroid, (2) Adrenal, (3) Growth Hormone and BOTTOM (4) Gonadal (5) Prolactin.

Abbreviations: thyroid releasing hormone (TRH), thyroid stimulating hormone (TSH), L-thyroxine (or T4), triiodothyronine (or T3), corticotropin releasing factor (CRF), adrenocorticotrophic hormone (ACTH), growth hormone releasing hormone (GHRH), somatostatin (SRIF), somatomedins (Soma-C), growth hormone (GH), luteinizing hormone-releasing hormone (LHRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), dopamine (DA).



demonstrates a peak in cortisol secretion in the morning with a trough at night. On the other hand, release of growth hormone is associated with non-REM sleep (Akil et al., 1999). In the following sections, each pituitary hormone will be discussed.

Thyroid Hormone

Thyroid hormone (TH) is involved in protein synthesis and metabolism and is necessary for normal development and brain function (Akil et al., 1999). TH comes in 2 forms, L-thyroxine (or T4) and triiodothyronine (or T3). TH is released in response to thyroid stimulating hormone (TSH) release from the anterior pituitary, which in turn is stimulated by thyroid releasing hormone (TRH) from the hypothalamus (Akil et al., 1999; Caldwell and Kayne, 1987). TRH was the first hypothalamic releasing factor identified (Reichlin, 1989). Free TH feeds back on the hypothalamus (to inhibit TRH release) and the anterior pituitary (to inhibit TSH release) to form a classic negative feedback loop. The relationship between free TH and TSH levels is very precise (Snyder and Utiger, 1973; Saberi and Utiger, 1975). As TH levels increase, TSH levels decrease and vice versa. TRH is responsible for establishing the set point for TSH secretion (Reichlin, 1966). TSH is also inhibited by dopamine, somatostatin, cortisol and thyroid hormone, while estrogen augments TSH response to TRH (Akil et al., 1999).

Adrenocorticotrophic Hormone

Stress is defined as the response to noxious stimuli (Selye, 1956). Hans Selye (1956) described the body's response to stress as a deviation from homeostasis and correction of this by the same system that mediates the stress response (the HPA axis). The stress response involves the HPA axis, involving the adrenal medulla, and the autonomic nervous system (ANS) (Akil et al., 1999). As a first step, corticotropin releasing factor (CRF) is released from the hypothalamus (Vale et al., 1981). The medial parvicellular region of the paraventricular nucleus (mpPVN) is the region that stains the most for CRF (Swanson et al., 1988). Neurons from the mpPVN project to the external layer of the median eminence where CRF is released into the portal bloodstream in the anterior pituitary (Akil et al., 1999). CRF stimulates the

production of pro-opio-melanocortin (POMC), which is cleaved into adrenocorticotrophic hormone (ACTH), the lipotropins, endorphins and enkephalins (Akil et al., 1999). CRF neurons also express vasopressin (AVP) (Caldwell and Kayne, 1987). The majority of AVP is expressed in the magnocellular region of the PVN that projects to the posterior pituitary. The corticotrophs bear G-protein coupled receptors for both CRF and AVP and their activation has a synergistic effect on these cells. These cells release ACTH into the systemic circulation and this binds to receptors on the adrenal cortex. The adrenal cortex, in response, makes glucocorticoids from cholesterol. The adrenal cortex does not store glucocorticoids at rest. ACTH is controlled by a diurnal rhythm (peak values in the early morning) as well as cortisol feedback at the level of the pituitary and hypothalamus. The role of the pituitary in stress will be explored in detail elsewhere in this thesis.

Growth Hormone

Growth hormone releasing hormone (GHRH) and somatostatin (SRIF) are both released from the hypothalamus (Frohman et al., 1999). GHRH stimulates release of growth hormone (GH) while SRIF inhibits secretion of GH (Mason et al., 1993; Tannenbaum, 1991). GH stimulates the release of insulin-like growth factors (IGF), or somatomedins (Salmon and Daughaday, 1956). The final effect of GH is mediated by these somatomedins (Salmon and Daughaday, 1956). The feedback mechanism that controls GH secretion is unclear (Frohman et al., 1999). Exercise, sleep, stress and hypoglycemia can all increase GH release (Frohman et al., 1999). Propranolol, clonidine, estrogen and dopamine can also stimulate GH release. Glucocorticoids (GC), hypogonadism, hypothyroidism, and obesity can lead to suppression of GH release (Frohman et al., 1999; Caldwell and Kayne, 1987).

Gonadal

Gonadal (or sex) hormones are modulated differentially in males and females (Caldwell and Kayne, 1987). In males, LHRH stimulates LH and FSH production and release. This in turn stimulates testosterone and sperm production. Testosterone feeds back on LH and FSH release. In females the story is more complex. Luteinizing

hormone-releasing hormone (LHRH) is released in a cyclical fashion over the course of about four weeks (Evans and Long, 1922; Fevold et al., 1931). This causes a cyclical secretion of the pituitary's luteinizing (LH) hormone and follicle-stimulating hormone (FSH) along with the ovarian estradiol (E2) and progesterone (PROG) hormones (Frohman et al., 1999). At the end of a menstrual cycle, E2 and PROG are reduced, causing there to be less feedback inhibition of FSH and LH. As FSH concentrations rise, there is a burst in E2 release. This causes a mid cycle burst in LHRH that is followed by a fast rise in LH and FSH, while E2 and PROG levels increase more gradually, ultimately inhibiting LH and FSH release (Frohman et al., 1999).

Prolactin

The primary release factor for prolactin is not known (Frohman et al., 1999; Caldwell and Kayne, 1987). Dopamine acting on the hypothalamus inhibits release of prolactin, as does prolactin feedback (Murai et al., 1989; Barraclough and Sawyer, 1959). Prolactin secretion is increased by sleep, stress (including exercise), pregnancy, nursing, nipple stimulation and in females, sexual intercourse. Pharmacologically, prolactin is stimulated by hypoglycemia, estrogen, TRH and antagonists of dopamine and serotonin (Freeman et al., 2000; Frohman et al., 1999). Prolactin is inhibited by dopamine, hyperglycemia, GCs, TH decrease and prolactin (Freeman et al., 2000; Frohman et al., 1999). Clinically, elevated prolactin is noted in conditions of hypothyroidism, renal failure and cirrhosis (Frohman et al., 1999).

Posterior Pituitary Hormones

There are two posterior pituitary hormones, AVP and oxytocin (Caldwell and Young, 2006; Caldwell and Kayne, 1987). These are synthesized in the nerve cell bodies of the supraoptic and paraventricular nuclei of the ventral hypothalamus. AVP and oxytocin are then transported along their axons to the storage sites in the posterior pituitary gland. Each hormone is attached to a carrier protein (or neurophysin or NP) that is co-released with the hormone (Iverson et al., 2000). AVP is released in response to increases in plasma osmolality. Changes in plasma osmolality are sensed

by osmoreceptors located mainly in the organum vasculosum of the lamina terminalis, from where the excitatory signals are sent to the magnocellular neurons in the supraoptic nucleus and PVN (Mangiapane et al., 1983; Johnson, 1985). Increases in plasma osmolality lead to increases in AVP release, while reductions lead to decreases in AVP release. The status of intravascular volume regulates AVP as well. AVP causes the kidney to reabsorb free water in the distal nephron, resulting in a reduction of plasma osmolality and an increase in urinary osmolality. Pharmacologically, enhancement of AVP function comes from drugs such as carbamazepine and morphine (Stephens et al., 1978; Aziz et al., 1981). Lithium, phenytoin and alcohol have the opposite effect (e.g., Hullin et al., 1979; Linkola et al., 1977). AVP release is stimulated by nausea, angiotensin, hypoglycemia, cortisol and hypothyroidism. Oxytocin is released in response to nipple stimulation and aids in breast milk secretion in conjunction with prolactin (Iverson et al., 2000).

COMMON PATHOLOGY OF THE PITUITARY GLAND

Pituitary tumors are the commonest lesions in the sella and suprasellar region (incidence 15-20/1, 000, 000 per year; Anderson et al., 1999). These include adenomas, craniopharyngiomas, aneurysms and meningiomas. Subclinical adenomas have an incidence of between 5 – 27% (Anderson et al., 1999). Pituitary disease is typically insidious in its development and often, in hindsight, may have been detected by the clinician earlier. The symptoms of hormonal hypersecretion in endocrinologically active tumors typically occur prior to suprasellar or parasellar extension. This means the hormonal effect is apparent first, then as the tumor progresses, the extremely abnormal increase in size is noted. In this thesis, I examined volumetric changes from normal that are typically smaller than those noted in pituitary adenomas, possibly reflecting more subtle changes in the relevant psychiatric diseases as compared with the typical very large volumetric changes in carcinomas.

Growth Hormone Adenomas

Somatic changes will usually cause a patient with a growth hormone adenoma to see a clinician, but so may optic nerve impairment, proximal myopathy, peripheral neuropathy and even psychological effects (mood and anxiety) of the adenoma (Anderson et al., 1999). It has been posited by Koibuchi et al. (1991) that the psychological/emotional effects associated with this condition are mediated by the excess growth hormone acting on the amygdala. Headaches are also common in acromegaly, a common result of GH adenomas, as are diabetes and hypertension.

Cushing's Syndrome

Patients with Cushing's syndrome present with microadenoma. In adolescents, symptoms may fluctuate over time (Anderson et al., 1999). Proximal myopathy, obesity, hypertension are often coupled with psychiatric disturbances, including anxiety or panic attacks.

Other Conditions

Prolactinomas and other hormonally inactive tumors are usually uncovered when they have grown to the point when they have a negative impact on the surrounding structures. Symptoms such as visual failure, ophthalmoplegia, trigeminal sensory loss and even hydrocephalus can occur (Anderson et al., 1999). Hypogonadism may present with weakness and fatigue and/or headache. In hypogonadism, the patient may also experience a hyperprolactinaemia as a result of the increase in pressure on the normal pituitary gland tissue. Hypothyroidism can present as carpal tunnel syndrome (van Dijk et al., 2003), myotonia and/or myopathy. In children, lesions to the pituitary and third ventricle can induce precocious puberty, a slowing in growth, diabetes insipidus and hydrocephalus.

DIAGNOSTIC IMAGING OF PITUITARY DISORDERS

Most diagnostic imaging of the pituitary gland is done in a qualitative manner (see chapter 2). Assessment is made visually, and findings are considered in the context of

neuroendocrine findings. MRI is also used for follow-up after intervention (either surgical or medication). MRI is emerging as one of the techniques of choice for diagnosis of pituitary-related problems. MRI is able to detect accurately microadenomas between 65 – 90% of the time (Stadnik et al., 1994). Most microadenomas appear hypointense on T1 weighted sequences as compared to healthy pituitary tissue. A false negative rate of about 13% occurs with MRI investigations of microadenomas. T1 weighted techniques that are gadolinium-enhanced are the most sensitive to small intrasellar lesions. The enhancement makes the normal tissue brighter, thus making the lesion area more apparent against the background. Pre-contrast images allow for the detection of lesions that may be isointense with the gland after enhancement. For macroadenomas, computed axial tomography (or CT) and MRI are equivalent for qualitative assessment (Anderson et al., 1999). Microadenomas are 400 times more common than macroadenomas (Anderson et al., 1999). Please see chapter 2 for more information on MRI techniques.

THE STRESS RESPONSE

Stress, from a biological viewpoint, is considered to be the body's response to noxious stimuli. Organisms typically strive towards homeostasis. In the classical view of stress, this balance is threatened by noxious stimuli. The stimuli may be physical or psychological in nature. These stimuli are called "stressors". These stressors trigger physiological and behavioral responses that tend to re-establish homeostasis. The role of stress in mental illness deserves particular attention as a risk factor. For example, depression is often described as a "stress-related" disorder and evidence exists that stress can be a contextual factor. Stress on its own, however, is not sufficient to cause depression as many people experience stress without developing depression. Severe stress certainly has a role in psychiatric illnesses such as post-traumatic stress disorder (PTSD). Although PTSD is quite distinct from MDD in its expression of the illness and its course over time, there appear to be similar neurobiological changes in PTSD and MDD, especially in the hippocampus (Bremner, 2002; Grossman et al., 2002).

Stress acts on the human body via the hypothalamic-pituitary-adrenal (HPA) axis. This system has been implicated by various neuroendocrine studies in MDD and may play a role in the expression of the disorder (Plotsky et al., 1998). In the HPA axis, neurons in the paraventricular nucleus (PVN) of the hypothalamus secrete corticotropin releasing factor (CRF) that in turn stimulates the release of adrenocorticotropin (ACTH) from the anterior pituitary. ACTH stimulates the release of cortisol (a glucocorticoid) from the adrenal cortex. Cortisol has a dynamic effect on metabolism and behavior via direct action at many brain regions (Cole et al., 2000). The HPA axis is controlled along many stages of its pathway. Two types of glucocorticoid receptors are thought to control negative feedback for the HPA Axis. Type I are also known as mineralocorticoid receptors (MRc) and Type II as glucocorticoid receptors (GR). MRcs are found in specific brain regions such as the amygdala (Goodrich and Lee, 1987). GR's are widely distributed in the brain, including the hippocampus, hypothalamus, and prefrontal cortex (De Kloet et al., 1984). MRc binds selectively to cortisol. GR also bind to cortisol but with a lower affinity compared to MRc. The hippocampus has an inhibitory influence on hypothalamic CRF neurons, while the amygdala exerts an excitatory influence. When high concentrations of cortisol are present, the MRc receptors saturate (become bonded to cortisol), and the GR receptors activate in order to move the body towards homeostasis. Basal levels of cortisol enhance hippocampal inhibition of HPA activity. Under conditions of sustained stress, the higher cortisol levels may damage hippocampal neurons. The nature of this damage has not been fully elucidated, but it may involve a decrease in dendritic branching and/or spines (McEwen, 2000; Sapolsky, 2000). The birth rate of new granule cells in the dentate gyrus may also decrease (Czeh et al., 2001). This damage may in turn diminish the inhibitory control the hippocampus has over the HPA axis. Should hippocampal neurons be damaged, particularly those with GR receptors, it would be more difficult for the body to return to homeostasis.

More recently, a second mode of operation related to the stress response has been uncovered. The fast initial 'fight or flight' response is mediated by CRH1 receptors. A

second, slower response is mediated by urocortins and the CRH2 receptors (Hsu and Hsueh, 2001; Lewis et al., 2001; Reyes et al., 2001). This response leads to adaptation and recovery (Hsu and Hsueh, 2001; Reul and Holsboer, 2002). Urocortin II (stress-related) and urocortin III (stresscopin) are distinct from other CRH peptides (Li et al., 2002). Urocortin I is formed in the Edinger-Westphal nucleus and acts on both CRH1 and CRH2 receptors. Urocortin II is formed in the PVN and locus ceruleus. Urocortin III is formed in the preoptic nucleus and amygdala. They are thought to play a role in the late recovery phase of the stress response, dealing with coping and behavioral adaptation. Urocortin II and III are anxiolytic while CRH is anxiogenic (Hsu and Hsueh, 2001). It is currently unknown if the urocortins play a role in psychiatric illness.

ANIMAL STUDIES OF PITUITARY TROPIC ACTIVITY

Nolan et al. (1998) studied male rats after surgical adrenalectomy and 14 days of dexamethasone treatment, examining mitotic and apoptotic bodies in the pituitary. They found that the male rat anterior pituitary cell either dies or divides as frequently as once every 60-70 days. Dexamethasone treatment of adrenalectomized rats produced a decline in the number of anterior pituitary cells, which continued for 2 weeks after the start of glucocorticoid treatment (Nolan et al., 1998). In the adult rat anterior pituitary, exogenous glucocorticoids, depending on timing and dose, induce apoptosis (Nolan and Levy, 2003). This response is enhanced by adrenalectomy (Nolan and Levy, 2003). This pituitary mitotic and apoptotic responsiveness following adrenalectomy is independent of PVN CRH input and relies more on direct glucocorticoid withdrawal at the level of the pituitary (Nolan et al., 2004).

Sex hormones also affect mitotic and apoptotic activity. Nolan and Levy (2006a) found that testosterone treatment was unable to reduce anterior pituitary mitotic activity in untreated, gonadal intact animals. It did suppress the early increase in mitotic activity induced by gonadectomy. Estrogen stimulated anterior pituitary mitotic activity in a time-dependent manner (Nolan and Levy, 2006a). When adrenalectomy and gonadectomy were combined, the amplitude of the pituitary

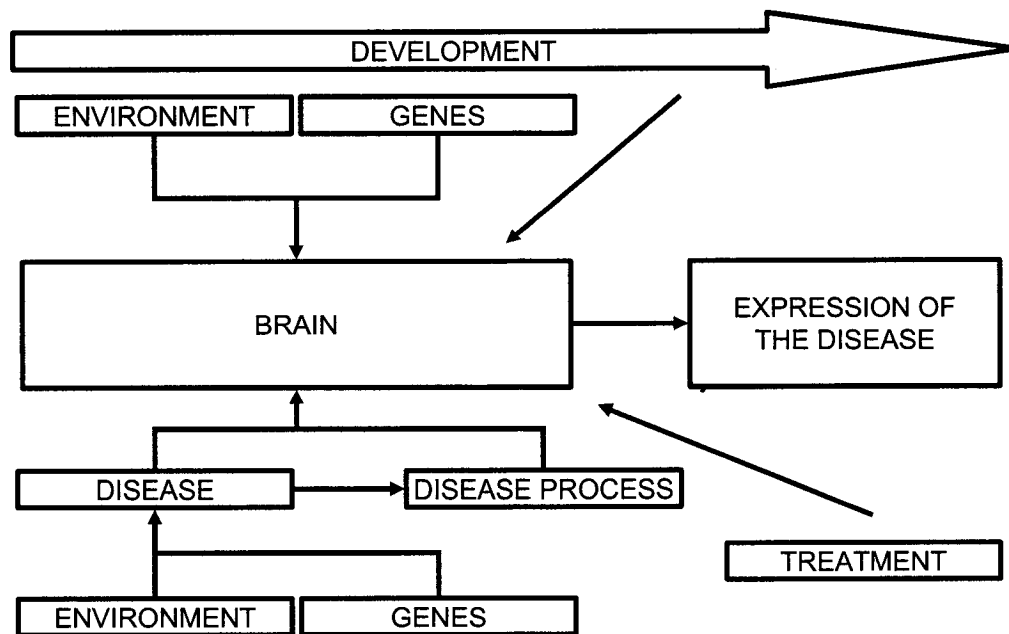
mitotic response did not change, indicating that the trophic stimuli were not additive (Nolan and Levy, 2006b). Despite the increase in mitotic activity, no increase in the number of LH cells after gonadectomy was detected (Nolan and Levy, 2006b). The non-additive pituitary mitotic and apoptotic responses indicated that the same progenitor cell population responded both to adrenalectomy and gonadectomy. The increase in the number of corticotrophs following adrenalectomy appears to be from differentiation of nascent cells (Nolan and Levy, 2006b). The authors suggest that it is progenitor cells that respond to hormonal stimuli (Nolan and Levy, 2006b). For the context of this thesis, these reports in animals provide direct evidence for the plausibility of trophic changes in the pituitary in response to stimuli.

RATIONALE FOR THESIS STATEMENT

Neurobiological mechanisms, if not directly responsible for the etiology of the mental disorders (a matter of considerable debate), are at least involved in the behavioral expression of the disorders. The disorders themselves, via neurobiological processes, may in turn be responsible for certain pathology in the brain ('wear and tear'). In the case of pediatric populations, the neurobiology involves a further complication. During childhood and adolescence, the brain undergoes a tremendous and complex maturational process. A healthy brain is affected by gene expression as well as environment, and these differ at various developmental stages. In a person with mental illness, the effects of the disease process itself are added to environmental and genetic aspects of development. Finally, if disease treatment is also considered, there are at least eight factors involved in a given pediatric mental illness (see figure 4). As stated above, all psychiatric diseases are understood and investigated from the vantage point offered by the expression of the disorder. The disorders, while complex overall, by necessity of the nature of the brain-to-body interface, can be considered to converge on certain key anatomical nexus points. One such nexus point, that is easily available for study *in vivo*, is the pituitary gland, the subject of this thesis.

Figure 4: Major Factors Involved in Pediatric Mental Illness

The brain is affected by multiple factors in pediatric mental illness. Development is responsible for numerous changes in the brain during childhood and adolescence. The disease may fundamentally change the brain as well as the disease process or course. Treatment can also affect brain function, chemistry and structure. All of the aforementioned factors would be moderated by genetic and environmental influences as well.



As stated earlier, there are three fundamental reasons for studying the pituitary gland in these disorders. First, one commonality among pediatric mental illnesses appears to be the involvement of the pituitary gland (the 'nexus point'). Second, the pituitary gland can change in volume under differing physiological conditions. Due to its physiological role in the body, the pituitary gland has to respond continuously to transient but often-recurrent stimuli and its physiology changes in response to activity. These stimuli include, but are not limited to, physical and psychological stress, and pregnancy. The pituitary can respond in at least two ways (Nolan and Levy, 2001); it can increase the secretory capacity of the pertinent cells or it can alter trophic activity levels by, for example, altering mitotic or apoptotic activity. Upon withdrawal of the stimulus, the pituitary can either return to its pre-stimulated state or change in some long-lasting manner that would allow for a prolonged enhanced response to a recurrent stimuli. The persistence of such hypothetical changes may predispose the pituitary not only to changes in secretory activity but also to trophic anomalies. Third, the pituitary can be evaluated *in vivo* in children and adolescents using magnetic resonance imaging.

In pediatric psychiatric illness, there have been three previously published reports on the pituitary gland. Enlarged pituitaries were found in post-traumatic stress disorder (PTSD) associated with suicidal ideation (Thomas and DeBellis, 2004). My previous work revealed enlarged pituitary glands in adolescent major depressive disorder (MDD) (MacMaster and Kusumakar, 2004a). Chen et al. (2004) reported finding no differences from controls in euthymic bipolar disorder subjects.

OUTLINE OF EXPERIMENTS

OVERALL HYPOTHESIS

Psychiatric diseases that affect or involve the function of the pituitary gland will exhibit abnormal morphology when compared to healthy pituitary glands.

In order to test this overall hypothesis, I have chosen four disorders to study: major depressive disorder (MDD), bipolar depression, obsessive-compulsive disorder (OCD) and attention deficit hyperactivity disorder (ADHD). These disorders provide a range of behavioral conditions around which to study the pituitary gland. The effect of therapeutic intervention is also examined to see if behavioral change (as mediated by medication) induces some return to normalcy for the pituitary gland. Some baseline knowledge is required before the clinical-control comparisons are undertaken, such as relative stability of the measure, and possible developmental and sex effects.

Experiment 1 - Development

As these disorders are being studied in pediatric populations, one must consider the possible effect of age on the pituitary gland. To address this, an MRI analysis was undertaken using data pooled from all available sources of healthy control subjects, resulting in 82 males and 82 females between the ages of 6 to 35 years of age forming a large sample with a heterogeneous distribution as to age. This experiment is detailed in chapter 3.

Hypothesis 1: Pituitary Gland Volume Increases with Age, Demonstrating a Growth Spurt during Adolescence

Experiment 2 – Sexual Dimorphism

Secondary to development, one must also consider the possibility of sex differences with regard to pituitary gland volume. 82 age- and sex-matched male-female pairs (6 – 35 years of age) were compared. This experiment is detailed in chapter 3.

Hypothesis 2: Females have Larger Pituitary Gland Volumes than Males

Experiment 3 - Stability

Prior to an examination of the effect of intervention on the pituitary gland, it is necessary to understand the temporal stability of the measure. Two samples were examined in this experiment: 1) 10 males and 6 females (6 – 12 years of age) had data

collected at baseline and again 8 weeks later (a typical clinical trial length) and 2) 10 males and 8 females (9 – 19 years of age) had data collected at baseline and again in a second scan 8 to 79 weeks later. This experiment is detailed in chapter 3.

Hypothesis 3: Pituitary Gland Volume does not vary Significantly over the Course of 8 Weeks of Normal Development

Experiment 4 – Major Depressive Disorder

I measured the volume of the pituitary gland in 25 MDD (10 to 17 years; 13M, 12F) patients and 25 age- and sex-matched healthy control subjects (9 to 17 years; 13M, 12F)

Hypothesis 4: Treatment-Naïve Youth with MDD will have Larger Pituitary Gland Volumes than Age- and Sex-Matched Healthy Controls

Experiment 5 – Treatment for Major Depressive Disorder

Thirteen MDD subjects were scanned at baseline and after 12 weeks of venlafaxine therapy for MDD (4 males and 9 females; 10 to 19 years of age).

Hypothesis 5: The Pituitary Gland will normalize in Volume after 12 Weeks of Venlafaxine Therapy for MDD

Experiment 6 – Bipolar and Psychotic Depression

Twelve age- and sex-matched case-control pairs (5 males, 7 females in each group; 14 to 20 years of age) were scanned.

Hypothesis 6: Youth with a Depressive Disorder (Bipolar Disorder or Psychotic Depression) will have Larger Pituitary Gland Volumes than Age- and Sex-Matched Healthy Controls

Experiment 7 – Obsessive-Compulsive Disorder

31 children with OCD who had not been exposed to psychotropic drugs, aged 8 to 17 years, and 31 case-matched healthy control subjects were analyzed to determine the volume of the pituitary gland.

Hypothesis 7: Treatment-Naïve Youth with Obsessive-Compulsive Disorder (OCD) will have Smaller Pituitary Gland Volumes than Age- and Sex-Matched Healthy Controls

Experiment 8 – Treatment for Obsessive-Compulsive Disorder

16 young people with OCD, aged 8 to 17 years, were scanned before and after 12 weeks of paroxetine therapy.

Hypothesis 8: The Pituitary Gland will Normalize with 12 Weeks of Paroxetine Treatment in Children with OCD

Experiment 9 – Attention Deficit Hyperactivity Disorder

Data for this experiment were derived from 17 age-matched case-control pairs (6 to 11 years of age).

Hypothesis 9: Treatment-Naïve Youth with Attention Deficit Hyperactivity Disorder (ADHD) will not Differ with Regard to Pituitary Gland Volumes from Age and Sex Matched Healthy Controls

Experiment 10 – Treatment for Attention Deficit Hyperactivity Disorder

Twelve ADHD subjects (8.22 ± 1.80 years of age) were scanned before and after treatment with methylphenidate for 8 weeks.

Hypothesis 10: There will be no Change in Pituitary Gland Volume with 8 Weeks of Methylphenidate Treatment in Children with ADHD

Table 5: Planned Contrasts for Thesis Experiments

Comparison	Planned Contrast
Male vs. Female	-1 1
MDD vs. Control	1 -1
MDD Pre- vs. Post-treatment	1 -1
BP vs. Control	1 -1
OCD vs. Control	-1 1
OCD Pre- vs. Post-treatment	1 1
ADHD vs. Control	1 1
ADHD Pre- vs. Post-treatment	1 1

CHAPTER TWO: GENERAL METHODOLOGY

INTRODUCTION

Given the hypotheses proposed above, the question remains as to how best to examine the pituitary gland. In pediatric psychiatric illness there are no established animal models of the disorders nor is there a strong post-mortem literature. However, emerging imaging technologies do offer insight into living brain structure, function and chemistry with a high degree of safety. In this chapter, I will first address the fundamentals of magnetic resonance (MR). The specific imaging parameters used in this thesis will be presented. Then I will discuss the basic principles of MR volumetrics, with a specific focus on the brain. The anatomic method used in this thesis will then be presented. Following that, the statistical rationale will be addressed. Finally, as some of the core experiments involve pre- versus post-intervention data sets, the temporal stability of the pituitary gland measure will be evaluated.

