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Polymorphic Regions of the Estrogen Receptor, Androgen Receptor and Serotonin Transporter Genes and their Association with Mood Variability in Young Women

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Abstract

The present study examined whether genetic polymorphisms on the estrogen receptor, androgen receptor, and serotonin transporter genes affect mood reactivity or mood variability in women. A total of 279 women from the university community completed a screening questionnaire (phase 1) for the study. During phase two, 146 women provided a buccal swab which was analyzed for Variable Number Tandem Repeat (VNTR) polymorphisms on the aforementioned genes. They also participated in an anxiety-inducing task. The Positive and Negative Affect Scale Expanded (PANAS-X), the Pleasantness-Unpleasantness Scale, as well as the Beck Anxiety Inventory (BAI) were completed prior to and following the completion of the mood induction task in order to assess mood reactivity. Mood variability was assessed in the third phase of the study where 62 women completed the PANAS and the Pleasantness-Unpleasantness Scale at eight one-hour intervals over the course of one 24-hour period. It was hypothesized that women's mood variability, either during the laboratory mood manipulation or over the 8-hour period, would differ based on number of repeats of (1) the 17 base pair element within intron 2 of the Serotonin transporter gene (5-HTT), (2) CAG repeat numbers on the Androgen Receptor (AR) gene, or (3) number of TA repeats on the Estrogen Receptor α (ERα) gene. Women's emotional variability did not differ significantly as a function of genotype. However, with respect to emotional reactivity, the following trends were observed: (1) a greater amount of overall mood change and PA change for the homozygous long group of the ERα allele, (2) a greater amount of NA change for the heterozygous group of the ERα allele, and (3) a greater amount of PA change for the 8/10-9/10 allele group of the 5-HTT gene. Additional predictors of affect are discussed.

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Polymorphic Regions of the Estrogen Receptor, Androgen Receptor and Serotonin Transporter Genes and their Association with Mood Variability in Young Women

The purpose of this study was to examine the effect of specific estrogenic, androgenic, and serotonergic gene repeats on mood variability in young women. There is much evidence that many mood and anxiety disorders are heritable (Middeldorp et al., 2005) and thus, somehow linked to genetic factors. However, very little research has been conducted to determine which genes play crucial roles in regulating mood and mood variability. Such information could have important implications for women's health. Particularly because affective disorders are nearly twice as prevalent in women than in men (APA, 2000). The detection of genetic predictors of mood variability would aid in the development of potential screening procedures that would allow for identification of women who are susceptible or genetically predisposed to mood and/or other affect disorders such as Depression, Bipolar Disorder, and Borderline Personality Disorder. It might also aid in the development of better pharmacological treatments. Previous research has linked mood with serotonin, estrogens, and androgens. The present study examined whether genes regulating these chemicals are linked to mood variability by examining a number of Variable Number Tandem Repeats (VNTRs). Specifically, the relationships between mood variability and the number of VNTRs on the estrogen receptor, androgen receptor, and serotonin transporter genes were investigated.

A tandem repeat is a short sequence of DNA that is repeated in a head-to-tail fashion at a specific chromosomal locus (Snustad & Simmons, 2000). Tandem repeats are interspersed throughout the human genome and the number of copies of each sequence present at a given site on a chromosome is highly variable. With regard to gene loci, one

VNTR region is inherited from each parent, making possible a number of allelic combinations. The polymorphic nature of VNTR regions therefore makes it possible to distinguish between individuals. When profiles of unrelated individuals are compared on a single VNTR locus, they are typically observed to be different. Though it is possible for two individuals to have the same genetic profile at one or two loci by chance, the likelihood of more than one person having the same DNA profile at four, five, or six different VNTR loci is extremely small. For example, when DNA profiles are used for forensic purposes, the match probability between unrelated people was estimated at less than 3×10^{-11} (using only a single multi-locus probe) and less than 5×10^{-19} (using two multi-locus probes) (Tamaki & Jefferies, 2005). These probabilities are so low that the only instance in which individuals would match on multiple loci would be in the case of monozygotic twins. Thus, individual differences in VNTRs may reflect phenotypic differences in specific traits and are worth examining in this context.

What follows is a review of the literature pertaining to the estrogen receptor gene, androgen receptor gene, and serotonin transporter gene as well as a description of how these genes may play a role in determining variability in affect. In addition, the role of the hormones associated with the aforementioned genes and their effects on abnormal psychological functioning will also be discussed. Finally, a short section will be presented on the current theoretical models of mood and affect, mood structure, and the various methods that have been employed in attempting to assess affective variability.

Serotonin (5-HT)

Serotonin (5-HT) is a peptide neurotransmitter that is derived from the amino acid tryptophan and is converted to its final structure in a two step process that is

characterized first by hydroxylation, forming 5-hydroxytryptophan (5-HTP), and then by decarboxylation, resulting in the end product serotonin (Stryer, 1997). The final step in the conversion, or the decarboxylation, is performed by the enzyme dopa-decarboxylase which is also involved in the synthesis of dopamine and norepinephrine.

Originally studied as a vasoconstrictor in blood plasma, serotonin derives its name from the Latin word serum, meaning watery animal fluid; and tonic, a Greek word referring to that which is characterized by muscular tension (Palfai & Jankiewicz, 1997). It was in 1953 that Twarog and Page first demonstrated the presence of 5-HT in the mammalian central nervous system, giving rise to the theory that 5-HT may play a role in regulating brain function (Cryan & Leonard, 2000). Indeed, research over the past few decades has demonstrated that decreased brain serotonin levels are associated with symptoms of major depression (e.g Nobler, Mann, & Sackeim, 1999). In fact, the serotonergic theory of depression has gained such prominence that it led Cowan (2002) to equate it with the status of "a textbook truism."

Evidence for the biochemical theory of depression first came about when reserpine was given to patients to treat high blood pressure (see discussion by Palfai & Jankiewicz, 1997). Following administration of the drug, many of these patients became severely depressed. It was later revealed that reserpine depletes stores of monoamines, or peptide neurotransmitters, including dopamine (DA), serotonin (5-HT), and norepinephrine (NE).

In the mid 1970s, the rate limiting enzyme in the conversion of 5-HT from tryptophan (tryptophan hydroxylase) was inhibited using parachlorophenylalanine in remitted depressed patients taking the antidepressants imipramine and tranylcypromine

(Marks, Marks, & Smith, 1996). These patients showed a rapid and robust return in depressive symptomatology which was reversed again relatively rapidly following cessation of the depletion exercise. Similarly, Delgado et al. (1990) depleted tryptophan, the amino acid precursor of 5-HT, using tryptophan-free diets and amino acid loading, in remitted depressed patients. He demonstrated a similar relapse into the depressive state in those patients who had previously responded to selective serotonin reuptake inhibitor (SSRI) treatment. The depressive symptoms were quickly reversed following replenishment of tryptophan stores.

Since the time of these studies, serotonin has been further implicated in the pathogenesis of affective disorders via experiments that have demonstrated the following: (a) reduced cerebrospinal fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of 5-HT in drug-free depressed patients; (b) reduced concentrations of 5-HT in postmortem brain tissue of depressed and/or suicidal patients; (c) decreased plasma tryptophan concentrations in depressed patients and a profound relapse in remitted depressed patients who have responded to a serotonergic antidepressant when brain tryptophan availability is reduced; (d) in general, all clinically efficacious antidepressants augment 5-HT neurotransmission following chronic treatment; (e) clinically efficacious antidepressant action by all inhibitors of 5-HT uptake; (f) increases in the density of 5-HT2 binding sites in postmortem brain tissue of depressed patients and suicide victims, as well as in platelets of drug-free depressed patients; and (g) decreased number of 5-HT transporter binding sites in postmortem brain tissue of suicide victims and depressed patients and in platelets of drug-free depressed patients (see review by Owens & Nemeroff, 1994).

As illustrated above, evidence of the role of serotonin in the development and maintenance of depression was bolstered considerably through the development of the SSRI family of antidepressants. SSRIs block the reuptake of serotonin by the vesicles in the pre-synaptic membrane and make the neurotransmitter available for a longer period of time at the synapse, thus relieving depressive symptoms (see discussion by Palfai & Janowicz, 1997). Perhaps the most commonly known SSRI is fluoxetine (Prozac). Another class of antidepressants, known as monoamine oxidase inhibitors (MAOIs) are sometimes used in the treatment of depression when SSRIs fail. MAOIs work by binding irreversibly to monoamine oxidase (MAO) molecules, which are the molecules that typically break down serotonin in the central nervous system. Clearly, there is a great deal of evidence suggesting 5-HT plays a role in the development and maintenance of depression.

The Serotonin Transporter (5-HTT) and the Serotonin Transporter Gene

Given the link between serotonin and depression, a number of studies have examined whether the serotonin transporter (5-HTT) gene is involved in the pathogenesis of mood disorders. Located on chromosome 17q11.1-17q12, the human 5-HTT gene spans 31 kilo-base pairs (kbp) and 14 exons (Ramamoorthy et al., 1993). A VNTR element of 17 bp (base pairs) has been identified in the second intron of the serotonin transporter gene (Lesch et al., 1994).

The serotonin transporter (5-HTT) is associated with the presynaptic membrane of the 5-HT neuron and regulates 5-HT neurotransmission through reuptake and removal of released 5-HT in the synaptic cleft (Rudnick & Clark, 1993). Although the precise mechanism is unclear, the serotonin transporter has been suggested to be involved in

mood disorders (Owens & Nemeroff, 1994). Experiential effects combined with differences in primary protein structure could potentially change the function of the protein and consequently affect such characteristics as binding affinity, thereby creating a vulnerability to dysfunctional affective processes..

Several studies have identified the serotonin transporter as a significant factor when attempting to differentiate between clinical and non-clinical mood disordered populations. One of the most prominent differences has been found in the level of activity of the transporter itself. Ichimiya et al. (1992) examined whether 5-HTT binding was altered in patients with mood disorders using positron emission tomography (PET). Thirteen antidepressant-naive patients with mood disorders, and 21 age-matched healthy control subjects participated in this study. The clinical group was composed of seven individuals with major depressive disorder and six individuals with bipolar disorder. PET scans were performed using a selective ligand for 5-HTT. The uptake was quantified in the thalamus and midbrain by graphical method with reference tissue, and binding potential was used for the index of 5-HTT binding. Binding potential in the thalamus was significantly increased in patients with mood disorders as compared to control subjects, whereas binding potential in the midbrain did not differ between the groups. Subgroup comparison showed that patients with major depressive disorder had significantly higher binding potential in the thalamus compared to control subjects. Binding potential in the thalamus was higher by approximately 22% in the combined patients and 23% in MDD patients relative to control subjects. These findings suggest that higher 5-HTT binding potential, particularly in the thalamus, may be a factor in the development and maintenance of mood disorders.

In 1996, Ogilvie and colleagues set out to identify polymorphisms of the 5-HTT gene to find out whether there was a relationship between specific polymorphisms and the occurrence of affective disorders. Patients with major affective disorder were recruited from inpatient and outpatient programs from the Royal Edinburgh Hospital in Scotland. A total of 83 patients were used in the experimental group. Of these 83 patients, 39 had single or recurrent major depressive episodes and 44 had bipolar disorder. The control group consisted of 122 anonymous blood donors and 71 volunteers who had been screened for psychiatric disorders. Three alleles of the 17 bp VNTR region in intron two of the 5-HTT gene were detected (STin2.9, STin2.10, and STin2.12) containing 9, 10 and 12 copies of the allele respectively. The frequencies of the different forms of the allele in the control group were compared with those in the affective disorders group. The STin2.9 allele frequencies were significantly higher among individuals in the affective disorders group than among individuals in the control group. When the experimental group was further separated into unipolar and bipolar groups and each was compared to the control group, it was found that a significantly higher proportion of the unipolar group had genotypes containing the STin2.9 allele.

Similar to the above study, an examination of whether the 5-HTT gene is linked to anxiety disorders was conducted by Ohara et al. (1999). The subjects were 103 patients who had met the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*) criteria for anxiety disorders and 106 healthy control subjects. The results indicated that there was a significant difference between the anxiety disorder group and the control group in the proportion of participants carrying the STin2.12 allele (12 repeats in intron 2 of the human 5-HTT gene) in that the frequency of the STin2.12 allele in the patient

group was significantly higher than in the control group. That is, patients diagnosed with obsessive compulsive disorder (OCD) and generalized anxiety disorder (GAD) had significantly higher proportions of the STin2.12 than did controls, and patients with a family history of either OCD or GAD tended to have the STin2.12 allele in higher numbers than did controls (Ohara et al.,1999). There was not a statistical difference according to gender.

Based on the research of Ogilvie et al. (1996) and Ohara et al. (1999), further investigation seems warranted on the frequency of VNTR polymorphisms within intron 2 of the serotonin transporter gene (5-HTT) and how these regions relate to mood and mood variability.

In addition to studies directly examining the 16/17 bp VNTR on the 5HTT gene, it has also been shown that human 5-HTT gene transcription is modulated by a common polymorphism in its upstream regulatory region. Lesch et al. (1996) reported a polymorphism in the transcriptional control region 1 kilo-base upstream of the 5-HTT coding sequence. This region is known as the 5-HTTPLR and it is composed of 16 repeat elements. The polymorphism consists of a 44 base pair insertion or deletion usually within repeat elements 6 to 8. Initial experiments demonstrated that the long (insertion) and short (deletion) variants of the 5-HTT gene-linked polymorphic region (5-HTTPLR) have different transcriptional efficiencies. The short (s) variant of the polymorphism reduces the transcriptional efficiency of the 5-HTT gene promoter, resulting in decreased 5-HTT expression and 5-HT uptake in lymphoblasts. Lesch and colleagues discovered that the basal activity of the long variant (l) of the gene promoter is more than twice that of the short variant of the promoter. Interestingly, uptake of serotonin in cells

homozygous for the long form of the promoter polymorphism was approximately two times that in cells carrying one or two copies of the short variant of the allele (Lesch et al., 1996). These results led to the hypothesis that clinical populations afflicted by disorders of the depression-anxiety spectrum should exhibit either the homozygous s/s or the heterozygous s/l genotype.

This hypothesis was recently confirmed in a Hungarian study which employed 139 unrelated Caucasian females as participants (Gonda et al., 2006). The women in the study presented with the absence of a history of current or lifetime Axis I psychiatric disorders. Using the Temperament Measure (TEMPS-A), which measures affective temperaments on five scales (depressive, cyclothymic, hyperthymic, irritable, and anxious), a significant association was found between the short (s) allele and the TEMPS scores of the depressive, anxious, irritable, and particularly the cyclothymic temperaments; no such association emerged however with respect to the hyperthymic temperament.

A German group (Hoefgen et al., 2005) set out to replicate the findings of the Lesch et al. (1996) study with the goal of employing a larger sample size that was ethnically homogeneous and systematically obtained and screened. A total of 466 patients diagnosed with Major Depressive Disorder (MDD) were recruited from consecutive admissions to the Department of Psychiatry at the University of Bonn in Germany. All participants necessitated inpatient hospitalization and met either *DSM III-R* or *DSM-IV* criteria for MDD. A total of 836 control subjects were randomly recruited from the list of registered inhabitants of the city with the support of the local Census Bureau of the City of Bonn. Patients and control subjects were all of German descent, the criteria of which

was met when both parents and all four grandparents originated from Germany. Results indicated that the short allele of 5-HTTLPR was significantly more frequent in patients (45.5%) than in control subjects (39.9%). Taken together, the results of the above three studies suggest that the short allele of the 5-HTTPLR is implicated in psychological dysfunction within the depression/anxiety spectrum and should be a strong candidate for future research.

Estrogen and the Estrogen Receptor Gene

Affective disorders are more prevalent in women than in men. MDD is the most commonly diagnosed psychiatric disorder among adults with U.S. lifetime prevalence rates of 20 to 25% for women and 9 to 12% for men; point prevalence rates are approximately 6% and 3% for women and men respectively (American Psychiatric Association, 2000). Thus, in terms of MDD there appears to be a sex bias in that rates of MDD in women are approximately twice those of men.

Although the reason for this gender difference is most likely due to a combination of biological, social, and environmental factors, it has been suggested that estrogen may play a key role in this phenomenon (Steiner, Dunn, & Born, 2003). For example, women are known to experience depressive episodes at times of hormonal change in their reproductive years. Affective disorders such as premenstrual syndrome, postnatal depression, and post-menopausal depression are all associated with low serum levels of estrogen (see review by Pearlstein, Rosen, & Stone, 1997). Furthermore, hormone replacement therapy has been shown to alleviate symptoms of postnatal and post menopausal depression (Best, Rees, Barlow, & Cowen, 1992). Thus, estrogen may be involved in the sex difference in rates of depression.

Given the link between estrogen and antidepressant effects, the estrogen receptor may play a role in mood change. The estrogen receptor (ER) consists of two subtypes: α and β, both of which are expressed predominately in limbic-related areas (Osterlund & Hurd, 2001). The ERα receptors are found primarily in the hippocampus and the amygdala (Osterlund & Hurd, 2001), two areas of the brain that are linked to mood and aggression, respectively. In comparison to ERα expression, ERβ mRNA expression is low and generally not detectable in several nuclei of the human amygdala (Osterlund & Hurd, 2001). The amygdaya therefore appears to be ERα dominant, suggesting that it is the α receptor that modulates neurons involved in emotional, affective and motivational behaviours mediated by the amygdala.

Research on the ERα gene indicates that specific polymorphisms of the TA (Thymine- Adenine) repeat may be associated with a number of mood variables. To test the potential contribution of genetic variations in this hormone receptor to anxiety, Comings, Muhleman, Johnson, and MacMurray (1999) examined the association between the alleles of the TA repeat on the ER α gene and the nine subscales and total score of the Symptom Checklist-90 (SCL-90) in a group of 179 adult males treated for substance abuse. The alleles were divided into two groups: short (s) and long (l). Alleles ranged from 179 base pairs to 213 base pairs. Short alleles included repeats 2 to 12 and long alleles included repeats 13 to 19. Analysis of the s/s, l/s, and l/l genotypes showed a significant association of the long alleles with three of the SCL-90 scores: anxiety, phobic anxiety, and total symptoms as measured by the global severity index. Of these, the anxiety score remained significant. There was a progressive decrease in the mean anxiety

score from the l/l genotype, the s/l genotype, to the s/s genotype. It was determined that the ER α gene accounted for 7% of the variance of the anxiety score.

Similar to the Comings et al. (1999) study, Westberg et al. (2003) investigated the repeat polymorphism in the ERα gene in 172 42-year-old women who had been assessed using the Karolinska Scales of Personality (KSP). As with the Comings et al. (1999) study, the alleles were divided into two groups according to base pair numbers. Seventeen alleles were found for the ERα gene, ranging from 183 to 215 base pairs. Alleles falling beneath 191 base pairs were classified as short while alleles corresponding to 191 base pairs and over were classified as long. In order to elucidate the possible influence of the ER alpha gene on the different aspects of personality as measured by means of the KSP, the possible associations between this gene and four different factors (neuroticism, psychoticism, non-conformity, and extraversion) were examined. Initially, neuroticism, psychoticism, and non-conformity all appeared to be associated with the short allele of the ER α gene. However, after correction for multiple comparisons, the associations with the non-conformity factor (including the indirect aggression and irritability subscales), as well as the psychoticism factor (including the suspicion subscale), remained significant. Thus, short alleles on the ERα gene may be associate with non-conformity and psychoticism.

The two studies on the ER α gene thus suggest that longer alleles seem to be associated with anxiety in men (Comings et al., 1999) but shorter alleles are associated with neuroticism, suspicion, irritability, and verbal aggression in women (Westburg et al., 2003). These findings suggest that a smaller number of TA repeats on the estrogen receptor α gene may be a predictor of mood variability in women.

Androgens and the Androgen Receptor

Like estrogen, the androgens are a group of steroid hormones derived from cholesterol. The principle androgens, in order of increasing potency, include dehydroepiandosterone (DHEA), dehydroepiandosterone sulfate (DHEAS), androstenedione, and testosterone (Davison & Davis, 2003). Testosterone is the most commonly referred to androgen and is responsible for the development of secondary sex characteristic in males. The actions of androgens in women remains unclear, however, with the body of research literature focusing primarily on their role in female sexual functioning and libido (Cameron & Braunstein, 2004). There is increasing evidence for widespread distribution of androgen receptors in women in areas such as breast, bone, and brain (Davison & Davis, 2003). This widespread distribution of receptors indicates that androgens and their metabolites may have important roles in pathologies such as breast cancer, osteoporosis, and even cognitive decline (Davsion & Davis, 2003).

In 2002, a group of international experts convened in New Jersey and formed the Princeton Consensus Statement on the definition, classification, and assessment of Androgen Insufficiency Syndrome in women (Cameron & Braunstein, 2004). This session was spawned by the need to clarify the ambiguous role of androgens in women's health. Bolstered by a substantial review of all the current literature, the Princeton Consensus Statement outlines a number of symptoms, both physiological and psychological, thought to be characteristic of androgen insufficiency syndrome. These include a diminished sense of well being or dysphoric mood; persistent unexplained fatigue; and sexual function changes, including decreased libido, sexual receptivity, and pleasure (Bachmann et al., 2002).