MAGNETIC RESONANCE IMAGING

BASIC PRINCIPLES

Magnetic resonance imaging, or MRI, can provide information about brain structure at high resolution and with a great deal of safety. MRI is commonly used in clinical practice to provide diagnostic and prognostic information about pathological conditions. Currently, there are over 1,000 1.5T MRI scanners in the United States alone.

The first experiment involving magnetic resonance was conducted over 60 years ago by Rabi et al., (1938), in which MR signals were detected off a beam of lithium chloride. Less than a decade later, two groups independently used MR to assess bulk matter (Bloch et al., 1946; Purcell et al., 1946). These experiments took advantage of the fact that each atomic nucleus has a spin angular momentum quantum number (or I , at half integer values: $1/2$, 1 , $3/2$...). In a magnetic field, symbolized by B_0 , non-zero nuclei will have $2I + 1$ equally-spaced energy levels separated by a specific amount of energy (ΔE) as determined by $\Delta E = \mu B_0/I$ (where μ is the nuclear magnetic moment). In MR experiments, energy is applied at a specific frequency to

the nucleus that causes a transition between energy levels (see figure 5). The resonance frequency is determined by $\nu_0 = \Delta E/h$, where h is Planck's constant. In the data represented in this thesis, the nucleus used was hydrogen (H), which has a spin quantum number of $I = \frac{1}{2}$ at 1.5T that results in a Larmor frequency of 63.87 MHz. This frequency, when applied to the sample, will induce the change in energy states from being aligned with the magnetic field to being aligned against the magnetic field.

Next, let's consider a collection of hydrogen nuclei. In an environment without a static magnetic field, the nuclei are aligned randomly. As a strong magnetic field is applied to the sample, the hydrogen nuclei distribute between parallel and anti-parallel orientations. This is known as the Boltzmann distribution. In human studies, this results in 10 in 1,000,000 hydrogen nuclei being aligned with the magnetic field (at 1.5T and 37°C). These collections of nuclei have a net magnetic moment, or M , which is the net difference in the number of nuclei in the two levels. The nuclei also tend to precess, or spin around the magnetic field. This is similar to the behavior of a gyroscope. This motion is called the rotating frame.

The net M is commonly represented as a motionless vector along the z – axis. Application of a radiofrequency (rf) pulse moves M off the z -axis and towards the xy plane. Maximum signal is achieved when M is completely on the xy plane with an equal number of nuclei in both high and low energy levels. After the rf pulse ceases, M will return to its primary state. The return in the transverse, or xy plane, is called spin-spin relaxation, or T2 relaxation. Recovery along the z -axis is called spin-lattice relaxation, or T1 relaxation. The rf signal (at the resonance frequency) released by T1 and T2 relaxation comprises the data used to generate MRI information (see figure 6).

The first MR image was published by Lauterbur (1973) using varied magnetic field gradients to localize two tubes of water in a phantom. The use of field gradients allows for the localization across a sample. The resonance frequency of a nuclear spin

Figure 5: Change in Energy States

In hydrogen (H), which has a spin quantum number of $I = \frac{1}{2}$ at 1.5T, results in a Larmor frequency of 63.87 MHz. This frequency, when applied to the sample, will induce the change in energy states from being aligned with the magnetic field to being aligned against the magnetic field. This is the basic premise of MRI.

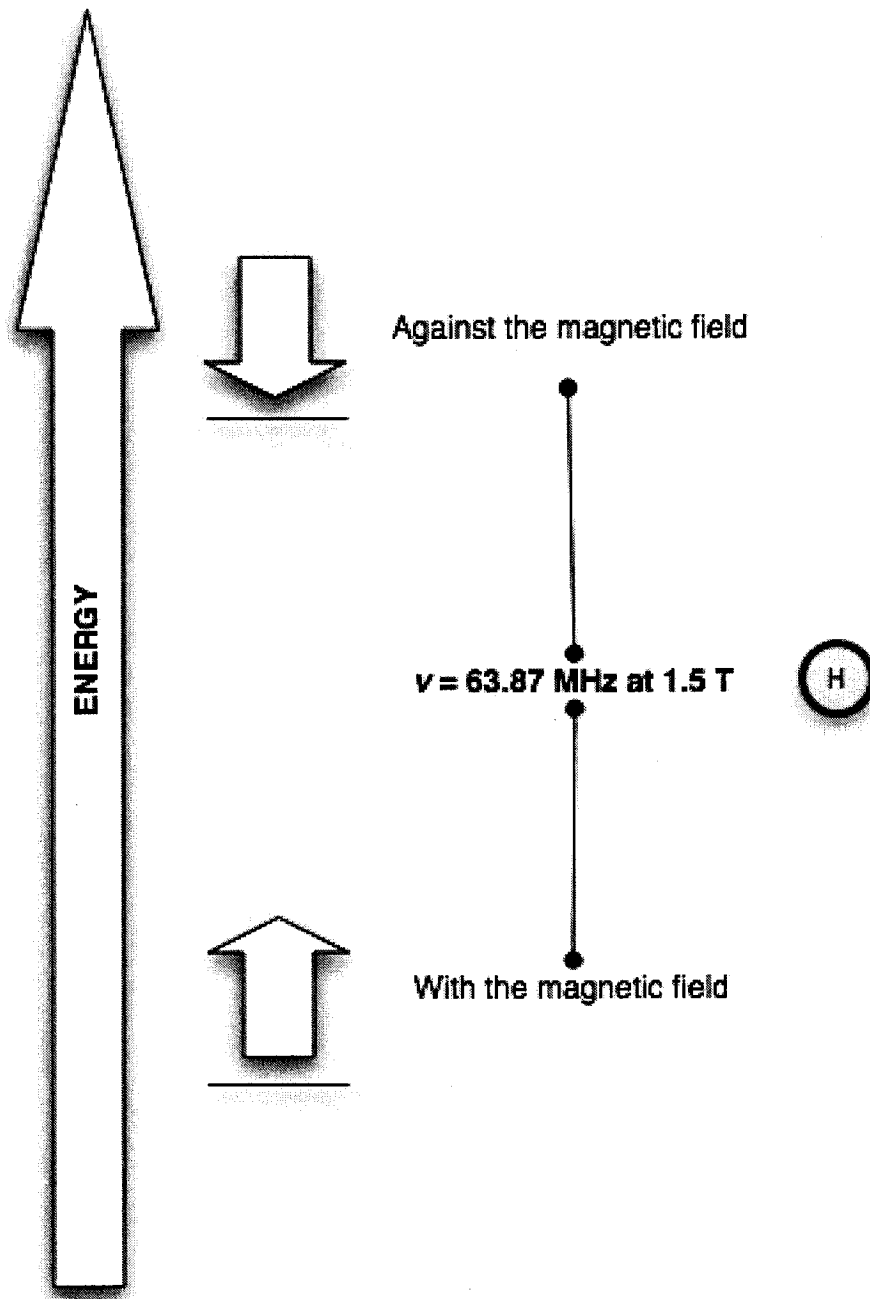
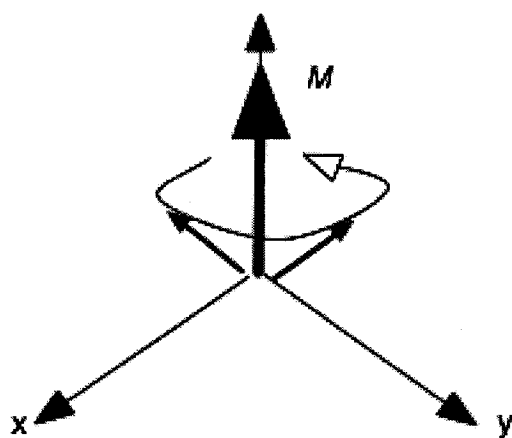


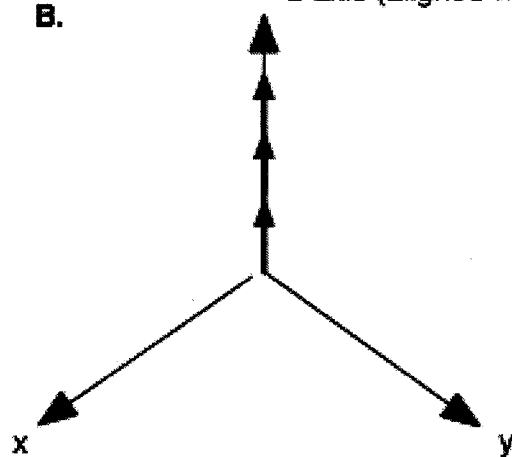
Figure 6: Relaxation

The net magnetic moment, or M , is commonly represented as a motionless vector along the z – axis. Application of a radiofrequency (rf) pulse moves M off the z -axis and towards the xy plane. Maximum signal is achieved when M is completely on the xy plane with an equal number of nuclei in both high and low energy levels. After the rf pulse ceases, M will return to its primary state. The return in the transverse, or xy plane, is called spin-spin relaxation, or T_2 relaxation. Recovery along the z -axis is called spin-lattice relaxation, or T_1 relaxation. The rf signal (at the resonance frequency) released by T_1 and T_2 relaxation comprises the data used to generate MRI information.

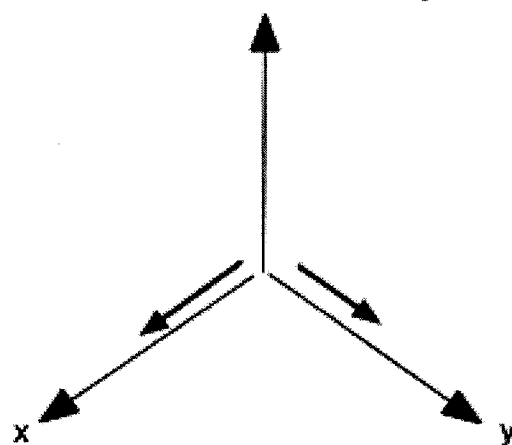
A. z-axis (aligned with magnetic field)



B. z-axis (aligned with magnetic field)



C. z-axis (aligned with magnetic field)



indicates its position along the gradient; this is known as frequency encoding. Using slice selective imaging, a single slice is excited (rather than the entire volume). This takes imaging from a 3 dimensional problem to a 2 dimensional problem. The excited spins at different points along the field gradients are at slightly different phases; this is known as phase encoding. Nuclei trapped in a solid matrix (like bone) cannot be affected by the rf pulse and thus result in a signal void in the image. The rate of rf application can be varied (TR or repetition time) as can the timing of the signal collected (TE or echo time). By varying the TE and TR, the resulting images may be either T1 weighted or T2 weighted. T1 weighted, as used in this thesis, results in a high contrast between gray and white matter and cerebral spinal fluid (CSF). In T2 weighted images, much common neurological pathology is distinguishable in high contrast between normal and healthy tissue.

INSTRUMENTATION

The MRI scanner has as its core a large electromagnet. Aside from this magnet, there are further smaller electromagnetic coils that allow for image production. The rf coils broadcast the rf signal into the subject. These same coils also act as antennas that receive the return signal from the subject. The rf coils can transmit and receive (transceivers) or receive only (in which case the body coil is used as the transmitter). There are many types of coils used in acquiring data from the brain. The 'birdcage' coil provides the best rf homogeneity and is commonly used for imaging the head. Most coils are designed with the intention of deriving the optimal signal from a given sample. For example, paired saddle coils are used to image data from the knees, Helmholtz pair coils are used for imaging the pelvis or cervical spine, and surface coils are used for imaging the spine, shoulder and other small body parts. By varying the magnetic field one can localize image slices as well as implement phase and frequency encoding. Gradient coils are used to produce deliberate variations in the magnetic field (B_0). Typically, there are three sets of gradient coils, one for each direction (X, Y, Z). The gradient coils used for the Z-axis are Helmholtz pairs and the X and Y-axis are paired saddle coils.

A computer is used to coordinate and direct the actions of the parts of the MR system. It is also used in processing the data. The computer tells the gradient amplifiers and rf transmitter when to start and turn off in order to run the correct sequence. The rf receiver amplifier relays the signal received by the rf coil from the subject (or sample) to a converter that digitizes the signal. From there it is sent back to the computer for reconstruction.

ACQUISITION OF BRAIN TISSUE DATA IN THIS STUDY

In this study, imaging parameters were based on a robust acquisition sequence that garnered high-resolution 3D data from the human brain. Such sequences are commonly used in neuropsychiatric morphometric studies (Rosenberg et al., 1997; Sassi et al., 2001; MacMaster and Kusumakar, 2004). Thus the data collected in the present study are easily comparable to previously published studies of the pituitary gland.

Data were collected from three MRI scanners, two in Halifax and one in Detroit. Images were collected in a similar manner that allowed for the data to be as comparable as possible from all three scanners. The following were commonalities among all the scans. The patients were provided with earphones and entertainment videos to reduce noise disturbances and alleviate possible boredom during the scan. A sagittal scout series of images was first obtained to verify patient position and image quality, to locate a midline sagittal image and for graphic prescription of the coronal and axial images. Then the coronal sequence was run (detailed below). Additionally, a double echo-spin echo sequence was used to obtain T2 and/or proton density images in the axial plane to screen for neuroradiological abnormalities. MRI scans were conducted in Halifax using a 1.5 Tesla Siemens Vision system (Erlington, Germany) at the Queen Elizabeth II Health Sciences Centre. Anatomical coronal fast low angle shot (FLASH) sequence parameters were as follows: TR = 25 ms, TE = 5.40 ms, flip = 40°, slice thickness = 1.5 mm, 124 slices, matrix = 256 x 256 pixels. In Halifax and Detroit, data were also collected on a 1.5 Tesla General Electric system. The coronal 3-dimensional spoiled gradient echo-pulse sequence

(SPGR) parameters were as follows: TR = 25 ms, TE = 5 ms, flip = 10°, slice thickness = 1.5 mm, 124 slices, matrix = 256 x 256 pixels. Anatomical measurements were conducted on an Apple workstation (Macintosh G4, G4 processor) using the semi-automated software NIH Image 1.62 (NIH, Bethesda, MD).

VOLUMETRIC ANALYSIS

PRINCIPLES OF VOLUMETRIC ANALYSIS

A 'structure' is defined as a “spatially contiguous region with a homogeneous representation in some descriptor which establishes a means of distinction, or contrast between the region and its neighborhood” (Kennedy et al., 1997). The “descriptor” could be cellular type, neurotransmitter receptor distribution or function. For example, amygdala could be a descriptor as it does have specific function and is spatially contiguous. From the functionality approach to the brain, one would want to determine where functionally specific structures are, what function they perform, and how the distributed processing of the function is organized (Zeki et al., 1991). As a measure, the volume of a given brain structure reflects the size, shape and density of its cellular constituents. With changes in functionality, it seems reasonable to assume that these changes would be reflected physiologically with alterations in cellular composition and activity. Hence, by examining the volumetric properties of a structure one can make inferences about its functionality.

In its first application to image the human brain, MRI was used much in the same way as classical radiological technologies, with images being assessed in a qualitative manner. Reports were made on the basis of qualitative observations such as brightness/darkness, size (small or large) and location of anatomical structures. The wealth of digital information collected by the MRI was not utilized to any great degree. The variation in data collection oftentimes prevented proper quantitative studies as the variation in imaging parameters rendered the data unsuitable for comparison. It did not take long for MRI to move forward to quantitative applications. Its potential in that regard is only now being effectively tapped, some twenty years after its initial introduction into the clinic. Gathering and analyzing

quantitative data takes more time than a qualitative analysis. The payoff is a reduction in potential bias, increase in reproducibility and more effective interpretation. Nevertheless the validity of these quantitative analyses must be evaluated by someone skilled in human brain anatomy (Caviness et al., 1999).

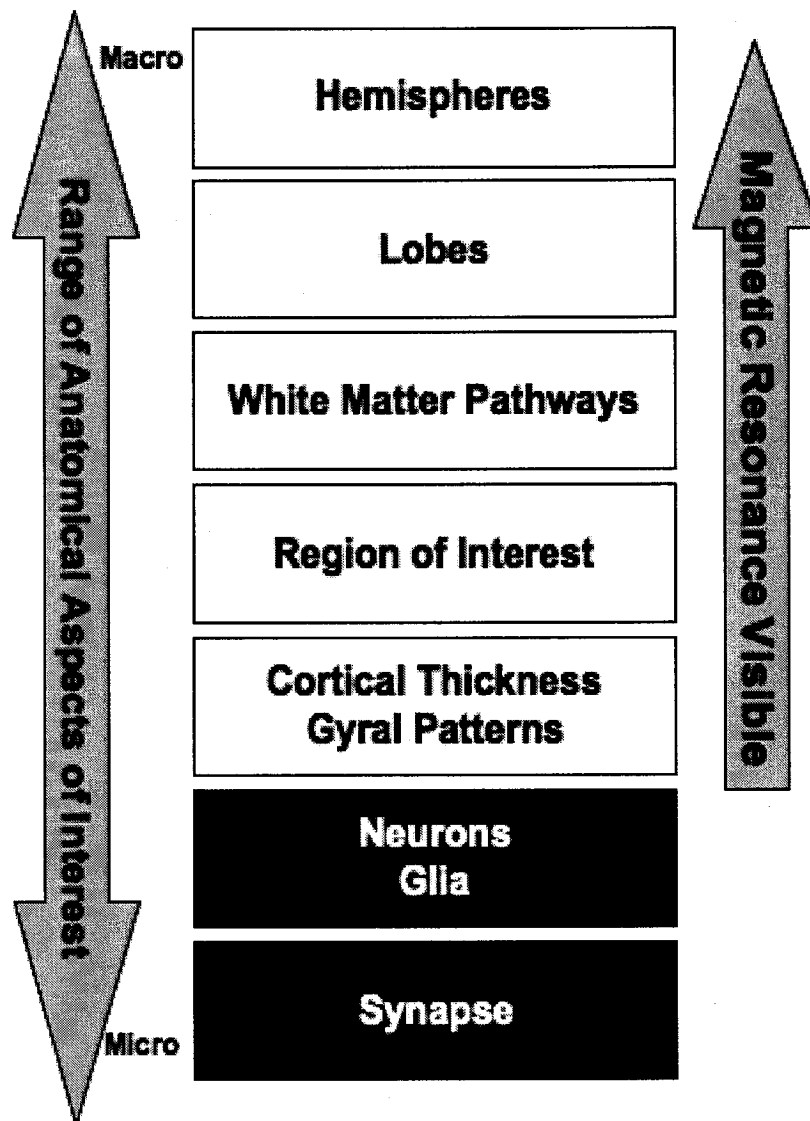
MRI based volumetrics is emerging as a viable tool in basic science studies (Caviness et al., 1999). As a scientific method, it has served many objectives such as mapping out developmental processes (Giedd, 1997), disease processes (e.g., Rosenberg et al., 1997) and treatment effects (Gilbert et al., 2000). MRI allows for *in vivo* examination of brain structures, repeated measures, and has a high degree of safety. It avoids virtually unknowable agonal changes that are part of the death process, and time between death and fixation and processing that are part of post-mortem studies (Caviness et al., 1999). Additionally, psychiatric diseases of childhood, like obsessive-compulsive disorder and attention deficit hyperactivity disorder, have virtually no post-mortem literature, and their study is benefiting from MRI analysis.

When measuring something, the measure should not only be valid and reliable but also reproducible and not subject to operator variations. Measurement of biological systems may be problematic in that many parameters have genuine biological variation over time. Biological systems, however, usually have a well-defined normal range. In the human brain, there is a wide range of “neuroanatomical effects” (Courchesne and Plante, 1997) and only a few are MR-visible currently (see figure 7). This is important, for when designing an experiment using MRI as the method, the effect the researcher is looking for must be of sufficient magnitude to be evaluated. The technology is always improving, though, and it may be possible one day to evaluate single cells *in vivo* using MRI based technologies. The structure must also be clinically of interest. In other words, it should be critically involved in a specific function affected by, or involved in, the disorder in question.

The three key steps in MR studies are 1) subject selection, 2) anatomic visualization and 3) anatomic quantification (Courchesne and Plante, 1997) . At each of these

Figure 7: Neuroanatomical Effects

Magnetic resonance is typically limited to large-scale effects and cannot discern individual synapses, neurons or glia.



points, bias and error can adversely affect the validity of the experiment. Gundersen and Jensen (1987) described bias as “irreversible, undetectable and potentially absolutely catastrophic”. Bias can not only obscure true effects, but also generate spurious findings. Error, on the other hand, consists of random variation in excess. Both can reduce statistical power and result in incorrect findings. There is no single accepted method for reducing bias and error in each of the three steps involved in MRI experiments. Despite brain structures being considered discrete entities, there is little consensus regarding how properly to execute the third step (anatomic quantification). With regard to the pituitary gland, anatomic quantification is described below; it draws upon a number of well-established methodologies. Non-biased stereological methods were not used because the necessary software, compatible with MRI imaging, was not available at the institution at the time of the study. The manual tracing technique (used in this study) is, in any case, much more common, followed by voxel based morphometry techniques. Validation of the manual tracing technique, as used here, has been achieved by a point-counting stereological approach based on Cavalieri’s theorem of systematic sampling, with both methods having documented validity and sensitivity (Keshavan et al., 1995).

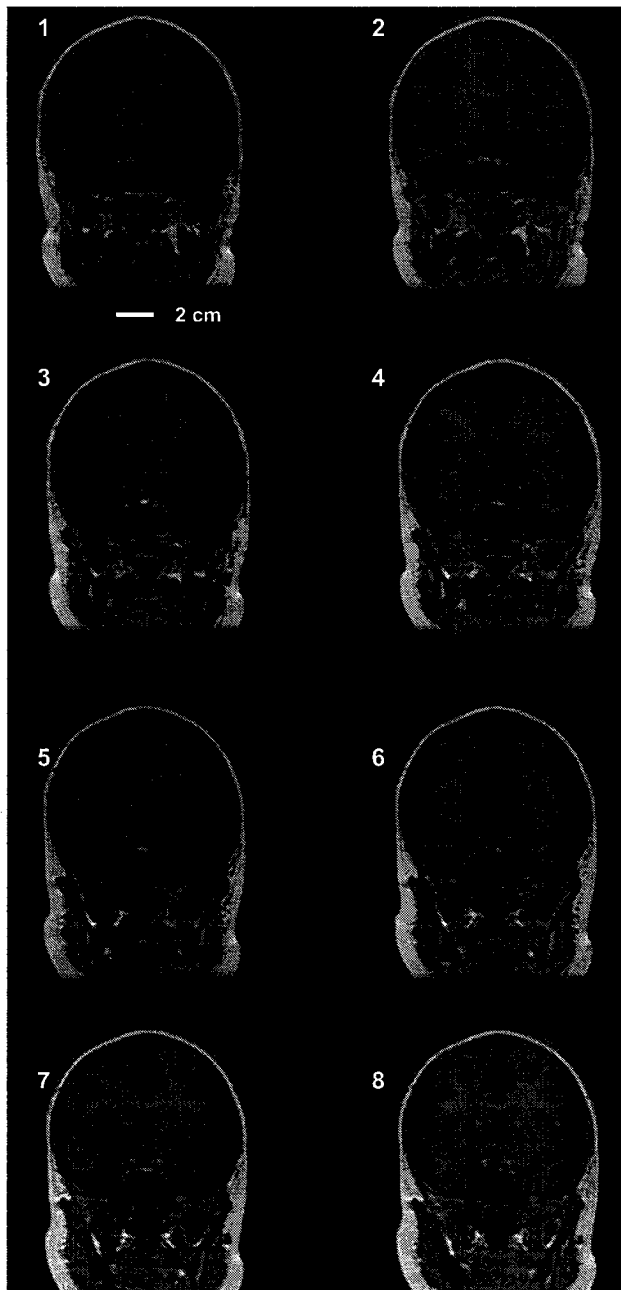
PITUITARY GLAND VOLUME METHOD USED IN THIS THESIS

Boundaries of the pituitary gland were determined by reference to standard neuroanatomical atlases (Daniels et al., 1987; Talairach & Tournoux, 1988), and measurement methods were adapted from previously published neuroimaging studies of the pituitary (Doraiswamy et al., 1990; 1991a; 1991b; Krishnan et al., 1991b; Tien et al., 1992; MacMaster et al., 1999; Sassi et al., 2001). The entire pituitary was measured using the manual tracing technique, and no attempt was made to separate it into the anterior and posterior lobes (see figure 8). Landmarks aiding the identification of the superior border of the pituitary were defined as the optic chiasm and infundibular recess of the third ventricle. The sella turcica and sphenoid sinus were used as a guide for the inferior border of the pituitary. The cavernous sinus and internal carotid artery provided guidance for determining the lateral boundary. It

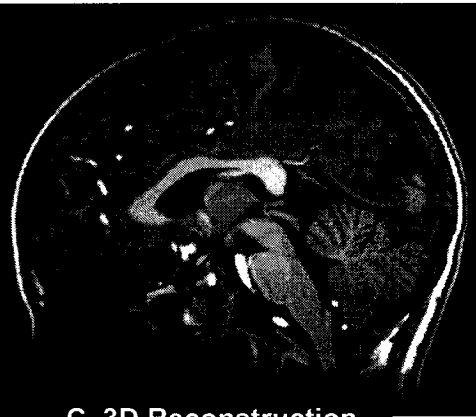
Figure 8: Manual Tracing Technique

(A) A sample tracing of the pituitary gland. Slices are numbers 1 through 8 starting from the posterior aspects and moving in the anterior direction. (B) A mid- sagittal slice is provided for reference. (C) A 3D reconstruction of the gland and brain along with a close-up (D) of the 3D reconstructed pituitary is also provided for reference.

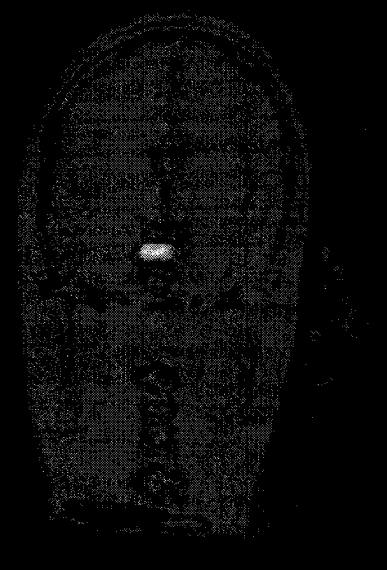
A. Serial Sections



B. Sagittal Slice



C. 3D Reconstruction



D. 3D Reconstruction Close-up



should be noted that boundary demarcation is a potential source of error in volumetric MRI studies. It is also critical to note that the pituitary is a small structure with regard to the resolution of the MR scan. There are two considerations to note with regard to the method used. First, the in plane resolution was 0.9 x 0.9 mm. As the volume is derived from the summed area measures for each slice, the technique employed here is fairly sensitive in-plane. Second, one cannot assume that differences noted between groups or conditions are uniform across the volume (e.g., a 3 mm² difference each slice). The pituitary is composed of multiple cell types and sub-structures and you cannot assume that all will be different between groups or change with intervention. Serial coronal slices (each slice 1.45 mm thick) were used for measuring the pituitary. The serial measures of pituitary area were summed and multiplied by the slice thickness. As the volume was acquired in a 3D mode, sagittal views were also used to guide measurements. A single trained rater made all measurements, and was blind to any identifying subject or clinical information. Data was provided to the rater on compact discs with all identifying information stripped. Linkage with clinical data occurred only after data was analyzed. The boundaries of the pituitary are very well defined using this method, and previously reported inter-rater and intra-rater reliabilities were very high for pituitary measurements (inter (and intra-) -rater reliability was $r = 0.92$ and 0.98 respectively)(MacMaster and Kusumakar, 2004).

INTRACRANIAL VOLUME METHOD USED IN THIS THESIS

Intracranial volume (ICV) was calculated by summing the areas of successive coronal slices and included both gray and white matter (Rosenberg et al., 1997). This was multiplied by the slice thickness to calculate the total volume. It included frontal, parietal, occipital and temporal lobes. Cerebellum and caudal brainstem were not included. ICV is used as a covariate in situations where ICV correlates with pituitary gland volume in either group, or in situations where ICV differs between the groups or conditions. As data could not be directly compared across experiments due to age and sex composition differences, we felt it was more robust to use ICV as a covariate (or not) as dictated by each experiment rather than standardized across all studies.

STATISTICAL METHODS

GROUP COMPARISONS

The core of this thesis involves group comparisons, i.e., clinical versus control or baseline versus post-treatment. There are many possible statistical methods for examining hypothesized differences between groups, with each being based on certain assumptions and each being appropriate for specific situations. The null hypothesis for group comparison is expressed as $H_0: u_1 - u_2 = 0$ and the experimental hypothesis as $H_1: u_1 - u_2 \neq 0$ (where u is the mean for each group or condition). Normand and Streiner (2000) describe statistical tests of groups/conditions as a means for examining signal (the true difference between groups or conditions) against noise (the variability in scores among individuals within each group or condition). In other words, one wants to maximize the ability to determine what $u_1 - u_2$ equals against the background of the variability in scores inherent in each group.

Analysis of Variance (ANOVA)

Analysis of variance (or ANOVA) is one of the most commonly used statistical techniques in biomedical research (Howell, 1992). ANOVA is a hypothesis-testing procedure used to determine if mean differences exist for two or more groups (or conditions). It uses sample data in order to make inferences about populations. In this study, ANOVA was used in comparisons such as clinical vs. control groups or males vs. females. It is also useful for evaluating more numerous comparisons such as male control versus female control versus male clinical versus female clinical subjects.

If, as in some experiments contained in this study, only two groups were being used for comparison rather than three or more, why would the ANOVA be used rather than a simple independent t-test? The reason is the control of the variation induced by certain variables. When dealing with populations that are influenced by a given variable, one may want to control for the effect or influence of that variable. The analysis of covariance (ANCOVA) allows a researcher to control for the influence of a particular (independent) variable on the (dependent) variable of interest. For

example, in the case of morphometric studies, this can be ICV (as mentioned earlier) or age.

Planned Comparisons

In cases where the hypothesis states an explicit direction of change (increase or decrease, greater or lesser, or equal) one can specify the nature of the comparisons. If a smaller volume is expected in the clinical population (for example) the planned comparison would be expressed as clinical = -1 and control = 1. If the clinical sample was expected to be larger for the given measure, then it would be expressed as clinical = 1 and control = -1. Statistically, this has the effect of increasing the statistical power of the test by specifying the directionality of the test (such as a one-tailed t-test).

Post-hoc Test

In situations where more than two groups or conditions are compared, there is a need for specific tests between each combination of groups (or conditions) following an ANOVA. These are called post-hoc tests. Post-hoc tests come in a variety of types, each with their own assumptions and utility. As this was a hypothesis-driven thesis with expressly stated planned comparisons (see above), the LSD post-hoc test for planned comparisons was chosen. This test allows the researcher to take advantage of, statistically, the planned comparisons in the post-hoc analysis.

Paired T-Test

In the experiments involving treatment, data are collected pre- and post-medication intervention. For these comparisons, paired t-tests were used. This is one of the simpler experiments in biomedical research: first you measure something, do something to make it “better”, and then measure again. The paired t-test is optimal in situations like this where small differences resulting from intervention are superimposed on large (but stable) differences between individuals. The stable nature of the measure of the pituitary gland is discussed in this chapter (see experiment 1). In

this case each individual subject's pituitary gland was measured at baseline and then compared to the measure taken after the medication trial. In the treatment experiments presented in this thesis, medications used included paroxetine for obsessive-compulsive disorder, venlafaxine for major depression and methylphenidate for attention deficit hyperactivity disorder. Paired t-tests are used rather than comparing treated and untreated samples for a number of reasons, most importantly a gain in precision and statistical power. If multiple data points are collected, a repeated measure ANOVA would be more appropriate, but as only two data points were collected in the treatment experiments in these studies, the paired t-test seemed a more logical choice. The typical null hypothesis would be that treatment had no effect (or true difference of zero). The H_1 would be that the treatment did affect pituitary gland volume. The direction would be toward normalcy and specific to each clinical condition. I felt that the effect of age and ICV on these samples (as they were the same people in each condition) was minimal and I did not need to co-vary for their possible effects, as the effect would be constant on both conditions.

Correlations

The correlation has become ubiquitous in biomedical research. The square of the correlation coefficient (r) gives the proportion of the variance in Y that is explained by X . This provides important context when interpreting the results of correlations. A strong correlation of $r = 0.6$ only explains 36% of the variance. A weaker correlation, which could still be statistically significant given a large sample size, such as $r = 0.3$ would explain less than 10% of the variance. In some disciplines such as epidemiology, correlations below $r = 0.7$ are considered somewhat suspect. In that field, they are looking for large effects in very large samples. In many aspects of brain research, acceptable correlations may be of a wider range given the degree of variation and the multitude of factors that can affect a given structure of chemical. For example, given the number of brain regions involved in emotions, expecting a clinical scale of depression to correlate strongly ($r \sim 0.8$) with a single brain region or

chemical may not be realistic. Each part of the circuit involved contributes to the variance of Y (the clinical scale). So each X explains an aspect of the variance.

Another way to think of it is in terms of scatter about Y (or standard deviation) (Norman and Streiner, 2000). Standard deviation represents the unexplained scatter. A correlation of $r = 1$ reduces the scatter about the line to zero. The standard deviation of Y at $r = 0.9$ is 43%. A correlation of $r = 0.5$ reduces the scatter in the Y's by only 13%. Of course, it is also critical to remember that correlation does not equal causation. You cannot predict Y from X, nor does X necessarily cause Y. It could be that an unknown factor causes both or Y causes X.

In the studies presented in this thesis, the Pearson correlation coefficient used was implemented in the SPSS software package. In the following chapter, the relationship between pituitary gland volume and age (in years) is examined. In subsequent chapters, Pearson correlations are used to examine the relation between pituitary gland volumes and clinical scale scores in the clinical populations. The relationship between pituitary gland volumes and age in the clinical populations are also investigated.

CHAPTER THREE: NORMAL DEVELOPMENT, SEXUAL DIMORPHISM AND TEMPORAL STABILITY OF THE PITUITARY¹

¹ A portion of this chapter has been accepted for publication in *Life Sciences* (2007)
doi:10.1016/j.lfs.2006.11.040

INTRODUCTION

Normal maturation of the brain is characterized by complex anatomic, molecular and organizational changes. This is to prepare the individual for optimal adaptive behavior. The developmental processes may also cause transient changes in brain structures that are purely developmentally related. Sexual dimorphism is also a critical consideration as there are critical differences between the sexes with regard to many aspects of psychiatric disorders (such as age of onset, prevalence, and symptomatology; APA, 1994).

Initial early *in vivo* studies of the pituitary gland were conducted using computed tomography (CT) imaging. Although typically of a lower resolution than MRI, CT evaluations of the pituitary gland have been shown to be roughly equivalent to MRI for gross measures of the gland (Wiener et al., 1985). Using coronal CT scans, Peyster et al (1983) examined the volume of the pituitary gland in 27 youths (8 – 21 years of age; 11 males and 16 females) and 27 adults (24 – 91 years of age; 11 males and 16 females). They found that the pituitary gland was larger in the adolescent subjects when compared to both the younger and older subjects. Peyster et al (1983) also noted that females had larger average pituitary gland volumes than males. In axial CT scans from 184 subjects (9 – 84 years of age; 98 males, 86 females), Peyster et al (1984) did not note any age or sex differences with regard to pituitary length. In a coronal CT study of pituitary gland height, Peyster et al (1986) examined 251 subjects (7 – 91 years of age; 92 males and 124 females). Females demonstrated a larger pituitary gland height than males. Pituitary gland height also demonstrated a decline with age.

Using sagittal MRI scans; Hayakawa et al (1989) examined the pituitary gland in 150 subjects (newborn to 60 years of age; 87 males and 63 females). A growth spurt was noted in the 10 – 15 year olds and a decline in volume in the 51 – 60 year olds. Otherwise, the relationship with age was linear. Elster et al (1990) studied 169 subjects (1 – 30 years of age; 84 males and 85 females) and noted that adolescents and adults had larger pituitary gland volumes than children. Lurie et al (1990) used

sagittal and coronal MRI scans to examine 35 subjects (26 – 79 years of age; 16 males and 19 females). Subjects over 59 years of age demonstrated smaller pituitary height, volume and cross sectional area. A reduction in pituitary gland volume with age was noted. No sex differences were seen in that study. Doraiswamy et al (1992) studied pituitary gland height and cross sectional area in 71 adults (21 – 82 years of age; 31 males and 40 females). Height and area reduced with age. Maximum height was seen in female subjects in the 20 – 40 year old age range.

In very young subjects (3 days to 4 years of age), Tien et al (1992) found no sex differences with regard to pituitary gland volume. Cox and Elster (1991) studied 48 neonates and infants and found an increase in length and a decrease in area over the first year of life. As these four studies did not include adolescent subjects, this may be why no sex differences were noted. Similar to Peyster et al (1986), Suzuki et al (1990) found greater pituitary gland heights in females as compared to males (213 subjects, 117 males, 96 females; newborn to 70 years). Maximum height was shown in both males and females in the 10 – 19 year olds. Argyropoulou et al (1991) studied 60 youths (8 days to 21 years of age; 30 males and 30 females) using sagittal MRI scans to examine pituitary gland height. A linear growth pattern was noted from 1 year of age to puberty followed by a plateau. In the largest study to date, using sagittal MRI scans; Tsunoda et al (1997) examined pituitary height in 1020 subjects (10 – 78 years of age; 533 males, 487 females). Pituitary height was greater in females than males. Pituitary height increased until age 29 years then declined. In females aged 50 to 59 years, an increase in pituitary height was noted. It was thought that this reflected changes in gonadotropin releasing hormone that occurs in women during this period. Finally, Takano et al (1999) studied pituitary gland volume in 199 subjects (newborn to 19 years of age; 90 males and 109 females). A strong growth spurt was noted during puberty, and was especially prominent in females. Posterior pituitary gland volume did not change with age. In the 5 – 9 year old age range, the posterior pituitary gland was larger in males than females. It should be noted that the resolution and contrast do not allow an investigator to robustly delineate the pituitary lobes and such distinctions are not based directly on the pituitary anatomy.

One of the core problems with many of these studies is that they did not use purely healthy controls, but patients referred for scanning for medical reasons (for example Peyster et al., 1983, 1984, 1986; Argyropoulou et al 1991; Tsunoda et al., 1997). Although the medical conditions did not have pituitary pathology as a primary concern, it is difficult to envision that, given the central role of the pituitary gland in body function, the various disease and injury states would not affect pituitary gland function in even a small way. Given that concern, data are presented here that utilize medically and psychiatrically healthy controls from a wide age range. Based on the previously presented studies, I hypothesized that a significant increase in pituitary gland volume during puberty would occur. I also hypothesized that healthy female subjects would have larger pituitary gland volumes than healthy male subjects.

EXPERIMENT 1 – DEVELOPMENT

SUBJECTS

In order to examine possible age related effects on the pituitary gland, data were used from 65 healthy males and 65 healthy females as control subjects. Overall mean age was 13.88 ± 5.32 years (males 14.35 ± 6.79 , females 13.41 ± 3.23). The overall age range was 6 to 55 years of age. All subjects were screened for mental illness using DSM-IV diagnostic criteria as determined by the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997), and confirmed in a clinical evaluation conducted by the attending psychiatrist (DDR; NC; VK). Exclusion criteria for participation in this study were a history of neurological illness, serious medical illness, claustrophobia, or the presence of a ferrous implant or pacemaker. Subjects had no personal history of psychiatric illness and no history of DSM-IV axis I disorder in their first-degree relatives. Adult subjects and legal guardians of children provided written, informed consent, and all children gave written assent before initiating all studies in compliance with the regulations of the Wayne State University Human Investigation Committee or the IWK Research Ethics Board.

DATA ANALYSIS

As an increase in pituitary gland volume during puberty was hypothesized based on previous research (Hayakawa et al., 1989; Argyropoula et al., 1991; Takano et al., 1999), for the purposes of significance testing adolescent subjects were considered 1 and other age groups (adults and children) were considered -1. The LSD post hoc test for planned comparisons was used to examine specific group differences. Data are split between two sets of comparisons. The first set includes groups based on the following age breakdown: 1) children 6 to 12 years of age, 2) adolescents 13 – 18 years of age and 3) adults 19 – 55 years of age. The second set is more detailed with the following age groups: 1) 6 – 9 years, 2) 10 – 12 years, 3) 13 – 15 years, 4) 16 – 18 years and 5) 19 – 55 years. Pearson correlation was used to examine the relationship between pituitary gland volume and age (in years).

RESULTS

In all subjects ($n = 130$), age strongly correlated with pituitary gland volume ($r = 0.29$, $p = 0.001$; see figure 9). In the comparison of the three age groups, adolescents had significantly larger pituitary gland volumes than children ($F_{1,127} = 16.23$, $p < 0.001$; post-hoc: children vs. adolescents $p < 0.001$, children vs. adults $p = 0.05$, adolescents vs. adults $p = 0.34$). This indicates a 34% larger mean pituitary gland volume in adolescents than in children. In the more detailed comparison, a significant difference in pituitary gland volume was still noted ($F_{1,125} = 25.94$, $p < 0.001$; see figure 10). This difference was limited to 6 – 9 year olds vs. 10 – 12 year olds ($p = 0.01$), 13 – 15 year olds ($p < 0.001$), 16 – 18 year olds ($p < 0.001$), and 19 – 55 years ($p = 0.007$), with the pituitary gland being smaller than all the other groups. The 10 – 12 year old pituitaries were smaller than the 13 – 15 year old ones ($p = 0.008$) and the 16 – 18 year old ones ($p = 0.001$), but as stated earlier, larger than the 6-9 year old ones ($p = 0.01$). No other differences were noted.

Figure 9: Age and Pituitary Gland Volume

In all subjects ($n = 130$), age strongly correlated with pituitary gland volume ($r = 0.29$, $p = 0.001$).

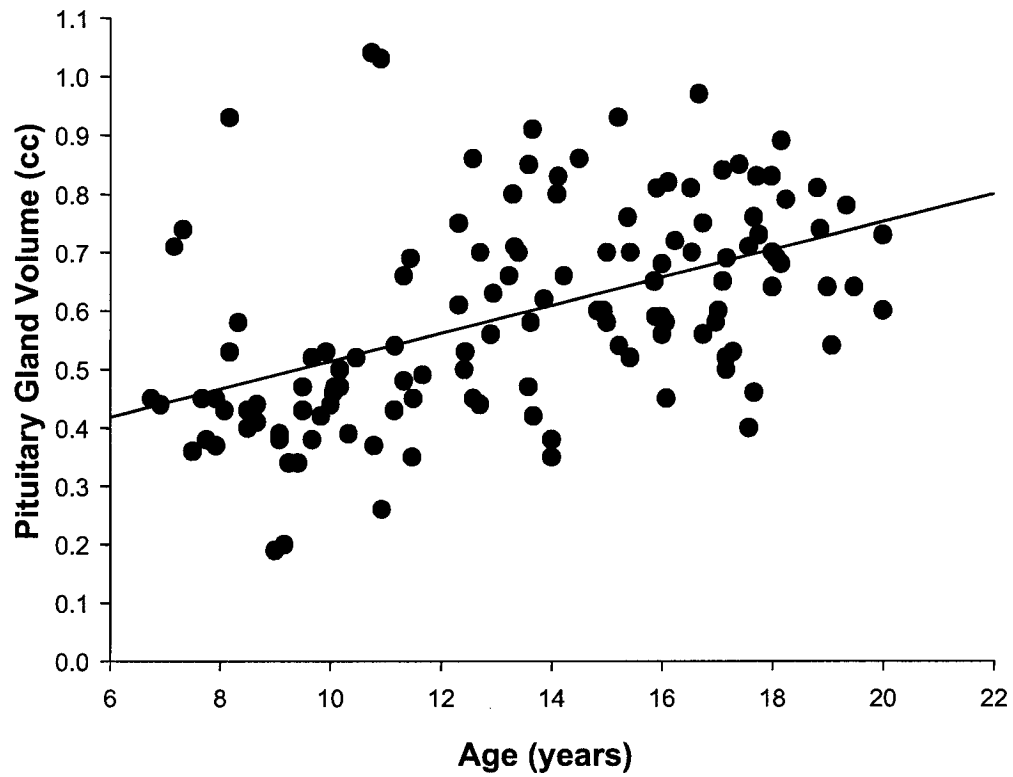
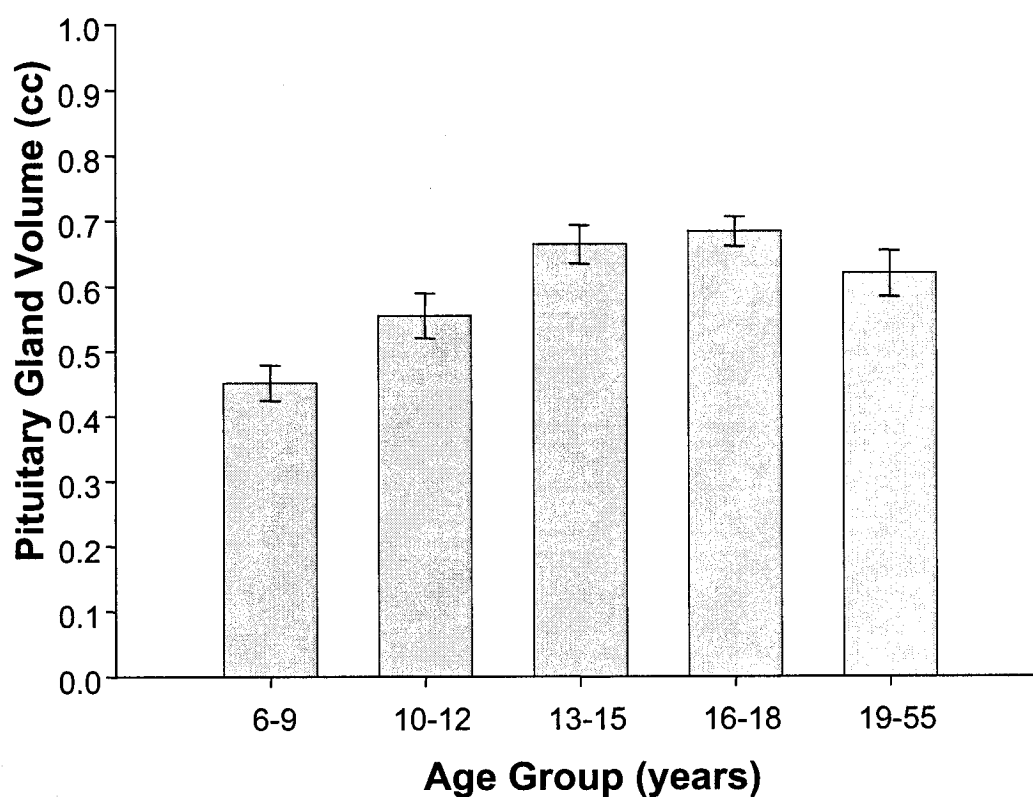


Figure 10: Age and Pituitary Gland Volume by Group

In the more detailed comparison, a significant difference in pituitary gland volume was still noted ($F_{1,125} = 25.94$, $p < 0.001$). This difference was limited to 6 – 9 year olds vs. 10 – 12 year olds ($p = 0.01$), 13 – 15 year olds ($p < 0.001$), 16 – 18 year olds ($p < 0.001$), and 19 – 55 years ($p = 0.007$), with the pituitary gland being smaller than all the other groups. The 10 – 12 year old pituitaries were smaller than the 13 – 15 year old ones ($p = 0.008$) and the 16 – 18 year old ones ($p = 0.001$), but as stated earlier, larger than the 6-9 year old ones ($p = 0.01$). No other differences were noted. Error bars indicate standard error.



EXPERIMENT 2 – SEX DIFFERENCES

SUBJECTS

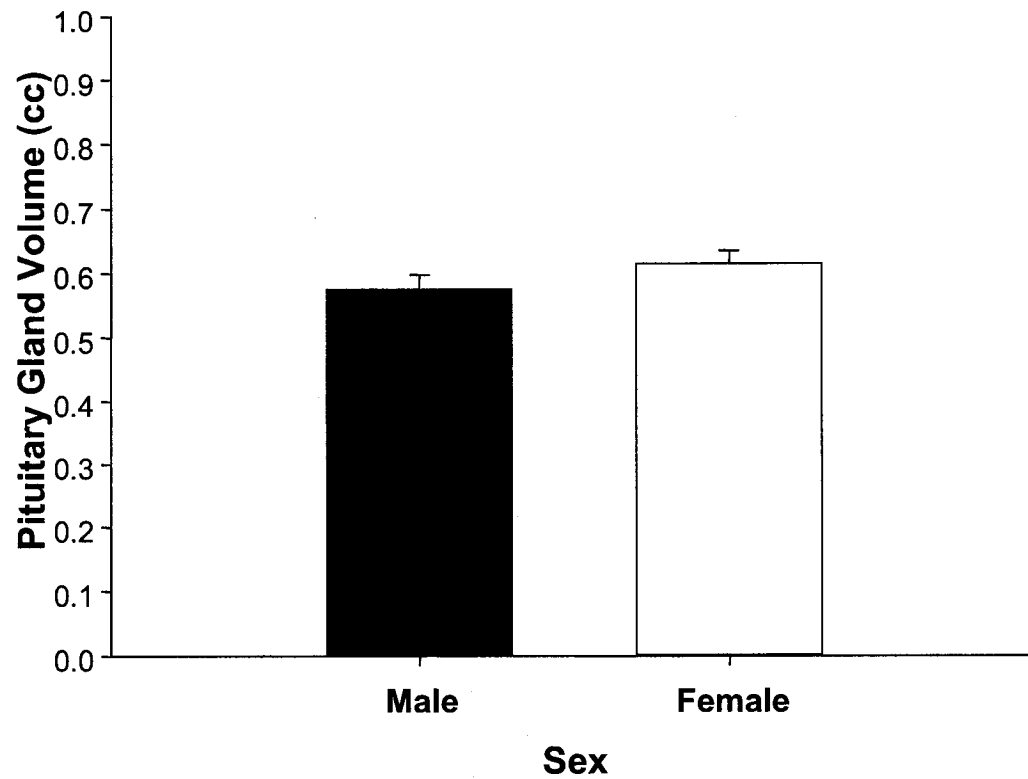
In order to examine possible sex differences in pituitary gland volume, I examined data from 49 pairs (age matched within 1 year) of male and female healthy controls. Mean age in years of the male subjects was 13.63 (\pm 3.57 years, standard deviation) and for the female subjects was 13.61 (\pm 3.41 years). All subjects were screened for mental illness using DSM-IV diagnostic criteria as determined by the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997), and confirmed in a clinical evaluation conducted by the attending psychiatrist (DDR; NC; VK). Exclusion criteria for participation in this study were a history of neurological illness, serious medical illness, claustrophobia, or the presence of a ferrous implant or pacemaker. Subjects had no personal history of psychiatric illness and no history of DSM-IV axis I disorder in their first-degree relatives. Adult subjects and legal guardians of children provided written, informed consent, and all children gave written assent before initiating all studies in compliance with the regulations of the Wayne State University Human Investigation Committee or IWK Research Ethics Board.

DATA ANALYSIS

Data were analyzed as detailed in chapter two. As I hypothesized a difference in pituitary gland volume between the sexes, based on previous literature (Peyster, 1983, 1986; Suzuki et al., 1990; Tsunodo et al., 1997; Takano et al., 1999), planned comparisons for the Analysis of Covariance (ANCOVA) were males = -1 and females = 1 were used. Due to the strength of the age – pituitary gland volume relationship outlined in the previous experiment, males and females were pairwise matched by age, and age was used as a covariate in the ANCOVA comparing pituitary gland volumes.

Figure 11: Sex and Pituitary Gland Volume

ANCOVA (covarying for age) revealed a significant difference in pituitary gland volume between males and females ($F_{1,95} = 9.47$, $p = 0.003$). Error bars indicate standard error.



RESULTS

ANCOVA (covarying for age) revealed a significant difference in pituitary gland volume between males and females ($F_{1,95} = 9.47$, $p = 0.003$; see figure 11). Females had significantly larger pituitary gland volumes than males ($0.63 \pm 0.16\text{cc}$ and $0.55 \pm 0.17\text{ cc}$ respectively, 14% larger in females). The age and pituitary gland volume correlation noted in the earlier experiment was found in both the male ($r = 0.49$, $p < 0.001$) and female ($r = 0.66$, $p < 0.001$) samples (see figure 12).

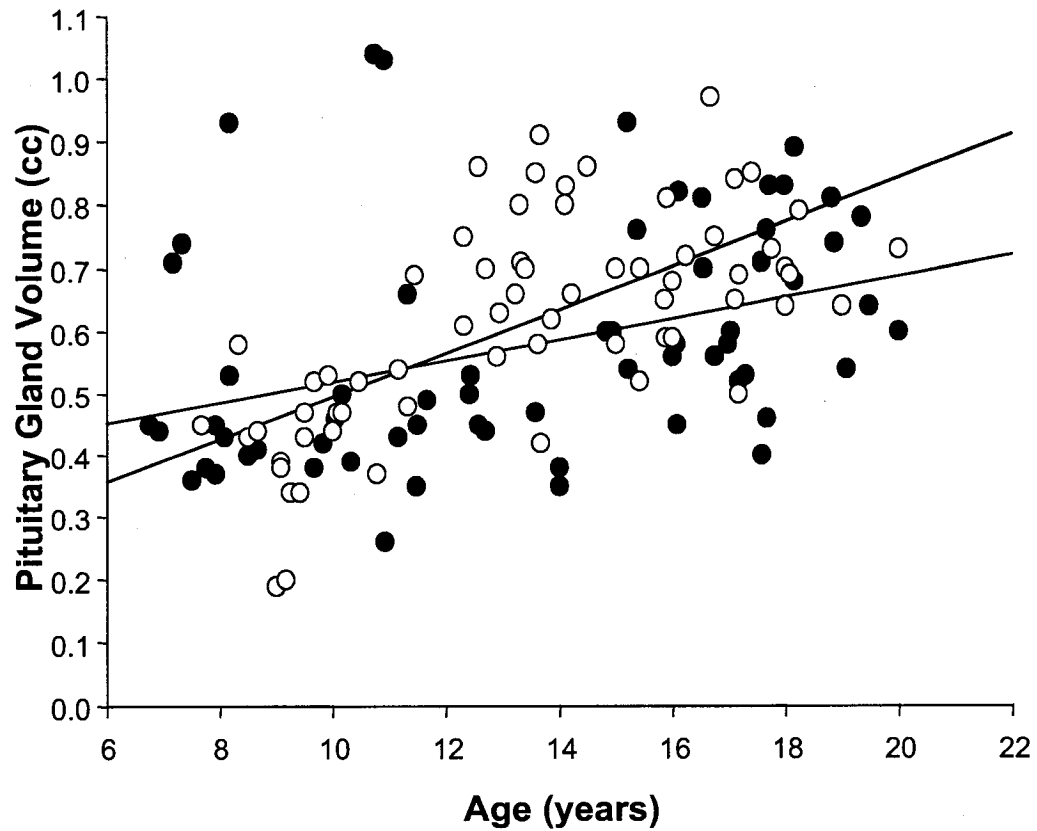
EXPERIMENT 3 – TEMPORAL STABILITY OF THE MEASURE

Volumetric or quantitative magnetic resonance imaging is being increasingly used to examine regional brain structures (Caviness et al., 1999). It is based on the premise that the differences noted are not due to technical factors or natural physiological fluctuations. This assumption is especially critical in longitudinal studies (such as development or illness course) and studies of clinical interventions. Surprisingly, there have been few reports on the temporal stability of MRI volumetric measures of regional brain volumes (Giedd et al., 1995). This is especially true for pediatric samples. One brain region that is known to fluctuate in size with physiological changes is the pituitary gland. The pituitary gland has a central role in endocrine function and can change volume in response to endocrine activity. Changes in neuroendocrine activity are reflected in alterations in pituitary gland morphology.