The number of CAG repeats on the Androgen Receptor (AR) gene is believed to reflect activity of the AR gene, with lower repeat numbers reflecting higher gene activation. Evidence of this comes from a study conducted by Chamberlain, Driver, and Miesfield (1994) whereby the CAG tract in both humans and rats was eliminated in vitro, resulting in increased transcription of the AR gene.

Recent research suggests that the CAG repeat may play a role in depression. Two studies have found relationships between CAG repeat number and depression in men (Harkonen et al., 2003; Seidman, Araujo, Roose, & Mikinlay, 2001). Harkonen and colleagues (2003) found that CAG repeat number was positively correlated with depression, as expressed by the wish to be dead ($r = .45$; $p < .0001$), depressed mood ($r = .23$; $p = .003$), anxiety ($r = 0.15$; $p < 0.05$), and deterioration of general well-being ($r = .22$; $p = .004$). These results suggest that low AR gene activation (e.g., high repeat numbers) is associated with depression. Although this research was conducted using male participants, CAG repeat number may also play a role in depression and mood variability in women.

Mood Versus Affect

As highlighted by Oinonen and Mazmanian (2002, p. 230), "mood and affect are closely related terms that are often used interchangeably in the literature." They are able to make the point however, that these two terms and their associated characteristics are actually different levels of similar constructs. The American Psychiatric Association describes affect as a pattern of observable behaviours that is the expression of a subjectively experienced feeling state or emotion (APA, 2003). Examples listed include sadness, elation and anger. This is in contrast to mood, which is described as a more

pervasive and sustained emotional state. Mood is likened to climate, while affect or emotion is likened to fluctuating changes in weather within that climate. For example, whereas a full emotion of anger might last for only a few minutes, an annoyed irritable mood might last for hours or days. The semantics surrounding these constructs can cause confusion. Even in the literature, the term *mood* is often used to subsume all affective and emotional states. However, they are in fact different concepts. For the purposes of this project, the terms "affect" and "emotion" will be used interchangeably.

In summarizing his own findings, Watson, in collaboration with Vaidya (2003) describes how mood differs from affect in three important respects. First, mood research focuses almost exclusively on subjective phenomenal experience, while affect or emotions have traditionally been viewed as multi-modal psycho-physiological systems, with at least four distinct components: (1) subjectivity (e.g., feelings of fear and/or apprehension), (2) the physiological (e.g., activation of the sympathetic nervous system), (3) the expressive (e.g., the facial expression of fear) and (4) the behavioural (e.g., flight away from danger) (Watson & Vaidya, 2003). Mood measurement, in contrast, typically involves only the assessment of subjective feelings (component 1), without consideration of the other three components. Second, affect or emotions, as already described by the APA, tend to be brief, lasting perhaps only a few seconds. Third, and finally, the concept of mood subsumes all subjective feeling states, not simply those experiences that accompany classical prototypical emotions such as fear and anger (Watson & Vaidya, 2003).

In a review of the literature, Larsen (2000) highlights another three important ways in which mood differs from affect. Moods, he states, are thought to differ from

emotions in both duration and intensity. Moods generally last longer than emotions, with some moods going on for hours, days, or weeks; while emotions tend to be shorter lived, but more intense. The author states that moods will *nag* at us, but emotions will *scream* at us. It is also argued that moods and emotions differ with regard to their course and how they unfold. Emotions typically have a distinct onset and offset in time, with a peak in between, whereas moods typically build gradually, such that it is difficult to say when they begin or end. This is because emotions often have a distinct cause or object of reference. With moods however, a causal incident or event is not always identifiable. The third and perhaps most compelling way in which moods differ from emotions is through the information that they convey (e.g., emotions generally produce immediate effects on the autonomic nervous system).

Much of the time, mood, as examined in the context of the psychological and medical literature, is examined primarily for the purpose of identifying disturbances outside of the normal range. Thus, clinical research is typically interested in describing the poles of a spectrum of mood that range from depression to mania. Far less research has focused on affect and how it varies in those whose mood does not deviate outside of the normal range. For example, an area of research that has been largely ignored is the examination of individual differences in affect variability. Research investigating individual differences in mood fluctuations throughout the day is important, as individual differences in these variations may help us understand underlying mechanisms involved in affect regulation. This will help our understanding and treatment of mood disorders.

The regulation of affect is central to being able to function in society on a daily basis. At the most basic fundamental level, the regulation of affect is critical to success

in the workplace, at home, or while attending school. Those who are incapable of emotional regulation would likely exhibit a wide range of interpersonal difficulties that may eventually come to interfere with basic task accomplishment and the development of competencies in several areas. For one whose affect has a tendency to fluctuate greatly, it would be very difficult to maintain relationships involving friends, colleagues, and even family. Not only would it be a challenge for such an individual to sustain current relationships, it would also be difficult to develop and maintain fulfilling relationships with new people. Thus, research on individual differences in affective variability and affect regulation is important.

Measurement of Affect

Early mood research tended to emphasize the importance of discrete specific types of affect such as fear-anxiety, sadness-depression, anger-hostility and happiness-joy (McNair, Lorr, & Droppleman, 1971). This approach was favored because structural analyses of mood terms repeatedly identified well-defined content factors corresponding to these specific affective states. Moreover, a common core of discrete affects including fear, sadness, and anger emerged consistently across factor analyses, reflecting different pools of items (Watson & Clark, 1994).

The problem with this approach lies in the fact that measures of different specific affects are strongly interrelated and tend to show questionable discriminant validity (Watson & Vaidya, 2003). In other words, correlations among similar affects tend to be quite strong. For example, those who experience significant levels of one type of negative affect (e.g., anger) tend to report elevated levels of other types of negative affects (e.g., fear, sadness, and guilt). Similarly, individuals who report one type of

positive affect, also tend to indicate higher levels of other types of positive affect. Thus, an individual who reports being joyful may also endorse items indicating energy, enthusiasm, or interest. Multi-trait multi-method analyses can therefore be said to demonstrate strong evidence for non-specificity in these affective states, or strong positive correlations among similarly valenced affects. This is in contrast to specificity which would indicate a unique relationship between items examining the same target affect.

Dimensional models of affect have been most prevalent over the past two decades with models proposed by both Russell (1980) and Watson and Tellegen (1985). Each model proposes that mood exists along a two dimensional circumplex. In each of the circumplex models, mood descriptors are represented along the outside of a circle. In the model presented by Russell, the circumplex is presented as four bipolar dimensions that are spaced 45 degrees apart: Pleasantness (pleasure vs. misery), Excitement (excitement vs. depression), Activation (arousal vs. sleepiness) and Distress (distress vs. contentment). Russell (1980) suggested that these interrelationships could be represented by a spatial model in which affective concepts fall in a circle in the following order: pleasure (0°), excitement (45°), arousal (90°), distress (135°), displeasure (180°), depression (225°), sleepiness (270°), and relaxation (315°).

Using a greater amount of self report data, Watson and Tellegen (1985) put forth their own circumplex model. Similar to the model of Russell (1980), their circumplex presented four bipolar dimensions that were also spaced 45 degrees apart, but with differing labels on the poles. The poles of Watson and Tellegen are instead labeled: Pleasantness (happy vs. sad), Positive Affect (excited vs. sluggish), Engagement (aroused

vs. still) and Negative Affect (distressed vs. relaxed). What is noteworthy about this model is the fact that Positive and Negative Affect each occupy their own dimension and that they are not simply on opposite ends of the same spectrum. According to Tellegen (1985), Positive Affect and Negative Affect are preferable dimensions to arousal and pleasantness-unpleasantness, in part because they relate more directly to major personality factors (Hepburn & Eysenck, 1989). Perhaps one of the most noteworthy aspects regarding the theoretical approach of Tellegen is the parallel illustrated between the two-dimensional mood space formed by Positive Affect and Negative Affect and the two-dimensional personality space formed between neuroticism and extraversion. (Hepburn & Eysenck, 1989; Tellegen, 1985). Specifically, Tellegen (1985) proposed that extraversion predicts positive affect, whereas neuroticism predicts negative affect. In a study where participants completed three mood questionnaires a day each weekday for three weeks, Hepburn and Eysenck (1989) found that mean positive affect levels were best predicted by extraversion, but that mean negative affect was strongly related to neuroticism. Interestingly, affective variability was related to both personality factors with neurotic extraverts exhibiting the most variability and stable introverts having the least mood variability.

Watson and Tellegen (1985) have further proposed that general dimensions (e.g., positive and negative affect) and discrete affects (e.g., anger, fear, and joy) are not mutually exclusive, but rather, reflect different levels of a single integrated hierarchical scheme. This means that the higher order dimensions of negative affect and positive affect can each be broken down into several correlated yet distinct affective states. For example, the dimension of negative affect can be decomposed into such specific negative

affective states such as fear, hostility, and sadness. The hierarchical scheme that is hypothesized proposes that the specific content of the mood descriptors (such as fear, hostility and anger as described above) exists at the lower levels. These include labels that describe distinctive qualities of the individual discrete affects. This is in contrast to the upper level of the hierarchy (e.g., negative affect or positive affect) which is said to reflect the valence of the descriptors – that is whether they reflect positive or negative affective states.

Watson and Clark (1992) published a paper supporting the existence of such a hierarchical arrangement of affect for negative affect. They conducted four independent studies examining the relationships among fear, hostility, sadness, and guilt through a series of multi-trait, multi-method matrices. Consistent with a hierarchical model, all four negative affects showed significant convergent validity and adequate discriminate validity, indicating that they represent meaningful and differentiable psychological constructs.

Studies Measuring Affect

While numerous studies have examined day-to-day affect over three or four week periods (e.g., Oinonen and Mazmanian, 2001), only a few studies have examined variability in affect during the course of the day. Clark and Watson (1988) conducted a study to assess the relationship between common events and two independent mood factors: positive affect and negative affect. A sample of 18 upper-class psychology students was recruited for the study. Participants were asked to record their moods three times a day (morning, afternoon, and evening) for 90 days. Subjects rated themselves on 57 mood terms using a five-point scale ranging from 1 (*applies very little or not at all*) to

5 (*applies very much*). In addition to the affect terms, each affect rating form contained six items querying subjects' daily experiences. Daily experiences were broken down into six categories including physical health problems, any drugs they had taken including alcoholic beverages, any notable experiences or events, things that were on their mind, number of hours they had slept the night before, and for females, whether they were experiencing their menstrual period. Positive affect was found to be associated with a wide range of daily events, whereas fewer correlations were found between these events and negative affect. The relation between high PA and reported social interactions (particularly physically active social events) was especially robust whereas the correlation between NA and physical problems was especially strong.

In another study examining the relationship between daily events and mood, Kennedy-Moore, Greenburg, Newman, and Stone (1992) examined the determinants of daily affect variability. Ninety–four subjects completed a mood diary every 15 minutes for one day, and subjects' emotions, activities, and locations were assessed. Although it did not contain a validated measurement tool (such as the PANAS or the Mood Adjective Checklist), the diary was designed to assess the psychosocial environment of the participants and consisted of a sheet divided into five sections, one of which examined affect. The first section asked for the time of day and physical position (sitting, standing, or reclining). The second section asked participants to indicate their location using response options (e.g., home, office, commuting). The third section gave participants 29 options to select with regard to current activities. The 29 options could be further divided into three categories consisting of physical, mental/social, and non-work. Participants could check multiple activities, however, the three most taxing were coded. A fourth

checklist was used for characterizing the social environment. The fifth and final section asked subjects to indicate their emotion by selecting an adjective from a list. It is not specified how these adjectives were derived or what methodology was used to select them. The adjectives used to assess affect consisted of the following: neutral, annoyed, angry, anxious, bored, excited, happy, rushed, sad, tense, tired, and stressed. The presence of linear trends suggested that mood increased or decreased throughout the day, partially as a function of activity and location. Negative linear trends were found for "annoyed, anxious, and rushed" indicating that as the day progressed, the occurrence of these moods decreased. The opposite was the case for positive moods in that their prevalence tended to increase as the day progressed. Despite not using a validated tool to measure affect, this study is noteworthy in that it is one of the few to examine change in affect within one day and to examine predictors of this change.

In a study conducted in Germany, Peters, Nicolson, Berkhof, Delespaul, and De Vries (2003) used experience sampling methodology (ESM) to investigate changes in NA and PA following minor daily events in those with MDD compared with healthy participants. The study was conducted because, although it is known that MDD is characterized by high negative affect (NA) and low positive affect (PA), little is known about emotional reactivity in daily life for individuals with MDD. ESM was used to collect data from participants at selected moments during their daily activities. Participants received auditory signals (beeps) from a wristwatch programmed to emit 10 beeps between 7:30 a.m. and 10:30 p.m. each day, at semi-random intervals of approximately 90 minutes. After receiving a beep, participants completed self-report forms concerning current mood, negative and positive events, and their appraisals.

Participants completed ESM reports for six consecutive days, including a weekend. Momentary mood states were assessed with 16 adjectives rated on 7-point scales ranging from 1 (*not at all*) to 7 (*very*). Two eight-item scales derived from a factor analysis were used to measure positive and negative affect. The items *anxious*, *irritated, restless*, *tense*, *guilty*, *irritable*, *easily distracted*, and *agitated* were averaged to form a NA scale; while the items *energetic*, *enthusiastic*, *happy*, *cheerful*, *talkative*, *strong*, *satisfied*, and *self-assured* were averaged to form a PA scale. The results showed that contrary to expectation, MDD participants did not report more frequent negative events, although they did report fewer positive events. Further analysis revealed that both NA and PA responses to negative events were blunted in the MDD group, whereas responses to positive events were enhanced. NA responses to negative events persisted longer in MDD participants. Depressed participants with a positive family history or a longer current depressive episode showed relatively greater NA response to negative events.

At least three studies have examined changes in affect throughout the course of one day. None of those studies have reported individual difference predictors of low versus high affect variability throughout the day. Furthermore, no previous studies have examined genetic predictors of individual differences in predisposition to low or high affective or emotional reactivity.

Affect Induction Procedures (AIPs)

One method used to explore individual differences in predisposition to affective reactivity involves inducing a positive or negative mood in participants and then examining predictors of low versus high emotional reactivity. In a review of nearly 250 studies spanning a ten-year period, Gerrards-Hesse, Spies, and Kordelia (1994) have

classified all mood or emotional induction procedures (MIPs) into five distinct categories. The first category of MIP is that which employs the free mental generation of emotional states. Included in this category is hypnosis and imagination. In the case of the hypnosis MIP, subjects enter a deep trance and then are instructed to remember and imagine a certain situation of their own choice in which they felt happy or sad. In studies working with the imagination MIP, subjects are instructed to imagine and re-experience situations or events in order to evoke the intended mood.

The second category of MIP is that which is based on the guided mental generation of emotional states. Included in this category are film and story MIPs, as well as music MIPs. For example, experimenters will typically present a story or a short description of a situation to their subjects and instruct them to imagine the situation and "get involved" in the feelings suggested. In the case of the MIPs involving music, subjects listen to a mood-suggestive piece of classical or modern music and are instructed to try to get into the mood expressed by the music using whatever means they find most effective.

The third category is much like the second except that participants are not instructed to try to put themselves in a mood that is congruent with the story or film. It is assumed that the participants' mood will change based on the emotional quality of the material being presented. This category of MIP is known as the presentation of emotion inducing material.

Category four is represented by MIPs that are related to the presentation of need-related emotional situations. Therefore procedures expose subjects to situations activating certain needs, such as the need for achievement or affiliation. An example is the

success/failure MIP, which relates the need for achievement. For example, in such a procedure participants may be given false-positive or false-negative feedback concerning their performance in a test alleged mainly to assess cognitive abilities.

The fifth and final category deals with MIPs aiming at the generation of emotionally relevant physiological states. This type of MIP typically involves the systematic variation of physiological states, usually in combination with a variation of situational stimuli to influence mood states. For example, to induce physiological arousal, studies applying the drug MIP use a drug (e.g., epinephrine), or a placebo introduced to subjects as a mood-inducing drug. Similar to this are the MIPs employing the facial feedback hypothesis which hypothesizes that facial expressions influence mood state. Accordingly, experimenters using the Facial Expression MIP instruct subjects to contract and relax different muscles to produce a frown or a smile, thereby inducing a negative or positive emotional state.

Previous research has not examined whether any genes are predictive of individual differences in response to MIPs. The present study employed an MIP that contained elements of both categories three and four. Participants were told that they would be given a test of their intelligence, and then the Mental Rotations Test (Vandenberg, 1971) was administered. The goal of this MIP was to attempt to induce a mood state characterized by anxiety, stress, or unpleasantness. It has been shown that tasks that are presented to participants relating to intelligence are generally believed to induce anxiety associated with evaluation and performance (Mogg, Matthews, Bird, & MacGreggor –Morris, 1990). Thus, In addition to activating the need for achievement, this MIP would also place the participant in a self perceived success/failure situation. It

was hoped that a situation that purported to assess their three-dimensional intellectual capacity would be especially stressful, inducing either anxiety or an increase in negative affect.

The Present Study

One hundred and forty-six women participated in the present study examining whether emotional variability and reactivity differ as a function of specific genetic polymorphisms. DNA samples were obtained in order to determine the number of VNTR polymorphisms on estrogen, androgen, and serotonin transporter genes. Women were then subject to an in vivo mood induction anxiety-producing task that was preceded and followed by the completion of an affective rating scales. Following the mood induction, women were asked to complete eight consecutive hourly mood surveys over the course of one eight-hour period. The focus was to examine the relationships between number of VNTR repeats on three specific genes and emotional and mood variability on two tasks.

Our hypotheses were threefold in this study. It was hypothesized that emotional variability and/or reactivity as measured using the "*in vivo*" laboratory mood manipulation and self-report affect measures would differ among the women according to genotype. Specifically it was hypothesized that numbers of: (1) 9 or 12 repeats in a VNTR polymorphism of a 17 base pair length element within intron 2 of the Serotonin transporter gene (5-HTT), (2) CAG repeat numbers on the Androgen Receptor (AR) gene, and/or (3) TA repeats on the Estrogen Receptor α (ERα) gene, would be significant predictors of emotional reactivity and/or emotional variability in women.

Method

Participants

Two-hundred-and-seventy-nine women (mean age = 19.60 years, *SD* = 2.40) from Lakehead University and the Thunder Bay community were recruited to participate in a study investigating *Genetic Factors Affecting Women's Health*. This study received approval by the Lakehead University Research Ethics Board. The majority of participants were students from introductory and upper year psychology courses. Those in introductory psychology received one bonus point of course credit for participation in the screening phase of the study. If participants met the criteria necessary to participate in additional phases of the study, they were eligible to receive up to two additional bonus points (one per phase).

Based on information provided by the screening questionnaire, 146 women (mean age = 19.63 years, *SD* = 2.40) were selected for the laboratory phase of the study (phase 2). These women were representative of three different groups: (1) *Mood Change Group* (*n* = 49). This group consisted of 23 previous oral contraceptive (OC) users who reported OC-related negative mood change and 26 current OC users who report previous or current negative mood change while taking OCs. (2) *No Mood Change Group* (*n* = 43). This group consisted of 26 current OC users and 17 previous OC users who reported no history of OC-related mood change. (3) *Control Group* (*n* = 54). The control group was comprised of women who had never taken hormonal contraceptives. The above groups were selected for methodological reasons relevant to a larger study (Oinonen, 2006). However, this type of sample likely included women who are particularly vulnerable to hormonal mood effects (i.e., the mood change group). This should increase the likelihood

of finding a relationship between mood variability and VNTRs on hormonal genes, if these genes do affect emotional variability. Of most relevance to the general population, however, may be the results from the control group, as these women were randomly selected based on their status as never-users of hormonal contraceptives.

A total of 62 women (mean age = 19.76, *SD* = 2.90) participated in phase three of the study, the hourly mood rating phase. The only exclusionary criterion used was the ongoing use of medication that could affect mood (e.g., antidepressants or sedative-hypnotics). These participants were excluded as such medications cause exogenous effects on mood and could produce a blunting effect in their affective response, which would subsequently have affected variability in the hourly mood surveys. This was an important exclusionary criterion given that the purpose of the hourly mood rating phase was to examine genetic predictors of daily affective/emotional fluctuation.