Differences in pituitary gland size have been noted between males and females and over the course of the lifespan of an individual. In the very young (1 to 4 years of age) no sex differences have been noted with regard to pituitary gland size (Tien et al., 1992) but from 5 to 9 years of age, the posterior pituitary is larger in males than females (Takano et al., 1999). For the remainder of development, females tend to have larger pituitary glands than males (Peyster et al., 1986; Suzuki et al., 1990; Tsunoda et al., 1997; Takano et al., 1999) although not all reports agree with this (see Lurie et al., 1990). A larger pituitary gland has also been noted in adolescence, indicating a specifically puberty-related increase in volume (Peyster et al., 1983; Hayakawa et al., 1989; Elster et al., 1990; Argyropoulou et al., 1991; Takano et al.,

Figure 12: Age and Pituitary Gland Volume by Sex

The age and pituitary gland volume correlation noted in the earlier experiment was found in both the male ($r = 0.49$, $p < 0.001$) and female ($r = 0.66$, $p < 0.001$) samples. Males are black circles and females are white circles.



1999).

As mentioned, the pituitary gland undergoes morphological changes in concert with changes in endocrine status. In pregnancy, for example, the pituitary increases in volume; this was suggested to involve lactotrophic hyperplasia (increase in secretory cell number; Gonzalez et al., 1988; Dinc et al., 1998). In children with shunted hydrocephalus, an enlarged pituitary and a reduction in plasma growth hormone have been reported (Lopponen et al., 1997). Levels of follicle stimulating hormone and luteinizing hormone (FSH and LH) have also been correlated with pituitary size. Furthermore, hypothyroid individuals have enlarged pituitary glands (Shimono et al., 1999). In dwarfs with a growth hormone releasing hormone receptor gene mutation, smaller than normal pituitaries were found (Murray et al., 2000). Smaller gland size was also noted in isolated growth hormone deficiency and in multiple pituitary hormone deficiency compared with healthy controls (Arslanoglu et al., 2001).

To date, few MRI studies have investigated pituitary volumes in psychiatric illness (Doraiswamy et al., 1990; Doraiswamy et al., 1991a; Krishnan et al., 1991b; Axelson et al., 1992; Schwartz et al., 1997; MacMaster et al., 1999; Beresford et al., 1999; Sassi et al., 2001; MacMaster and Kusumakar, 2004; Thomas and DeBellis, 2004; Pariante et al., 2004). Krishnan et al. (1991) found enlarged pituitary volumes in acutely depressed adult patients as compared to healthy controls. Axelson et al., (1992) found a positive correlation between post-dexamethasone plasma cortisol concentration and pituitary volumes in a group of psychiatric inpatients, which consisted mainly of MDD patients. More recently, Schwartz et al., (1997) did not find any differences in pituitary volumes between patients with seasonally affective disorder and healthy controls. Sassi et al. (2001) did find a significant decrease in pituitary volume in bipolar adults compared to controls, but no size effects in their unipolar sample. MacMaster and Kusumakar (2004) found larger pituitary glands in adolescent MDD subjects as compared to healthy controls. Beresford et al. (1999) reported a trend towards enlarged pituitaries in subjects with alcohol dependence. Decreased pituitary volumes have been noted in bulimic patients (Doraiswamy et al.,

1990; Doraiswamy et al., 1991a) and in treatment-naïve pediatric obsessive-compulsive disorder (MacMaster et al., 1999). In post traumatic stress disorder (PTSD), Thomas and DeBellis (2004) found that pituitary volumes were significantly larger in pubertal and postpubertal maltreated subjects with PTSD than in control subjects, but pituitary sizes were similar in prepubertal maltreated subjects with PTSD and control subjects. Pituitary volumes were larger in the PTSD subjects with a history of suicidal ideation. Finally, in schizophrenic patients, Pariante et al. (2004) found that, compared with the control group, the people with first-episode psychosis had pituitary volumes that were 10% larger, whereas those with established schizophrenia had pituitary volumes that were 17% smaller than controls.

Due to its physiological role in the body, the pituitary gland has to respond continuously to transient but often recurrent stimuli. These stimuli include, but are not limited to physical and psychological stress, pregnancy and the like. The pituitary can respond in at least two ways (Nolan and Levy, 2001). First, it can alter the secretory capacity of individual pertinent cells. Second, it can change the trophic activity level (i.e. alter the number of pertinent cells), by increasing mitotic or apoptotic activity. Upon withdrawal of the stimulus, the pituitary can either return to its pre-stimulated state, or change in a manner that would allow for an enhanced response to a recurrent stimuli. The persistence of changes may predispose the pituitary not only to changes in secretory activity, but also to trophic anomalies. The reason for this experiment is to establish the level of stability of the measure of the pituitary gland. Knowledge of the temporal stability of the measure will provide a necessary context for the experiments that study the effects of intervention in clinical studies. Given the hypothesized role of the pituitary gland in psychiatric illness as outlined above, studies of intervention and illness course in clinical populations are indicated. However, the temporal stability of pituitary gland volumetric measures in healthy control populations is not known. I hypothesized that there would not be a significant change over eight weeks in pituitary gland volume in normal healthy young individuals.

SUBJECTS

The first sample was scanned at the IWK Health Centre (Halifax, Nova Scotia, Canada) and consisted of 10 males and 6 females between the ages of 6 to 12 years (mean \pm standard deviation = 9.37 ± 1.60 years). This sample was scanned at baseline and then 8 weeks later (8.67 ± 0.26 weeks). This allowed for the examination of the temporal stability of the pituitary gland measurement over a time frame similar to that of a clinical intervention with psychotropic medications.

The second sample was scanned at Wayne State University (Detroit, Michigan, USA). This sample consisted of 10 males and 8 females between the ages of 9 and 19 years (14.50 ± 2.84 years). This sample was scanned at varying time intervals (8.86 to 79.71 weeks; 29.06 ± 18.64 weeks). This allowed for the investigation of the effect of longer times between scans on pituitary gland volume measurement. Prior to all studies, legal guardians gave written informed consent, and all subjects provided written assent.

DATA ANALYSIS

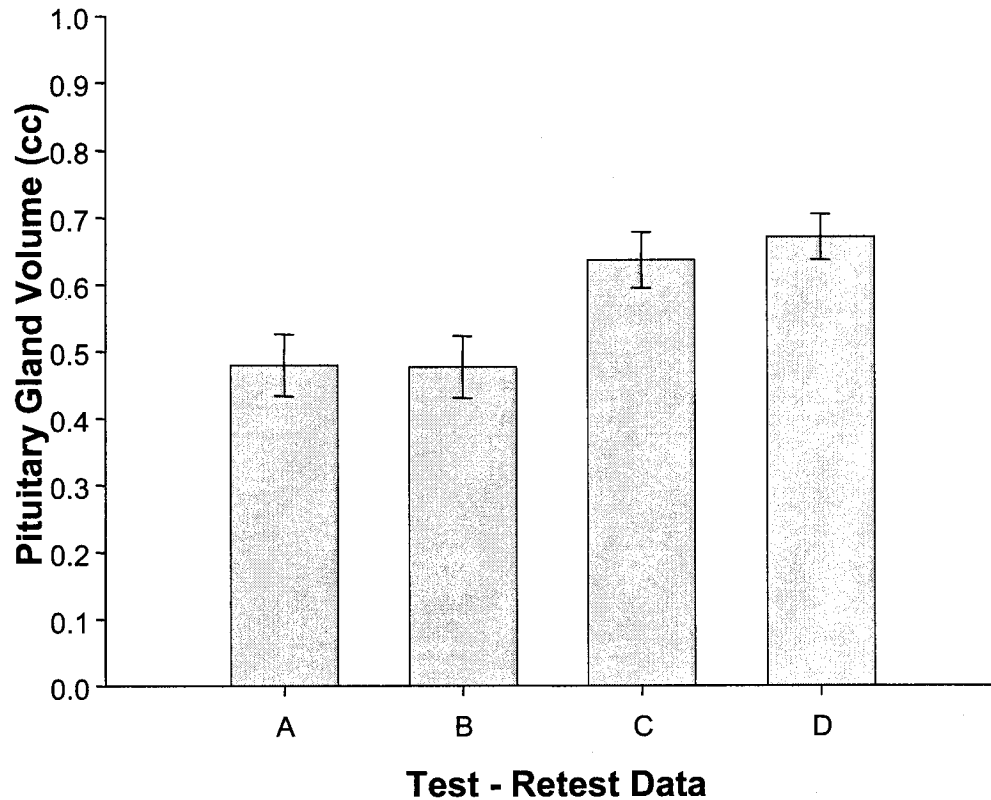
An average of 5.94 ± 1.57 slices was used for the eight week sample and 6.83 ± 0.99 for the second sample. A paired t-test was used to determine the extent of changes in the pituitary gland volume from baseline to the second scan. Intraclass correlations were also used in each sample. An independent t-test was used to compare the percent change in pituitary gland volume between the two samples. In the second sample, a linear and quadratic regression was fitted to relate weeks between scans to percent change in pituitary gland volume.

VALIDATION OF METHOD

In the eight-week sample, no significant change was noted in pituitary gland volume ($t_{15} = 0.30$, $p = 0.77$; mean percent change was $0.17 \pm 8.93\%$; see figure 13). In the second sample, there was also no significant difference found ($t_{17} = 1.19$, $p = 0.25$; mean percent change was $9.54 \pm 23.73\%$; see figure 13). There was no significant

Figure 13: Temporal Stability of the Measure

Graph of pituitary gland volume (cc, y-axis, mean \pm standard error) and the time point (baseline and retest, x-axis) for both groups of healthy volunteers. Time between scans (mean \pm standard deviation) was 8.67 ± 0.26 weeks for groups A and B and 29.06 ± 18.64 weeks for groups C and D. In the eight-week sample, no significant change was noted in pituitary gland volume between A and B ($t_{15} = 0.30$, $p = 0.77$; mean percent change was $0.17 \pm 8.93\%$). Error bars indicate standard error.



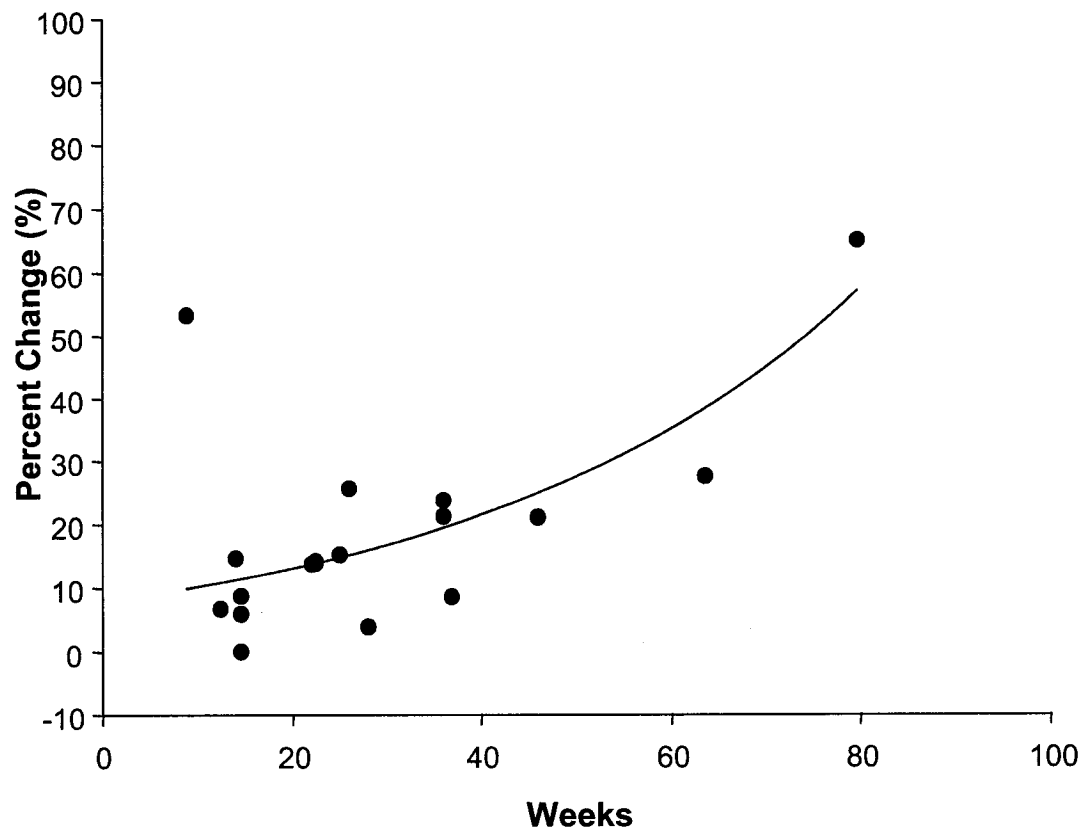
difference in percent change between groups ($t_{32} = 1.54$, $p = 0.13$). In the second sample, the linear regression revealed a significant relationship between the percent change in pituitary gland volume and the number of weeks between scans ($F_{1,16} = 7.18$, $p = 0.02$, $r^2 = 0.31$; see figure 14). The quadratic fit of the data was also significant ($F_{1,15} = 8.54$, $p = 0.003$, $r^2 = 0.53$).

In summary, the results showed no significant differences from time 1 to time 2 with regard to pituitary gland volume in either the short term (eight week) or long-term samples. Given the small percent change noted in the 8-week study, measures of the pituitary gland should be sensitive to fairly subtle changes due to changes in clinical state (e.g., ill versus remission) or due to the intervention itself. The variance inherent in the measure requires a reasonably large (for pediatric neuroimaging studies) sample size ($N \geq 10$). Indeed, power analysis revealed a Cohen's d of 0.02, a very small effect size difference in the healthy controls from time one to time two. A moderate to large effect in the clinical samples would be more than evident given that backdrop.

In conclusion, it appears that short-term studies examining pituitary gland volume with MRI at 1.5T are valid. Given the findings, longer duration of illness, and illness course studies may be more problematic than short-term studies, especially when using developmental samples, and especially during puberty. The marked age vs. pituitary gland volume relationship noted earlier may be at the root of this increase in the degree of change noted with longer times between scans. A longitudinal study of the pituitary gland in a clinical condition would necessitate a longitudinally collected sample of healthy controls. This experiment does establish the validity of using test/retest paradigms of clinical intervention studies involving the pituitary gland. As stated earlier, critical effects such as age and gender require further investigation, and this will be explored in chapter three.

Figure 14: Temporal Stability over Longer Periods

In the second sample, the linear regression revealed a significant relationship between the percent change in pituitary gland volume and the number of weeks between scans ($F_{1,16} = 7.18$, $p = 0.02$, $r^2 = 0.31$). The quadratic fit of the data was also significant ($F_{1,15} = 8.54$, $p = 0.003$, $r^2 = 0.53$).



DISCUSSION

In the first two experiments, I have noted a striking significant increase in pituitary gland volume with age, mainly due to a possible puberty-related growth spurt, and a significant difference in gland volume with regard to sex. Two fundamental questions arise from these findings: 1) why does the gland volume change during puberty and 2) why does the volume differ between the sexes?

The terms puberty and adolescence are sometimes confused (Sisk and Foster, 2004). Puberty is the activation of the hypothalamic–pituitary–gonadal (HPG) axis that results in gonadal maturation. Adolescence, on the other hand, refers more commonly to the maturation of social and cognitive behaviors to the adult levels. The two are inextricably linked to each other, however. Given the hormonal changes that occur as a result of puberty during adolescence (and development in general), changes in both cell volume and number in the pituitary are distinct possibilities. In central precocious puberty, a larger than normal pituitary gland has been noted (Kao et al., 1992; Sharafuddin et al., 1994; Van Beek et al., 2000). In female mice, the number of somatotrophs and lactotrophs increased with age as well (Sasaki, 1988). The pituitary gland is not the only brain region affected by puberty related processes. For example, frontal cognitive efficiency decreases at the onset of puberty (McGivern et al., 2002). Puberty is also a time of increased risk for developing a psychiatric illness (APA, 1994). The number of cases of OCD in females is much higher in puberty than in pre-pubertal individuals. MDD sharply increases from a low prevalence in children to rise, during puberty, quickly to adult levels. The onset of ADHD occurs prior to adolescence (diagnostic criteria specify that symptoms must occur prior to age seven) (APA, 1994).

Other sexually dimorphic brain regions have been noted in developing populations. The largest study was conducted by Giedd et al (1997) examining 121 healthy control subjects between the ages of 4 to 18 years of age. Overall, males had a 9% larger cerebral volume than females. The basal ganglia also demonstrated sex differences in

brain structure volume with the caudate being relatively larger in females while the globus pallidus was relatively larger in males. The lateral ventricles demonstrated a prominent sex difference in brain maturation with markedly large ones in males. A significant change in the linear regression slope of lateral ventricular volume in males after age 11 years was not seen in females. Finally, amygdala and hippocampal volume increased for both sexes over development, but the amygdala increased significantly more in males than females, while hippocampal volume increased more in females. Killgore et al (2001) found that females, in response to ‘affective faces’, showed a progressive increase in prefrontal cortex relative to amygdala activation in the left hemisphere, whereas males failed to show a significant age related difference.

There are a number of limitations to the experiments described in this chapter. One is the lack of direct hormone assays. The rationale for this is described in the final chapter. Hence, direct confirmation of puberty was not obtained. Tanner staging would also offer insight but was not available with this data set. Naturally, a longitudinal study like Giedd’s (1997), where healthy controls were scanned repeatedly over the course of years, would be optimal compared to a cross-sectional design such as that used here. The costs involved in such a study, however, precluded this kind of design for the current study. Second, given the robust nature of the age and sex effects found, it seems unlikely that the findings from a longitudinal study would differ greatly from those presented here.

Based on the data from all the healthy controls, and assuming equal variances in a two-group comparison (not a test/retest paradigm), in order to detect a very small difference (5%) between groups, very large samples are needed ($n = 446$ in each group). For moderate differences (15%) sample sizes of $n = 50$ per groups are needed. For large differences (25 – 30%) less than twenty subjects per group are needed. It is important to remember that these are just estimates of what would be needed in order to detect a significant difference at $p = 0.05$, power = 0.80, in a one tailed hypothesis driven test.

In summary, these findings emphasize that puberty has a very significant effect on pituitary gland morphology and that there is a strong sexual dimorphism with regard to pituitary gland volume. These two findings have implications for the remaining experiments and must be considered during experimental design for future studies. Pair-wise age and sex matching is critical for successful determination of clinically relevant differences in pituitary gland volume between psychiatric populations and controls.

The above findings are consistent with the notion proposed in this thesis that the pituitary gland is a critical nexus point in mood disorders. Development and sex certainly play critical roles in psychiatric disorders (APA, 1994), and the pituitary gland has a strong relationship with age and sex. Pituitary gland volumetric differences described in this chapter are consistent with this. Due to its role in the body, especially in response to stress, the pituitary is well positioned to play a role in the etiology of, or at least be affected by, mental illness.

CHAPTER FOUR: MOOD DISORDERS²

² Portions of this chapter has been accepted for publication in *Biological Psychiatry* (2006) doi:10.1016/j.biopsych.2006.04.013

INTRODUCTION

Major depressive disorder (MDD) is a severe, common and debilitating illness with alarming rates of morbidity and mortality. Evidence suggests that child and adolescent MDD is continuous with adult MDD (Lewinsohn et al., 1999). The lifetime prevalence of MDD in youth is approximately 15-20%, which is consistent with adult rates of MDD (Lewinsohn et al., 1993). Structural neuroimaging studies in adult MDD have explored the role of prefrontal cortex, basal ganglia and temporal limbic circuitry in the pathophysiology of the disorder (Soares and Mann, 1997). To date, there have been few investigations into the morphology of the hypothalamic-pituitary-adrenal (HPA) axis in MDD. The pituitary gland plays a pivotal role in endocrine function. Developmentally, in humans it is bulbous at birth, but shifts to a flatter superior surface after 2 months of age (Tien et al., 1992). MRI studies of pituitary volume have demonstrated a period of growth during puberty (Takano et al., 1999; MacMaster and Kusumakar, 2004), with a gradual decrease with age over adulthood (Lurie et al., 1990; Schwartz et al., 1997). Previous studies have demonstrated that changes in endocrine activity are associated with changes in the morphology of the pituitary (Gonzalez et al., 1988; Chakeres et al., 1989; Dinc et al., 1998). There are sex differences in the size of the pituitary; women tend to have larger pituitaries than men (Takano et al., 1999), even during adolescence (MacMaster and Kusumakar, 2004).

Disruption of the normal functioning of the HPA axis has been noted in depression. Hypercortisolemia and a failure to suppress serum cortisol after the administration of dexamethasone has been reported in MDD patients (Carroll et al., 1981; Krishnan et al., 1985). In adolescents with MDD, the evidence of cortisol abnormalities has been less compelling, with one study failing to note any differences in cortisol levels (Dahl et al., 1989), while two studies did report an increase in cortisol after sleep onset in MDD youths (Dahl et al., 1991; Kutcher et al., 1991). Typically, after sleep onset plasma cortisol levels are usually low. It was hypothesized that this change (the increase after sleep onset) reflects more of a change in the diurnal variation of cortisol than an increase in overall cortisol activity (Dahl et al., 1991; Kutcher et al., 1991).

The classic cortisol hypercortisolemia is thought to be rare in MDD children and adolescents and the changes in cortisol at sleep onset appear to be more of a dysregulation of cortisol and not hyperactivity of cortisol production.

To date only five studies have assessed pituitary sizes *in vivo* with neuroimaging techniques in mood disorders (Krishnan et al., 1991; Axelson et al., 1992; Schwartz et al., 1997; Sassi et al., 2001; MacMaster and Kusumakar, 2004). We have noted a larger pituitary gland volume in adolescent MDD patients as compared to age- and sex-matched controls (MacMaster and Kusumakar, 2004). Larger pituitary glands have also been noted in elderly MDD subjects (Krishnan et al., 1991). Considering the evidence of HPA dysfunction in adult MDD, HPA dysregulation in adolescent MDD, and our previous finding of a larger pituitary gland in adolescent depression (MacMaster and Kusumakar, 2004), I hypothesized that MDD is associated with an larger pituitary volume that possibly reflects these changes in HPA activity. Second, I expected to find a larger pituitary gland volume in the healthy females as compared to the healthy males (Takano et al., 1999). This hypothesized sex difference has not been found in MDD patients (MacMaster and Kusumakar, 2004). Thus, we performed an MRI study in treatment-naïve pediatric MDD patients focusing on the size of the pituitary.

EXPERIMENT 4 – MAJOR DEPRESSIVE DISORDER

SUBJECTS

Twenty-five DSM-IV MDD patients (mean age \pm S.D. = 14.06 ± 2.32 years; 13 male {M}, 12 female {F}) and 25 age- and sex-matched healthy controls (mean age \pm S.D. = 14.10 ± 2.39 years; 13M, 12F) were studied. Mean (\pm SD) age of onset of the first clinical presentation in the patients with MDD was 12.52 ± 2.91 years. Duration of illness in the MDD subjects was 14.43 ± 12.49 months. Depression symptom severity was assessed using the Childhood Depression Rating Scale (CDRS; mean \pm SD score, 57.60 ± 8.03 ; Poznanski et al., 1985). All depressed subjects had a CDRS score above 44, indicative of significant dysfunction. Thirteen of the 25 MDD subjects had a family history of a mood disorder. All case-control pairs were matched for age (± 12

months) and sex. The inclusion criteria were a diagnosis of MDD, with age between 7-18 years. All patients met the DSM-IV diagnostic criteria for MDD as determined by the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997), and confirmed in a clinical evaluation conducted by the attending psychiatrist (DDR). Exclusion criteria for participation in this study were a history of neurological illness, serious medical illness, claustrophobia, age greater than 18 years, or the presence of a ferrous implant or pacemaker. Healthy control subjects had no personal history of psychiatric illness and no history of DSM-IV axis I disorder in their first-degree relatives. Psychiatric illness in first-degree relatives was assessed by Family History-Research Diagnostic Criteria (Andreasen et al 1977). Legal guardians provided written, informed consent, and all children gave written assent before initiating all studies in compliance with the regulations of the Wayne State University Human Investigation Committee.

DATA ANALYSES

An analysis of covariance (ANCOVA) was performed to compare the values of the pituitary volumes among the age- and sex-matched groups, with age and ICV used as covariates. Given the previous finding of a sex difference in pituitary gland volume (Takano et al., 1999), and our finding of a similar sex difference in our adolescent control but not our MDD sample (MacMaster and Kusumakar, 2004), I decided to use four groups (Healthy Males, Healthy Females, MDD Males and MDD Females) for the core comparison. ICV's were used as a covariate in the ANCOVA as they demonstrated a significant difference between MDD patients and controls ($F_{1,46} = 9.78, p = 0.003$; MDD patients: $1240.07 \pm 155.46\text{cc}$, Controls: $1150.09 \pm 114.94\text{cc}$). Age was used as a covariate since a strong correlation between pituitary volume and age was noted in the control males ($r = 0.52, p = 0.03$) and females ($r = 0.54, p = 0.04$). The planned comparisons for pituitary volume were as follows: Control Males (-1) vs. MDD Males (1); Control Females (-1) vs. MDD Females (1); Control Males (-1) vs. Control Females (1); MDD Males (1) vs. MDD Females (1). A LSD post-hoc test for planned comparisons was used. Pearson's correlations were used to examine pituitary gland volume and age (one-tailed, MacMaster and Kusumakar, 2004) and

pituitary gland volume and clinical variables in the MDD patients (two-tailed). Results are stated in terms of mean \pm standard deviation unless otherwise indicated.

RESULTS

The analysis of covariance (ANCOVA; age/ICV) revealed an overall difference in pituitary volumes when comparing the four groups ($F_{3,44} = 4.82$, $p = 0.005$). Control males had smaller pituitary gland volumes than MDD males ($p = 0.03$; controls: $0.51 \pm 0.13\text{cc}$, MDD patients: $0.63 \pm 0.23\text{cc}$). No significant differences were noted between control females and MDD females. Control females had larger pituitary gland volumes than control males ($p = 0.01$; Males: $0.51 \pm 0.13\text{cc}$, Females: $0.65 \pm 0.08\text{cc}$). No significant differences were noted between MDD females and MDD males (figure 15). No significant effects of age on pituitary volumes were found in MDD males and females. However, a strong correlation between pituitary volume and age was noted in the healthy controls (males: $r = 0.52$, $p = 0.03$; females: $r = 0.54$, $p = 0.04$). In the MDD sample, pituitary volumes did not correlate with CDRS or HAM-D scores. As stated earlier, ICV was significantly larger in the MDD patients as compared to the healthy controls ($F_{1,46} = 9.78$, $p = 0.003$; MDD patients: $1240.07 \pm 155.46\text{cc}$, Controls: $1150.09 \pm 114.94\text{cc}$).