Measures

Screening Questionnaire (SQ). The screening questionnaire (SQ) (see Appendix A) includes several different sections. The screening questionnaire was used to select participants based on the three groups described above. It included six sections including demographics, reproductive history, contraceptive history, medical and health history, psychiatric history, eating habits, and personality/mood. Many of the questions were taken from the questionnaires developed for Oinonen's two previous projects on hormonal effects, as reliability and validity statistics were available. The demographic section included questions relating to age, education, relationship status, and ethnicity. Questions about reproductive history included information about age of menarche, parity, length of menstrual cycle, length of menses, premenstrual symptoms, and cycle regularity

(both before and during hormonal contraceptive use). The section on contraception contained questions about sexual activity, contraceptive history, reasons for specific contraceptive choices, types of OCs taken, reasons for discontinuing specific contraceptives, duration of contraceptive use, and specific questions about the experience of certain OC side effects, including positive and negative mood change. The medical and health history questions included information regarding medical and hormonal conditions, medication use, alcohol and drug use, smoking status, and family history of hormonal medical conditions (e.g., thyroid disorders, diabetes, breast cancer) or fertility problems. A section on personal and family psychiatric history contained questions about postpartum depression, schizophrenia, alcoholism, unipolar depression, and bipolar disorder. Finally, scales from four questionnaires were included to collect information about mood, personality, eating habits, and sociosexuality. These included the Depression Scale from the Symptom Checklist 90-Revised (SCL-90) (Derogatis, 1994), the PANAS (Watson, Clark & Tellegen, 1988), the Neuroticism and Extraversion Scales from the Revised NEO Personality Inventory (NEO-PI-R; Costa & McRae, 1992), the Eating Disorders Inventory (Garner, 1991) and the Sociosexual Orientation Inventory (SOI) (Simpson & Gangestad, 1991). The latter two scales are not relevant to the present study. The personality section of the SQ assessed the participants' tendency toward neuroticism and extraversion, two dimensions of personality that have been found to correlate highly with mood (Egloff, 1998). These characteristics were assessed using the NEO-PI R. It was estimated that the questionnaire would take approximately 45 minutes to complete.

Mental Rotations Test (MRT) Anxiety Induction Paradigm. In order to examine individual differences in mood reactivity, an anxiety induction paradigm was employed.

Participants were told they would be completing an intelligence test and were then given the Mental Rotations Test. Mood/affect ratings were completed both before and after the mood induction to assess any change in affective states. The Vandenberg (1971) adaptation of Shepard and Metzler's (1971) mental rotations test (see Appendix B) is a measure of visuospatial ability that involves mentally rotating a target three-dimensional shape and matching it to other three-dimensional shapes. The participant is required to choose which two of four drawings (presented as possible answer choices) depict the target drawing in a rotated position. The test consists of two sections, each containing ten items, making the total number of items twenty. Each item consists of a criterion figure, two correct alternatives, and two "distracter" items. The correct alternatives are structurally identical to the criterion but are shown in a rotated position. For half of the items, the distracters are rotated mirror-images of the criterion while distracters for the other half of the items are rotated images of one or two of the other structures. In total, there are 40 correct responses. Standard test instructions were employed. However, given that the test was used for the purpose of inducing an anxious state, participants were initially told they would be timed and their responses later scored. Prior to beginning the MRT, participants were given approximately two- and -a-half minutes to complete three sample problems, which were provided for the purpose of allowing participants the opportunity to familiarize themselves with the format of questions on the test. Following the completion of the three sample problems, the women were given three minutes to complete each of the two parts (10 items each). Thus, total testing time was six minutes. As the test consists of 20 questions, and each question has two correct answers, the test was scored out of 40 possible points. Participants were allotted one point

for each correct answer they selected. For the MRT, Vandenberg and Kuse (1971) report adequate Kuder-Richardson-20 internal consistency (.88), test-retest reliability (.83), and split-half reliability corrected by the Spearman-Brown formula (.79).

Laboratory Mood Questionnaires. Two similar questionnaires were used to assess mood reactivity in the laboratory session. One questionnaire was administered prior to the completion of the mental rotation test (MRT) anxiety induction paradigm and the second questionnaire was completed after the MRT. The first questionnaire was entitled the Pre-MRT Questionnaire (see Appendix C). It consisted of three individual tests: the Beck Anxiety Inventory (BAI) (Beck, 1990), the Pleasantness-Unpleasantness Scale (Diener, Larsen, Levine, & Emmons, 1985), and the Positive-Negative Affect Scale Expanded (PANAS-X) (Watson & Clark, 1994) (see below for a description of these scales). The second questionnaire administered to participants was entitled the Post-MRT Questionnaire (see Appendix D). This test also contained the BAI, the Pleasantness-Unpleasantness Scale, and the PANAS-X. In addition, the Post-MRT Questionnaire contained specific questions asking the participants how well they thought they performed on the test and what percentage of items they thought they answered correctly on the test.

Beck Anxiety Inventory (BAI). The BAI measures the severity of self-reported anxiety (Beck, 1990). It consists of 21 descriptive anxiety symptoms which are rated on a four-point scale with the following correspondence: 0 (*not at all*), 1 (*mildly, it did not bother me much*), 2 (*moderately, it was very unpleasant, but I could stand it*), and 3 (*severely; I could barely stand it*). The 21 items include *numbness or tingling, feeling hot, wobbliness in legs, unable to relax, fear of the worst happening, dizzy or light headed,*

heart pounding or racing, unsteady, terrified, nervous, feelings of choking, hands trembling, shaky, fear of losing control, difficulty breathing, fear of dying, scared, indigestion or discomfort in abdomen, faint, face flushed, and sweating (not due to heat). Analysis has revealed that the BAI correlates highly with other measures of anxiety, including the Hamilton Anxiety Scale Revised (r = .51) and the anxiety subscale of the Cognition Check List (CCL-A) (r = .51) (Beck, Epstein, Brown, & Steer, 1988). The BAI was constructed to measure symptoms of anxiety that are minimally shared with those of depression (Beck & Steer, 1990). With their diagnostically mixed sample of 160 outpatients, Beck, Epstein, Brown, and Steer (1988) reported that the BAI had a high internal consistency (Cronbach coefficient alpha = .92). This same study reported a test retest reliability of .75.

Pleasantness-Unpleasantness Scale. The second mood measure on both of the Laboratory Mood Questionnaires assessed the pleasantness component of positive affect. The pleasantness scale contained the words *happy, joyful, content, at ease,* and *calm* (Diener et al., 1985). Conversely, the unpleasantness scale contained the words *sad, blue, downhearted, alone,* and *lonely* (Diener et al., 1985). Participants were asked to indicate how they felt at the moment they were completing the surveys. They rated each adjective on a five- point likert-type response scale which was anchored by the values 1 (*very slightly or not at all)*, and 5 (*extremely*).

Positive and Negative Affect Scale Expanded (PANAS-X). The PANAS-X is an expansion of the original PANAS that attempts to identify the higher order dimensions of Positive Affect (PA) and Negative Affect (NA). To assess these specific emotional states, Watson and Clark (1994) created a 60-item expanded version of the PANAS. In

addition to the two original higher order scales (PA and NA), the PANAS-X measures 11 specific affects: fear, sadness, guilt, hostility, shyness, fatigue, surprise, joviality, self-assurance, attentiveness, and serenity. The PANAS-X thus provides for mood measurement at two different levels. Internal consistency reliabilities (Cronbach's coefficient alpha) for both scales are high, generally ranging from .83 to .90 for Positive Affect, and from .85 to .90 for Negative Affect. Test retest reliability indicates that scores on all of the scales are quite stable, with coefficients ranging from .51 (for Serenity) to .71 (for general Negative Affect in the larger sample) (Watson & Clark, 1994). The stability coefficients based on general instructions are consistently higher than those based on "past week" instructions, which further validates the use of different time instructions with the PANAS-X scales. The PANAS-X was completed in the laboratory both before and after the mental rotations test anxiety induction paradigm (along with the other two mood measures).

Hourly Mood Questionnaire. The Hourly Mood Questionnaire (see Appendix E) is a take home questionnaire that participants completed eight times over the course of one eight-hour period. The questionnaires were completed approximately one hour apart. The questionnaire was designed with the intention of monitoring daily mood variability. It consisted of the Positive and Negative Affective Scale (PANAS) (see below) and the Pleasantness-Unpleasantness questionnaire (see above). The first seven questionnaires given to the participants were identical. The final questionnaire, in addition to consisting of the PANAS and the Pleasantness-Unpleasantness questionnaire, also contained several additional questions (see Appendix F). The questions inquired as to whether anything occurred during the course of the day that may have affected their mood; how many

hours of sleep they acquired the night before; and whether or not they exercised, took any drugs or medications, drank alcohol, or drank coffee.

Positive and Negative Affect Scale (PANAS). The PANAS (Watson, Clark, & Tellegen, 1988) consists of two scales: one for positive affect (PA) and one for negative affect (NA). High positive affect reflects a state of high energy, full concentration, and pleasurable engagement; whereas low PA is characterized by sadness and lethargy. In contrast, NA is a general dimension of subjective distress and unpleasant mood states, including anger, contempt, and disgust. Low NA indicates a sense of calm and security. The PA PANAS items include *attentive, interested, alert, excited, enthusiastic, inspired, proud, determined, strong,* and *active,* while the PANAS scale for NA includes the items *distressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery,* and *afraid.* In the current study, participants were asked to indicate how they felt at the moment they were completing the surveys. They were asked to rate each adjective on a five-point response scale ranging from 1 *(very slightly or not at all)* to 5 (*extremely).* The PANAS was also used in the take home phase of the study in which participants were required to complete an affect survey at eight intervals during one 24-hour period.

Genotyping . The DNA samples were genotyped in the Paleo-DNA Laboratory at Lakehead University under the supervision of Dr. Carney Matheson. For the purposes of this study, genomic DNA was isolated from the buccal tissue using the chelex extraction method which has been shown to be as efficient or more efficient than using proteinase K and phenol-chloroform extraction (Walsh, Metzger, & Higuchi., 1991). The following three different repeat regions were quantified: CAG repeats on the androgen receptor (AR) gene, TA repeats on the estrogen receptor α (ERα) gene, and the 16/17 base pair

repeat in the serotonin transporter (5-HTT) gene. The different regions were subsequently amplified by polymerase chain reaction (PCR) using fluorescently labeled primers. The fluorescently labeled DNA fragments were analyzed by size with automated capillary electrophoresis using an ABI 3100 Automated Sequencer (Applied Biosystems) and analyzed using genescan software. This method provided measures of the number of repeats at each polymorphic region for both alleles for each participant.

Procedure

Screening Phase. In the screening phase of the project, women were recruited from Lakehead University and the surrounding community of Thunder Bay. Recruitment was primarily targeted at undergraduate psychology classes. The women were asked to participate in a study on genetic factors in women's health. All participants completed Consent Form A (see Appendix G), a Screening Questionnaire, and received Debriefing Form A (see Appendix H). Based on information obtained from the screening questionnaires, three groups of women were selected: (1) Mood Change Group, (2) No Mood Change Group, and (3) Control Group (as discussed above). Each woman deemed appropriate for the subsequent laboratory phase was contacted by telephone. The telephone call was used to convey the requirements of the next stage of the study, and also to determine whether the women were interested in participating. If the participant indicated interest, an appointment for a laboratory session was then scheduled.

Laboratory Phase. Each laboratory session lasted approximately 35 minutes and involved the completion of consent form B (Appendix I); the collection of a DNA sample by buccal swab, the measurement of height, weight and finger lengths; the counting of digit hairs (for the larger study); and completion of the 'in vivo' mood manipulation task.

Buccal swabs of DNA were collected by having the research assistant rotate a collection swab on the inside of each woman's cheek. Once collected, the swab was dried at room temperature, placed in a plastic bag, and then stored in a freezer set to -34 degrees Celsius.

With regard to body measurements, a fixed tape measure was used to measure height and a digital scale was used to measure weight. Mitutoyo Electronic Digital calipers (Model MIT-500-171) were employed in the measurement of the length of digits 2 to 5. Both left- and right-sided traits were measured twice to reduce measurement error and to assess test-retest reliability. Each digit on both the left and right hands was measured on its ventral surface from the basal crease to the tip, using the electronic digital calipers measuring to 0.01 mm. These measurements have shown a high degree of test-retest reliability (Manning et al., 1998). The number of hairs on the back of the second phalanges of each digit were also counted, as these digits are differentially sensitive to testosterone and hair number appears to be an indicator of individual differences in testosterone sensitivity or exposure (Manning, 2002). All of the body measurements were collected for the purposes of the larger study.

Upon entering the lab, the buccal DNA swab and the body measurements of the participants were taken. Following the collection of body measurements, participants were asked to complete the first Laboratory Questionnaire or the Pre-MRT test. Then the mood induction procedure was initiated. Participants were presented with the Mental Rotations Test and they were instructed that they would be completing a brief test which would assess their level of intelligence. They then completed the MRT. The intention of the instructions used with the MRT test was to induce a negative or anxious mood state in

participants which could be measured by examining the change between the Pre-MRT and Post- MRT Test affect scores. After completion of the MRT, participants completed the Post- MRT laboratory Questionnaire. Following the administration of the Post MRT questionnaire, women received a full debriefing about the mood induction procedure and further information about the experiment. They were also provided with Debriefing Form B (see Appendix J) which explained the purpose of the study and also provided a listing of various mental health resources within the community. In addition, participants were given the opportunity to complete Consent Form D (see Appendix M) which involved providing consent to allow the experimenters to contact them for a possible follow-up study within the next five years (if such a project receives ethical approval by the Research Ethics Board).

Hourly Mood Ratings. After participants received Debriefing Form B, they were asked whether they would like to participate in the third and final stage of the study. Those interested in participating received Consent Form C (see Appendix K). Participants were asked to complete the Hourly Mood Questionnaire once every hour for eight consecutive hours. They were instructed to complete these questionnaires as soon as possible after the laboratory phase. The women were provided with two packages of the eight questionnaires. The second package acted as a spare in case they were unable to complete the first as instructed. They were also provided with a summary of the instructions (see Appendix E) indicating that if they failed to complete the assessment in one day, they could simply start a new assessment the next day using the extra materials. The Hourly Mood Questionnaire was used to indicate the amount of variability in participant affect over the course of an eight hour period. When complete, participants

returned their questionnaires to the lab where they were provided with Debriefing Form C (see Appendix L) and a description of the entire study.

Genetic Analyses

Extraction. Genomic DNA was isolated from the buccal tissue using a Chelex extraction method (Walsh et al., 1991). A 10% Chelex solution was added to each sample, after which samples were incubated at 56 degrees Celsius and mixed at 500 rpm for approximately 3 hours.

Amplification. Amplification was achieved via polymerase chain reaction (PCR). To a 2.0 µL sample of genomic DNA the following solutions were added: 5.0 µL PCR buffer, 2.0 µL dNTP's, 0.5 µL each of forward and reverse primers, 1.5 µL MgCl2, 0.2 µL polymerase, and 38.3 µL H20 to a total of 50.0 µL. Samples were then transferred to the Eppendorf Mastercycler for a Hot Start at 94°C for 3 minutes. The conditions for 45 cycles consisted of denaturation at 94°C for 1.5 minutes, annealing at 57.7°C for 1 minute, and extension at 72°C for 2 minutes. Final extension occurred at 60°C for 1 hour, at which point samples were held at 4°C until sequencing.

G*enetic Analysis*. To each amplified sample 0.3 µl of size standard and 9 µl of Hi-Di Formamide were added. Samples were then denatured at 95°C for 3 minutes and then immediately chilled on crushed ice for 2 minutes. The labeled DNA fragments were analyzed by size using automated capillary electrophoresis using an ABI 3100 Automated Sequencer (Applied Biosystems) and analyzed using the Genescan software. This measured the number of repeats at each polymorphic region for both alleles for each participant.

Data Reduction and Statistical Analyses

The analyses consisted of six multivariate analyses of variance (MANOVAs) with follow-up univariate ANOVAs to determine whether women differed in mood variability or reactivity based on the Androgen Receptor (AR) gene, the Estrogen Receptor α gene (ERα), or the Serotonin Transporter Gene (5HTT). Each MANOVA included genotype group (from one gene region) as the independent variable. The dependent variables were Overall Mood Change, Overall Positive Affect Change, and Overall Negative Affect Change for the laboratory emotional reactivity analyses; and Positive Affect Variability, Negative Affect Variability, Pleasantness Variability, and Unpleasantness Variability for the hourly affect variability analyses. For the AR and ERα genes, genotype groups were determined by performing a median split based on the frequency of the number of repeats for each allele. For these genes, alleles with repeat numbers falling beneath the median were labeled as short(s), while those falling above the median were labeled as long (l). As the genotype of each gene of each participant includes two alleles, a median split of the data resulted in three allelic combinations: homozygous short (s/s), heterozygous (s/l), and homozygous long (l/l). For the AR genotype, CAG repeats corresponding to ≤ 244 base pairs were categorized as short, while those that corresponded to ≥ 245 base pairs, were categorized as long. For the ERα gene, TA repeats that corresponded to ≤ 191 base pairs were categorized as short, while those that corresponded to ≥ 192 base pairs were categorized as long. Genotypes containing both a short and long allele were heterozygous and those that were either short/short and long/long were termed homozygous.

The five 5HTT genotype groups were based on actual genotype: 8/10, 9/10, 10/10, 10/12 and 12/12. Because the 8/10 and 9/10 groups contained few participants (n

= 1 and n = 3 respectively), they were combined to form one group. Thus, the two MANOVAs conducted with 5HTT genotype as the independent variable, employed four groups (actual genotype) within the independent variable rather than three (e.g., s/s, s/l or l/l).

The seven dependant variables were measures of emotional reactivity that were obtained in the laboratory, and measures of affect variability determined from the hourly mood surveys. Overall Lab Mood Change was calculated by transforming the absolute values of the pre-post difference scores from the eleven scales of the PANAS-X into z scores and then adding them together. Positive and Negative affect change within the lab was determined by subtracting pre-administration PANAS scores from post-administration PANAS scores. For the hourly mood survey variables, a score was derived for each hour that a survey was completed. Thus, at the end of the take home exercise, each of Positive Affect, Negative Affect, Pleasantness, and Unpleasantness had eight scores, one for each hour. Variability for each of these four DVs was determined by grouping all eight scores for one variable together and calculating the variance. MANOVAs were then performed using the ERα, AR, and 5HTT genotypes as independent variables and laboratory affect change scores and hourly mood rating variability scores as dependent variables.

Results

Data screening

Prior to the main analyses, the distributions of the three laboratory dependent variables (DVs) (Overall Lab Mood Change, Lab Positive Affect Change, and Lab

Negative Affect Change) and the four DVs from the take-home exercise (Hourly Positive Affect Varibility, Hourly Negative Affect Variability, Hourly Pleasantness Variability, and Hourly Unpleasantness Variability) were examined as a function of genotype group for the presence of univariate and multivariate outliers (e.g., Tabachnick & Fidell, 2001).

Using the criteria of $\geq |3.29|$ (e.g., Tabachnick & Fidell, 2001), one univariate outlier was found for Lab Negative Affect Change for the homozygous long allele group on the AR gene. Another outlier was found in the heterozygous allele group for Negative Affect Variability on the same gene. A logarithmic transformation of negative affect variability was consequently employed to decrease the influence of outliers in the AR gene analysis.

Two univariate outliers were found for the ERα gene distribution of scores, each within the heterozygous allelic group. The first outlier was found for the Lab Negative Affect Change distribution and the second outlier was found for the Overall Lab Mood Change distribution. These outliers were dealt with using logarithmic transformations.

For the 5HTT gene, an additional two univariate outliers were found. The first outlier was found in the 10/10 genotype group within the Lab Negative Affect Change distribution, and the second was found in the 12/12 genotype group within the Overall Lab Mood Change distribution of scores. As was the case with the outliers found on the other genes, these distributions were outlier-free following a logarithmic transformation.

Following screening for univariate outliers, Mahalanobis distances ($p < .001$ criterion) were used to screen for multivariate outliers. For the purposes of this screening exercise, the three measures of affect variability obtained from the laboratory session (Overall Lab Mood Change, Change in Lab Positive Affect, Change in Lab Negative

Affect) were examined together and the four affect variables obtained from the take home exercise (Hourly Positive Affect Variability, Hourly Negative Affect Variability, Hourly Pleasantness Variability, and Hourly Unpleasantness Variability) were examined. Distances were calculated across variables for each genotype group within each gene for both sets of variables. Analyses revealed only one multivariate outlier which corresponded to participant number 141 within the heterozygous genotype group of the AR gene for the laboratory group of variables. It was decided that this individual would be deleted from the MANOVA examining the effect of AR genotype on laboratory affect change.

Assessing Multivariate Assumptions

Before undertaking analyses to test the main hypotheses, the data were examined to ensure that the assumptions of MANOVA were met. Graphical checks of linearity using bivariate scatterplots indicated that linearity was adequate for all dependent variables. Criteria for normality included passing a visual check of the distribution of scores as well as using the following formulas: [(skewness ÷ standard error of skewness) < 3] and [(kurtosis ÷ standard error of kurtosis) < 3]. The assumption of normality was judged adequate for all distributions.

Box's M multivariate test for homogeneity of variance-covariance matrices found adequate homogeneity of variance-covariance matrices for the first three analyses using genotypes on the AR, ERα, or 5HTT genes as the independent variable (IV), and variables from the laboratory anxiety induction procedure as DVs. Box's M results for these anxiety induction lab analyses variables were adequate: F (12, 16525) = 1.08, p > .05, for AR genotype; F (12, 5358) = 1.54, p >.05. for ERα genotype; and F (18, 500) =

0.64, $p > .05$ for the 5HTT gene. All three MANOVAs using the variables from the take home hourly mood ratings as IVs and genotype as DVs produced significant values for the Box's M test, ($p < .001$) indicating heterogeneity of variance–covariance matrices. Examination of cell sample sizes and cell variances and covariances indicated that smaller sample sizes were sometimes associated with larger variances. This suggests the possibility that significance tests may be liberal (Tabachnick & Fidell, 2001).

For the purposes of examining multicolinearity, correlations between the seven dependent variables are listed in Table 1. With regard to the three variables assessed in the laboratory anxiety induction procedure, Overall Lab Mood Change was found to be negatively correlated with Lab Positive Affect Change, indicting that greater lab mood change was associated with a decrease in PA. With regard to the variables assessed in the take home hourly affect ratings, significant positive correlations were found between Positive Affect Variability and Pleasantness Variability, and Negative Affect Variability and Unpleasantness Variability. The relationships between these dependent variables have not been examined in previous research and are of interest here. Due to both the robustness of MANOVA to correlations below .90 (Tabachnich & Fidell, 2001) and the relevance of these correlations to the current study, multicollinearity was not judged to be a problem and no adjustments were made to the variables.

The following statistics and statistical criteria were used for the analyses. For each of the three sets of main analyses, MANOVAs were performed on the affect scores using an alpha level of .025. Significant MANOVAs were followed up with univariate ANOVAs. The conservative Pillai's criterion for evaluating multivariate significance

Table 1

Intercorrelations Between Dependant Variables from the Anxiety Induction Procedure and the Take-Home Daily Affect Ratings

	Overall Lab Mood Change	Lab PA Change	Lab NA Change	PA Variability	NA Variabil y	Pleasantness Variability	Unpleasantness Variability
			Laboratory Anxiety Induction Procedure Variables				
Overall Lab Mood Change	1.00	-.34*	.15				
Lab PA Change		1.00	-.13				
Lab NA Change			1.00				
			Take Home Daily Affect Ratings Variables				
PA Variability				1.00	.09	.42**	.09
NA Variability					1.00	.23	.63**
Pleasantness Variability						1.00	.24
Unpleasantness Variability							1.00

Note. Ns range from 58-142.
$^{t}p < .65$, $*p \leq .05$, $**p < .01$

was used in all analyses. Using an alpha level of .025, Tukey's Honestly Significant Difference (HSD) post hoc comparisons were conducted on any significant effects.

Manipulation Check for the Anxiety Induction Procedure

In order to asses whether the laboratory anxiety induction procedure was effective, a paired samples *t*-test was conducted comparing pre-and post-anxiety-induction BAI scores. The *t*-test revealed that the anxiety-induction procedure was indeed effective, with the mean level of anxiety having increased from the pre-test ($M = 5.41$, $SD = 4.70$) to the post-test ($M = 6.05$, $SD = 5.20$), $t(121) = -2.29$, $p = .02$.

Allele Frequencies

The number and frequency of each allele for the three gene regions are reported in table 2 (see Table 2). For the AR CAG repeat region, repeats 21, 23, 19 and 25 are most frequent. This distribution of allele frequencies is comparable to other reports in the literature, though the range of repeats in the Harkonen et al. (2003) and Seidman et al. (2001) studies was greater than that of the present study, extending slightly beyond the highest number of repeats that was found in the present study. For the ERα TA repeat region, repeats 14 and 15 were most common. Similar frequencies were reported by both Comings et al. (1999) and Westburg et al. (2003). For the 16/17 5-HTT base-pair repeat region, repeats 10 and 12 were most frequent. Consistent with previous research, repeat 9 occurred very infrequently (1.85%). Of particular interest, however, is the discovery of one allele with the 8 repeat. This is the first report of this short 8 repeat allele.

Table 2

Allele Frequencies for the Androgen Receptor (AR), Estrogen Receptor α (ERα), and Serotonin Transporter (5HTT) Genes

Gene	Repeat	Base Pairs	Repeat Numbers	Frequency	(%)
1. AR	CAG	192	4	1	0.45
		221	14	1	0.45
		227	16	3	1.32
		230	17	2	1.35
		233	18	16	7.46
		236	19	24	10.09
		239	20	16	7.46
		242	21	46	20.18
		245	22	21	9.21
		247.5	23	28	12.28
		250	24	20	8.77
		253	25	23	10.09
		256	26	15	6.58
		259	27	6	2.63
		261	28	2	0.90
		264	29	1	0.45
		267	30	1	0.45
2. ERα	TA	180	12	3	1.80
		182	13	3	1.35
		184	14	25	11.26
		186	15	94	42.34
		188	16	21	9.01
		190	17	9	4.05
		192	18	7	3.15
		194	19	2	0.90
		196	20	5	2.25
		198	21	4	1.80
		200	22	21	9.46
		202	23	12	5.41
		204	24	11	4.95
		206	25	4	1.80
		212	28	1	0.45

(Table 2 continues)

Table 2 (continued)

Allele Frequencies for the Androgen Receptor (AR), Estrogen Receptor α (ERα), and Serotonin Transporter (5HTT) Genes

Gene	Repeat	Alleles (Repeat Numbers)		Frequency	(%)
3. 5HTT	16/17 bp	228	8	1	0.46
		244	9	4	1.85
		261	10	85	39.51
		294	12	126	58.33

5-HTT Genotype Frequencies

Genotype	Frequency	%
8/10	1	1
9/10	3	3
10/10	27	25
10/12	27	25
12/12	49	46

Main Analyses

Between Group Comparisons for the Laboratory Emotional Reactivity Variables

Three one-way between-subjects MANOVAs were performed on three dependent variables reflecting affect change as a result of the laboratory anxiety induction paradigm: Overall Lab Mood Change, Change in Lab Positive Affect, and Change in Lab Negative Affect. The independent variable for each analysis was the genotype of participants on one of either: the AR gene, the ERα gene, or the 5HTT gene.

For the MANOVA comparing the three AR genotypes, the means and standard deviations of the three mood change variables are listed in Table 3 (see Table 3). Using Pillai's criterion, it was found that the three dependent variables were not significantly affected by AR genotype, $F(6, 202) = 0.50$, $p = .81$, $\eta^2 = .02$, power = .13. Thus, when the three laboratory mood variables were combined, mood reactivity was found not to differ as a function of AR genotype.

The means and standard deviations for the three lab affect change variables as a function of ERα genotype are listed in the top panel of Table 4 (see Table 4). The MANOVA comparing the three ERα genotypes found a strong trend indicating that the three genotypes differed in emotional reactivity, $F(6, 144) = 2.43$, $p = .029$, $\eta^2 = .09$, power = .81. Thus, a follow-up univariate ANOVA was computed on each dependent variable. No significant group differences were found with respect to any of the dependent variables. However, strong trends for the ERα genotype were exhibited for all three dependent variables. Among the strongest trends were those for Lab PA change,

Table 3

Means and Standard Deviations for the Emotional Reactivity Variables in the Lab Session as a Function of Androgen Receptor (AR) Genotype

	s/s genotype (n = 21)		s/l genotype (n = 60)		l/l genotype (n = 24)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Lab Mood Change	0.88	(4.70)	-0.82	(5.60)	1.03	(6.65)
Lab PA Change	-3.28	(3.66)	-3.15	(3.13)	-3.63	(3.51)
Log Lab NA Change	1.39	(0.05)	1.40	(0.04)	1.40	(0.05)

Repeats falling at 244 base pairs and below are coded as short (s), while those falling at 245 base pairs and above are coded as long (l).

*$p < .025$

Table 4

Means and Standard Deviations for the Emotional Reactivity Variables in the Lab Session as a Function of Estrogen Receptor Alpha (ERα) Genotype

	s/s genotype (n = 44)		s/l genotype (n = 20)		l/l genotype (n = 12)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Log Lab Mood Change[t]	1.40	(0.10)	1.36	(0.07)	1.44	(0.08)
Lab PA Change[t]	-3.70	(3.39)	-2.05	(2.95)	-4.83	(3.34)
Log Lab NA Change	1.39	(0.05)	1.41	(0.04)	1.37	(0.07)

	s/s or l/l genotype (n = 56)		s/l genotype (n = 20)	
	Mean	(SD)	Mean	(SD)
Log Lab Mood Change[t]	1.40	(0.10)	1.36	(0.07)
Lab PA Change[t]	-3.95	(3.35)	-2.05	(2.95)
Log Lab NA Change[t]	1.38	(0.05)	1.41	(0.04)

Repeats falling at 191 base pairs and below are coded as short (s), while those falling at 192 base pairs and above are coded as long (l).

*$p < .025$, [t]$p < .07$.

$F(2,73) = 3.08, p = .05$, $\eta^2 = .078$, power = .58, and Overall Lab mood change $F(2,73) = 2.77, p = .069$, $\eta^2 = .07$, power = .53. The trend for lab NA change was not as robust $F(2,73) = 2.70, p = .07$, $\eta^2 = .07$, power = .52. Tukey HSD post hoc tests revealed trends indicating less overall lab affect change for the s/l group when compared with the l/l group $q(df = 73) = 0.08$, p = .06, as well as less change in lab PA for the s/l group when compared to the l/l group $q(df = 73) = 2.78$, p = .06. With respect to lab NA change, there was no evidence of any trends indicating group differences between the s/s and the l/l group for any of the dependant variables. These results suggest that individuals homozygous for the long ERα alleles experienced more overall affect change and a greater decrease in PA in anxiety arousing situations than women with the short/long heterozygous alleles.

The above results and the direction of the means suggest that women possessing a heterozygous ERα genotype differ in affect reactivity from those with homozygous genotypes following an anxiety induction procedure. Therefore, further exploratory analyses were performed. A MANOVA was performed in which the two homozygous groups were combined and compared with the heterozygous group on the three emotional variables measured in the lab (see group means in the bottom panel of Table 4). The MANOVA indicated an overall group effect, $F(3, 72) = 4.04, p = .01$, $\eta^2 = .14$, power = .82. Follow-up one-way ANOVAs were conducted for each of the three dependent variables. A strong trend suggested that homozygotes exhibited a greater change in PA following the AIP than the heterozygotes, $F(1, 74) = 5.00, p = .03$, $\eta^2 = .06$, power = .60 The direction of this change was in a negative direction indicating homozygotes experience more of a decline in PA following anxiety arousing situations. Scores for

Negative Affect change indicated that those with heterozygous alleles exhibited a greater change in negative affect as a result of the anxiety induction procedure, $F(1, 74) = 4.48$, $p = .04$, $\eta^2 = .06$, power = .55. In other words, this result suggests that those with heterozygous (s/l) alleles on the AR gene may be prone to increases in negative affect in the face of threatening or anxiety provoking situations. A weaker trend was found for Overall Lab Mood Change Scores as homozygotes experienced more overall change in affect following the anxiety inducing procedure, $F(1, 74) = 3.66$, $p = .06$, $\eta^2 = .05$, power = .47. Thus, following the AIP, there were trends for homozygotes to show greater mood change, a greater decrease in PA, and less of an increase in NA compared to heterozygotes.

Means and standard deviations for the mood reactivity variables within each of the 5HTT genotypes are presented in Table 5 (see Table 5). Results for the MANOVA on the three dependant variables were similar to those of the AR gene, indicating no significant group differences in affect change across the four genotypes, $F(9, 285) = 0.98$, $p = .45$, $\eta^2 = .030$, power = .38. Although this result was not significant, the direction of the means suggested the possibility of group differences for PA. Thus, follow-up ANOVAs were conducted for exploratory purposes. Those revealed a trend indicating group differences in lab positive affect change, $F(3, 95) = 2.65$, $p = .053$ $\eta^2 = .08$, power = .52. Thus, women with either the 8/10 or 9/10 genotype exhibited an increase in positive affect while those with all other genotypes exhibited a decrease in positive affect following the anxiety induction procedure. Despite the small sample size for the 8/10 and 9/10 group, Tukey HSD post hoc tests revealed trends indicating that women with the 8/10 or 9/10 genotypes differed form the other three genotypes in terms of PA change,

Table 5

Means and Standard Deviations for the Emotional Reactivity Variables in the Lab Session as a Function of Serotonin Transporter (5HTT) Genotype

	8/10-9/10 genotypes (n = 4)		10/10 genotype (n = 27)		12/10 genotype (n= 25)		12/12 genotype (n = 43)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Log Lab Mood Change	1.33	(0.92)	1.40	(0.09)	1.39	(0.11)	1.39	(0.09)
Lab PA Change[t]	1.00	(2.58)	-3.33	(3.51)	-3.24	(3.09)	-3.65	(3.01)
Log Lab NA Change	1.39	(0.04)	1.39	(0.06)	1.40	(0.05)	1.39	(0.05)

Actual repeat numbers used to create the four genotype groups.

[t] $p < .06$

10/10, $q(95) = 4.33, p = .072$; 12/10 $q(95) = 4.24, p = .087$; 12/12 $q(95) = 4.65, p = .035$. Although based on a small sample, these results suggest that those possessing either the 8/10 or 9/10 genotype may differ from the other genotypes in susceptibility to positive affect change.

Between Group Analyses for the Eight Hour Affect Variability Scores

In order to test hypotheses relating to daily affect variability, three one-way between-subjects MANOVAs were performed on the four dependent variables derived from the take home hourly affect ratings: Hourly Positive Affect Variability, Hourly Negative Affect Variability, Hourly Pleasantness Variability, and Hourly Unpleasantness Variability. Once again, the independent variable for each analysis was the genotype of participants on the AR gene, the ERα gene, or the 5HTT gene.

Means and standard deviations for the four hourly affect ratings variables as a function of AR genotype are reported in Table 6 (see Table 6). The MANOVA indicated that the four dependent variables were not significantly affected by AR genotype, $F(8, 88) = 0.88, p = .54, \eta^2 = .07$, power = .28. Thus, when affect variability scores from the hourly mood rating exercise were compared across AR genotypes, there was no significant difference in change in affect variability across the four measures.

Means and standard deviations for the four hourly affect rating variables as a function of ERα genotype are reported in Table 7. Despite a pattern in the means suggesting that the homozygous l/l genotype was associated with greater affect variability, the four dependant affect variability variables did not differ significantly according to ERα genotype, $F(8, 60) = .47, p = .87, \eta^2 = .06$, power = .20. Heterogeneity of variance-covariance matrices cannot account for the lack of a significant group

Table 6

Means and Standard Deviations for Daily Affective Variability as a Function of Androgen Receptor (AR) Genotype

	s/s genotype (n = 11)		s/l genotype (n = 30)		l/l genotype (n = 8)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Log Negative Affect Variability	0.38	(0.35)	0.69	(0.47)	0.77	(0.48)
Positive Affect Variability	34.36	(19.63)	43.31	(36.81)	58.82	(103.72)
Pleasantness Variability	9.95	(8.71)	13.49	(13.81)	19.25	(24.95)
Unpleasantness Variability	0.37	(0.81)	3.61	(5.50)	3.11	(5.89)

Repeats falling at 244 base pairs and below are coded as short (s), while those falling at 245 base pairs and above are coded as long (l).

*$p < .025$

Table 7

Means and Standard Deviations for Daily Affective Variability as a Function of Estrogen Receptor Alpha (ERα) Genotype

	s/s genotype (n = 19)		s/l genotype (n = 10)		l/l genotype (n = 6)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Negative Affect Variability	7 27	(10.76)	7.86	(9.04)	10.59	(18.60)
Positive Affect Variability	44.33	(39.76)	35.24	(31.63)	71.95	(119.23)
Pleasantness Variability	13.28	(15.51)	11.99	(11.52)	27.12	(27.71)
Unpleasantness Variability	2.50	(3.99)	3.43	(5.70)	3.59	(6.80)

Repeats falling at 191 base pairs and below are coded as short (s), while those falling at 192 base pairs and above are coded as long (l).

*$p < .025$

difference as smaller sample sizes were associated with larger variances, indicating a liberal significance test and that null hypotheses can be retained with confidence (Tabachnick & Fidell, 2001).

Means and standard deviations for the four hourly affect ratings variables as a function of 5-HTT genotype are reported in Table 8. The MANOVA was not significant, $F(12, 129) = 0.75$, $p = .70$, $\eta^2 = .07$, power = .31. These results indicate that affective variability measured over an eight hour period does not differ with respect to AR, ERα, or 5HTT genotype in women.

Supplementary Analyses

Given that very little research has examined predictors of affect variability, two sets of supplemental analyses were conducted to further investigate the potential relationships between affect variability and both genetic and hormonal indicators. Chi-square analyses were used to examine the relationships between affect variability and genetic variables for three dichotomous variables. Spearman correlations were used to investigate any potential relationships between affect variability and some continuous hormonal variables.

For both sets of analyses, variables used to represent affective change/variability were: Overall Lab Mood Change, Daily Positive Affect Variability, and Daily Negative Affect Variability. For the chi-square analyses, these variables were each split into low and high groups based on a median split of the total affect change or variance scores. Thus, those participants exhibiting a small change in overall mood in the laboratory or a minimal amount of variability in daily PA or daily NA were classified as the low affective variability group within their respective variable, while those with a greater

Table 8

Means and Standard Deviations for Daily Affective Variability as a Function of Serotonin Transporter (5HTT) Genotype

	8/10-9/10 genotype (n = 3)		10/10 genotype (n = 11)		12/10 genotype (n = 13)		12/12 genotype (n = 21)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Negative Affect Variability	4.55	(7.32)	6.83	(5.17)	6.77	(12.80)	7.99	(13.42)
Positive Affect Variability	41.12	(37.38)	43.33	(32.94)	68.50	(86.69)	30.01	(16.07)
Pleasantness Variability	16.06	(14.54)	9.86	(11.07)	20.03	(23.48)	11.75	(9.79)
Unpleasantness Variability	3.52	(5.61)	2.61	(2.88)	1.87	(4.19)	3.50	(6.42)

Actual repeat numbers used to create the four genotype groups.

*$p < .025$

amount of overall mood change in the lab and higher amounts of PA and NA variability were classified as the high affective variability group within their respective variable.

Genetic and hormonal variables were chosen from the screening questionnaire. The dichotomous variables that were selected as potential predictors of a genetic contribution to emotional variability/reactivity were: personal diagnosis of depression (yes/no), family history of depression (yes/no), and family history of bipolar disorder (yes/no). Results of the Chi-Square analyses indicated only one significant result. Those participants who had a family member diagnosed with depression were more likely to exhibit high positive affect variability as measured by the take home daily affect rating exercise, χ^2 (df = 1) = 5.24, p = .02. Thus, a genetic predisposition toward depression may be associated with high PA variability.

Continuous variables that were selected from the screening questionnaire as potential hormonal indicators of affect variability/change were: (1) perceived severity of pre-menstrual syndrome, (2) perceived severity of oral contraceptive negative mood effects, (3) length of menstrual cycle, (4) length of menses, (5) age at first menstruation, (6) regularity of menstrual cycle, and (7) history of acne relative to peers. Using two-tailed Spearman's rho correlational tests, (see correlations in Table 9) three interesting findings emerged. First, women reporting more severe pms symptoms showed greater overall lab mood change (r = .27, p = .002, N = 128), and greater NA daily variability (r = .35, p = .005, N = 62). Second, women reporting a history of oral contraceptive negative mood change showed a trend toward greater variability in daily PA (r = -.34, p = .046, N = 35). Third, women with shorter menstruation day lengths experienced a trend toward greater variability in daily PA (r = -.27, p = .038, N = 61). These

Table 9

Spearman's Inter-correlations Between Hormonal Variables and Overall Laboratory Mood Change, Positive Affect Variability and Negative Affect Variability

	Severity of PMS	Oral Contraceptive Mood Change	Length of Menstrual Cycle	Length of Menses	Cycle Regularity	Age at first Menstruation	Acne History
			Laboratory Variable				
Overall Lab[a] Mood Change	.27**	-.15	-.02	-.09	-.14	.10	.03
			Hourly Affect Ratings				
Daily PA[b] Variability	.08	-.34[t]	.20	-.27[t]	.02	-.10	-.10
Daily NA[b] Variability	.35*	-.17	.06	.05	-.11	.09	-.03

Note. [a] Ns range from 84 to 130. [b] *Ns* range from 35 to 62.
[t] $p < .05$, * $p < .025$, ** $p < .01$, *** $p < .001$

correlations suggest that pms severity, oral contraceptive mood change, and menstruation length are potential predictors of affect variability and vice-versa.