EXPERIMENT 5 – THE EFFECT OF TREATMENT

SUBJECTS

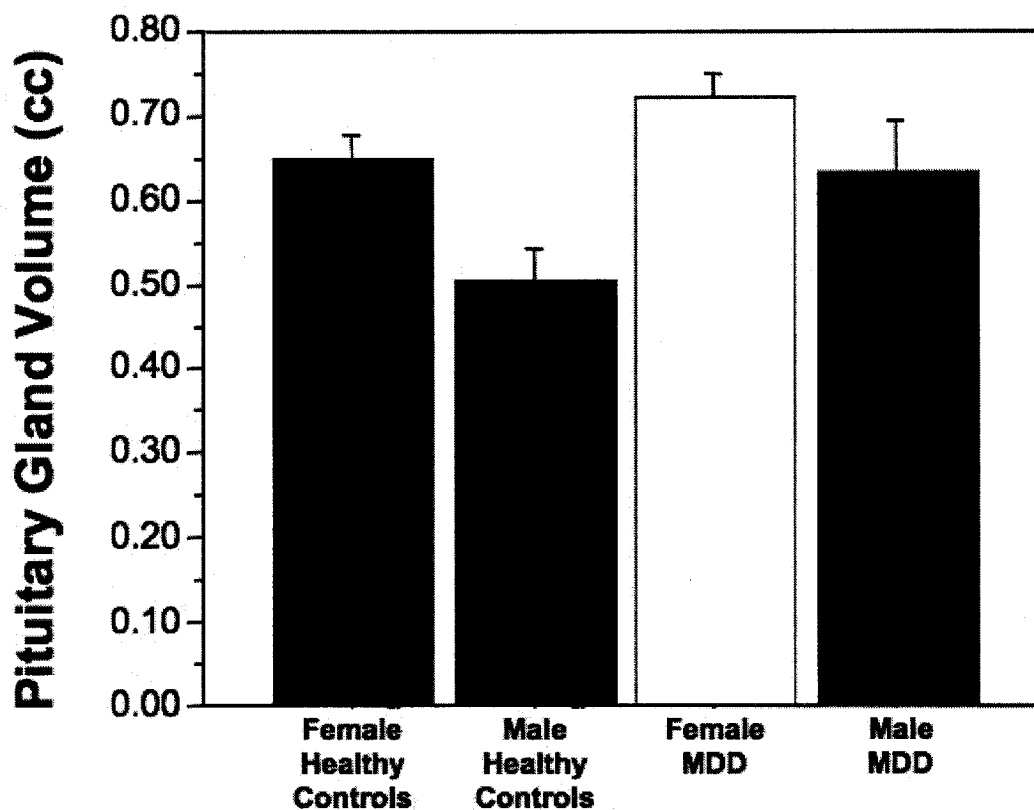
Thirteen MDD subjects were scanned at baseline and after 12 weeks of venlafaxine therapy for MDD (4 males and 9 females; 10 to 19 years of age, 14.16 ± 2.43 years).

DATA ANALYSIS

As the data represented a comparison between treatment-naïve and treated conditions in individual subjects, paired t-tests were used to compare data regarding the intervention (venlafaxine). There was very little change over 8 weeks in healthy control children (see chapter 2), therefore I expect that this comparison should be

Figure 15: Pituitary Gland Volume in Major Depression (MDD)

The analysis of covariance (ANCOVA; age/ICV) revealed an overall difference in pituitary volumes when comparing the four groups ($F_{3,44} = 4.82$, $p = 0.005$). Control males had smaller pituitary gland volumes than MDD males ($p = 0.03$; controls: $0.51 \pm 0.13\text{cc}$, MDD patients: $0.63 \pm 0.23\text{cc}$). No significant differences were noted between control females and MDD females. Control females had larger pituitary gland volumes than control males ($p = 0.01$; Males: $0.51 \pm 0.13\text{cc}$, Females: $0.65 \pm 0.08\text{cc}$). No significant differences were noted between MDD females and MDD males. HC = healthy controls. Error bars indicate standard error.



sensitive enough to detect even relatively subtle changes in gland volume in the clinical sample over the 12 weeks of medication therapy. As a significant difference was hypothesized at baseline between healthy controls and MDD subjects (larger gland volume in MDD), I also hypothesized a reduction in pituitary gland volume after venlafaxine treatment.

RESULTS

Subjects demonstrated symptom improvement over 12 weeks with medication (CDRS: $t_{12} = 8.38$, $p < 0.001$; HAM-D: $t_{12} = 7.19$, $p < 0.001$; HAM-A: $t_{12} = 4.98$, $p < 0.001$). A paired t-test revealed no significant difference in pituitary gland volume after 12 weeks of medication therapy for MDD ($t_{12} = 0.59$, $p = 0.57$, 3% increase) (figure 16).

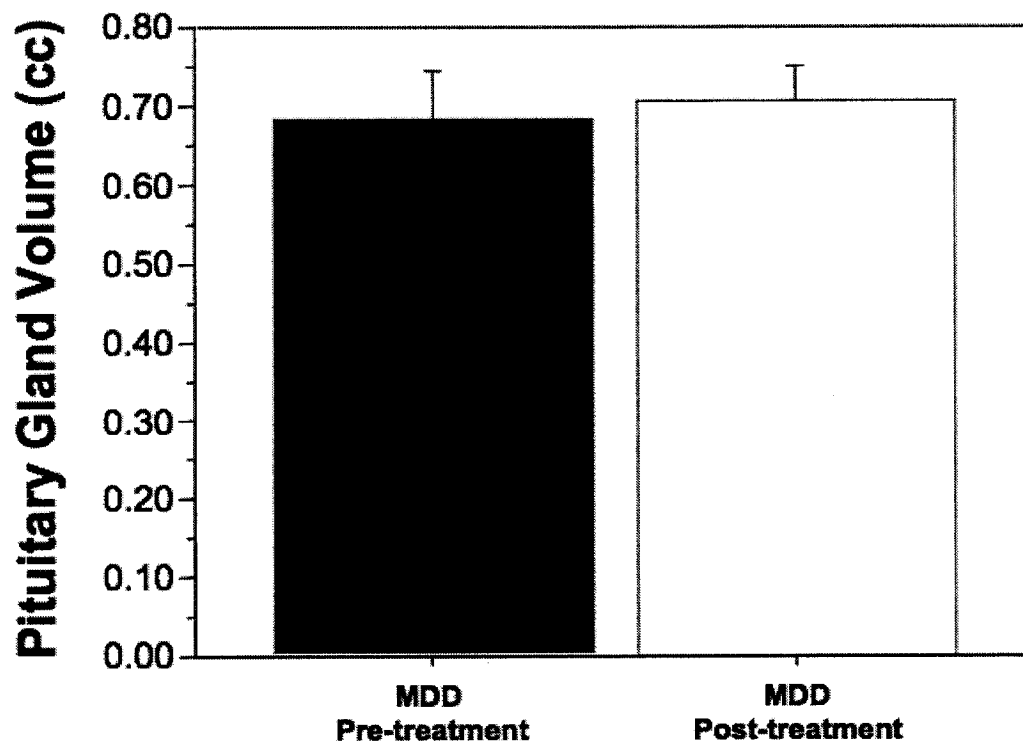
EXPERIMENT 6 – BIPOLAR DISORDER

SUBJECTS

Data for this experiment were derived from 12 age- and sex-matched case-control pairs (5 males, 7 females in each group; 14 to 20 years of age, controls: 16.50 ± 1.93 years, bipolar depression: 16.92 ± 1.62 years). All case-control pairs were matched for age (± 12 months) and sex. The inclusion criteria were a diagnosis of bipolar illness, with age between 7-18 years. All patients met the DSM-IV diagnostic criteria for bipolar disorder (depressed phase) as determined by the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997), and confirmed in a clinical evaluation conducted by the attending psychiatrist (Vivek Kusumakar). Exclusion criteria for participation in this study were a history of neurological illness, serious medical illness, claustrophobia, age greater than 20 years, or the presence of a ferrous implant or pacemaker. Healthy control subjects had no personal history of psychiatric illness and no history of DSM-IV axis I disorder in their first-degree relatives. Legal guardians provided written, informed consent, and all children gave written assent before initiating all studies in compliance with the regulations of the research ethics board of the IWK Grace Health Centre.

Figure 16: Pituitary Gland Volume in Major Depression (MDD) Before and After Treatment

A paired t-test revealed no significant difference in pituitary gland volume after 12 weeks of medication therapy for MDD ($t_{12} = 0.59$, $p = 0.57$, 3% increase). Error bars indicate standard error.



DATA ANALYSIS

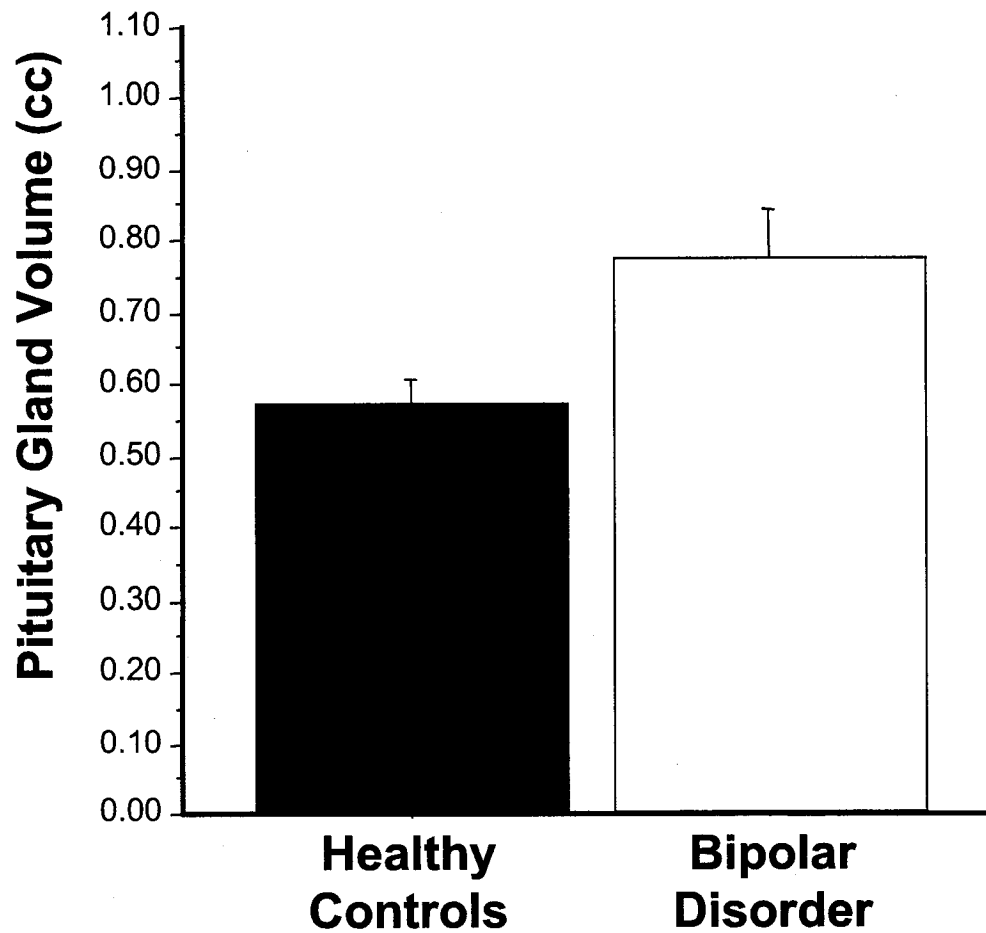
Neither ICV nor age correlated with pituitary gland volume in either group. ICV also did not differ between groups. Hence, an analysis of variance (ANOVA) was used to compare the two groups. Planned comparisons were as follows: bipolar disorder = 1, healthy control = -1. In order to render the results between experiments more comparable, I also ran the data as 4 groups (male control, female control, male bipolar depression, females bipolar depression) using an ANCOVA (age and ICV as covariates). The planned comparisons for pituitary volume were as follows: control males (-1) vs. MDD males (1); controls females (-1) vs. MDD females (1); control males (-1) vs. control females (1); MDD males (1) vs. MDD females (1). A LSD post-hoc test for planned comparisons was used. Pearson's correlations were used to examine pituitary gland volume and age (one-tailed, MacMaster and Kusumakar, 2004) and pituitary gland volume and clinical variables in the MDD patients (two-tailed). Results are stated in terms of mean \pm standard deviation unless otherwise indicated.

RESULTS

ANOVA revealed a significant difference in pituitary gland volume between healthy controls and patients with bipolar disorder ($F_{1,22} = 7.08$, $p = 0.01$; healthy controls: $0.57 \pm 0.12\text{cc}$ and bipolar disorder: $0.77 \pm 0.23\text{cc}$) (figure 17). This larger volume was found mainly in the males (overall: $F_{3,18} = 3.35$, $p = 0.04$; post-hoc test control males versus bipolar disorder males: $p = 0.03$) rather than the females (control females versus bipolar disorder females: $p = 0.13$). Males with bipolar disorder did not differ from females with bipolar disorder ($p = 0.47$). In control subjects, females demonstrated a trend for larger pituitary gland volumes than males ($p = 0.09$). No significant correlation was noted between pituitary gland volume and age (in years) in either the controls ($r = 0.47$, $p = 0.12$) or the bipolar disorder subjects ($r = 0.21$, $p = 0.52$). No significant correlation with clinical variables was noted in the patient group.

Figure 17: Pituitary Gland Volume in Bipolar Disorder

ANOVA revealed a significant difference in pituitary gland volume between healthy controls and patients with bipolar disorder ($F_{1,22} = 7.08$, $p = 0.01$; healthy controls: $0.57 \pm 0.12\text{cc}$ and bipolar disorder: $0.77 \pm 0.23\text{cc}$). Error bars indicate standard error.



DISCUSSION

The main findings of the present study are as follows: (1) a larger pituitary volume in male MDD patients compared to age matched healthy controls was found. No difference between female controls and female MDD patients was found. (2) Control females had larger pituitary gland volumes than control males. This sex difference was not seen in the MDD patients. A robust correlation between pituitary volume and age was noted in the male and female healthy controls, consistent with previous findings (Takano et al., 1999). This was not seen in the MDD male and female patients. Again, this is consistent with previous findings (MacMaster and Kusumakar, 2004). It may be, therefore, that pathological enlargement of the pituitary in such mood disorders may mask or 'overwhelm' normal physiological patterns of enlargement during development. The larger ICV is interesting, as this was not seen in our previous study (MacMaster and Kusumakar, 2004). This could be due to the fact that the subjects in the present study were younger than those in the earlier report. Additionally, Lampe et al. (2003) found that depressive states may lead to changes in global cerebral gray matter volume over time (and in turn ICV), and that may account for the loss of this difference as MDD patients get older.

There have been 5 previous reports using MRI studies to evaluate pituitary gland volumes in mood disorder patients. Krishnan et al. (1991) found enlarged pituitary volumes in acutely depressed adult patients as compared to healthy controls. Axelson et al. (1992) found a positive correlation between post-dexamethasone plasma cortisol concentration and pituitary volumes in a group of psychiatric inpatients, which consisted mainly of MDD patients. More recently, MacMaster and Kusumakar (2004) noted a larger pituitary gland volume in adolescents with MDD as compared to age- and sex-matched healthy controls. Two negative reports exist, however. First, Schwartz et al. (1997) did not find any differences in pituitary volumes between seasonally affective disorder (SAD) patients and healthy controls. Second, Sassi et al. (2001) did find a significant decrease in pituitary volume in bipolar adults compared to control subjects, but no difference between control subjects and MDD patients. Key methodological differences between our study and the ones by Schwartz et al.

(1997) and Sassi et al. (2001) may account for the different results found amongst these studies. Subjects in our sample of MDD patients were much younger than any previously studied, in fact even younger than those in our own previous pediatric MDD study (MacMaster and Kusumakar, 2004). It is possible that the normal increase in pituitary volume during youth might mask depression-related changes in older patients. This suggestion is consistent with our finding of a robust correlation between age and pituitary volume in control subjects. Another interesting possibility is that as subjects age and become elderly, the decrease in pituitary size in healthy people as noted previously (Lurie et al., 1990) might be again able to reveal larger pituitary volumes in MDD subjects. Also, the unipolar (MDD) sample studied by Sassi et al. (2001) was composed mostly of women; this would likely have influenced their results, since we did not find an effect in the size of the pituitary in female MDD subjects as compared to the healthy females. Finally, half of the MDD subjects in Sassi et al. (2001) were euthymic at the time of the MRI scan, whereas all our MDD patients were acutely depressed at the time of scanning. It is uncertain that this is an important consideration, however, as Schwartz et al. (1997) did not show any influence of affective status on pituitary size. It should be noted that the sample used in the study by Schwartz et al (1997) was comprised of seasonal affective disorder (SAD) patients and not MDD patients. Finally, it is unlikely that the differences were due to differences in the analysis technique utilized to measure pituitary gland volume. In all three studies (Sassi et al., 2001; Schwartz et al., 1997; MacMaster and Kusumakar, 2004), serial coronal images were utilized to measure the area of the gland in all slices where it could be identified, and the total area was multiplied by the slice thickness to obtain the volume.

At least 9 MRI studies have previously examined pituitary volumes in psychiatric illness (Doraiswamy et al., 1990; Doraiswamy et al., 1991; Krishnan et al., 1991; Axelson et al., 1992; Schwartz et al., 1997; MacMaster et al., 1999; Beresford et al., 1999; Sassi et al., 2001; MacMaster and Kusumakar, 2004). Beresford et al. (1999) reported a trend towards enlarged pituitary in subjects with alcohol dependence but as none of our MDD subjects reported any substance abuse, the effect noted here is

unlikely to be due to that. Anxiety disorders tend to demonstrate a smaller pituitary volume (e.g., bulimic patients: Doraiswamy et al., 1990; Doraiswamy et al., 1991a) and treatment-naïve pediatric obsessive-compulsive disorder subjects: MacMaster et al., 1999). Hence, comorbid (or sub-clinical) anxiety would conceivably decrease the pituitary volume effect noted in MDD patients rather than add to it.

The current study has a few methodological limitations that need to be addressed in future studies of the pituitary gland in MDD. All the MDD patients studied here were acutely depressed. Studies of euthymic patients should be conducted in order to determine if pituitary gland size changes as noted here are state-related or a trait of depressive disorders in youth. This may be important when considering the discrepancy between this study and that of Sassi et al., (2001) and Chen et al., (2004). A more critical confound is the fact that one cannot reliably delineate the anterior from the posterior pituitary using the methodology outlined here. Hence, I could not specify the region of hypertrophy as being related to the anterior or posterior (or both) lobes of the pituitary with any degree of certainty. This is important, as the function of the anterior and posterior lobes is markedly different.

In summary, these results provide evidence of enlarged pituitary glands in early onset MDD patients. This replicates and expands upon a previous report in adolescents (MacMaster and Kusumakar, 2004) and one in elderly MDD patients (Krishnan et al., 1991). This larger gland volume in depression may represent an attempt by the organ at adaptation to chronically high plasma cortisol levels or a change in the diurnal variation, which is more typically seen in adolescents. However, these suggestions must be experimentally tested, as there were no endocrinological measurements made in the subjects of the current study. Future studies with larger sample sizes as well as direct endocrine measures will be informative.

CHAPTER FIVE: ANXIETY DISORDERS³

³ A portion of this chapter has been accepted for publication in *Biological Psychiatry* (2006) doi:10.1016/j.biopsych.2005.06.028

INTRODUCTION

Anxiety disorders are common debilitating conditions affecting a great number of children and adolescents. Obsessive-compulsive disorder (OCD) is characterized by repetitive, ritualistic thoughts, ideas and behaviors. Its lifetime prevalence is 2-3% (Valleni-Basile et al., 1994; Hanna, 1995; Flament et al., 1988) with as many as 80% of all cases beginning during childhood and adolescence (Pauls et al., 1995). OCD involves the body's response to stress and this may be reflected in changes in brain structure. The identification of biomarkers in pediatric mental illness is needed in order to advance our understanding of the pathophysiology of the disorders and to help better define phenotypes for genetic studies. One possible biomarker of interest is the morphology of the pituitary gland, which has not been studied in any detail in OCD.

Dysregulation of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis has been reported in patients with OCD (Altemus et al., 1992; Catapano et al., 1992; Monteleone et al., 1995, 1997). Elevated urinary and plasma cortisol levels and levels of corticotropin-releasing factor have been reported in OCD patients (Gehris et al., 1990; Catapano et al., 1992; Altemus et al., 1992) although contradictory reports exist (Michelson et al., 1996; Weizman et al., 1990; Lucey et al., 1993). A role for increased posterior pituitary oxytocin levels has also been suggested in OCD, as oxytocin-mediated grooming in animals may have parallels with OCD in humans (Leckman et al., 1994). Alterations in posterior pituitary arginine vasopressin have also been observed in OCD patients (Altemus et al., 1992).

These studies provide a peripheral window into brain function in OCD. I examined pituitary volumes using volumetric magnetic resonance imaging (MRI) analysis in children and adolescents with OCD before and after treatment with the selective serotonin reuptake inhibitor (SSRI) paroxetine, as compared to age- and gender-matched healthy pediatric controls. To my knowledge, this is the first study to directly examine pituitary volumes in patients with OCD.

EXPERIMENT 7 – OBSESSIVE-COMPULSIVE DISORDER

SUBJECTS

All OCD patients were medication naive. All children and their parent(s) were assessed with the Kiddie Schedule for Affective Disorders and Schizophrenia (Kaufman et al 1997) with DSM-IV criteria. All patients and their parent(s) were interviewed by a board-certified child psychiatrist (DRR) to confirm diagnostic criteria. Exclusion criteria for all subjects included any lifetime history of psychotic disorder, bipolar disorder, posttraumatic stress disorder, conduct disorder, substance abuse/dependence, Tourette's syndrome, eating disorders, significant medical or neurologic disease, intelligence quotient less than 80, autism, or learning disability. Healthy control subjects had no personal history of psychiatric illness and no history of DSM-IV axis I disorder in their first-degree relatives. Psychiatric illness in first-degree relatives was assessed by Family History-Research Diagnostic Criteria (Andreasen et al 1977). Legal guardians provided written, informed consent, and all children gave written assent before initiating all studies in compliance with the regulations of the Wayne State University Human Investigation Committee.

Thirty-one OCD patients (10 males and 21 females), aged 8 to 17 years, and 31 case-matched healthy comparison subjects (10 males and 21 females), aged 8 to 17 years, underwent MRI scans. In the OCD patients, the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Scahill et al 1997) was used to measure OCD symptom severity (mean score 27.29, SD = 6.03). All patients had a CY-BOCS score of at least 16, indicative of significant dysfunction. Severity of anxiety was measured by the Hamilton Anxiety Rating Scale (HAM-A; Hamilton 1959) (mean score 9.74, SD = 6.15), and severity of depression was measured by the Hamilton Depression Rating Scale (HAM-D; Hamilton 1967) (mean score 9.19, SD = 6.16). Mean duration of illness was 24.48 months (SD = 29.15) with a wide range (1 to 120 months).

DATA ANALYSIS

Analysis of covariance (ANCOVA) was used to examine the differences between groups with regard to pituitary gland volume. Sex was used as a covariate as there have been consistent results showing a sex difference between males and females with regard to pituitary gland volume (Takano et al., 1999; MacMaster and Kusumakar, 2004). As I hypothesized that OCD patients would have smaller pituitary gland volumes based on my finding of a reduction in pituitary gland midsagittal areas in ODC patients (MacMaster et al., 1999), I used planned comparisons (controls = 1, OCD patients = -1). Age was used as a covariate as it correlated with pituitary gland volume in both groups (healthy comparison subjects $r = 0.72$, $p < 0.001$; OCD patients $r = 0.62$, $p < 0.001$). ICV did not correlate with pituitary gland volume in either controls or OCD patients, but there was a trend suggesting a correlation with age in the OCD patients ($r = 0.33$, $p = 0.07$). Therefore ICV and age was used as a covariate as well. Sex differences in the two groups were also explored, and I hypothesized larger pituitary glands in females as compared to males (planned comparisons: females = 1, males = -1) (Takano et al., 1999; MacMaster and Kusumakar, 2004).

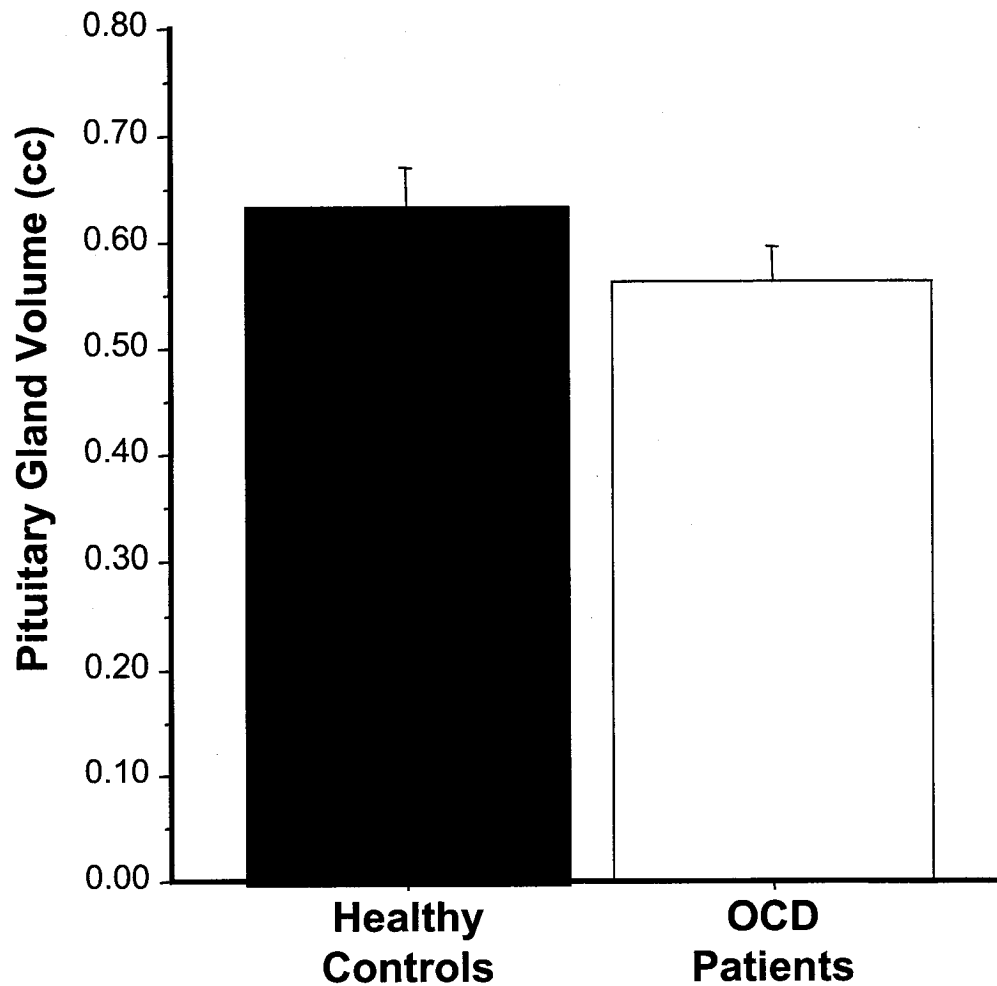
As I hypothesized a relationship between age and pituitary gland volume (Takano et al., 1999; MacMaster and Kusumakar, 2004) a one-tailed Pearson correlation was used in order to assess the relationship between pituitary gland volume and age. As I did not have a specific directional hypothesis with regard to clinical variables and pituitary gland volume, a two-tailed Pearson correlation was used to investigate the relationship between pituitary gland volume and clinical measures (CY-BOC, CDRS, HAM-A, HAM-D)

RESULTS

Pituitary gland volumes were significantly smaller in the patients with OCD ($0.56 \pm 0.19\text{cc}$) than in control subjects ($0.64 \pm 0.20\text{cc}$) ($F_{1,57} = 4.62$, $p = 0.036$, 11% decrease) (see figure 18). In the controls, females had larger pituitary gland volumes than males ($F_{1,27} = 5.75$, $p = 0.02$), as did the OCD females when compared to the

Figure 18: Pituitary Gland Volume in Obsessive-Compulsive Disorder (OCD)

Pituitary gland volumes were significantly smaller in the patients with OCD ($0.56 \pm 0.19\text{cc}$) than in control subjects ($0.64 \pm 0.20\text{cc}$) ($F_{1,57} = 4.62$, $p = 0.036$, 11% decrease). Error bars indicate standard error.