Discussion

Summary of Findings

The first phase of the study examined women's emotional reactivity in response to an anxiety induction procedure, as a function of CAG repeats on the AR gene, TA repeats on the ERα gene, and the 16/17 base pair repeat on the 5HTT gene. When the AR genotype was examined, women did not differ in terms of their overall lab mood change, lab positive (PA) change, or lab negative affect change (NA). The analysis examining ERα genotype, however, indicated a trend suggesting that those women who were heterozygous for the ERα genotype (s/l) experienced more lab NA change in an anxiety-arousing situation. The analysis further revealed a trend indicating that those women who were homozygous long (l/l) for the ERα genotype showed more overall lab mood change and more PA change than the heterozygous (s/l) and homozygous short (s/s) genotypes. Subsequent comparisons of the homozygous (s/s and l/l) and heterozygous (s/l) genotypes served to substantiate the first analysis and indicated trends suggesting that homozygotes exhibited a greater amount of overall affect change as well as a greater amount of PA change than the heterozygous genotype group, while those women heterozygous for the ERα genotype experienced a greater amount of NA change in the laboratory. Comparisons of the 5HTT genotypes revealed a trend suggesting that the groups differed in PA change. Follow-up ANOVAs revealed that those individuals with the 8/10 and 9/10 genotype exhibited an increase in PA while those with the other genotypes exhibited a decrease in positive affect. Thus, overall, in response to the mood

induction, women with the s/l ERα genotype showed a trend toward greater increases in NA, while women with the l/l or s/s ERα genotypes exhibited trends toward greater decreases in PA as well as greater amounts of overall lab mood change. Also, women with the 8/10 and 9/10 genotypes on the 5-HTTgene experienced a greater increase in PA in response to the anxiety induction procedure.

The second phase of the study examined whether women's emotional variability (PA, NA, pleasantness and unpleasantness) over the course of the day differed as a function of genotype for the three genes. None of these analyses produced significant findings, nor were any observable trends present in the data. These results suggest that daily mood variability cannot be predicted based on AR, ERα, or 5HTT genotype.

The supplemental analyses examined the ability of self-report genetic and hormonal indicator variables to predict emotional reactivity or variability. Examination of the genetic variables revealed that women with a relative with a diagnosis of depression exhibited greater PA variability throughout the day, compared to those without a family history of depression. Correlational analysis between the hormonal variables and the indicators of emotional variability revealed three interesting findings. First, women reporting more severe pre-menstrual syndrome (PMS) symptoms experienced greater overall mood change in response to the anxiety induction (r = .27), and greater daily NA variability (r = .35). Second, women reporting a history of greater oral contraceptive negative mood change experienced a trend toward greater variability in daily PA (r = -.34). Finally, women with a shorter average menstruation duration showed a trend toward greater variability in daily PA (r = -.27). These correlations suggest that PMS severity, oral contraceptive mood change, and menstruation length are potential

predictors of affect variability, and that women with greater affect reactivity /variability are at increased risk for both PMS and oral contraceptive mood change. Moreover, these correlations provide additional evidence that hormones play a role in affective variability.

Findings for the AR Gene

With regard to the CAG repeat on the AR gene, there was no evidence of an effect of genotype on the affect variables assessed in the laboratory anxiety induction procedure. Similarly, there was no evidence of an effect of AR genotype on emotional variability as measured by the hourly affect rating scales. Very little is known about the action of androgens in women. Two studies have linked CAG repeat numbers to depression in men (Harkonen et al., 2003; Seidman et al, 2001). In the case of each of the above studies, samples were made up exclusively of men, thus, it is unclear whether any of these results can be generalized to women. While the present study did not examine depression, the results suggest that affect variability is not related to androgen receptor genotype in women.

To test the hypothesis that CAG repeat numbers are linked to depression, scores from the SCL-90 depression scale were compared across AR genotype groups using an ANOVA. The results of this analysis indicated that CAG repeat numbers were not associated with depression scores in women, $F(2, 109) = .36$, $p = .70$. Thus, in addition to having no effects on emotional reactivity or variability, CAG repeat numbers were not associated with depression in the group of women who participated in this study.

Given the findings of the main analyses on the AR gene, as well as the findings of the above ANOVA, it is possible that the psychological effects of polymorphisms on the AR gene are manifested differently in men and women. Comparisons of allele

frequencies between our sample and those in the Harkonen et al. (2003) and Seidman et al. (2001) studies indicate little difference in AR CAG repeat allele frequency. Most frequent in the present study was the 21 CAG repeat allele, accounting for 20.18% of the alleles in the sample. This corresponds well with the Harkonen et al. (2003) study in which the most frequent allele was also the 21 base pair repeat. Similarly, Seidman et al. (2001) reported that 41% of their alleles fell between 21 and 23 repeats. The only notable difference between the two groups of men and the group of women used in the present study was in the range of alleles. Though similar at the bottom of the range, the studies employing male samples reported higher maximum repeat numbers. The repeat numbers in the present study ranged from 5 to 28, whereas the repeat numbers in the studies employing male participants ranged from 13 to 31 for Harkonen et al. (2003) and 4 to 40 for Seidman et al. (2001). It is plausible that because women have fewer long alleles of the CAG repeat polymorphism, they are less susceptible to androgen-related anxious and depressive symptoms. This is supported by the results of Harkonen and colleagues (2003), who found that CAG repeat number was positively correlated with depression in men (e.g., longer alleles were associated with depression).

In addition, Chamberlain et al. (1994) reported that several human genetic diseases have recently been associated with CAG trinucleotide repeat expansion, including X-linked spinal and bulbar muscular atrophy (Kennedy's disease), Huntington's disease, and spinocerebellar ataxia type 1 (SCA1). Moreover, they report that the size range of the repeat in affected individuals is approximately twice the range in the normal population. More recent research has indicated that polyglutamine diseases are due to CAG repeat expansions. Everett and Wood (2004) state that the polyglutamine disorders

are the result of a toxic gain of function of mutant expanded proteins. This toxic process is characterized by protein misfolding, interference with DNA transcription and RNA processing, activation of apoptosis, and dysfunction of cytoplasmic elements, and that the end result is apoptotic cell death with many aspects of neuronal function being perturbed. Though few studies have been conducted examining psychological disorders and CAG repeat numbers, it would appear that more research in this area is warranted given the effects of CAG repeat expansion in populations with polyglutamine disorders.

Findings for the ERα Gene

The results of the analyses for the ERα gene provided some support for the hypothesis that TA repeat numbers are associated with emotional reactivity in women. The findings indicated a trend ($p < .03$) suggesting that women with different ERα genotypes differed with respect to affect change following the laboratory anxiety induction procedure. The results suggested that the homozygous (l/l) genotype reacted differently than the heterozygous (s/l) genotype with respect to all three dependant variables. The homozygous (l/l) genotype group did not show as great an increase in lab negative affect change as the heterozygous (s/l) group, yet exhibited a greater amount of overall laboratory mood change as well as a greater reduction in lab positive affect following the AIP. Additional exploratory analyses comparing the s/l genotype with a combined group of homozygotes (s/s and l/l) revealed a similar trend indicating that the homozygotes exhibited a greater decrease in PA, a greater change in overall lab mood change and a smaller amount of NA change than the heterozygous group following the AIP.

The finding that positive affect decreased most and overall lab mood change increased most in the homozygous long group is somewhat consistent with the findings of Comings et al. (1999). These researchers found that ERα genotype was significantly associated with the anxiety scales of the Symptom Checklist-90, accounting for seven percent of the variance in total anxiety scores. They found that there was a progressive decrease in the mean anxiety scores from the l/l to the s/l to the s/s ERα genotype groups. As the Comings et al. study was conducted using only male participants, the authors suggested that because estrogen levels are much higher in women than in men, the relationship between the ERα genotype and anxiety may account for the increased frequency of anxiety in females. Thus, individuals with the longer alleles experienced more anxiety. In the present study, women with the longer alleles showed the greatest decrease in PA and the greatest increase in overall affect (made up of fear, sadness, guilt, hostility, shyness, fatigue, surprise, joviality, self-assurance, attentiveness, and serenity) in response to an MIP designed to induce anxiety. Showing a decrease in PA and increase in overall mood may be similar to showing increased anxiety.

In order to test the possibility that women who differ in terms of anxiety before or after the MIP also differ on the ERα gene, two one way ANOVAs were performed. Both ANOVAs used the genotypes of the women as the IV, with the first ANOVA using the pre-BAI score as the DV, and the second using the post-BAI score as the DV. Neither the BAI pre score ANOVA, $F(2, 71) = 0.13, p = .88$, nor the BAI post score ANOVA were found to be significant, $F(2, 69) = 0.23, p = .79$. This finding indicates that, in our sample, women did not differ in level of anxiety as a function of ERα genotype.

The range and frequencies of alleles in the present study were similar to those in the Comings et al. (1999) study, with the 14 repeat allele being the most frequent in their study and the 15 repeat allele being most common in the present study. This difference did not affect the grouping of long and short alleles. Thus, allele frequencies in the Comings et al. study appear similar to those of the women in the present study. However, while Comings et al. reported that long ERα alleles are associated with anxiety in men, the present study does not suggest this association in women.

The finding that the heterozygous (s/l) genotype group exhibited a greater increase in negative affect following the AIP is difficult to explain given what has been found in the literature. However, no previous studies have examined affect change with respect to ERα genotype. It has been found that longer alleles are associated with anxiety (Comings et al., 1999), but that shorter alleles are associated with neuroticism and suspicion (Westburg et al., 2003). Neuroticism, depression and anxiety have shown intercorrelations above .55 (Jardine, Martin, & Henderson, 1984).

Thus, it is possible that the heterozygous combination of alleles creates a predisposition in which one is more susceptible to negative affect change following stressful or anxiety-provoking situations because the long allele predisposes one to anxiety and the shorter one to neuroticism and suspicion. This explanation is problematic however in that each individual would be susceptible to some variety of psychological instability because each possible genotype is associated with some level of dysfunction. While it seems illogical from an evolutionary perspective for each genotype combination to be associated with dysfunction, regardless of the degree of severity, research on other VNTRs have indicated similar patterns (see discussion of the 44 base pair VNTR later).

Perhaps the TA repeat on the ERα gene acts in concert with other genes to predispose one to anxiety, neuroticism, and negative affect change. It may be that all genotype groups are associated with some affective dysfunction if and only if another allele on another gene is present. Given that the heterozygous group differed from the homozygous groups in NA yet there was no pattern indicating that either short or long alleles are dominant or recessive, this finding is difficult to interpret. Clearly, this finding needs to be replicated.

Given the present findings, one could speculate that the ERα gene may play a role in affect regulation. Future research could examine how this VNTR interacts with other genes and the environment to regulate affect. Due to the nature of conflicting findings associated with polymorphisms on the ERα gene, further investigation into its role as a contributor to psychological dysfunction is warranted in both men and women, not only in the case of emotion and emotional variability and dysregulation, but also in the case of anxiety, depression, and personality traits. This research is especially important given the number of estrogen-related mood problems women tend to experience over the course of their lives (e.g., PMS, menopause, oral contraceptive mood change, and post partum depression).

Findings for the 5HTT Gene

Overall, there was no strong evidence that the 16/17 base pair allele on the serotonin transporter (5HTT) gene was associated with emotional reactivity or variability. However, a trend indicated that women with either the 8/10 or 9/10 genotype experienced an increase in PA following the MIP, while those women with 10/10, 10/12 and 12/12 genotypes showed an overall decrease in PA. This trend is worth examining as the effect size was quite large and low power likely accounted for the lack of significance. The 8/10

and 9/10 genotypes are very infrequent and the latter have generally been excluded from analyses in previous studies (e.g. Jernej et al., 2004).

It was hypothesized that increases in PA in the combined 8/10-9/10 genotype group could perhaps be attributed to actual confidence in their ability with respect to three- dimensional visuo-spatial tasks due to their higher ability (i.e. these women may have better visuo-spatial ability and therefore show an increase in PA). To test this hypothesis, an independent-groups t-test was conducted in which the 8/10-9/10 genotypes were combined into one group and compared with all of the other genotypes on their Mental Rotations Intelligence Test (MRT) scores. This analysis was not significant, $t(97) = -1.67$, $p = .76$. The mean score on the MRT of the 8/10-9/10 genotype group was 14.67 ($SD = 6.43$), while that of the group composed of the remaining genotype was 20.23 ($SD = 5.65$). Thus the 8/10-9/10 genotype group actually performed worse (but not significantly so) on the MRT than did those women in the other group.

Because actual visuo-spatial test scores did not account for the Lab PA increase in the 8/10-9/10 genotype group, it was then hypothesized that perceived performance could have accounted for the increase in Lab PA. In other words, individuals with the 8/10-9/10 genotype may have performed equally well on the test as those exhibiting the other genotypes, however, their perception of their performance may have been better than it actually was, thereby increasing their PA from administration one to administration two of the PANAS-X. Once again, t-test revealed that this was not the case $t(103) = -0.37$, $p = .57$. Thus, perceived performance on the MRT test did not account for the increase in positive affect after the MRT test in the 8/10-9/10 group.

Thus, a trend indicated that women with the 8-10-9/10 genotype group exhibited a predisposition toward greater PA change. This result must be interpreted with caution given that the total number of participants in this group was four. It is not surprising the effect was not significant. A sample of four participants generally does not have adequate power to produce statistical significance. It is worth noting, however, that both the 8/10 and the 9/10 genotype occurs very rarely in the population. It has been estimated that the nine repeat allele occurs in only one percent of the population, while the eight repeat allele has not yet been reported in the literature. Consequently, it would be difficult to establish a large group of exclusively 8/10 or 9/10 genotypes.

The trend toward group differences in PA change indicated that the 8/10-9/10 genotype group differed most notably from those participants with the 12/12 genotype. While the 8/10-9/10 women showed an increase in positive affect, the 12/12 women exhibited the greatest positive affect decrease following the laboratory anxiety induction procedure. This result is somewhat consistent with other studies examining the effects of the 5-HTT genotype on affective instability. For example, Ohara et al. (1998) reported that the 12 repeat allele was significantly correlated with the risk of anxiety disorders. Thus, taken together, the present study and the Ohara study suggest that the 12/12 genotype predisposes one to anxiety and decreased PA, while the 8/10 and 9/10 genotypes may protect against the experience of anxiety.

One possible reason for the involvement of the 12 repeat allele in anxiety over the other alleles relates to its level of 5-HTT activation. Finkerstrand, Lovejoy, and Quinn (1999) found increased reporter gene expression mediated by the 12 repeat allele of the 5-HTT gene in embryonic stem cells. Similarly, MacKenzie, and Quinn (1999) found that

both the 10 and 12 repeat alleles acted as strong and consistent positive transcriptional regulatory elements. Moreover, the two alleles were found to differ in the strength of their transcriptional-inducing abilities within the developing rostral hindbrain of rat embryos. The 12 repeat allele seemed to be significantly stronger than the 10 repeat allele (MacKenzie & Quinn, 1999). These findings are supported by data from the same study in which additional in vitro data showed that the 12 repeat polymorphism acted as a significantly more potent positive regulator of marker gene expression than the 10 repeat polymorphism when transformed into embryonic stem cells deprived of leukemia inhibitory factor. Their data suggest that the 10 and 12 repeats of the 17 base pair polymorphism act as transcriptional regulators and have allele dependent differential enhancer-like properties in the embryonic stage of development. Thus, individuals with the 12 repeat allele may have higher 5-HTT activity leading to less 5 HT availability in the synaptic cleft and increased susceptibility to anxiety.

Certainly, the structure of the 5HTT gene warrants further investigation as several different studies have indicated that another VNTR element in the promoter region is linked to anxiety. This is known as the 5-HTTPLR region. With regard to this region, Ohara and colleagues (1998) found that the 12 repeat allele on the 44 base pair VNTR element was associated with anxiety, while Ogilvie et al. (1996) found the 9 repeat allele to be associated with anxiety. Similarly, the 10 repeat allele has been associated with impulsive, suicidal behaviours, such as those exhibited by individuals with Borderline Personality Disorder (Lopez de Lara et al., 2006; Ni et al., 2006). Thus, the role of the 5-HTT gene in anxiety warrants further study.

As illustrated above, findings with respect to different polymorphisms on different areas of the 5-HTT gene are varied and the implications of their effects not clear. One approach that may be worthwhile in future studies would involve sequencing the whole gene region, as primary protein structure within the VNTR regions may affect the regulation of the gene (Snustad & Simmons, 2000). In principle, one or two nucleotide changes in a consensus sequence can either specify for a specific transcription factor complex or could alter binding affinity (Snustad & Simmons, 2000). Therefore, it is conceivable that both the number of repeats comprising the VNTR and the sequence of the individual base-pairs within it may be major determinants of VNTR activity (Finkerstrand, Lovejoy, & Quinn, 1999). Thus, the VNTR domain can therefore act as a transcriptional regulator, a property which could potentially contribute to disease susceptibility.

Genetic Predictors of Emotional Reactivity

The exploratory analyses examining potential self-report genetic predictors of emotional reactivity indicated that having a relative with a diagnosis of depression was associated with greater daily positive affect variability. At first, this finding seems paradoxical, as one might intuitively expect that having a family history of depression would, if anything, predispose one to exhibit a greater amount of negative affect variability or lower PA. However, this finding may make sense, given the nature of the measure that was used to assess affect. The PA scale of the PANAS is more reflective of terms indicating arousal than happiness or excitement. Thus, the label "PA" might not be optimal, as the dimension contains a large activation component. The scale does not include terms such as happy, content, or at ease. Consequently, if one employs the

PANAS as a measure of PA, the absence of pure pleasantness items and the large activation component of the scale might account for some unexpected results (Egloff, 1998). Given that the pleasantness scale used in the present study contains terms that are more reflective of happiness and enjoyment rather that arousal, a chi-square test was done to examine whether women with and without a family history of depression differ in their daily pleasantness variability. There was no significant result. Thus, it may be that variability arousal/activation is associated with a history of depression.

Emotions inform the individual of his or her responses to internal or external events and are thus considered an important source of information regarding the meaning of a given event. Although emotion is generally regarded to have an adaptive function within the humanistic/experiential tradition, Misserlian, Toukmanian, Warwar and Greenburg (2005) suggest that affect also plays a key role in psychological dysfunction by way of underlying maladaptive emotion schemas. Defined as "complex cognitive–affective structures [that]. . .store our experienced reactions plus the salient features of the situations that elicited the emotions" (Greenberg & Korman, 1993, p. 259), variability in emotion schemas are seen to develop out of one's experiences and interactions with the world and serve to inform one's perception, experiencing, and anticipation of future events. Emotion schemas are generally useful for dealing with the world (Greenberg & Safran, 1987). These schemas provide an efficient means for the individual to save time and effort in future perceptual activities. However, they can also be maladaptive when they generate emotional reactions that are not congruent with the current situation (e.g., they are over-generalized, or misapplied, and, therefore no longer adaptive). Thus, in depressed individuals, the sympathetic nervous system might become more easily

aroused or activated. This arousal may then consequently affect thoughts, behaviours, and interpretation of events, leading to depressive symptoms.

Another possible explanation for the link between increased PA and family history of depression is an environmental one. That is, individuals with a depressed relative may share less PA variability due to the difficulties associated with having a depressed relative (e.g., life experiences or family reinforcement patterns). Forbes and Dahl (2005) further illustrate how environmental factors associated with PA could lead to depression. They do this by relating the criteria for depression with fluctuations in positive affect. They begin by stating that depressed mood itself is often experienced or expressed as decreased positive affect. Other fundamental characteristics such as anhedonia and fatigue are marked by diminished capacities to experience enjoyment and diminished motivation and/or decreased energy to pursue enjoyable and goal related activities. Social withdrawal, another feature of depression (but not a criteria), may indicate reduced enthusiasm for interactions with others or difficulty obtaining enjoyment from those interactions. The authors go on to say that several components of affective processes may be disrupted with depression and that many of these components are related to positive affect systems. Notably, core aspects of depression may include any or all of the following: reduced motivation to engage in pleasant activities, reduced opportunities to experience rewarding situations that generally activate positive emotions, difficulty activating positive emotions, and difficulty sustaining positive emotions once they are activated. Thus, when one experiences a reduction in PA or when one has a tendency to vacillate between high and low levels of PA, one may be more susceptible to depression.

Hormonal Predictors of Emotional Variability and Reactivity

An examination of correlations between self-report hormonal variables and emotional variability/reactivity revealed three interesting findings. First, women who reported more severe pre-menstrual symptoms experienced greater overall lab mood change and greater daily NA variability. Second, women reporting a history of worse oral contraceptive negative mood change experienced a trend toward greater variability in daily PA. Third and finally, women with a shorter menstruation duration experienced a trend toward greater variability in daily PA. These correlations suggest that PMS severity, oral contraceptive mood change, and length of menstruation are potential predictors of affect variability. Furthermore, high daily affect variability may predict risk for pre-menstrual mood symptoms and oral contraceptive mood change.

Premenstrual syndrome (PMS) is a cyclical syndrome characterized by mood-related and somatic symptoms that occur during the luteal phase of the menstrual cycle and disappear at or soon after the onset of menstruation (Schmidt, Nieman, Danaceau, Adams, & Rubinow, 1998). There is no consistent or convincing evidence in the literature indicating that PMS is characterized by abnormal circulating plasma levels of gonadal steroids, although several studies suggest that levels of estrogen, progesterone, or serotonin may correlate with symptom severity in women with PMS (Rubinow, Schmidt, & Roca, 1998). Elimination or premature termination of the luteal phase of the menstrual cycle and suppression of ovulation via the use of gonadotropin releasing hormone (GNrH) are two of the experimental techniques used to elucidate the effects of gonadal hormones on PMS (Rubinow, Schmidt, & Roca, 1998). In the later stages of the luteal phase, the level of estrogen increases. At this time, both progesterone and estrogen cause

the lining of the uterus to thicken in preparation for menstruation. Thus, prior to menstruation, there is a fairly high level of estrogen production. Although the elimination of the luteal phase has been shown to be effective in relieving symptoms of PMS, two studies have demonstrated the return of PMS symptoms in women undergoing these treatments (Mortola, 1991; Schmidt et al., 1998). Rubinow et al. (1998) state that, although not a direct cause of PMS, serotonin may interact with estrogen in the luteal phase and aid in conveying a vulnerability to PMS by interacting with changing levels of gonadal steroids.

In an attempt to reconcile the discrepancies of the above studies, Schmidt, Nieman, Danaceau, Adams, and Rubinow (1998) conducted a study in which ovulation was eliminated using a GNrH treatment in both women who endorsed symptoms of PMS and those who did not. After a period of time, the authors re-introduced concentrations of estrogen and progesterone in order to determine the effects of each hormone on the onset of PMS. The most striking finding of this study was that although women with premenstrual syndrome had few symptoms during ovarian suppression and recurrence of symptoms during ovarian steroid hormone replacement, the normal women had no perturbation of mood during either manipulation. It was concluded that normal plasma concentrations of gonadal steroids can trigger an abnormal response in women susceptible to PMS. In line with the previous study, Rubinow, Schmidt, and Roca, (1998) hypothesize that women with PMS are differentially sensitive to the mood-perturbing effects of gonadal steroids, since similar steroid manipulations in women without a history of PMS are without effect. Thus, it appears that the differential sensitivity hypothesis may be valid given that both women with and without a history of PMS have

similar levels of circulating gonadal hormones. In the case of the present study, in the context of an anxiety-arousing situation, women who experience PMS due to increased hormonal sensitivity may also experience greater overall lab mood change and daily NA variability. For the same reason, observed connections between hormones and mood (e.g., mood symptoms during the premenstrual period) are due to increased hormonal sensitivity, the present findings suggest that women who experience more emotional variability/reactivity do so because of an increased hormonal sensitivity.

With regard to the second finding of a correlation between PA variability and a history of oral contraceptive negative mood effects, Oinonen and Mazmanian (2002), in a comprehensive review, outline a number of factors that research has suggested may predispose some women to negative mood or affect change during OC use. These factors include a history of depression, symptoms of psychological distress, a history of mood symptoms related to pregnancy, a family history of OC-related mood symptoms, age, being in the postpartum period, and dysmenorrhea and premenstrual mood complaints prior to using OCs. A study by Joffe, Cohen, and Harlow (2003) found that a previous diagnosis of depression was a significant predictor of OC-related premenstrual mood deterioration. Mood improvement was predicted by both dysmenorrhea, and premenstrual mood disturbance with an early onset. The above papers provide a substantial amount of evidence for hormonal involvement in OC related mood change, as nearly all of the predisposing factors for OC negative mood change are biological in nature.

The finding that women with a history of OC-related negative mood change experience more variability in PA may seem somewhat paradoxical to individuals who tend to think of negative mood as only involving changes in NA. However, as was

described earlier, this could be reflective of the fact that the PA scale tends to encompass items reflecting level of arousal, and women who are more susceptible to negative mood side effects may be more predisposed to a greater range of physiological arousal. This theory is in line with the differential sensitivity hypothesis put forth by Schmidt et al. (1998) in the case of PMS. Furthermore, two other studies have suggested a link between OC use and PA variability. Oinonen and Mazmanian (2001) found increased PA variability in first time monophasic OC users during the pill-free week. Similarly, unpublished findings of Jarva and Oinonen (2006) indicated that OC users experience decreased PA variability than non-users in response to four MIPs. Taken together, these findings, as well as those of the present study, suggest that the hormones in OCs may affect PA variability.

There is very little research examining the effect of menstruation duration on affect variability or mood in general. Our correlation suggests that those women with shorter menstruation day lengths experience a trend toward greater variability in daily PA. One of the only reported studies examining menses duration length and affect was that of Levy (1941) who reported that women who displayed more maternal behaviours tended to have shorter menstrual day lengths. Those whose menstrual period lasted for six or more days viewed themselves as more maternal as defined by play with dolls, role most frequently selected in doll play, voluntary mothering in childhood, number of children anticipated and general maternal fantasies, general response to babies, anticipation of self care, and self-rating on a scale of maternal feeding toward children. One other study has linked a longer duration of menstruation to more feminine interests and value patterns (Peskin, 1968). Since this time, very little research has been

conducting in examining behavioural or personality characteristics as a function of menstruation duration. Certainly this area of research warrants further investigation.

Strengths and Limitations of the Present Study

With regard to strengths, this study is of note primarily because no previous studies have examined affect variability or emotional reactivity as a function of genotype. Moreover, to increase the likelihood of determining which genes and genotypes are linked to emotional variability and reactivity, both an in vitro experimental mood induction procedure and an in vivo take home measure was used. No prior studies have incorporated both take home, as well as laboratory assessment measures. Though the present study offers the previous strengths, it is also not without limitations, among which include small groups of participants used in some of the genotype comparisons and, possibly, the multiplex fragment analysis used to determine the number of repeats on the AR, ERα, and 5-HTT genes.

In the case of nearly all of the analyses, participant numbers differed across groups. The issue of small sample size was especially prevalent in both analyses examining the effect of 5-HTT genotype on affective reactivity and variability. Despite the fact that the 8/10 and 9/10 5-HTT genotype groups were combined, the sample size for the MANOVA examining emotional reactivity in the AIP was only four, while the sample size for this cell within the MANOVA examining the effect of 5-HTT genotype on daily affective variability was three. In an experiment such as this in which a variable is examined as a function of genotype, the problem of small cell size can be difficult to circumvent. The 5-HTT gene is a prime example of this as the 9/10 allele is found in only 1.85% of the population and to date, aside from this study, there has been no known

report on 8/10 genotype. Thus, assembling a large group of participants falling into each of these categories or even a combined category would prove very difficult.

With regard to the genotyping, a multiplex procedure was used to determine the number of VNTR base pair repeats for all of the genes of interest at one time. The multiplex procedure produced graphical outputs from which the alleles then had to be extrapolated via visual analysis. Though the interpretations of these graphs were performed by experienced laboratory technicians, a procedure in which each region was sequenced individually would perhaps have been more reliable. Such a sequencing procedure is planned for the samples employed in this study in the near future.

Another potential limitation with respect to this study is the generalizability of the sample. This sample of women was obtained almost exclusively from the university community and contained educated women between the ages of 19 and 25. It is not known whether the results of the present study could be generalized to older women, men, or less educated populations. In addition, the genes of the women are not homogeneous with respect to ethnicity. Many of the participants in the study indicated that they were either first or second generation Canadians with parents or grandparents originating from different countries. Women presented with several different ethnic backgrounds ranging from all areas of Europe, to Asia, to South America. An ethnically heterogeneous sample could pose a problem in that the genes could differ with respect to the area of the world in which they originated and may exert different effects on affective reactivity and variability.

Three potential limitations relate to the take-home hourly affect surveys. The first is concerned with those problems that are typical of studies relying on self-report data. It

is difficult to know whether the women observed the proper intervals when recording their affect. Second, participants did not complete the affect ratings at the same time of day, or on the same day. In the future, it may be worthwhile to have all participants complete momentary affect ratings at on the same day at the same times. This would reduce the likelihood of biases associated with the day of the week or the time of day. For example, some individuals chose to complete their affect ratings on a weekend day, while others chose to complete their affect ratings during the week. Because some individuals' affect depends largely upon the context in which they find themselves, we may have introduced noise into the data by allowing some women to complete the affect ratings on a relaxing weekend day rather than a hectic work or school day. Similarly, some participants chose to begin their ratings in the afternoon while other chose to begin theirs in the morning or early evening. Allowing participants to select their own times to begin the affect ratings could have affected PA in that people may have chosen to complete the ratings at the time of the day they felt best. Thus, an individual who is happy and content in the early hours of the day would perhaps have produced different ratings and consequently different variability indices if they were made to complete the affect ratings in the evenings. This would also be the case if a so called "night owl" was made to begin completing their ratings at 7 a.m. The third potential limitation relates to the method of measuring affect variability. Larsen (1987) highlights a particular problem that may be of note when using self report data to monitor affect variability. He indicates that although self or other-reported variability is important because it reflects one's own or others' view of oneself, it can nonetheless be influenced by judgment bias and might therefore not reflect "true" variability of affect. Larsen (1987) goes on to say that while intra-individual

standard deviations (or variances) are easy to calculate, are face valid, and do not depend on restrictive assumptions, they do not reproduce the process of change, and may consequently confound the frequency and extremity of change. Therefore, another possible way to examine the present data would be to examine whether genotype affects one's affect range as opposed to variability.

A final limitation of the present study concerns a subgroup of women who were not included in the analyses. This group consists of women who experienced positive OC mood effects. It is possible that this reduced the power of the study design as some extreme alleles (i.e., particularly short or long) may have been under-represented.

Conclusions and Directions for Future Research

This study suggests some directions for future research. There is obviously much work to be done in determining the effect of hormonal and monoamine (MA) transporter genotypes on affect and affective variability, but also on mood at the clinical level. This is particularly the case with respect to the ERα and 5-HTT genes, where strong trends indicating differences in affective reactivity between genotype groups were noted using conservative alpha levels. These trends may be especially important because they were observed in a sample of women that was largely non-clinical with respect to psychological functioning.

Future research should be directed at trying to further elucidate the role played by the ERα gene in women and its influence on mood and affect-related phenomena. Many mood-related phenomena in women have been linked to periods of hormonal fluctuation (e.g., menopause, premenstrual phase, postpartum period). Thus, future studies could focus on comparing ERα genotypes of women who are depressed or women who have

had or are currently in the midst of a post-partum episode. Future research could also focus on monitoring affect variability/reactivity in non-clinical samples of women across the duration of the menstrual cycle, as little research has examined to how factors such as cycle length, days of menstruation and age at first menstruation contribute to emotional variability.

Studies involving the role of VNTR polymorphisms on mood and affect variability should also be carried out in which participants from clinical populations are compared to those from non-clinical populations. Of interest would be those clinical populations whose hallmark feature is vacillation of affect, such as those with Borderline Personality Disorder or Bipolar disorder.

Understanding the contribution of MA and hormonal transporter polymorphisms to human behavior, disease susceptibility and response to pharmacotherapies will involve further progress in genetic research that will be aided by both the identification of highly specific phenotypes and the usage of a large number of polymorphic markers. Determination the relationships of genetic polymorphisms to psychological phenomena will help clarify the means by which MA transporter and hormonal genetic variability contribute to our individuality.

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Appendix A

Subject Number _____

SCREENING QUESTIONNAIRE

---

1. Age: _____

2. Sex (Circle your answer): male female

3. Please check the box that best describes the **highest level of education** that you have completed:

[ ] some elementary
[ ] completed grade 8
[ ] some high school
[ ] completed high school
[ ] some college
[ ] completed college
[ ] some university
[ ] completed a university degree
[ ] some graduate studies
[ ] completed a graduate degree

4. Today's date: _____ _____ _____
Day of Week (e.g. Monday) Day of Month (e.g. 5th) Month (e.g. May)

5. Height: _____ (feet & inches) or _____ (cm)

6. Weight: _____ (pounds) or _____ (kg)

7. Check the box that best describes your current relationship status:

[ ] married or living with partner
[ ] no partner (single)
[ ] casually dating
[ ] one partner, but living apart
[ ] more than one partner
[ ] other: _____

8. If you are currently in a steady relationship, how long have you and your partner been together (in years and months)? _____ years and _____ months.

9. If you are currently in a steady relationship, rate your happiness/satisfaction with your current romantic partner:

Extremely Unhappy or Dissatisfied 1 2 3 4 5 6 7 Extremely Happy or Satisfied

10. a. Were you raised in Thunder Bay? (circle one) YES NO Part of the Time

b. Were you raised in Northwestern Ontario? (circle one) YES NO Part of the Time

c. If you were not raised in Northwestern Ontario, was the city/town that you grew up in larger in population than Thunder Bay? (circle one) YES NO Same Size

d. Are your biological parents together (married or in a relationship)? (circle one)

YES NO

11. Are you currently taking any medication? (circle one) YES NO

If YES, what medications are you taking? (please list)

_____

_____

12. Please list any medical or psychological conditions that you have been diagnosed with (e.g. hypothyroidism, depression, asthma, cancer, diabetes, etc.)

_____

_____

13. Have you ever been diagnosed with or treated for depression? (circle your answer)
YES NO MAYBE

14. Have you ever been diagnosed with or treated for bipolar disorder or manic depression? (circle your answer)
YES NO MAYBE

15. Have you ever been diagnosed or treated for an eating disorder? (circle your answer).
YES NO MAYBE

16. Do you think any of your relatives (i.e. parents, siblings, children, grandparents) have had any mental health problems (i.e. depression, anxiety, schizophrenia, alcoholism, eating disorders)? (circle your answer) YES NO MAYBE

17. For each of the following, please check the box if you think that one of your **biological relatives** has been diagnosed with or treated for this psychological problem. Also, on the line beside each mental health problem, please indicate the relationship of the family member(s) to you (e.g., mother, father, sister, grandmother, uncle).

[ ] Depression _____
[ ] Personality Disorder _____
[ ] Schizophrenia _____
[ ] Anxiety Disorder _____
[ ] Bipolar Disorder/Manic Depression _____
[ ] Eating Disorder _____
[ ] Alcoholism _____
[ ] Drug Abuse _____
[ ] Other: _____

18. To the best of your ability, please record your **biological parents'** ethnicity and the percentage or fraction for each different ethnicity (please leave blank if you do not know the information about your biological parents). If you are unsure of the percentages or fractions, simply record the ethnicities.

a) F

b) M

The following two **examples** are possible ethnic percentages. Use them as **guidelines** to fill in your biological mother and father's ethnicity in (a) and (b) below these examples.

a) Father's Ethnicity 1: Finnish
Percentage (or fraction): 75%

Father's Ethnicity 2: Aboriginal
Percentage (or fraction): 25%

Father's Ethnicity 3: _____
Percentage (or fraction): _____

Father's Ethnicity 4: _____
Percentage (or fraction): _____

b) Mother's Ethnicity 1: Irish
Percentage (or fraction): 1/8

Mother's Ethnicity 2: French
Percentage (or fraction): 2/8 or 1/4

Mother's Ethnicity 3: Jamaican
Percentage (or fraction): 1/8

Mother's Ethnicity 4: Scottish
Percentage (or fraction): 4/8 or 1/2

Ethnicity 2: _____ Ethnicity 4: _____
Percentage (or fraction): _____ Percentage (or fraction): _____

19. Check the box of the statement that best describes you:
[ ] I feel happiest and most productive in the morning hours of the day.
[ ] I feel happiest and most productive in the evening hours of the day.
[ ] I feel equally happy and productive in the morning and evening.
[ ] I feel that none of the above statements apply to me.

20. For each of the following, please check the box if you think that one of your **biological relatives** has been diagnosed with or treated for this medical problem. Also, on the line beside each medical problem, please indicate the relationship of the family member(s) to you (e.g., mother, father, sister, grandmother, uncle).

[ ] Breast Cancer _____
[ ] Ovarian Cancer _____
[ ] Cervical Cancer _____
[ ] Autism _____
[ ] Heart Disease _____
[ ] Prostate Cancer _____
[ ] Testicular Cancer _____
[ ] Other Cancer (Please specify): _____
[ ] Fertility Problems _____
[ ] Thyroid Disorder (Specify Type if known): _____

21. This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate to what extent you have felt this way *today*. Use the following scale to record your answers.

1	2	3	4	5
very slightly or not at all	a little	moderately	quite a bit	extremely

____ interested
____ distressed
____ excited
____ upset
____ strong
____ guilty
____ scared
____ hostile
____ enthusiastic
____ proud
____ irritable
____ alert
____ ashamed
____ inspired
____ nervous
____ determined
____ attentive
____ jittery
____ active
____ afraid

22. Current and Recent Alcohol Use (Within the Past 6 months)

a) Currently, how often do you normally consume alcohol? Circle the number under the best answer.

never	once or twice a month or less	once or twice a week	three to four times a week	almost every day
0	1	2	3	4

b) Currently, what is the average number of drinks you have when/if you drink? Circle your answer.

none	one to three	four to seven	eight to twelve	more than 12
0	1	2	3	4

c) Currently, how often do you wake up in the morning with a "hangover" due to alcohol use the previous night?

never	one to three times a month or less	four to eight times a month	eight to twelve times a month	most mornings
0	1	2	3	4

d) Within the past six months, how many times have you drunk so much alcohol that you vomited? Circle the number under the best answer.

never	one to three	four to seven	eight to twelve	more than 12 times
0	1	2	3	4

e) Currently, how often do you skip meals when you consume alcohol? Circle the number under the best answer.

never	25% of the time or less	50% of the time	75% of the time	every time I drink
0	1	2	3	4

23. Past Use of Alcohol

a) About how old were you when you first took one or more full drinks of alcohol? _____

b) About how old were you when you first became intoxicated? _____

c) In the course of one evening, what is the highest number of alcoholic drinks that you have **ever** consumed? _____(# of drinks) (1 drink = 1 beer, 1 glass of wine, or 1.25 ounces of liquor)

How many times have you consumed this many drinks (give best estimate)? _____

d) How many times in your life have you drank so much alcohol that you vomited? (put 0 if never and estimate if unsure of the exact number) _____

e) How many times in your life has your drinking resulted in you having a seizure or convulsions? (put zero if never) _____

f) When you were in the following different **age** groups, **how often did you normally consume alcohol**? Circle the number that best describes your frequency of alcohol use during that time period. Please leave blank the age groups that are older than your current age.

Age Group	never	once or twice a month or less	once or twice a week	three to four times a week	almost every day
7-8	0	1	2	3	4
9-10	0	1	2	3	4
11-12	0	1	2	3	4
13-14	0	1	2	3	4
15-16	0	1	2	3	4
17-18	0	1	2	3	4
19-20	0	1	2	3	4
21-22	0	1	2	3	4
23-24	0	1	2	3	4
25-26	0	1	2	3	4
27-28	0	1	2	3	4
29-30	0	1	2	3	4

g) When you were in the following different age groups, **what was the average number of drinks you had when/if you drank?** Circle the number that best describes your typical consumption of alcohol during that time period (note that 2 = four to seven and 4= more than 12). Please leave blank the age groups that are older than your current age.

Age Group	none	one to three	four to seven	eight to twelve	more than 12
7-8	0	1	2	3	4
9-10	0	1	2	3	4
11-12	0	1	2	3	4
13-14	0	1	2	3	4
15-16	0	1	2	3	4
17-18	0	1	2	3	4
19-20	0	1	2	3	4
21-22	0	1	2	3	4
23-24	0	1	2	3	4
25-26	0	1	2	3	4
27-28	0	1	2	3	4
29-30	0	1	2	3	4

h) When you were in the following different age groups, **how many times did you drink so much alcohol that you vomited?** Circle the number under the response that best describes your typical consumption of alcohol during that time period (note that 2 = four to seven and 4= more than 12). Please leave blank the age groups that are older than your current age.

Age Group	never	one to three times/year	four to seven times/year	eight to twelve times/year	more than 12 times/year
7-8	0	1	2	3	4
9-10	0	1	2	3	4
11-12	0	1	2	3	4
13-14	0	1	2	3	4
15-16	0	1	2	3	4
17-18	0	1	2	3	4
19-20	0	1	2	3	4
21-22	0	1	2	3	4
23-24	0	1	2	3	4
25-26	0	1	2	3	4
27-28	0	1	2	3	4
29-30	0	1	2	3	4

24. OTHER DRUGS

a) Within the past six months, how often have you typically used recreational/illegal drugs such as marijuana, hash, cocaine, LSD, ecstasy, etc.?

never	once or twice a month or less	once or twice a week	three to four times a week	almost every day
0	1	2	3	4

b) Approximately how many times have you tried ecstasy? _____ times

c) Approximately how many times have you tried magic mushrooms (psilocybin)? _____ times

d) Check the box that best describes your smoking status:

[ ] never smoked [ ] previous casual/social smoker [ ] previous smoker
[ ] previous smoker [ ] current casual/social smoker [ ] current smoker

e) If you are a current cigarette smoker, how many cigarettes on average do you smoke per day? _____

f) If you have ever been a cigarette smoker, how many years did you smoke for? _____ years

25. FAMILY HISTORY

a) Using the following scale, select the number that best describes the **current and highest** drinking level of your biological parents. If your parent is deceased, please leave the current level of drinking blank:

Drinking Levels
0= I don't know
1= nondrinker (abstainer)
2= occasional or light social drinker
3= moderate or average social drinker
4= frequent or heavy social drinker
5= problem drinker
6= alcoholic

biological mother:
current level of drinking: _____, highest level ever: _____

biological father:
current level of drinking: _____, highest level ever: _____

b) Do you believe that you have a family history of alcoholism? (Circle answer) Yes No Maybe

c) Have any of your four biological grandparents ever been problem drinkers or alcoholics?
(Circle answer): Yes No Maybe

26. Please answer all of the following questions honestly. For the questions dealing with behaviour, write your answers in the blank spaces provided. For the questions dealing with thoughts and attitudes, circle the appropriate number on the scales provided.

a) With how many different partners have you had sex (sexual intercourse) within the past year? _____

b) How many different partners do you foresee yourself having sex with during the next five years? (Please give a *specific, realistic* estimate) _____

c) With how many different partners have you had sex on *one and **only one*** occasion? _____

d) How often do (did) you fantasize about having sex with someone other than your current (most recent) dating partner? (Circle one. **If you have not been in a dating relationship then leave this question blank.**)

1. Never
2. Once every two or three months
3. Once a month
4. Once every two weeks
5. Once a week
6. A few times each week
7. Nearly every day
8. At least once a day

e) Sex without love is OK.