OCD males ($F_{1,27} = 10.36$, $p = 0.003$). The difference noted between OCD patients and controls in the primary comparison was more prominent in the males ($F_{1,16} = 3.47$, $p = 0.08$) than females ($F_{1,38} = 2.22$, $p = 0.14$). In this experiment, age and pituitary gland volumes correlated in both control subjects ($r = 0.72$, $p < 0.001$) and OCD patients ($r = 0.62$, $p < 0.001$). Pituitary gland volume did not correlate with any clinical variables measured in the OCD patients.

EXPERIMENT 8 – THE EFFECT OF TREATMENT

SUBJECTS

Sixteen OCD patients (6 males and 10 females), aged 8 to 17 years, underwent MRI scans before and after paroxetine therapy. CY-BOCS was used to measure OCD symptom severity (mean score 29.75, SD = 5.65). HAM-A scores were similar to OCD subjects in experiment 1 (mean score 10.13, SD = 8.04), but the HAM-D scores were somewhat less (mean score 8.50, SD = 5.94). Mean duration of illness was 29.13 months (SD = 31.23) and, again, demonstrated a wide range (3 to 96 months).

After completion of the baseline clinical assessment and MRI scan, 16 of the 31 patients with OCD were treated with the SSRI paroxetine hydrochloride. Paroxetine was started at a dose of 10 mg/d and titrated to a maximum dose of 60mg/d (mean {SD} paroxetine dose, 38.13 ± 17.97 mg/d; range, 10 – 60mg/d). During treatment, patients were monitored for side effects and adverse experiences and then underwent a follow-up MRI after 12 weeks of treatment with paroxetine. No significant side effects were observed. At the follow-up MRI scan, the same clinical scales used at the baseline assessment were used again for assessment. All children receiving paroxetine received no other treatment, e.g., cognitive behavioral therapy, family therapy or any psychotherapy other than supportive therapy.

DATA ANALYSIS

Paired t-tests were used to compare pituitary volumes before and after 12 weeks of paroxetine therapy. I hypothesized an increase in pituitary gland volume with a reduction in OCD symptom severity in the OCD patients.

RESULTS

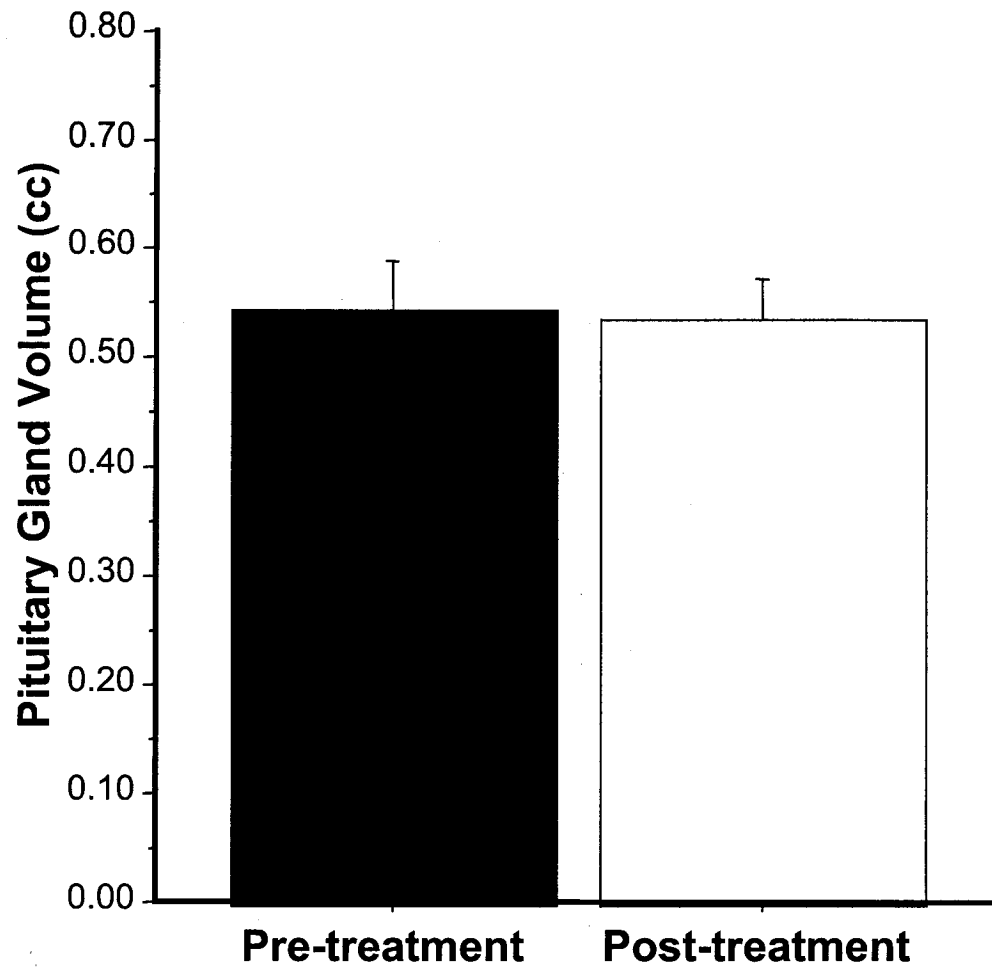
Paroxetine therapy was not associated with any change pituitary gland volume in the OCD patients (baseline: $0.54 \pm 0.19\text{cc}$, post-treatment: $0.53 \pm 0.16\text{cc}$; $t_{15} = 0.262$, $p = 0.80$) (figure 19). Significant reductions in clinical symptoms were noted in CY-BOCS total score ($t_{15} = 4.65$, $p < 0.001$) and both the obsession subscale ($t_{15} = 5.43$, $p < 0.001$) and the compulsion subscale ($t_{15} = 3.91$, $p = 0.001$). The Hamilton anxiety scores were also reduced with treatment ($t_{15} = 3.09$, $p = 0.007$). Hamilton depression scale scores demonstrated a trend for a reduction with treatment as well ($t_{15} = 2.55$, $p = 0.022$). Intracranial volume did not change with paroxetine treatment in the OCD patients ($t_{15} = 0.11$, $p = 0.92$). Pituitary gland volume did not correlate with any clinical variables at baseline or post treatment.

DISCUSSION

To my knowledge, this is the first neuroimaging study to demonstrate reduced pituitary gland volume in psychotropic-naïve OCD patients near the time of illness onset. The finding of a smaller pituitary gland volume in the OCD patients as compared to healthy controls is consonant with my previous study of midsagittal pituitary gland area in similar patients (MacMaster et al., 1999) and reductions in pituitary gland size noted in eating disorders (Doraiswamy et al., 1990; Doraiswamy et al., 1991a). No change in pituitary gland volume was noted in the OCD patients after 12 weeks of paroxetine therapy. Furthermore, in post-traumatic stress disorder (PTSD), pituitary volumes were found to be significantly larger in pubertal and postpubertal maltreated subjects with PTSD than control subjects, but were similar in prepubertal maltreated subjects with PTSD and control subjects (Thomas et al., 2004). Larger pituitary gland volumes have been noted in elderly and pediatric mood disorders (Krishnan et al., 1991; MacMaster and Kusumakar, 2004) with negative

Figure 19: Pituitary Gland Volume in Obsessive-Compulsive Disorder (OCD) Before and After Treatment

Paroxetine therapy was not associated with any change pituitary gland volume in the OCD patients (baseline: $0.54 \pm 0.19\text{cc}$, post-treatment: $0.53 \pm 0.16\text{cc}$; $t_{15} = 0.262$, $p = 0.80$). Error bars indicate standard error.



reports in adult populations (Schwartz et al., 1997; Sassi et al., 2001). Axelson et al. (1992) suggested that activation of the HPA-axis in psychiatric patients, as manifested by elevated post-dexamethasone cortisol concentrations, might influence pituitary volume. Beresford et al. (1999) reported a trend towards enlarged pituitary glands in subjects with alcohol dependence. As none of the subjects in the current study reported any substance abuse, the effect noted here is unlikely to be affected due to that.

A striking positive correlation between age and pituitary area was observed in both control groups and OCD patients in both experiments. The robust correlation between pituitary volume and age noted in the healthy controls is consistent with previous findings of an increase in pituitary volume with puberty (Takano et al., 1999) that is age related (MacMaster and Kusumakar, 2004). In the OCD patients, age and pituitary gland volume correlated strongly as well. These findings suggest a similar developmental trajectory in the OCD patients as in the healthy controls. However, the perturbations in pituitary anatomy in OCD patients suggest an early event developmentally in these patients that results in a smaller pituitary volume when compared to healthy children of the same age and sex. Following this hypothesized event, a similar course for growth is followed. This suggestion should be investigated in a longitudinal study.

A sex difference in healthy subjects noted in this study is similar to those found in previous studies (Takano et al., 1999; MacMaster and Kusumakar, 2004). Interestingly, the sex difference was more robust in the OCD patients, with females demonstrating larger pituitary gland volumes than males. The difference in volume noted between OCD patients and healthy comparison subjects was also more prominent when comparing OCD males to healthy male controls than between OCD females and control females.

As stated earlier, MRc and GR receptors are distributed throughout the brain. For example, the PFC is rich in GR (DeKloet et al., 1988). Given the modulatory

relationship between PFC and the HPA-axis, a disruption to this relationship may affect the PFC and disrupt its ability to appropriately activate HPA responses to stress and therefore compromise negative feedback regulation of the HPA-axis. Indeed, I found that pituitary gland volume correlated with prefrontal cortex gray and white matter content in healthy controls but not in the OCD subjects. Pituitary gland volume did not correlate with hippocampus or amygdala in either the control or OCD groups.

Alterations in the HPA axis have been noted in previous studies of OCD. For example, increases in cortisol have been noted (Monteleone et al., 1995, 1997; Catapano et al., 1992). Increases in oxytocin (Leckman et al., 1994), arginine vasopressin and corticotropin releasing factor (Altemus et al., 1992) have also been reported. Negative findings have also been demonstrated, however (Lucey et al., 1992; Michelson et al., 1996; Coryell et al., 1989; Weizman et al., 1990). As changes in endocrine function can affect pituitary morphology (Gonzalez et al., 1988; Chakeres et al., 1989) even in psychiatric disease (Axelson et al., 1992), it may be that alterations in cortisol function caused the volumetric differences noted here between the OCD patients and control subjects. The lack of endocrinological measurements in this study limits its ability to directly link the anatomical pituitary findings to possible perturbations in the HPA-axis.

No changes in pituitary gland volume in OCD patients were found despite a reduction in OCD symptoms. Interestingly, Gerhis et al (1990) found that elevated cortisol levels in OCD patients were lowered after administration of clomipramine or placebo while OCD symptoms were only reduced in the clomipramine group. Monteleone et al (1995) reported elevated cortisol levels in OCD patients that persisted after 2 months of fluoxetine therapy despite a reduction in OCD symptoms. The implications of these findings coupled with those of the current study are unclear.

As noted above, these findings have a few potential methodological limitations, which need to be addressed in future studies of the pituitary gland in OCD. A

confound is the fact that, in this study I could not reliably delineate the anterior from the posterior pituitary. Therefore, I could not specify the region of hypertrophy with any degree of certainty. However, hormones related to the anterior pituitary have been implicated in OCD (Monteleone et al., 1995, 1997; Catapano et al., 1992; Altemus et al., 1992), as are those associated with the posterior pituitary (Leckman et al., 1994; Altemus et al., 1992), so it may not be the case that the effect is lobe-specific. Clearly, a direct assessment of anterior and posterior lobe changes in concert with endocrine measures would provide useful further information relevant to OCD.

As this is a preliminary cross sectional study with a wide age range, further investigation, including longitudinal studies, is indicated. Functional and metabolic neuroimaging studies may further elucidate the developmental neurobiology of pituitary abnormalities in OCD. In summary, this is the first evidence of decreased pituitary gland volume in pediatric OCD patients. This change in volume may reflect an adaptation to neuroendocrine levels. Future studies with larger case-control matched patient samples and direct endocrine measures will be needed to further elucidate neuroendocrine involvement in this illness.

CHAPTER SIX: BEHAVIORAL DISORDERS

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a serious public health problem that affects between 3 – 6% of children and accounts for as many as 30% to 50% of child referrals to mental health services. Children with ADHD have developmentally higher than normal levels of activity and impulsivity or inattentiveness, which significantly impairs their family, school, and social functioning .

A core deficit in response inhibition has been posited for ADHD (Barkley, 1997; Quay, 1997). Response inhibition is thought to be part of the behavior inhibition system (or BIS; Quay, 1997). Deficits in BIS are thought to affect working memory, self-regulation of affect, internalization of speech and goal directed behavior. More directly related to ADHD, BIS also mediates signals of punishment and frustrative non-reward. This aspect of BIS physiologically involves the hypothalamic-pituitary adrenal (HPA) axis. For example, in patients with ADHD, a loss of the normal diurnal variation in cortisol release has been noted (Kaneko et al., 1993). A striking (40%) reduction in epinephrine excretion in response to the stress of standard intelligence testing was also noted in ADHD males as compared to healthy controls (Kaneko et al., 1993). King et al., (1998) noted that ADHD subjects who maintained their diagnosis over 1 year had a blunted cortisol response to mental stress as compared to ADHD subjects who did not maintain their diagnosis over the year. More recently, Hong et al., (2003) noted that blunted HPA responses were linked with impulsivity in ADHD subjects. This is consonant with previous studies linking impulsivity and blunted HPA axis in other psychiatric disorders (Moss et al., 1995).

Given the findings noted above, I examined pituitary gland volume using magnetic resonance imaging (MRI) in treatment –naïve males with ADHD as compared to healthy controls. I also examined pituitary gland volume after treatment with methylphenidate in the ADHD subjects. Given the weak findings of pituitary involvement in ADHD, I hypothesized that no differences between ADHD patients and control subjects, or between ADHD patients before and after methylphenidate treatment, would be found. In light of the overall thesis hypothesis that pituitary

abnormalities will be evident only in disorders with strong evidence for pituitary dysfunction, the ADHD experiments will offer critical data in support (or against) that premise.

EXPERIMENT 9 – ATTENTION DEFICIT HYPERACTIVITY DISORDER

SUBJECTS

Data for this experiment were derived from 17 age-matched case-control pairs (6 to 11 years of age, controls: 8.49 ± 1.40 years, ADHD: 8.06 ± 1.71 years). All case-control pairs were matched for age (± 12 months) and all were males. The inclusion criteria were a diagnosis of ADHD, with age between 6-12 years. All patients met the DSM-IV diagnostic criteria for ADHD as determined by the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997), and confirmed in a clinical evaluation conducted by the attending psychiatrist (NC) and all ADHD children needed to meet criteria on the Hyperactivity and ADHD Index subscales (T-score of 70 or above) on the Parents and Teacher's Conners Rating Scale (CPRS and CTRS). Exclusion criteria for participation in this study were a history of neurological illness, serious medical illness, claustrophobia, age greater than 12 years, or the presence of a ferrous implant or pacemaker. Healthy control subjects had no personal history of psychiatric illness and no history of DSM-IV axis I disorder in their first-degree relatives. Legal guardians provided written, informed consent, and all children gave written assent before initiating all studies in compliance with the regulations of the research ethics board of the IWK Grace Heath Centre.

DATA ANALYSIS

Although the age range was small and only a single sex was examined, age was used as a covariate in the analysis of covariance (ANCOVA) as a strong correlation of pituitary gland volume with age was noted in the ADHD subjects ($r = 0.64$, $p = 0.003$). As ICV was also significantly smaller in the ADHD subjects ($t_{32} = 2.34$, $p = 0.03$, ADHD ICV volume: 1279.33 ± 127.34 and healthy control ICV volume: 1367.55 ± 88.92), ICV was also used as a covariate. As no significant difference was

hypothesized between groups, the planned comparisons were as follows: ADHD = 1, healthy controls = 1. Pearson's correlations were used to examine pituitary gland volume and age (one-tailed; MacMaster and Kusumakar, 2004). Results are stated in terms of mean \pm standard deviation unless otherwise indicated.

RESULTS

When covarying for age and ICV, no significant difference in pituitary gland volume was noted between ADHD and healthy control subjects ($F_{1,30} = 0.65$, $p = 0.43$) (figure 20). However an ANCOVA, using only age as a covariate, indicated that the pituitary was significantly smaller in the ADHD subjects as compared to controls ($F_{1,31} = 4.63$, $p = 0.04$). There was no difference in the number of slices of pituitary measured between the two groups ($t_{32} = 1.45$, $p = 0.16$; ADHD = 5.71 ± 1.16 and controls = 6.47 ± 1.84 slices). ADHD subjects demonstrated a correlation of pituitary gland volume with age ($r = 0.64$, $p = 0.003$) while the healthy controls did not ($r = 0.14$, $p = 0.29$).

EXPERIMENT 10 – THE EFFECT OF TREATMENT

SUBJECTS

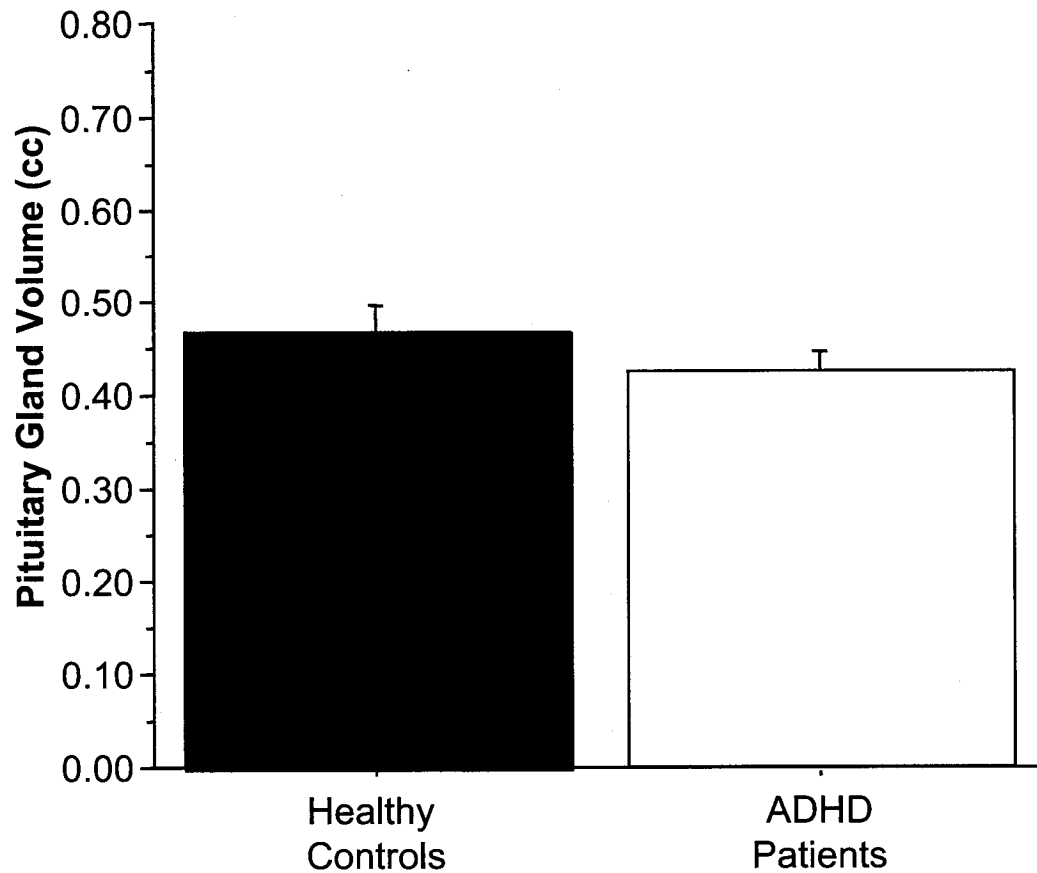
Twelve ADHD subjects (8.22 ± 1.80 years of age) were scanned before and after treatment with methylphenidate for 8 weeks. ADHD children were treated after the first scan with methylphenidate (MPH) from 0.3 to 0.6 mg/kg t.i.d. A second set of MR images was collected from the twelve ADHD patients at 8 weeks while on their stimulant medication, and within 1.5 hour of taking their morning dosage.

DATA ANALYSIS

As the data comprised a comparison between treatment-naïve and treated conditions in individual subjects, paired t-tests were used to determine the effect of intervention with methylphenidate. There was very little change in pituitary gland volume over 8 weeks in healthy control children (see chapter 2), so this comparison was deemed to

Figure 20: Pituitary Gland Volume in Attention Deficit Hyperactivity Disorder (ADHD)

No significant difference in pituitary gland volume was noted between ADHD and healthy control subjects ($F_{1,30} = 0.65$, $p = 0.43$). Error bars indicate standard error.



be sensitive enough to detect even subtle changes in gland volume. As no difference in pituitary gland volume was expected at baseline between healthy controls and ADHD children, I also did not expect to find a difference after treatment in the ADHD patients.

RESULTS

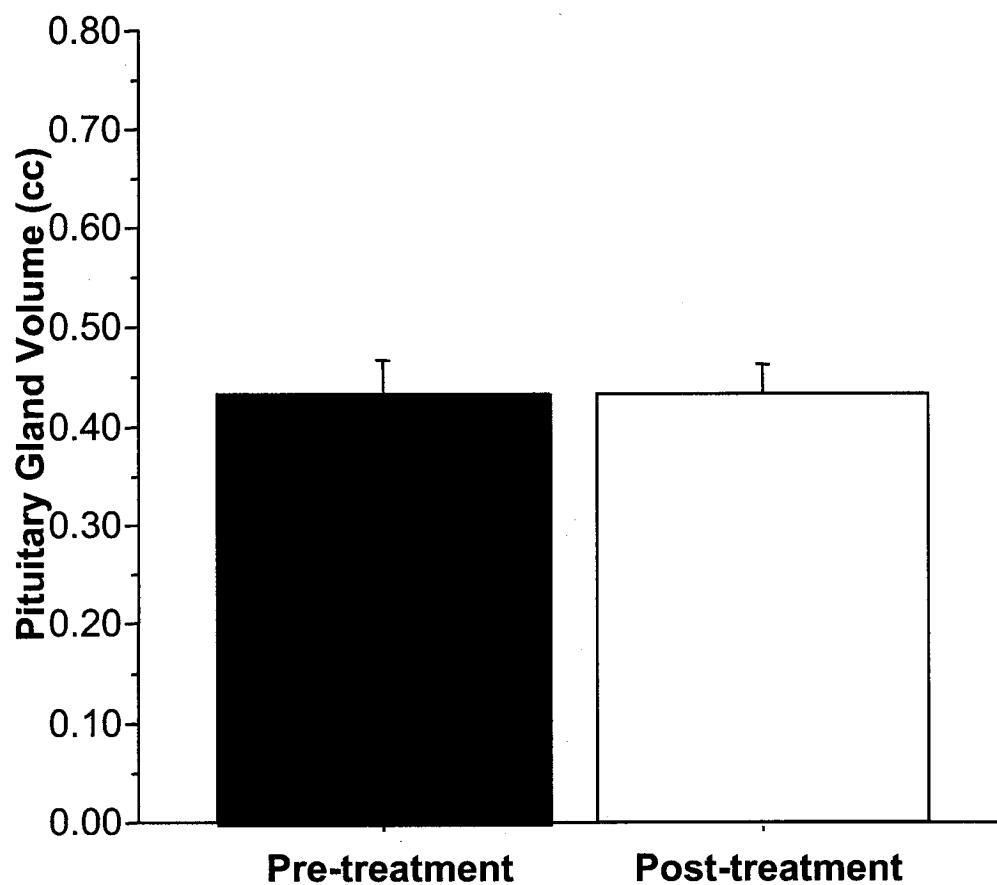
After 8 weeks of MPH treatment the behavior of all 12 ADHD children improved and they no longer met criteria for ADHD on the KSADS and on the CTRS and CPRS. More specifically, all 4 subscales of the CTRS (oppositional, cognitive/inattentive, hyperactivity and Conner's ADHD index) and the CPRS showed decreases in mean T-scores significant at the $p = 0.005$ level. CGI scores (Clinical Global Improvement) indicated that 4 subjects had healthy ratings, 5 subjects were 'borderline mentally ill', one subject was 'mildly ill' and one subject was 'moderately ill'. Eight children had no side effects as a result of methylphenidate administration, and 4 children had minimal side effects (transient loss of appetite not interfering with functioning). No significant differences were found in pituitary gland volume after 8 weeks of methylphenidate treatment ($t_{11} = 0.02$, $p = 0.99$, less than 1% change) (figure 21). There was no significant difference in the number of slices of pituitary gland measured before and after treatment ($t_{11} = 1.15$, $p = 0.28$).

DISCUSSION

In these experiments, no differences in pituitary gland volume were found between ADHD patients and healthy controls or following methylphenidate treatment in the ADHD patients. When uncontrolled for ICV (the variance in pituitary volume resultant from differences in ICV), however, the pituitary was smaller in ADHD subjects as compared to controls. A smaller ICV was found in the ADHD patients as compared with control subjects. Smaller ICV volumes are one of the most consistent findings in ADHD imaging studies (Castellanos et al., 2002; Durston et al., 2004). It is believed (Castellanos et al., 2002) that the smaller ICV in ADHD is indicative of an overall developmental lag in brain development children in ADHD. Hence, the

Figure 21: Pituitary Gland Volume in Attention Deficit Hyperactivity Disorder (ADHD) Before and After Treatment

No significant differences were found in pituitary gland volume after 8 weeks of methylphenidate treatment ($t_{11} = 0.02$, $p = 0.99$, less than 1% change). Error bars indicate standard error.



smaller pituitary noted in ADHD, when not controlling for ICV, may be more indicative of the global (smaller brain and substructures overall) nature of the deficit.

In ADHD patients, pituitary gland volume correlated with age while that in healthy control males did not. As a pituitary growth spurt was found in control pubertal subjects (see chapter 3), this strong prepubertal correlation between pituitary gland volume and age suggests a deviation from the standard growth trajectory for the ADHD subjects. As the reduction in caudate nucleus volume seen in ADHD subjects disappeared with increasing age (Castellanos et al., 2002), such a difference in size, and subsequent 'normalization' may be true of the pituitary gland in ADHD as well. The pituitary may increase in volume over time (as noted in the strong correlation with age in ADHD subjects) and "catch up" to the gland volume found in healthy children. This may mean that, while no overall difference was noted between the two groups, the pituitary in ADHD subjects may indeed be developing along a different time course than that of normal individuals, as has been hypothesized for the caudate nucleus in ADHD (Castellanos et al., 2002).