I Strongly Disagree — I Strongly Agree

1 2 3 4 5 6 7 8 9

f) I can imagine myself being comfortable and enjoying "casual" sex with different partners.

I Strongly Disagree								I Strongly Agree
1	2	3	4	5	6	7	8	9

g) I would have to be closely attached to someone (both emotionally and psychologically) before I could feel comfortable and fully enjoy having sex with him/her.

I Strongly Disagree								I Strongly Agree
1	2	3	4	5	6	7	8	9

h) How frequently do you think about sex?

Virtually Never								Almost all of the time
1	2	3	4	5	6	7	8	9

i) During the past year, with how many different partners have you had **only one occasion** of sexual contact (e.g., hands to genitals, hands to breasts, oral-genital) that did not include sexual intercourse? _____

27. Rate your sexual orientation on the following scale:

I am only attracted to people of the opposite sex				I am equally attracted to people of both sexes				I am only attracted to people of the same sex as me
1	2	3	4	5	6	7	8	9

28. Emotions and Personality

a) The following items ask about your attitudes, feelings, and behaviour. Some of the items relate to food or eating. Other items ask about your feelings about yourself.

For each item, decide if the item is true about you ALWAYS (A), USUALLY (U), OFTEN (O), SOMETIMES (S), RARELY (R) or NEVER (N). Circle the letter in the adjacent column that corresponds to your rating. For example, if your rating for an item is OFTEN, you would circle the O for that item.

Respond to all of the items, making sure that you circle the letter for the rating that is true about you. If you need to change an answer, make an "X" though the incorrect letter and then circle the correct one.

A = ALWAYS, **U** = USUALLY, **O** = OFTEN, **S** = SOMETIMES, **R** = RARELY, **N** = NEVER

1.	I eat sweets and carbohydrates without feeling nervous.	A U O S R N
2.	I think that my stomach is too big.	A U O S R N
3.	I eat when I am upset.	A U O S R N

A = ALWAYS, **U** = USUALLY, **O** = OFTEN, **S** = SOMETIMES, **R** = RARELY, **N** = NEVER

4.	I stuff myself with food.	A U O S R N
5.	I think about dieting.	A U O S R N
6.	I think that my thighs are too large.	A U O S R N
7.	I feel extremely guilty after overeating.	A U O S R N
8.	I think that my stomach is just the right size.	A U O S R N
9.	I am terrified of gaining weight.	A U O S R N
10.	I feel satisfied with the shape of my body.	A U O S R N
11.	I exaggerate or magnify the importance of weight.	A U O S R N
12.	I have gone on eating binges where I felt that I could not stop.	A U O S R N
13.	I like the shape of my buttocks.	A U O S R N
14.	I am preoccupied with the desire to be thinner.	A U O S R N
15.	I think about bingeing (overeating).	A U O S R N
16.	I think my hips are too big.	A U O S R N
17.	I eat moderately in front of others and stuff myself when they're gone.	A U O S R N
18.	If I gain a pound I worry that I will keep gaining.	A U O S R N
19.	I have the thought of trying to vomit to lose weight.	A U O S R N
20.	I think that my thighs are just the right size.	A U O S R N
21.	I think that my buttocks are too large.	A U O S R N
22.	I eat or drink in secrecy.	A U O S R N
23.	I think that my hips are just the right size.	A U O S R N

b) Please read each of the following statements carefully and circle the one answer that best corresponds to your agreement or disagreement. Circle "sd" if the statement is definitely false or if you **strongly disagree**. Circle "d" if the statement is mostly false or if you **disagree**. Circle "a" if the statement is mostly true or if you **agree**. Circle "sa" if the statement is definitely true or if you **strongly agree**. There are no right or wrong answers, and you need not be an "expert" to complete this questionnaire. Describe yourself honestly and state your opinions as accurately as possible.

SD = strongly disagree, **D** = disagree, **N** = neutral, **A** = agree, **SA** = strongly agree

1. I am not a worrier.	sd	d	n	a	sa
2. I really like most people I meet.	sd	d	n	a	sa
3. I often get angry at the way people treat me.	sd	d	n	a	sa
4. I shy away from crowds of people.	sd	d	n	a	sa
5. I rarely feel lonely or blue.	sd	d	n	a	sa
6. I am dominant, forceful, and assertive.	sd	d	n	a	sa
7. In dealing with other people, I always dread making a social blunder.	sd	d	n	a	sa
8. I have a leisurely style at work and play.	sd	d	n	a	sa
9. I rarely overindulge in anything.	sd	d	n	a	sa
10. I often crave excitement.	sd	d	n	a	sa
11. I often feel hopeless and want someone else to solve my problems.	sd	d	n	a	sa
12. I have never literally jumped for joy.	sd	d	n	a	sa
13. I am easily frightened.	sd	d	n	a	sa
14. I don't get much pleasure from chatting with people.	sd	d	n	a	sa

15. I'm an even-tempered person.	sd	d	n	a	sa
16. I like to have a lot of people around me.	sd	d	n	a	sa
17. Sometimes I feel completely worthless.	sd	d	n	a	sa
18. I sometimes fail to assert myself as much as I should.	sd	d	n	a	sa
19. I seldom feel self-conscious when I'm around people.	sd	d	n	a	sa
20. When I do things, I do them vigorously.	sd	d	n	a	sa
21. I have trouble resisting my cravings.	sd	d	n	a	sa
22. I wouldn't enjoy vacationing in Las Vegas.	sd	d	n	a	sa
23. I feel I am capable of coping with most of my problems.	sd	d	n	a	sa
24. I have sometimes experiences unique joy or ecstasy.	sd	d	n	a	sa
25. I rarely feel fearful or anxious.	sd	d	n	a	sa
26. I'm known as a warm and friendly person.	sd	d	n	a	sa
27. I am known as hot blooded and quick tempered.	sd	d	n	a	sa

SD = strongly disagree, **D** = disagree, **N** = neutral, **A** = agree, **SA** = strongly agree

28. I usually prefer to do things alone.	sd	d	n	a	sa
29. I am seldom sad or depressed.	sd	d	n	a	sa
30. I have often been a leader of groups I have belonged to.	sd	d	n	a	sa
31. At times, I have been so ashamed, I just wanted to hide.	sd	d	n	a	sa
32. My work is likely to be slow but steady.	sd	d	n	a	sa
33. I have little difficulty resisting temptation.	sd	d	n	a	sa
34. I have sometimes done things just for "kicks" or "thrills."	sd	d	n	a	sa
35. When I'm under a great deal of stress, sometimes I feel like I'm going to pieces.	sd	d	n	a	sa
36. I am not a cheerful optimist.	sd	d	n	a	sa
37. I often feel tense and jittery.	sd	d	n	a	sa
38. Many people think of me as somewhat cold and distant.	sd	d	n	a	sa
39. I am not considered a touchy or temperamental person.	sd	d	n	a	sa
40. I really feel the need for other people if I am by myself for long.	sd	d	n	a	sa
41. I have sometimes experiences a deep sense of guilt of sinfulness.	sd	d	n	a	sa
42. In meetings, I usually let the others do the talking.	sd	d	n	a	sa
43. It doesn't embarrass me too much if people ridicule and tease me.	sd	d	n	a	sa
44. I often feel as if I'm bursting with energy.	sd	d	n	a	sa
45. When I am having my favourite foods, I tend to eat too much.	sd	d	n	a	sa
46. I tend to avoid movies that are shocking or scary.	sd	d	n	a	sa
47. I keep a cool head in emergencies.	sd	d	n	a	sa
48. Sometimes I bubble with happiness.	sd	d	n	a	sa
49. I'm seldom apprehensive about the future.	sd	d	n	a	sa
50. I really enjoy talking to people.	sd	d	n	a	sa
51. I often get disgusted with people I have deal with.	sd	d	n	a	sa
52. I prefer jobs that let me work alone without being bothered by other people.	sd	d	n	a	sa
53. I tend to blame myself when anything goes wrong.	sd	d	n	a	sa
54. Other people often look to me to make decisions.	sd	d	n	a	sa

55. I often feel inferior to others.	sd	d	n	a	sa
56. I'm not as quick and lively as other people.	sd	d	n	a	sa
57. I seldom give into my impulses.	sd	d	n	a	sa
58. I like to be where the action is.	sd	d	n	a	sa
59. It's often hard for me to make up my mind.	sd	d	n	a	sa
60. I don't consider myself especially "light hearted."	sd	d	n	a	sa
61. I often worry about things that might go wrong.	sd	d	n	a	sa
62. I find it easy to smile and be outgoing with strangers.	sd	d	n	a	sa
63. It takes a lot to get me mad.	sd	d	n	a	sa
64. I'd rather vacation at a popular beach than at an isolated cabin in the woods.	sd	d	n	a	sa
65. I have a low opinion of myself.	sd	d	n	a	sa
66. I would rather go my own way than be a leader of others.	sd	d	n	a	sa
67. I feel comfortable in the presence of my bosses or other authorities.	sd	d	n	a	sa
68. I usually seem to be in a hurry.	sd	d	n	a	sa
69. I sometimes eat myself sick.	sd	d	n	a	sa
70. I love the excitement of roller coasters.	sd	d	n	a	sa
71. I can handle myself pretty well in a crisis.	sd	d	n	a	sa
72. I am a cheerful, high-spirited person.	sd	d	n	a	sa
73. I have fewer fears than most people.	sd	d	n	a	sa
74. I have strong emotional attachments to my friends.	sd	d	n	a	sa
75. At times I have felt bitter and resentful.	sd	d	n	a	sa
76. Social gatherings are usually boring to me.	sd	d	n	a	sa
77. Sometimes things look pretty bleak and hopeless to me.	sd	d	n	a	sa
78. In conversations, I tend to do most of the talking.	sd	d	n	a	sa
79. If I have said or done the wrong thing to someone, I can hardly bear to face them again.	Sd	d	n	a	sa

SD = strongly disagree, **D** = disagree, **N** = neutral, **A** = agree, **SA** = strongly agree

80. My life is fast paced.	sd	d	n	a	sa
81. Sometimes I do things on impulse I later forget.	sd	d	n	a	sa
82. I am attracted to bright colours and flashy styles.	sd	d	n	a	sa
83. When everything seems to be going wrong, I can still make good decisions.	sd	d	n	a	sa
84. I rarely use words like "fantastic!" or "sensational!" to describe my experiences.	sd	d	n	a	sa
85. Frightening thoughts sometimes come into my head.	sd	d	n	a	sa
86. I take a personal interest in people I work with.	sd	d	n	a	sa
87. Even minor annoyances can be frustrating to me.	sd	d	n	a	sa
88. I enjoy parties with lots of people.	sd	d	n	a	sa
89. Too often, when things go wrong, I get discouraged and feel like giving up.	sd	d	n	a	sa
90. I don't find it easy to take charge of a situation.	sd	d	n	a	sa
91. When people I know do foolish things, I get embarrassed for them.	sd	d	n	a	sa
92. I am a very active person.	sd	d	n	a	sa

93. I am always able to keep my feelings under control.	sd	d	n	a	sa
94. I like being part of a crowd at sporting events.	sd	d	n	a	sa
95. I'm pretty stable emotionally.	sd	d	n	a	sa
96. I laugh easily.	sd	d	n	a	sa

c) Below is a list of problems that people sometimes have. Please read each one carefully, and check the box that best describes HOW MUCH THAT PROBLEM HAS DISTRESSED OR BOTHERED YOU DURING THE PAST 7 DAYS INCLUDING TODAY.

	not at all	a little bit	moderately	quite a bit	extremely
Loss of sexual interest or pleasure	[ ]	[ ]	[ ]	[ ]	[ ]
Feeling low in energy or slowed down	[ ]	[ ]	[ ]	[ ]	[ ]
Thoughts of ending your life	[ ]	[ ]	[ ]	[ ]	[ ]
Crying easily	[ ]	[ ]	[ ]	[ ]	[ ]
Feelings of being trapped or caught	[ ]	[ ]	[ ]	[ ]	[ ]
Blaming yourself for things	[ ]	[ ]	[ ]	[ ]	[ ]
Feeling lonely	[ ]	[ ]	[ ]	[ ]	[ ]
Feeling blue	[ ]	[ ]	[ ]	[ ]	[ ]
Worrying too much about things	[ ]	[ ]	[ ]	[ ]	[ ]
Feeling no interest in things	[ ]	[ ]	[ ]	[ ]	[ ]
Feeling hopeless about the future	[ ]	[ ]	[ ]	[ ]	[ ]
Feeling everything is an effort	[ ]	[ ]	[ ]	[ ]	[ ]
Feelings of worthlessness	[ ]	[ ]	[ ]	[ ]	[ ]

29. <u>Reproductive Questions</u>:

1) a) Have you ever been pregnant? (Only say YES if you were 100% sure) YES NO

b) If yes, how many times have you been pregnant? _____

c) How many children have you given birth to? _____

d) How many times have you miscarried? _____

e) How many times have you had an abortion? _____

f) Are you currently pregnant? (Circle your answer) YES NO MAYBE

g) Some women report experiencing an increase in negative mood, irritability, or weepiness in the week or days prior to starting their period each month (during the premenstrual phase). To what extent do you experience such negative mood changes prior to your period? (Circle your answer)

1	2	3	4	5
not at all	a little	moderately	quite a bit	extremely

2) a) Have you ever taken oral contraceptives? (Circle your answer) YES NO

b) If you have ever taken oral contraceptives, look at the following side effects that some people might experience when taking oral contraceptives. Put a check beside any and all of the following **side effects that you experienced** when taking oral contraceptives. Please indicate what **oral contraceptive brand** you were taking when you experienced the side effect. Check all that apply. (see question 3f on p. 11 for a list of some oral contraceptive brands)

[ ] Nausea/Vomiting: _____
[ ] Breast size increase _____
[ ] Decreased ability to orgasm _____
[ ] Weight gain _____
[ ] Increased sex drive/arousal _____
[ ] Fewer menstrual cramps _____
[ ] Positive Mood change _____
[ ] Tiredness/fatigue _____
[ ] High blood pressure _____
[ ] Irregular heartbeat _____
[ ] Clearer complexion _____
[ ] Complete loss of periods _____
[ ] Heavier periods (↑ bleeding) _____
[ ] Desire to become pregnant _____
[ ] Too hard to use _____
[ ] Too expensive _____
[ ] Conflicts with another medication _____
[ ] Breakthrough bleeding (bleeding between periods) _____

[ ] Headaches: _____
[ ] Breast size decrease _____
[ ] Increased ability to orgasm _____
[ ] Weight loss _____
[ ] Decreased sex drive/arousal _____
[ ] More menstrual cramps _____
[ ] Negative mood change _____
[ ] Dizziness/Faintness _____
[ ] Painful or tender breasts _____
[ ] Swelling of breast or abdomen _____
[ ] Complexion Problems (e.g., acne) _____
[ ] Sexual relationship ended _____
[ ] Lighter periods (↓ bleeding) _____
[ ] Concerned about hormones _____
[ ] Medical condition (Specify:) _____

c) At what age did you start using oral contraceptives?_____years

d) If you have ever taken oral contraceptives, complete the following:

I believe that oral contraceptives have affected my mood (Circle the best answer)

Very Negatively	Slightly Negatively	In no way at all	Slightly positively	Very positively
0	1	2	3	4

e) If you believe that you have ever experienced a **change in your mood when taking oral contraceptives**, check any of the following changes that you noticed in yourself:

Check any of those that apply:

____Slept more than usual
____Slept less than usual
____Depression
____More pessimistic
____More irritable
____More jealous
____Less jealous
____Sadness
____More optimistic
____Less irritable
____More moody
____Less moody
____Lower self-esteem
____Higher self-esteem
____Cried more than usual

___Feelings of inferiority	___More sensitive to criticism	___Cried less than usual
___Disrupted sleep	___Less sensitive to criticism	___More self-critical
___More content/happy	___Less trust in partner (fidelity)	___Less self-critical
___ More Aggressive	___More trust in partner (fidelity)	___ Less Aggression

f) Have negative mood side effects ever influenced you to stop taking oral contraceptives? (Circle best answer) Yes No Somewhat

g) If you have ever discontinued oral contraceptives due to mood side effects, approximately how many days or months did you experienced these negative mood side effects before discontinuing use?

_____months and _____days

h) If you have ever experienced **negative mood side effects** when taking oral contraceptives, what was/were the name of the oral contraceptive(s) that you were taking when you experienced these effects? (see list on next page (3g) if you need help remembering the different types)

_____

_____

3) a) Are you currently taking oral contraceptives? (Circle your answer) YES NO

b) If you are currently taking oral contraceptives, for how many years and months have you been taking your **current** oral contraceptive?_____years and_____months

c) How long in total have you taken **any** oral contraceptive?_____years and_____months

d) Why did you **start** taking oral contraceptives? (Check all that apply)

[ ] Birth Control [ ] Treat acne
[ ] For cycle regularity [ ] Other: _____
[ ] Due to a hormonal medical condition (Specify): _____
[ ] I was taking another medication that could have produced birth defects

e) Why are you currently taking oral contraceptives? (Check all that apply)

[ ] Birth Control [ ] Treat acne
[ ] For cycle regularity [ ] Other: _____
[ ] Due to a hormonal medical condition (Specify): _____
[ ] I am currently taking another medication that could produce birth defects

f) If you are currently taking oral contraceptives, please put an X beside the type of oral contraceptive you are currently taking.

Alesse	___	Ortho-Cept	___
Brevicon 0.5/35	___	Ortho 7/7/7	___
Brevicon 1/35	___	Ortho 10/11	___
Cyclen	___	Synphasic	___
Demulen 30	___	Tri-Cyclen	___
Loestrin	___	Triphasil	___
Marvelon	___	Triquilar	___
MinEstrin	___	Demulen 50	___
Min-Ovral	___	Norlestin 1/50	___
Norinyl	___	Ovral	___

Ortho 1/35 ___ Ortho-Novum 1/50 ___
Ortho 0.5/35 ___ Diane 35 ___
Other (Please Specify): _____

4) a) How many different types/brands of oral contraceptive have you taken? _____ types/brands

b) Please list all of the different types of oral contraceptives have you used? (Please list all. Refer to above list in 3f.)

_____
_____
_____
_____

c) If you have **previously taken oral contraceptives** but are not taking them right now, how many years and months has it been **since you last took oral contraceptives**? _____years and_____months

d) If you ever stopped a type of oral contraceptive, **why did you stop** taking that oral contraceptive? Check the boxes for all that apply and indicate the oral contraceptive brand that you stopped taking due to the side effect (list continued on next page).

[ ] Nausea/Vomiting: _____
[ ] Breast size increase _____
[ ] Decreased ability to orgasm _____
[ ] Weight gain _____
[ ] Increased sex drive/arousal _____
[ ] Fewer menstrual cramps _____
[ ] Positive Mood change _____
[ ] Tiredness/fatigue _____
[ ] High blood pressure _____
[ ] Irregular heartbeat _____
[ ] Clearer complexion _____
[ ] Complete loss of periods _____
[ ] Heavier periods (↑ bleeding) _____
[ ] Desire to become pregnant _____
[ ] Too hard to use _____
[ ] Too expensive _____
[ ] Conflicts with another medication _____
[ ] Breakthrough bleeding (bleeding between periods) _____
[ ] Physician recommended discontinuation _____
[ ] Other: _____

[ ] Headaches: _____
[ ] Breast size decrease _____
[ ] Increased ability to orgasm _____
[ ] Weight loss _____
[ ] Decreased sex drive/arousal _____
[ ] More menstrual cramps _____
[ ] Negative mood change _____
[ ] Dizziness/Faintness _____
[ ] Painful or tender breasts _____
[ ] Swelling of breast or abdomen _____
[ ] Complexion Problems (e.g., acne) _____
[ ] Sexual relationship ended _____
[ ] Lighter periods (↓ bleeding) _____
[ ] Concerned about hormones _____
[ ] Medical condition (Specify:) _____

e) Do you have a biological mother or sister who has experienced negative mood effects while taking oral contraceptives? (Circle answer): YES NO UNSURE

f) Have you ever taken a contraceptive that contained hormones but that was not oral contraceptives (e.g., contraceptive patch, vaginal ring, DepoProvera, hormonal implants, etc.)? (circle answer)
YES NO UNSURE

5) a) What is the average length of your menstrual cycle right now (i.e., How many days are there from the first day of one period to the first day of your next period – most people range between 25 and 35)? _____ days

b) What is your average length of menstruation/bleeding **when you are not taking oral contraceptives**? (i.e., how many days does your period last? Most people's periods last between 1 and 10 days.) _____ days

c) Which statement best describes your menstrual cycle **when you are not taking oral contraceptives**? (Check the box with an "X" beside the appropriate response.)