Few studies have previously reported on pituitary-related function in ADHD. No abnormalities have been detected in thyroid hormone levels, and generalized resistance to thyroid hormone is rare in ADHD (Elia et al., 1994). Thyroxine concentrations were found not to be related to hyperactivity by Stein and Weiss (2003). Toren et al (1997) and Spencer et al (1995) also reported no differences from controls regarding thyroid function in ADHD. In adults believed to have had ADHD as children, generalized resistance to thyroid hormone was strongly associated with ADHD (Hauser et al., 1993). This may indicate that changes in resistance in thyroid hormone seen in adult ADHD may be an effect of illness chronicity rather than that of an etiologically relevant factor, such as primary pituitary dysfunction.

Salivary cortisol has been found to be lower in a group of ADHD and ODD children as compared to healthy controls (Kariyawasam et al., 2002). More recently, it was noted that stress response differences are more common in ODD, and ADHD children

appear not to differ from healthy children in this respect (Snoek et al., 2004). It may be that the previous studies (Kaneko et al., 1993; King et al., 1998; Hong et al., 2003) included some subjects with ODD comorbidity or that there was some misdiagnosis of ADHD in their samples that skewed their findings.

In a longitudinal study of ADHD children over 2 – 3 years of age, the prolactin response to fenfluramine challenge was found to be stable across the evaluations ($r = 0.58$), although the magnitude of the response decreased significantly with age (Pick et al., 1999). No difference in prolactin response to fenfluramine was noted between ADHD patients and controls (Halperin et al., 1997), but levels of aggression may play a role in prolactin response to fenfluramine challenge (Halperin et al., 1994).

No effect of methylphenidate on pituitary gland volume was found in this study. In a previous report, Toren et al (1997) found that methylphenidate did not affect fasting serum growth hormone levels, growth hormone binding activity, or insulin like growth factor levels. Shaywitz et al (1990) also found that growth hormone and prolactin levels were poor measures of brain catecholaminergic response to methylphenidate in ADHD.

In conclusion, ADHD pituitary gland volumes did not differ from that of healthy controls, nor were they affected by methylphenidate treatment. As noted above, the evidence for endocrine changes in ADHD is not compelling, and hence the lack of a significant finding pituitary gland volume in this study is not surprising. The relationship between age and pituitary gland volume does suggest a difference in developmental trajectory in the ADHD children. This developmental difference may be reflective of more global differences in brain development thought to underlie ADHD.

CHAPTER SEVEN: DISCUSSION

SUMMARY OF FINDINGS

The overall hypothesis of this thesis is that pituitary glands of sufferers of psychiatric diseases that affect or involve the function of the pituitary gland (Boyer, 2000; Arborelius et al., 1999) will exhibit abnormal morphology when compared to healthy pituitary glands. Indeed, pituitary abnormalities were noted in the disorders with known HPA axis involvement (mood and anxiety disorders; Boyer, 2000; Arborelius et al., 1999) while in a disorder without a strong HPA axis component (ADHD), no abnormalities were noted. In the mood disorders MDD and bipolar disorder, a larger pituitary volume was noted while in the anxiety disorder OCD a smaller pituitary gland was noted. Pharmacological intervention, albeit successful in reducing the symptoms, did not affect these morphological differences in the pituitary gland. In the behavioral disorder ADHD, which does not have a large HPA axis component, no differences were noted in pituitary gland size as compared to healthy control subjects or in patients after pharmacological intervention.

Three experiments were also conducted in order to provide much needed context for the clinical experiments. In experiment one, I noted a strong relationship between pituitary gland volume and age. This was especially apparent upon examination of adolescent subjects, who had larger pituitary gland volumes than children. In experiment 2, a striking sex difference in pituitary gland volume was noted. Experiments one and two not only demonstrated developmental and sex effects, but also provided critical experimental design information for the analysis and interpretation of the clinical experiments. Experiment three revealed the strong temporal stability in the pituitary gland measure over the limited time periods employed in these studies.

TECHNICAL CONSIDERATIONS

The most critical consideration when evaluating these results involves the small sample sizes used. As noted by Giedd et al (1997), in general, large sample sizes are needed for definitive morphological work, as there is age- and/or sex-related variance

Table 6: Summary of Pediatric Psychiatric Findings

Group	Effect Size (d)	Treatment	Effect Size (d)
Males vs. Females	-0.48	Time 1/2	0.02
Controls vs. MDD	-0.64	Pre/Post TX	-0.13
Controls vs. MDD (MacMaster and Kusumakar, 2004b)	-0.70	-	-
Controls vs. BP Depressed	-1.09	-	-
Controls vs. BP Euthymic/Treated (Chen et al., 2004)	-0.24	-	-
Controls vs. OCD	0.41	Pre/Post TX	0.06
Controls vs. ADHD	0.37	Pre/Post TX	0.08
Controls vs. Psychosis	0.51	Pre/Post TX	-0.40

inherent in many of the measures. Certain research design techniques were used in the present study to minimize those confounds. By age- and sex-matching each clinical case with a healthy control ('case-control matching'), these effects have been controlled for, to some extent. Age- and sex-matching by group (similar sex breakdown, mean age) is not as stringent as case-control matching and was not used for that reason. Upon examination of the effect sizes (see table 6), the findings in the bipolar depressed subjects as compared to controls are the most robust. As the MDD vs. control findings demonstrated a large effect size, and replicate earlier work (MacMaster and Kusumakar 2004b) using similar methods, they should be considered fairly robust as well. However, the chance of types I and II error, due to insufficient power, especially for the OCD and ADHD experiments, is a distinct possibility. Replication of the OCD and ADHD findings with independent samples, as was done with the MDD findings (MacMaster and Kusumakar 2004b) is required. It should be noted that the findings of a smaller pituitary gland area in pediatric OCD in a previous sample (MacMaster et al., 1999) do lend some credence to the findings noted in this thesis regarding pituitary volume in OCD. As stated above, the small sample sizes used in this thesis may not be of sufficient power to be definitive. The treatment studies that did not find significant differences had effect sizes that were quite small and even with larger samples would most likely not achieve significance. A second sample-related limitation is the small age range covered. The age range comprised children and adolescents (6 – 19 years of age) for the clinical /control comparisons overall. Also, the samples differed in age and had different sex composition in each experiment. Hence the controls were matched to their respective clinical groups with regards to age and sex. Given the changes noted during development and between the sexes, the mean pituitary volumes differed across studies for the controls. This clustering around differing ages in each study also negated the ability to group data across studies. Given the lack of size changes found in the pituitary gland by Sassi et al (2001) in adult MDD, compared with the significant changes observed in younger individuals in the present study, there may indeed be differences in pituitary volume in clinical states across the lifespan. A much more expensive study, employing many more subjects, would be needed to

investigate those questions, however. Finally, in the present set of studies, all subjects were ill at the baseline time point. Subjects in remission from psychiatric symptomatology may differ from those who are suffering from active symptoms at the time of the scan. Given the lack of effect of medication on pituitary volume (despite symptom improvement) in any of the clinical populations studied here, this may not prove to be the case, however.

A number of other technical issues must be considered: (1) Are the acquisition parameters sufficient to provide enough resolution, contrast, etc. for the structure of interest? (2) Can the structure be reliably measured? (i.e., are intra-rater and inter-rater variations sufficiently small?) (3) Are the definitions of the boundaries used acceptable in comparison to the past literature? As the answers to these questions with regard to volumetric analysis of the subdivisions of the pituitary gland (i.e., anterior and posterior lobes considered separately) were mostly no, I decided it was best to evaluate the pituitary using total volume measures. However, the resolution of measures in this thesis (total gland volume) may still be a limitation. As stated earlier, the pituitary is composed of multiple cell types and sub-structures and one cannot assume that all will be different between groups or change with intervention and that each slice will change by the same percentage. Those caveats provided, we can critique the resolution based on these assumptions: (1) if we assume that the length does not differ greatly after infancy (Cox and Elster, 1991) and (2) that the shape resembles a prolate spheroid. As to the first assumption, for example the number of slices does not differ significantly between OCD patients and controls (6.74 vs. 6.87 respectively). As to the second assumption, while not a sphere, a prolate spheroid is somewhat closer to the true shape of the pituitary. If we assume that pituitary length does not differ/change, the differences noted must be due to a difference in cross-sectional area. If a prolate spheroid model, one can estimate the linear difference at the widest point of the pituitary. For the difference between the bipolar depressed patients and healthy controls, the volume is larger in the patients (0.57 vs. 0.77 cm³). The linear difference is approximately 1.7 mm, which is well within the 0.9 mm resolution. Considering the smaller difference reported for the anxiety patients as

compared to healthy controls, the change in cross-sectional area on each slice may have been below the resolution of the method. In other words, the method may have been insensitive to small changes in cross-sectional area. This may explain why, even with a larger sample size, the effect size was weaker than in the mood disorder studies. The difference in the OCD patients and controls may have been below the threshold of this method to robustly capture it. In the studies presented here, a 3-dimensional high-resolution coronal acquisition was used for evaluation of the pituitary. A slice thickness of 1.5 mm, with an in-plane resolution of 0.9 x 0.9 mm, was used in order to provide detail. The thickness of 1.5 mm was considered optimal as it made the results comparable to those from other labs conducting pediatric morphological studies using MRI.

Reliability at or above 0.90 is generally considered acceptable for morphological measures. Reliability was directly assessed for each of the three scanners. Data were analyzed after data collection was completed for each study, allowing for a more compressed time frame for analysis than would be the case if analysis proceeded as the data came in. If a break (a week or so) in analysis occurred, some previously analyzed scans were included with the remaining data to allow for a re-check of reliability after time away from measuring. There have been many published studies with reliabilities of a lesser magnitude (~0.80) but it was felt that enough variance was present due to age and sex in this sample that reliabilities should be set at a higher level. The pituitary gland is a fairly simple structure to evaluate with MRI scans due to its location in the brain; definitions for this structure were derived from the literature (Doraiswamy et al., 1990; 1991a; 1991b; Krishnan et al., 1991b; Tien et al., 1992; Sassi et al., 2001; MacMaster and Kusumakar, 2004). As a high-resolution 3D acquisition was used for the current studies, measures could be evaluated in axial, coronal and sagittal planes. This offered a very useful alternate visualization of the measures, especially on a small structure like the pituitary; simple changes in perspective within the data set offer increased opportunities for accurate delineation of the pituitary. The measurement of the subdivisions of the pituitary, however, has yet to be truly optimized. In an MRI scan, the resolution and contrast (at any level)

are simply not sufficient to allow for the accurate delineation of the pituitary into its component lobes. This problem is not unique to MRI and is present in many methods (both *in vivo* and post-mortem) unfortunately. With enhanced computer power and better image analysis software applications being developed, the possibility of an automated method for pituitary evaluation may someday be available. While great effort was taken to match the acquisition sequences between the scanners, it must be noted that some differences will still exist due to idiosyncrasies of the scanners themselves, as well as how the manufacturers set up and execute the particular sequences. Another concern is the possible effect of susceptibility artifact. The artifact has the effect of brightening the pixel signal intensity of the affected region. The attachment of the sphenoid septum to the sellar floor can cause some susceptibility artifact; this can be minimized by using a broad bandwidth (Sakurai et al., 1992). As we used a broad bandwidth and selected all tissue within the traced area on each slice and did not rely on signal intensity to delineate tissue, this susceptibility artifact would have had minimal influence on these data. It is also worth noting that even with the more problematic acquisition sequences, the susceptibility artifact is not common to all scans (14%; Elster, 1993).

A major limitation of these experiments is the lack of direct endocrine measures. Ethical considerations make it problematic to employ invasive procedures (such as blood draws, PET imaging) in a pediatric population. Non-invasive methods of assessing endocrine function (e.g., saliva, urine) were not available at the time. However, the results of the current studies provide intriguing clues about neuroendocrine disturbances in some psychiatric conditions that will provide a rich vein for design of future research projects.

COMPARISON WITH THE ADULT LITERATURE

There are no reports of pituitary volume changes in adult OCD and ADHD. However, the finding of decreased pituitary gland size in pediatric OCD is concordant with findings in anxiety spectrum disorders (Hollander et al., 1996) such as eating disorders (Doraiswamy et al 1990, 1991). Larger pituitary volume in male pediatric

patients with MDD is consistent with prior reports of hyperactivity of the LHPA in unmedicated patients with MDD (Nemeroff, 1998) and healthy subjects at high familial risk for MDD (Holsboer et al., 1995; Birmaher et al., 2000; Rao et al., 1996; Franz et al., 1995). In adults with bipolar disorder, Sassi et al., (2001) found a smaller pituitary volume as compared to control subjects. However, Chen et al., (2004) found no evidence of size abnormalities in the pituitary gland in child and adolescent bipolar patients, contrary to Sassi et al., (2001). In this thesis, I reported a larger pituitary volume in bipolar adolescents. Unlike the report by Chen et al., (2004), whose subjects were largely euthymic at the time of their scan, all bipolar adolescents investigated here were depressed at the time of their scan. Thus, the discordance in the findings of Chen and the current study may indicate that the larger volume noted here in bipolar disorder might be state-related.

To provide perspective on my pituitary findings in pediatric psychiatric disorders, consider the following. In mood disorders, no difference in pituitary gland size was noted between adult patients and controls in the study by Sassi et al., (2001). However, an enlarged pituitary gland was noted in elderly MDD patients (Krishnan et al., 1991). It may be that the normal growth patterns of the pituitary mask any clinical differences during early adulthood but not during the childhood/adolescence and elderly life stages. As for possibly related conditions, similar to my findings in MDD, Thomas and DeBellis (2004) reported an enlargement of the pituitary in PTSD; this was more pronounced in pediatric patients with PTSD that was co-morbid with suicidal ideation. Also, Beresford et al., (1999) found enlarged pituitaries in people with substance abuse problems. In people at risk for developing psychosis, Garner et al (2005) found an increase (12%) in pituitary volume in subjects who later actually developed psychosis. It should be noted that, in this sample, a large number of subjects of these subjects had symptoms of MDD and this may have influenced the results. Indeed, conflicting reports regarding pituitary volume in schizophrenia have been published in the literature (Pariante et al., 2005; 2004; Upadhyaya et al., Appendix I). Increased cortisol has been noted in first episode psychosis (Ryan et al., 2004; Sachar et al., 1970). Increased volume of the pituitary was noted in first

episode psychosis (Pariante et al., 2004, in press), while in chronically ill schizophrenics, Upadhyaya et al (Appendix I) found a smaller pituitary volume as compared to controls. Hence, it may be that with the initial onset of illness, a larger pituitary gland occurs followed by a decrease in volume with illness chronicity. Thus the pituitary enlargement effect is not specific to MDD alone (Thomas and DeBellis, 2004; Garner et al., 2005; Pariante et al., 2004, 2005), nor is a smaller pituitary specific to OCD alone (Sassi et al., 2001; Doraiswamy et al., 1990, 1991; Upadhyaya et al., 2007, Appendix A). Hence, the diagnostic specificity of the measure is low.

VARIATIONS IN PITUITARY GLAND VOLUME ASSOCIATED WITH SEX DIFFERENCES

There are compelling differences between males and females with regard to the time course and other detailed characteristics of both MDD and OCD (APA, 1994). This may mean that the biological substrates of these diseases may differ between the sexes. Given that the differences in pituitary volume seen in the current study were more pronounced in the male patients than the female patients, it is interesting to consider possible reasons for this.

Sex differences appear to be very pronounced in OCD (Lochner et al., 2004). In males, OCD is associated with an earlier onset and with the presence of tics (Eichstedt and Arnold, 2001; Rasmussen and Eisen, 1990; Lochner et al., 2004). Conflicting reports on this subject do exist, however, with data from the Epidemiological Catchment Area (ECA, Karno et al., 1988) finding no difference in the age of onset between males and females with OCD. This may be the result of differences between affected and healthy samples. Males are also thought to often have a more severe form of the illness with a worse prognosis than females (Rasmussen and Tsuang, 1986; Ravizza et al., 1997; Rosario-Campos et al., 2001). Males with OCD commonly have obsessions that revolve around sexuality, exactness and symmetry, and compulsions centered on checking and symmetry (Lensi et al., 1996). Females with OCD more commonly perform washing rituals and have contamination fears (Castle et al., 1995; Rasmussen and Eisen, 1988). It should be

noted that these are not strict differences, as some investigators have reported males having more washing/contamination-centered OCD (Fischer et al., 1996). Genetically, the low activity variant of the catechol-O-methyltransferase (COMT) gene has been associated with OCD in males (Karayiorgou et al., 1997; 1999). The excess presence in the brain of a high activity variant of monoamine oxidase, MAO-A, was also associated with OCD in males (Camarena et al., 2001; Karayiorgou et al., 1999). These findings have not been widely replicated (Alsobrook et al., 2002).

Recently, Lochner et al., (2004) noted that, in females, regular changes in OCD symptoms occurred with the premenstrual and menstrual periods, during and after pregnancy, and during menopause. These findings are consistent with past reports (Rasmussen and Eisen, 1988; Williams and Koran, 1997; Neziroglu et al., 1992; Altshuler et al., 1998; Maina et al., 1999). Hence, there does seem to be an endocrinologically related flux to the OCD symptoms in females. With a waxing and waning course related to hormonal output, it would seem that, despite no volumetric differences being noted in females with OCD as compared to controls, the pituitary may still play a role in the expression of the illness. The time point of the menstrual cycle was not controlled for in the work discussed in this thesis, and this would be a fruitful area to examine in future studies. More theoretically, the effect behind the overall larger pituitary volume seen in healthy females in this study may actually 'buffer' the females with OCD against the effect causing smaller volume noted in males with that disorder. Metaphorically, it would be considered a "basement effect", as it can only get 'so' small in the females. It may also be that the increased severity and nature of the disorder in males may push males over a threshold that results in a pituitary abnormality. The sex-related effect noted in OCD in this thesis does lend further credence to the existence of sex differences in OCD.

A sex difference in the rate of MDD is one of the most consistent findings in psychiatry (Nolen-Hoeksema et al., 1999). Mean gender ratios for lifetime prevalence is 2:1, females:males (Kuehner, 2003). In females an increase in occurrence of anxiety symptoms in MDD has been noted (Breslau et al., 1995;

Marcus et al., 2005) with substance abuse being more common in males (Kessler et al., 1997; Marcus et al., 2005). The relationship of substance abuse and MDD in males is noteworthy because a larger pituitary volume has been noted previously in individuals who indulge in substance abuse (Beresford et al., 1999). Since none of the MDD males in this study reported substance abuse, this may not be a factor, or perhaps the conditions that lead to an enlarged pituitary may be similar in substance abuse and MDD. Sex differences in MDD symptoms have also been noted previously, with women demonstrating more somatic concerns, appetite and weight increases and hypochondrias and males demonstrating more weight loss (Kornstein et al., 2000; Young et al., 1990). Frank et al., (1988) did not note any differences in severity, length of illness or functional impairment between the sexes. Marcus et al., (2005) found an earlier age of onset of MDD in females. They proffered that this finding may be linked to an earlier onset of puberty in women. Indeed, Angold et al., (1999) found that advanced Tanner stage and onset of menses are associated with MDD. Sexual abuse is also a potential consideration in the onset of MDD, particularly in females (Bifulco et al., 1998). This where the line between PTSD and MDD can become blurred, especially when considering that an enlarged pituitary has been noted in PTSD previously (Tomas and De Bellis, 2004). Indeed, with regard to the pituitary volume seen in MDD, there may be a “ceiling effect” that precludes the pituitary from being larger in females.

Unlike MDD, bipolar disorder is equally prevalent in men and women (Angst, 1998; Bebbington and Ramana, 1995). Females may have more depressive than manic episodes as compared to males (Taylor and Abrams, 1981; Robb et al., 1998). There is also evidence that the first episode in males tends to be a manic episode, while a depressive episode is more common in females (Viguera et al., 2001; Hendrick et al., 2000). More recently, Kawa et al., (2005) found no gender differences with regards to clinical characteristics between males and females with bipolar disorder. As for MDD however, females did report more changes in weight and appetite during depressive episodes compared with healthy subjects (Carter et al., 2000).

To date, there has been little consensus regarding potential gender differences in ADHD. Biederman et al., (2005) did not find differences in DSM-IV sub-typing, comorbidity or treatment history. They considered that previously reported differences were the result of referral bias. That said, recruitment for the ADHD arm for the current study provided a much greater number of males than females, such that only males were used for the study. Thus, the issue of possible sex differences in ADHD with respect to pituitary gland function remains unresolved.

DEVELOPMENT

Sobin et al., (2000) put forth the idea that early (childhood) onset OCD and later onset OCD may indeed be different clinical entities. As mentioned earlier, Angold et al., (1999) found that advanced Tanner stage and onset of menses are associated with MDD in females. More recently, Forbes et al., (2005) found that peri-sleep onset cortisol (i.e., cortisol in the period surrounding sleep onset) was elevated in children with anxiety disorders but not in adolescents with anxiety disorders. Conversely, peri-sleep onset cortisol elevations were noted in the adolescents with depression but not in the depressed children. Given the divergent findings noted in the present study of a smaller pituitary in the anxiety disorder OCD, and a larger pituitary noted in the depressive disorders MDD and bipolar disorder, the report by Forbes et al., (2005) is especially interesting. Adaptation to stress and anxiety could entail a gradual lowering of plasma cortisol and, by the time the anxious children become adolescents, it may be regulated back to normal levels. One mechanism by which this may occur may involve a decrease in the size and/or number of corticotrophs. This may explain the smaller pituitary gland volume seen in OCD, as the sample in the present study covers the ages of 7 to 18 years. The chronic nature of the anxiety experienced may lead to the morphological changes noted in OCD. In MDD, Forbes et al., (2005) proffered that the HPA axis may be 'protected' from the factors that cause depression by an unknown mechanism in childhood depression but it may be more vulnerable to these changes during adolescence. It may also be that childhood MDD and adolescent MDD may be separate entities. Indeed, the question does arise: *'is the developmental process itself a risk factor for mental illness and does the timing of the*

dysfunction (i.e., change in pituitary volume) impact which disorder develops'? Our limited sample sizes did not allow for the proper testing of childhood versus adolescence differences in MDD and OCD in pituitary volume. However, this is a compelling direction for future research.

WHY IS THE PITUITARY GLAND AFFECTED IN PEDIATRIC MENTAL ILLNESS?

Larger pituitary gland volume in pediatric patients with MDD is consistent with existing reports of hyperactivity of the HPA in adults with MDD (Nemeroff, 1998) and healthy subjects at high familial risk for MDD (Holsboer et al., 1995; Birmaher et al., 2000; Rao et al 1996; Franz et al., 1995). In children and adolescents, Dahl et al., (1991) and Kutcher et al., (1991) found overall cortisol levels were not elevated in mood disorders but were high prior to sleep onset, when levels are at their lowest in healthy children. This was interpreted as indicating a dysregulation in the circadian rhythm in children and adolescents with mood disorders. My findings of larger pituitary gland volume in MDD are intriguing and may be consistent with prior investigations demonstrating prefrontal abnormalities in pediatric MDD (Nolan et al 2002; Farchione et al 2002). Stress has been shown to induce dendritic spine loss and reorganization in the prefrontal cortex (Radley et al., 2004, 2005), a brain region which plays an important role in the regulation of stress-induced HPA activity (Sullivan and Gratton 2002; Spencer et al., 2004). Alternatively, these gland size changes may be due to a loss or reduction in negative feedback from the hippocampus in pediatric MDD. Indeed, I have recently noted a reduction in hippocampal volume in pediatric MDD (MacMaster and Kusumakar, 2004b) and have replicated this finding in an independent sample (MacMaster et al., manuscript in review). Although speculative, these interpretations are suggestive of further types of study that will contribute to a growing body of knowledge concerning neurobiologic effects of the HPA-axis in pediatric MDD.

Dysregulation of the HPA-axis has been reported in patients with OCD (Altemus et al., 1992; Catapano et al., 1992; Montelone et al., 1995, 1997). Elevated urinary and plasma cortisol levels and levels of corticotropin-releasing factor have been

reported in patients with OCD (Gehris et al., 1990; Catapano et al., 1992; Altemus et al., 1992) although contradictory reports exist (Michelson et al., 1996; Weizman et al., 1990; Lucey et al., 1993). Alterations in pituitary arginine vasopressin have also been observed in patients with OCD (Altemus et al., 1992). There are no reports on endocrine measures in pediatric OCD. Frontal-striatal abnormalities have been noted in pediatric OCD (Rosenberg et al., 1997; Rosenberg et al., 2000). A similar prefrontal dysregulation to that suggested for adult OCD patients may be involved in pediatric OCD.

POTENTIAL NEUROBIOLOGICAL MECHANISMS

There are numerous possible explanations for pituitary gland volume alterations in certain psychiatric disorders. For example, decreases in pituitary volume may involve at least three different possibilities (Nolan and Levy, 2001). First, increased apoptosis in the gland may be responsible for increased rates of cell death. Second, rates of cellular proliferation may be reduced. Third, individual cell volumes may be reduced. In mood disorders, a larger pituitary size may arise from any of the opposite of those three possibilities (i.e., it may be reduced apoptosis or a larger cell number or volume). Animal models, such as the rodent olfactory bulbectomy model of MDD, may offer insight as to why the pituitary gland volume is changing in mood disorders as more direct assessments of cell number and composition can be conducted in animal models. Given the data available from adult MDD and bipolar disorder studies (Sassi et al., 2001 as well as the recent report by Pariante et al., 2004), there may also be larger scale changes in pituitary volume due to ongoing development or feedback mechanisms that change over time.

WHY NO CHANGE WITH MEDICATION?

In experiment three, it was noted that there was less than 1% change in pituitary gland volume over 8 weeks in healthy controls. Hence, even relatively subtle volume changes over a treatment trial (8 – 12 weeks) should be apparent with this measure. None of the drug treatments examined in this thesis (paroxetine, venlafaxine and methylphenidate) significantly affected pituitary gland volume over 8 – 12 weeks in

the study patients despite their improvement in clinical symptoms. In mood disorders, larger pituitary gland volumes were found in males with major depressive disorder and subjects (both males and females) experiencing bipolar depression as compared to age- and sex-matched healthy controls. These findings are consistent with my previous findings in young mood disorder patients (MacMaster and Kusumakar, 2004).

The lack of detection of any volumetric changes due to the drugs investigated in the current studies is probably not simply due to MRI methodological limitations, however, as MRI has been able to detect volumetric changes due to pharmacological intervention of other types in previous studies. For example, changes in thalamus volume in pediatric OCD (Gilbert et al., 2000) and caudate nucleus volume in schizophrenia (Chakos et al., 1994; Scheepers et al., 2001; Keshavan et al., 1994) have been noted following psychotropic treatment. Given the small degree of variance over 12 weeks seen in experiment one and the previous studies detecting medication related differences with MRI, the method used in the current studies appears to be sensitive enough to have detected any volumetric changes that would have occurred.

In fact, in a previous study (MacMaster et al., 2007; Appendix B) I found evidence for volumetric changes in pituitary gland volume in schizophrenia resulting from pharmacological treatment. Following one year of risperidone treatment for schizophrenia, the pituitary gland increased in volume on average by 13%. One year, of course, is a much longer duration of treatment than 8 – 12 weeks. Hence, some volumetric differences of this nature may only become evident following chronic rather than more acute (or 'sub-chronic') treatment trials. Risperidone is also thought to directly involve the disinhibition of prolactin (Hummer and Huber, 2004), so the changes I found in the schizophrenia study may have been due to a lactotrophic hyperplasia similar to the one noted in pregnancy (Dinc et al., 1998; Gonzalez et al., 1988). The fact that treatment with the prolactin-sparing drug olanzapine in the

schizophrenic patients resulted in only a 3% increase in pituitary volume may be relevant to this issue.

FUTURE DIRECTIONS

The most obvious future direction is to conduct neuroendocrine assessments in concert with MRI measures in clinical and control populations in order to more directly link the two measures. This work is beginning, with vasopressin and MR signal intensity appearing to be correlated in the posterior pituitary (see review by Fujisawa, 2004). It is important to note that any endocrine abnormalities may not be simply hyper- or hypo-functioning, but a dysregulation of circadian-related control mechanisms. Indeed, Dahl et al. (1991) and Kutcher et al. (1991) noted that overall (entire 24 hour period) cortisol levels were not elevated in mood disorders but that levels were high prior to sleep onset in affected individuals, when cortisol levels are at their lowest in healthy children. This was interpreted as indicating a dysregulation in the circadian rhythm in the children and adolescents with mood disorders.

In order to determine if changes in the pituitary play a role in development of the illness or are a result of the illness itself, high-risk populations need to be studied. For mood disorders, children of parents with mood disorder offer a potentially rich vein of research. For OCD, siblings of patients offer a similarly valuable study population. High-risk strategies are fraught with limitations, however, as only a fraction of the samples go on to develop illness. This necessitates large samples to be studied longitudinally. That said, it is currently the best way to resolve the question as to when changes in neuroendocrine function, as indicated by pituitary gland size differences, arise.

A further future direction would involve the development of high resolution anatomical scans, possibly at higher magnetic field strengths, that will allow for the accurate delineation of anterior and posterior pituitary glands. Ultra-high resolution imaging is a developing field. Current work ongoing in our laboratory on the evaluation of olfactory bulb volume in schizophrenia relies upon increased volumetric

resolution made possible with high-field MRI, for example. The MRI contrast required to delineate anterior and posterior pituitary lobes is, however, not yet available.

Given my findings (see appendix B) of changes in pituitary volume in schizophrenia with risperidone treatment over one year, longer-term assessment of clinical interventions is merited. The effect of SSRIs given to MDD patients may, like risperidone for schizophrenia, take more than 12 weeks to alter pituitary gland volume. Symptom-alleviating clinical effects may take place in the absence of detectable underlying endocrine changes as well. Indeed, in OCD, Monteleone et al. (1995) found that SSRIs reduced symptomatology without apparent changes in endocrine findings. Such medications may merely alleviate symptoms without affecting the underlying pathophysiology.

Another future direction would be to link the macro-morphological changes as seen in the current studies with histological changes. This may be done with either animal model studies or human post-mortem work. For example, with increases in plasma cortisol levels, are there increases in corticotroph number or size? Does risperidone really affect lactotroph number or volume? The findings of the MRI studies in this thesis suggest avenues for many basic science and pre-clinical studies that will elucidate our understanding of neuroendocrine effects in psychiatric illnesses.

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APPENDIX A⁴

Pituitary Volume in Schizophrenia: A Structural MRI Study

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⁴ A portion of this appendix has been published in *Schizophrenia Research* (2007)
doi:10.1016/j.schres.2006.09.033

INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis and specifically the stress response has been a subject of great discussion in terms of understanding neuropsychiatric illness. The hypothalamus releases corticotropin-releasing hormone (CRH) to induce ACTH secretion by the anterior pituitary, which in turn stimulates the adrenals to release cortisol as part of the stress response. Cortisol exerts negative feedback on the release of both CRH and ACTH, thus completing the feedback loop of HPA regulation. Dysregulation of the normal HPA axis as a factor in the development and maintenance of psychosis has become a subject of increased scientific scrutiny.

In the neuroendocrine literature, there have been numerous reports indicating HPA over activity in schizophrenia. Elevated plasma cortisol levels have been reported in several patient samples with schizophrenia (Walsh et al 2005; Muck-Seler et al 2004; Ryan et al 2004). Elevated ACTH levels either independently measured or in response to an acute metabolic stressor such as 2-deoxy-D-glucose (2-DG) have also been seen in schizophrenic populations in comparison to healthy controls (Ryan et al 2004; Elman et al 1998). Walsh et al (2005) also reported augmented release of both cortisol and ACTH in response to metoclopramide-induced release of arginine vasopressin (AVP) by the posterior pituitary in male schizophrenic patients. Moreover, in addition to simply the presence of an enhanced stress response, there is evidence to suggest that the elevated cortisol which is present leads to various adverse affects. For example, Gold et al (2005) describes frontal lobe atrophy in individuals with HPA overactivity. Multiple studies have reported increased symptom severity in psychotic populations with elevated cortisol levels (Walder et al 2000, Lammers et al 1995, Tandon et al 1991). Such studies allow for the reasonable speculation that the HPA axis in schizophrenia may be abnormally sensitized to stress. This leads to exaggerated hormone levels that can be clinically manifested as the symptomatology of psychosis.

Also reported in the neuroendocrine literature is the role of adrenal steroids other than cortisol in psychosis. For example, Dehydroepiandrosterone (DHEA) and its sulfate

derivative (DHEA-S) are neuroactive steroids similar to cortisol that are produced by the adrenals and affect the brain. However, in contrast to cortisol, elevated levels of DHEA and DHEA-S have been shown to be efficacious in enhancing mood, energy levels and confidence in schizophrenic patients (Strous et al 2004; Harris et al 2001). Predictably, the baseline cortisol/DHEA ratio is increased in patients with schizophrenia compared to healthy controls (Ritsner et al 2004). Thus, there seems to be substantial evidence documenting the association between HPA dysfunction and schizophrenia.

In addition to neuroendocrine studies, structural neuroimaging can provide ancillary information regarding the role of the HPA axis in schizophrenia. The pituitary gland is perhaps the most suitable region of interest (ROI) for brain morphometric study of the HPA (MacMaster et al 2005; MacMaster and Kusumakar, 2004; Sassi et al 2001). Unlike the hypothalamus, which is very difficult to delineate in terms of its anatomical boundaries, the pituitary gland can be measured volumetrically reliably. Changes in neuroendocrine activity are also reflected in pituitary gland morphology. In pregnancy, for example, the pituitary gland increases in volume. This volumetric change was suggested to involve lactotrophic hyperplasia (increase in secretory cell number; Dinc et al 1998; Gonzalez et al 1988). In children with shunted hydrocephalus, an enlarged pituitary and a reduction in plasma growth hormone have been reported (Lopponen et al 1997). Hypothyroid individuals have enlarged pituitary glands (Shimono et al 1999). In dwarfs with a growth hormone releasing hormone receptor gene mutation, smaller than normal pituitaries were found (Murray et al 2000). Smaller gland size was also noted in isolated growth hormone deficiency and in multiple pituitary hormone deficiency compared with healthy controls (Arslanoglu et al 2001).

To date, the only MRI studies looking at pituitary volume in psychotic populations have been performed by Pariante et al (2004 and 2005) and found increased pituitary volume in first-episode psychotics compared to healthy control subjects. The first of these studies (Pariante et al 2004) was confounded, as the clinical subjects were not

medication naïve. A repeat study (Pariante et al 2005) looked at a limited number of medication naïve first episode subjects but not classified schizophrenics. To our knowledge, there has been no structural MRI study examining pituitary volume in a large sample of schizophrenic patients who are medication naïve. Herein, we present pituitary volumetric findings of a sample of neuroleptic naïve schizophrenic patients compared to healthy control subjects.

MATERIALS AND METHODS

SUBJECTS

A total of 106 individuals comprised the study sample. Of these, 51 were patients with a DSM-IV diagnosis of schizophrenia (SCZ) and 55 were age and sex matched healthy controls (HC). There was no significant difference in age between the two groups ($t = 0.07$; $p = 0.95$). While there was a trend of more women in the healthy control group compared to patients, this gender proportion was not statistically significant (chi square = 2.90, $df = 1$, $p = 0.1$).

Duration of illness data was also available for the SCZ group. Illness duration was divided into two components: (1) time in the prodromal phase and (2) time in the psychotic state. Both time periods were deemed relevant to illness duration. The two components were added in order to determine total duration of illness. Patients spent an average of 209 weeks in the prodromal phase and an average of 122 weeks in the psychotic state with an average total duration of illness of 282 weeks.

This research study was approved by the University of Pittsburgh Biomedical Internal Review Board. Prospective patients for the study were evaluated by the Structured Clinical Interview for DSM IV, Patient-Edition (SCID-P) (First et al 1995). Patients were eligible to be included in the study if they had an IQ > 75, were between the ages of 15-45, had no previous treatment with neuroleptics, had no significant medical or neurological comorbidities, had no previous head injury with loss of consciousness which may have been temporally related to the onset of psychosis and had no current substance abuse or dependence. Patients deemed by a psychiatrist to

be too ill to understand the nature of the study and to provide informed consent were not included.

Healthy comparison subjects for the study were recruited through local advertisements. Upon completion of an initial telephone screening, they were further evaluated in person by a trained psychiatrist or psychologist through use of the Structured Clinical Interview for DSM-IV-Non-Patient Edition (SCID-1/NP) (First et al 1996). Healthy comparison subjects had no current or previous history of an Axis I disorder, no prior exposure to any psychotropic medication within six months of their baseline assessment, no history of any neurological or other medical disorders which could affect neurologic function, IQ > 75 and no reported history of schizophrenia or major mood disorders in first degree relatives. Patients and controls (or their guardians) provided informed consent for their participation in the research study. Subjects younger than 18 also provided informed consent.

MAGNETIC RESONANCE IMAGING PROCEDURE

MRI scans were acquired on a 1.5 T GE scanner (General Electric Medical Systems, Milwaukee, WI, USA). A three dimensional spoiled gradient recall acquisition in the steady state pulse sequence was utilized to obtain 1.5 mm coronal images (TE = 20 msec, TR = 40 msec, acquisition matrix = 256 x 192, FOV = 20 cm, flip angle = 10°). Neuroanatomical measurements were conducted using the BRAINS2 image analysis software (Magnotta et al 2001). Volumetric measurements were obtained by two trained raters who were blind to group assignment, subject identity and all other demographic information regarding the subjects. The interrater reliability of the two raters based on a training set of 20 scans was $r = 0.83$ (intraclass correlation coefficient). All volumes were reported in cm^3 .

PITUITARY TRACING METHODOLOGY

The pituitary gland was measured with the tracing methodology described in Sassi et al (2001). The only modification in the method was that the pituitary was first identified in a midsagittal slice in order to clearly identify the anterior and posterior

extent of the structure. Subsequently, as described in Sassi et al (2001), the gland was identified in the coronal plane at the sella turcica and traced in all coronal slices in which it was viewed. The infundibular stalk was excluded from the tracings.

STATISTICAL ANALYSES

All analyses were conducted using the Statistical Package for the Social Sciences (SPSS version 13.0) and two-tailed statistical significance level was set at $p \leq .05$. We performed ANCOVA with age, gender and ICV as covariates in order to compare pituitary volumes between the groups. Pearson's correlation coefficients were used to perform correlational analyses.

RESULTS

The mean \pm SD pituitary volumes for the two groups were: SCZ = $0.58 \pm 0.14 \text{ cm}^3$, HC = $0.66 \pm 0.17 \text{ cm}^3$. Using an independent samples *t* test, we found that patients with schizophrenia had significantly smaller pituitary volumes than the healthy control subjects ($p = 0.009$). This significant volumetric difference remained when using ANCOVA with ICV, gender and age as covariates: ($F = 5.8635$; $df = 1, 83$; $p = 0.02$).

In terms of gender, when comparing all 106 subjects (patients and controls), females had significantly larger pituitary volumes: (66 males, $0.59 \pm 0.15 \text{ cm}^3$; 40 females, $0.66 \pm 0.17 \text{ cm}^3$; ANCOVA, $F = 11.53$; $df = 1, 84$; $p = 0.001$). However, when using an ANOVA, post-hoc Scheffe test comparing four groups: male patients, female patients, male controls and female controls, there was no significant volume differences between the groups with the exception of male patients and female controls ($p = .02$) where female controls had significantly larger volumes. When using gender as the only covariate, the volumetric difference between SCZ and HC persisted: ($F = 5.46$; $df = 1, 103$; $p = .02$). Male controls (MC) demonstrated a trend towards a larger pituitary volume compared to male patients (MP): (MC = $0.63 \pm 0.18 \text{ cm}^3$, MP = $0.56 \pm 0.12 \text{ cm}^3$; ANOVA, $F = 2.74$; $df = 1, 52$; $p = 0.10$). A similar trend

was found when comparing female controls (FC) versus female patients (FP): (FC = $0.69 \pm 0.15 \text{ cm}^3$; FP = $0.61 \pm 0.18 \text{ cm}^3$; ANCOVA, $F = 2.21$; $df = 1, 31$; $p = 0.15$).

Illness duration data was available for 45 out of the 51 patients in the sample. Measured in weeks, the mean \pm s.d. illness duration for the patient sample was 281.9 ± 292.2 . Using Pearson's r , no correlation between total illness duration and pituitary volume was found ($r = -0.19$; $p = 0.22$). For further analysis, the patient sample was subdivided into two groups: those patients ($n=29$) with total illness duration below the mean, labeled short duration patients (SDP); and those patients ($n=16$) with total illness duration above the mean, labeled long duration patients (LDP). When comparing these two subgroups, there was a trend towards lower pituitary volume for the LDP subgroup. This difference achieved statistical significance (ANCOVA; $F = 4.05$, $df = 1, 36$; $p = 0.05$). Finally, there were no correlations found between age and pituitary volume (total sample: $r = -0.03$, $p = 0.74$; SCZ: $r = 0.15$; $p = 0.37$; HC: $r = -0.1$; $p = 0.5$).

DISCUSSION

These findings provide new evidence of reduced pituitary volume in patients with schizophrenia. We hypothesize that during both the prodromal phase as well as the psychotic phase of illness, the HPA axis is repetitively stimulated to produce a stress response. Inevitably, this will lead to increased cortisol in the circulation. In first episode/early onset populations, such as psychosis (Pariante et al 2004, 2005) and major depression (MacMaster and Kusumakar, 2004), HPA activity is acutely elevated leading to an increase in pituitary gland volume. However, in a schizophrenic population, there is a certain chronicity to the illness that is absent in the broadly defined first-episode psychosis group. Just as acute activation of the HPA axis is related to increases in pituitary volume, it has been hypothesized that chronically elevated levels of cortisol may ultimately lead to inhibitory effects on pituitary corticotrophs with an eventual reduction in gland size and a corresponding reduction in structure volume (Sassi et al 2001). Pariante et al (2004) reported increased pituitary volume in a first-episode psychosis population. The average

duration of illness of first-episode patients in that sample was approximately 10 weeks. In a replication/extension study of medication naïve first-episode patients, Pariante et al (2005) again found increased pituitary volume among first-episode psychotics. The average duration of illness for that group was approximately 27 weeks (Carmine Pariante, *personal communication*). Thus, our finding of decreased pituitary volume can be expected given the extended average duration of illness (282 weeks) of our patient sample.

There is a growing body of literature of pituitary morphometric studies in psychiatric populations (Doraiswamy et al 1990, 1991; Krishnan et al. 1991; Axelson et al 1992; Schwartz et al 1997; Beresford et al 1999; Sassi et al 2001; MacMaster and Kusumakar 2004; Chen et al 2004; Thomas and Debellis 2004; Pariante et al 2004, 2005; Garner et al 2005; MacMaster et al 2005). Increased pituitary volume has been noted in depressed patients (Krishnan et al 1991; MacMaster and Kusumakar 2004), post-traumatic stress disorder (PTSD; Thomas and Debellis 2004) and alcohol dependence (Beresford et al 1999). It should be noted that two studies failed to note a difference in pituitary volume in depressed mood disorder patients (Schwartz et al 1997 and Sassi et al 2001). Including this study of schizophrenics, decreases in pituitary volume have been noted in eating disorders (Doraiswamy et al 1990, 1991), obsessive-compulsive disorder (MacMaster et al 2005) and bipolar disorder (Sassi et al 2001) and may represent a morphometric finding that cuts across diagnostic boundaries. The commonality of these disorders is the chronicity of the stress aspect of the patient symptomatology.

There are, however, a few potential limitations of the present study, which are worth addressing. First, we are only able to theorize what are the functional consequences of our structural findings as there were no hormone levels acquired in this sample. A more precise examination of HPA axis dysregulation in schizophrenia would necessarily include levels of cortisol, ACTH, CRH and potentially other hormones that may influence both physiology and phenotype in psychotically ill individuals. These levels would then be correlated with the volumetric findings to effectively

examine the utility of the structural differences observed between patients and healthy subjects. This is not to state that the structural findings alone are inconsequential. Indeed, we believe that the volumetric differences found in this dataset are quite noteworthy as we have not found elsewhere in the literature another examination of pituitary volume on such a large sample of schizophrenic patients, which are medication naïve.

An additional limitation of the study is that though we speculate that the decreased pituitary volume observed in our patient sample is due to chronically elevated cortisol levels secondary to a long illness duration, there was no observed correlation between illness duration and pituitary volume in our analysis. However, we assert that it is probably too simplistic to observe a perfectly linear inverse correlation between pituitary volume and illness duration. It is probably more reasonable to expect that pituitary volume will gradually increase under acute hyper-activation of the HPA axis early in psychotic illness. This enlargement will continue until a variable threshold point after which persistently elevated cortisol levels will lead to pituitary hypoplasia. Indeed, when our analysis did examine the subgroup of patients with a shorter versus a longer duration of illness, the more chronically ill patients did exhibit an even more substantial decrease in pituitary volume.

Finally, one may also feel that the study is weakened due to the presence of a greater proportion of women in the HC sample than the SCZ group. This may be significant as it has been reported that otherwise healthy females tend to have larger pituitaries than males (Takano et al 1999; MacMaster and Kusumakar 2004). Moreover, increases in pituitary size are observed during pregnancy (Gonzalez et al 1988; Dinc et al 1998). Therefore, it is tempting to infer that the enlarged pituitary volumes in HC may be due to a gender effect. However, the distribution of women in the HC sample was not significantly greater than those in the SCZ sample. Also, when using gender only as a covariate, the volumetric differences between HC and SCZ remained. Finally, when comparing male controls versus male patients and female

controls versus female patients, both male and female controls demonstrated a trend of larger pituitary volumes than their respective male and female patient counterparts.

The principal finding of decreased pituitary volume appears to be consistent with other studies examining pituitary volume in patients with a chronic mental disorder. To our knowledge, this is the first study of pituitary volume in a medication-naïve schizophrenic population. The neuroleptic-naïve nature of this sample is significant as it lends credibility to the idea that the volumetric findings are secondary to the disease process and not to pharmacologic effects. Future studies will need to attempt to replicate the mentioned anatomical findings and establish some parameters of statistical correspondence between measured hormonal levels and structural MRI data.

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APPENDIX B⁵

Effect of Antipsychotics on Pituitary Gland Volume in Treatment Naïve First Episode Schizophrenia: A Pilot Study

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⁵ A portion of this appendix is published in *Schizophrenia Research* (2007)
doi:10.1016/j.schres.2007.01.022

INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis has been implicated in schizophrenia. For example, elevated plasma cortisol levels have been reported in schizophrenia (Ryan et al., 2004). Psychotic symptom severity has been linked with elevated cortisol levels (Walder et al., 2000). Elevated adrenocorticotrophic hormone (ACTH) levels have also been seen in schizophrenic populations (Ryan et al., 2004). To date, there are only four MRI studies looking at pituitary volume in psychotic populations (Pariante et al., 2004, 2005; Garner et al., 2005; Upadhyaya et al., in press). Three studies found increased pituitary volume in first-episode psychotics compared to healthy control subjects (Pariante et al., 2004, 2005; Garner et al., 2005). In the first of these studies, the clinical subjects were not medication naïve (Pariante et al., 2004). A replication study (Pariante et al., 2005) looked at a limited number of medication naïve first episode and already treated subjects but not classified schizophrenics. Pariante et al. (2005) noted larger pituitary glands in subjects who were receiving antipsychotic treatment. In a neuroleptic naïve population, Upadhyaya et al. (in press) found smaller pituitary gland volumes in schizophrenic subjects as compared to controls. However, to date, no pre/post treatment study has examined the effect of antipsychotics on pituitary volume or the specificity of such effects. Changes in neuroendocrine activity are known to influence pituitary gland morphology (Dinc et al., 1998; Gonzalez et al., 1988). In addition, antipsychotics have been shown to reduce ACTH and cortisol secretion in healthy subjects (Cohrs et al., 2006). Hence, the pituitary may be a suitable biomarker for evaluating the effect of medication. Given the previous literature (Pariante et al., 2005, Upadhyaya et al., in press), we hypothesized that pituitary volume would increase with antipsychotic treatment in the schizophrenic patients. No change over time is expected in controls.

MATERIALS AND METHODS

SUBJECTS

Sixteen patients with a DSM-IV diagnosis of schizophrenia (SCZ; mean \pm standard deviation; 28.51 ± 6.72 years) and 12 were healthy controls (HC; 23.75 ± 5.03 years)

comprised the study sample. Prospective patients for the study were evaluated using the Structured Clinical Interview for DSM IV, Patient-Edition (SCID-P) (First et al., 1995). Patients were eligible to be included in the study if they had: 1) IQ > 75, 2) between 15-45 years, 3) no previous treatment with a neuroleptic, 4) no significant medical or neurological comorbidities, 5) no previous head injury with loss of consciousness which may have been temporally related to the onset of psychosis and 6) no current substance abuse or dependence. Patients deemed by a psychiatrist to be too ill to understand the nature of the study and to provide informed consent were not included. Symptoms were assessed using the Brief Psychiatric Rating Scale (BPRS)(Overall and Gorham, 1982), the Scale for Assessment of Positive symptoms (SAPS) and the Scale for Assessment of Negative Symptoms (SANS)(Andreasen et al., 1982). Subjects were then scanned at baseline before the initiation of treatment. Eight subjects received risperidone (final dosage: $3.07 \pm 1.37\text{mg}$) and eight received other antipsychotics (5 olanzapine (final dosage: $10.50 \pm 5.12\text{mg}$), 1 haloperidol (2 mg), 1 on clozapine (400mg) and haloperidol (3mg) and 1 on ziprasidone (120mg)). All medications were given as monotherapy. All schizophrenic subjects were treated for 12 months then scanned a second time. Healthy comparison subjects for the study were recruited through advertisements. Healthy comparison subjects had no current or previous history of an Axis I disorder, no prior exposure to any psychotropic medication within six months of their baseline assessment, no history of any neurological or other medical disorders which could affect neurologic function, IQ > 75 and no reported history of schizophrenia or major mood disorders in first degree relatives. Patients and controls (or their guardians) provided informed consent for their participation in the research study. Subjects younger than 18 provided informed assent. The University of Pittsburgh Biomedical Internal Review Board approved this research study.

DATA ACQUISITION AND ANALYSIS

All MR data were acquired on a 1.5 T GE scanner (General Electric Medical Systems, Milwaukee, WI, USA). Volumetric data was obtained from a three-dimensional spoiled gradient recall coronal acquisition (parameters as follows: TE =

20 msec, TR = 40 msec, acquisition matrix = 256 x 192, FOV = 20 cm, flip angle = 10°; 1.5mm thick). BRAINS2 image analysis software (Magnotta et al., 2002) was used for volumetric analysis. Two trained raters obtained volumetric measurements. Raters were blind to group assignment, subject identity and all other demographic information regarding the subjects. The inter-rater reliability of the two raters based on a training set of 20 scans was $r = 0.83$ (intra-class correlation coefficient). Volumes are reported in cm^3 . Pituitary gland measurement was described previously (Sassi et al., 2001). The only change in this study was that the pituitary was first identified on a midsagittal slice to clearly identify the anterior and posterior extent of the structure. The gland was identified in the coronal plane at the sella turcica and traced in all coronal slices in which it was viewed. The infundibular stalk was excluded from the tracings. Intracranial volume (ICV) measurement was described previously (Upadhyaya et al., in press). No change in ICV was expected in either group. Time one and time two pituitary and ICV measures were evaluated using a paired t-test. The data from controls is provided as context (temporal stability of the pituitary measure) for the core comparison of pituitary volume before and after treatment in the patients. In an exploratory analysis, the effect sizes for the patients receiving risperidone ($N = 8$) and those receiving another antipsychotic ($N = 8$) were also calculated. Pearson correlation was used to examine the relationship between symptom severity and pituitary gland volume.

RESULTS

Pituitary volume significantly increased in the schizophrenic subjects after treatment ($t_{15} = 2.58$, $p = 0.02$, baseline = 0.589 ± 0.145 , post treatment = 0.661 ± 0.197 , 12% increase, $d \sim 0.42$). This appears to be driven by patients receiving risperidone (18% increase, $d \sim 0.54$) as compared to other antipsychotics (8%, $d \sim 0.28$). In controls, pituitary volume did not change significantly ($t_{11} = 0.37$, $p = 0.72$, time 1 = 0.617 ± 0.139 , time 2 = 0.600 ± 0.090 , 3% decrease, $d \sim 0.15$). We also compared the percent change scores between schizophrenic subjects and controls. The groups do indicate a trend towards significance despite the small sample ($X = 7.20$, $df = 2$, $p = 0.055$). ICV did not change in the schizophrenic patients ($t_{15} = 0.01$, $p = 0.99$) or controls ($t_{11} =$

0.14, $p = 0.89$). Negative symptoms (SANS: $t_{15} = 2.89$, $p = 0.01$; BPRS negative: $t_{15} = 2.71$, $p = 0.02$) and positive symptoms (BPRS positive: $t_{15} = 2.91$, $p = 0.01$) were reduced over 1 year in schizophrenic subjects. Pituitary volume did not correlate with any clinical variables. The change in pituitary volume was not correlated with change in symptom severity.

DISCUSSION

We have found that pituitary volume increases following antipsychotic treatment in schizophrenic subjects. This appears to be specific to antipsychotic treatments, especially prolactin elevating drugs (e.g., risperidone) in contrast to prolactin sparing drugs (e.g., olanzapine). No previous study, to our knowledge, has documented effects of these medications on pituitary volume. This observation raises the question of whether these structural changes might be reversible or not, and also brings antipsychotic use as part of the differential diagnosis of pituitary hyperplasia often seen in clinical practice. There is growing evidence that antipsychotic drug treatment may be responsible for increases in pituitary volume (Pariante et al., 2005 and this report). This report serves to clarify a critical controversy in the recent literature with reports of both larger and smaller glands in schizophrenia (Pariante et al., 2004, 2005; Upadhyaya et al., in press). Our data may explain the conflicting studies as previous reports used both naïve and medicated patients. The marked increase in pituitary volume with treatment noted here may be related to the fact that risperidone elevates prolactin (Zhang et al., 2005). It may be that the disinhibition of prolactin (via D2 receptor antagonism) by the medications may induce an increase in prolactin secreting cells, their volume or some combination of these two possibilities. Indeed, the volumetric change noted in pregnancy was suggested to involve lactotrophic hyperplasia (increase in secretory cell number) (Dinc et al., 1998; Gonzalez et al., 1988). This study provides further evidence of the effect of psychotropic medication on regional brain volume. To date, only a handful of reports have investigated this potential effect of medication. Medication history/status may be a critical confound in studies of regional brain volumes in psychiatric populations. The current study involved well-classified schizophrenics who were treatment-naïve at the time of their

baseline scan. This study is limited, however, by the small sample sized used, the mix of medications, the long period of treatment, possible influences of comorbidity, and the lack of direct measures of pituitary gland function. Future studies should combine neuroendocrine measures with pituitary gland volumetric measures.

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