[ ] I never have my period.
[ ] Some months I get my period and some months I don't.
[ ] I usually get my period every month, but it is irregular and I cannot predict when it will start.
[ ] I usually get my period within two or three days of when I expect it.
[ ] My period is like clockwork and the same number of days elapse between periods each month.

d) How old were you when you first started menstruating (started your period)? _____years old

e) As a teenager and young adult, how did/does you acne/pimples compare to your same-age peers? I had _____ acne compared to most girls/women my age (circle the best response).

Significantly Less	Slightly Less	About the same	Slightly More	Significantly More
0	1	2	3	4

6) a) Have you ever purposely tried to lose weight? YES NO MAYBE

b) If yes, at what age did you first attempt to lose weight? _____ years

c) At the current time, are you purposely trying to lose weight? (circle your answer)
YES NO MAYBE

d) During the past five years, how much of the time have you been purposely trying to lose weight? (circle your answer)

Virtually never								Almost all of the time
1	2	3	4	5	6	7	8	9

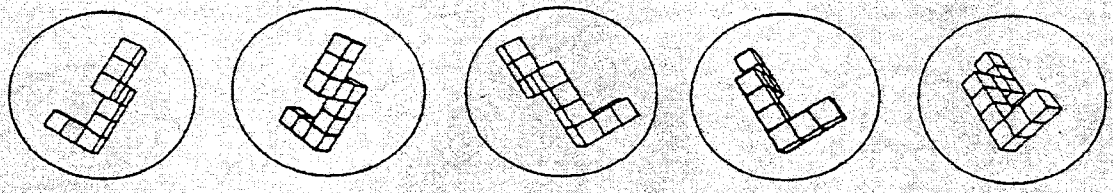
Appendix B

Name _____

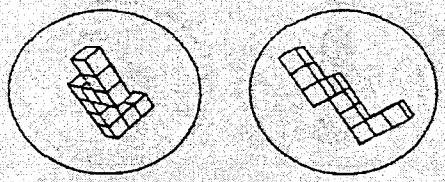
M.R.T. Test Date _____

_____

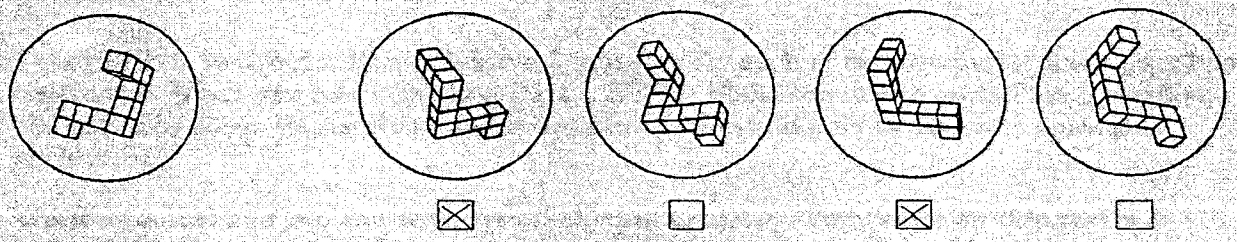
This is a test of your ability to look at a drawing of a given object and find the same object within a set of dissimilar objects. The only difference between the original object and the chosen object will be that they are presented at different angles. An illustration of this principle is given below, where the same single object is given in five different positions. Look at each of them to satisfy yourself that they are only presented at different angles from one another.



Below are two drawings of new objects. They cannot be made to match the above five drawings. Please note that you may not turn over the objects. Satisfy yourself that they are different from the above.



Now let's do some sample problems. For each problem there is a primary object on the far left. You are to determine which two of four objects to the right are the same object given on the far left. In each problem always two of the four drawings are the same object as the one on the left. You are to put Xs in the boxes below the correct ones, and leave the incorrect ones blank. The first sample problem is done for you.

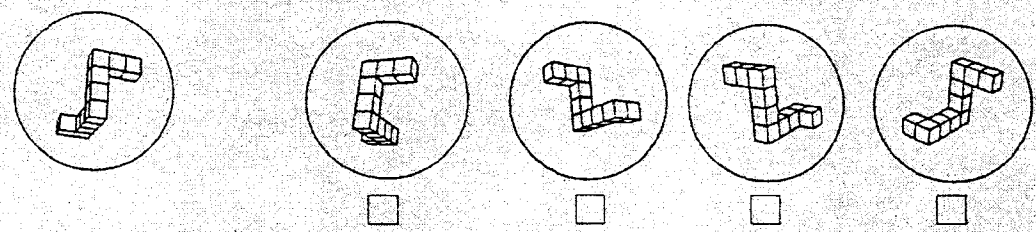


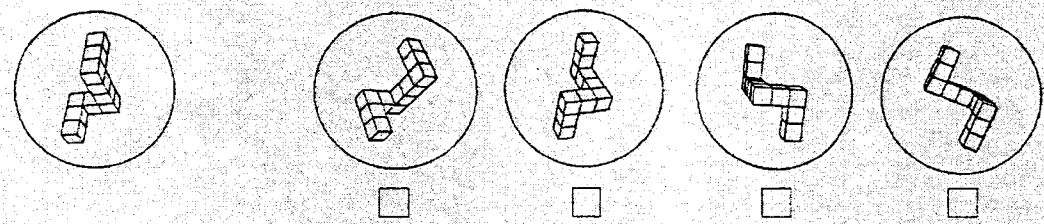
Go to the next page

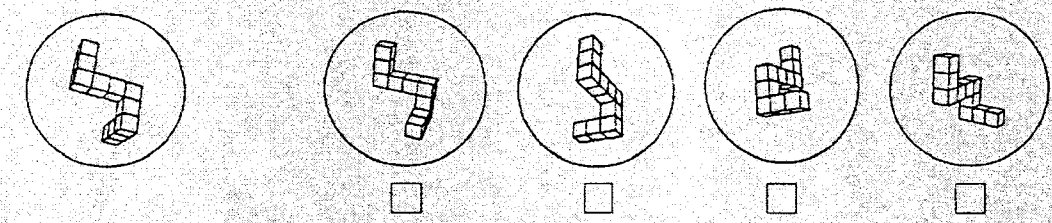
Adapted by S. G. Vandenberg, University of Colorado, July 15, 1971
Revised instructions by H. Crawford, U. of Wyoming, September, 1979
Images digitized and reprinted by Susanna Douglas, University of Texas, March, 1996

page 2

Do the rest of the sample problems yourself. Which two drawings of the four on the right show the same object as the one on the left? There are always two and only two correct answers for each problem. Put an X under the two correct drawings.

1. 

2. 

3. 

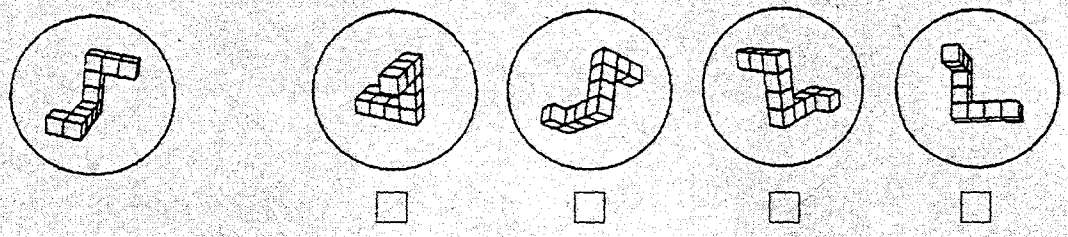
Answers: 1. first and second drawings are correct
2. first and third drawings are correct
3. second and third drawings are correct

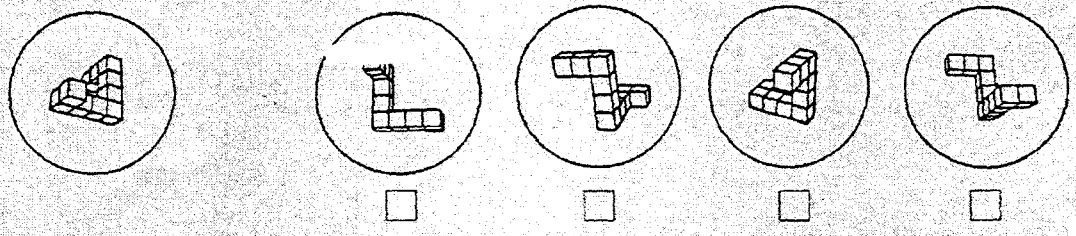
This test has two parts. You will have 3 minutes for each of the two parts. Each part has two pages. When you have finished Part I, STOP. Please do not go on to Part 2 until you are asked to do so. Remember: There are always two and only two correct answers for each item.

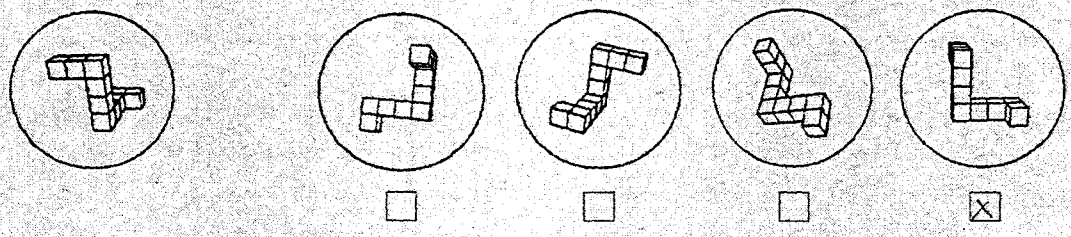
Work as quickly as you can without sacrificing accuracy. Your score on this test will reflect both the correct and incorrect responses. Therefore, it will not be to your advantage to guess unless you have some idea which choice is correct.

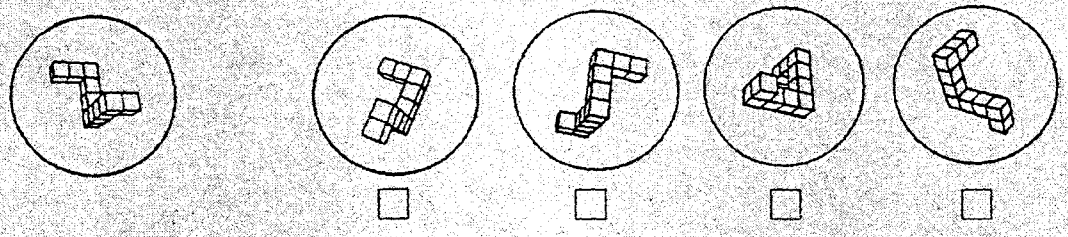
DO NOT TURN THIS PAGE UNTIL ASKED TO DO SO.

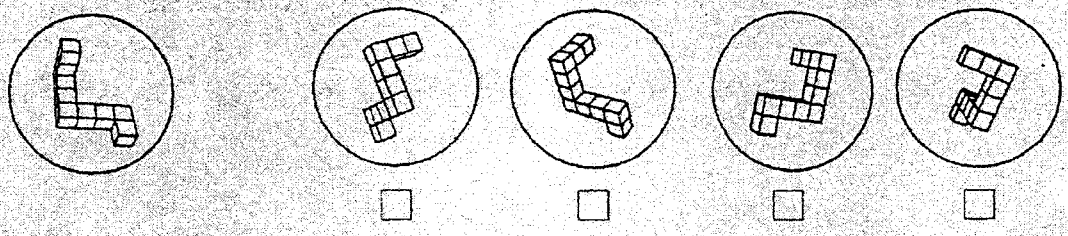
PART I page 3

1. 

2. 

3. 

4. 

5. 

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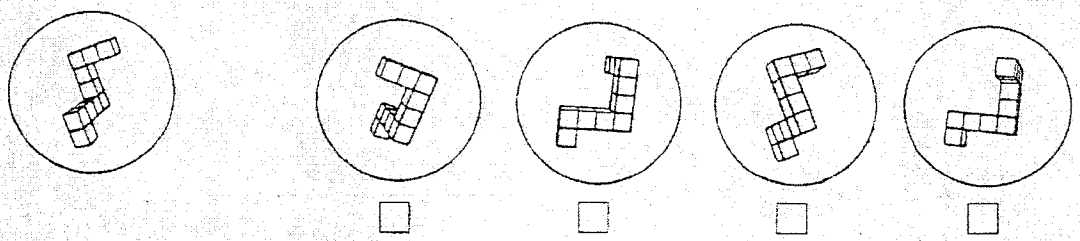
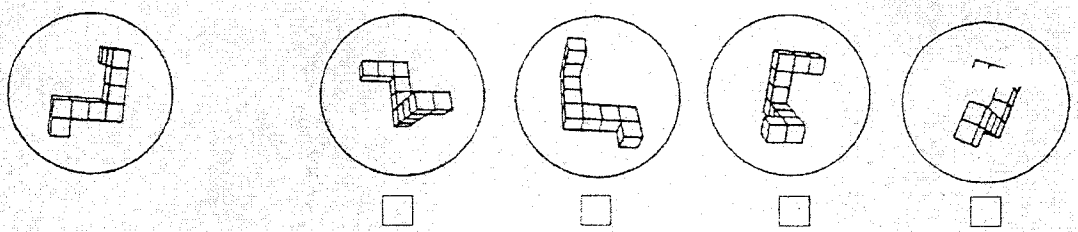
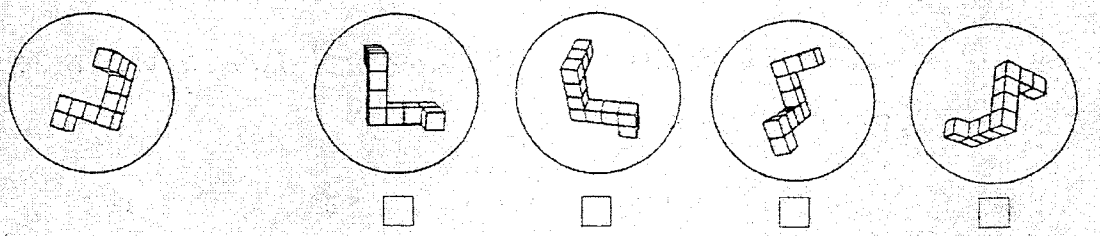
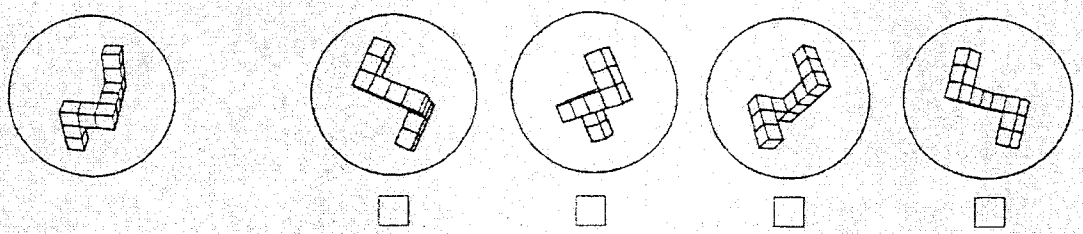
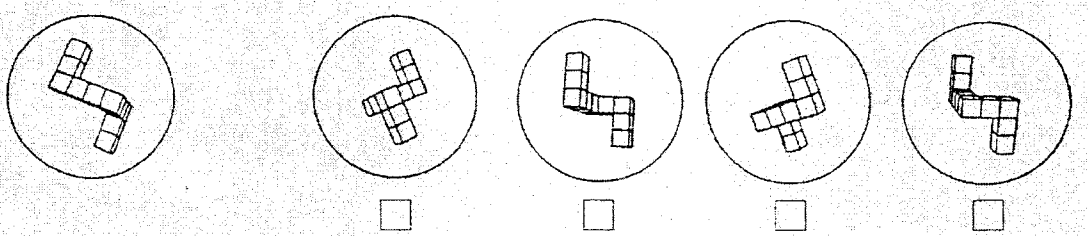
6. 

7. 

8. 

9. 

10. 

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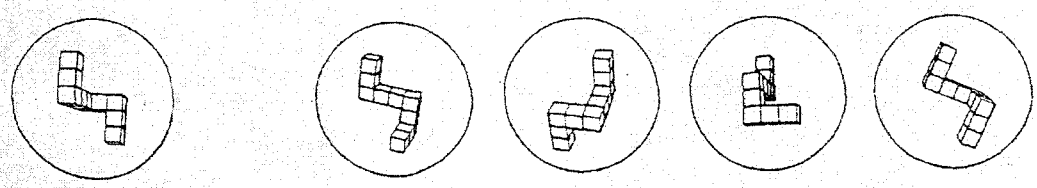
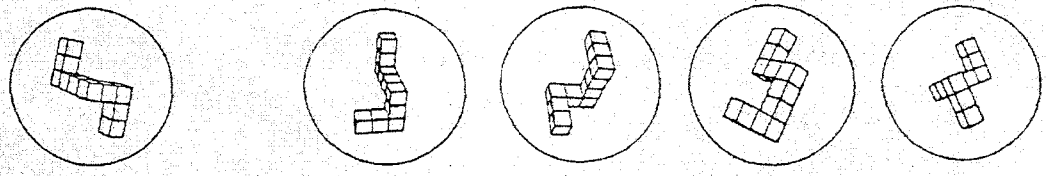
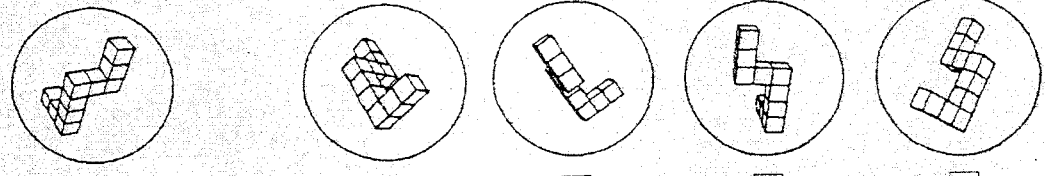
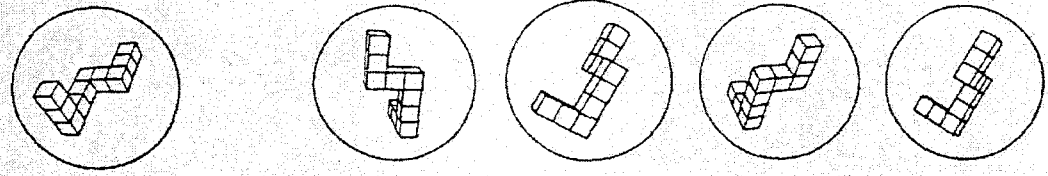
PART II

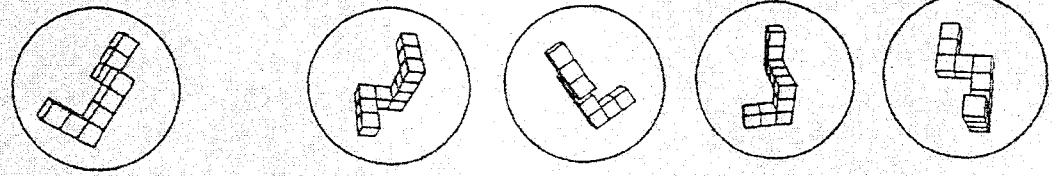
page 5

11. 

12. 

13. 

14. 

15. 

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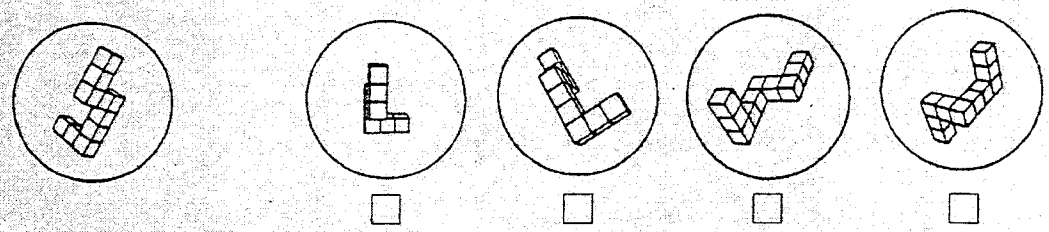
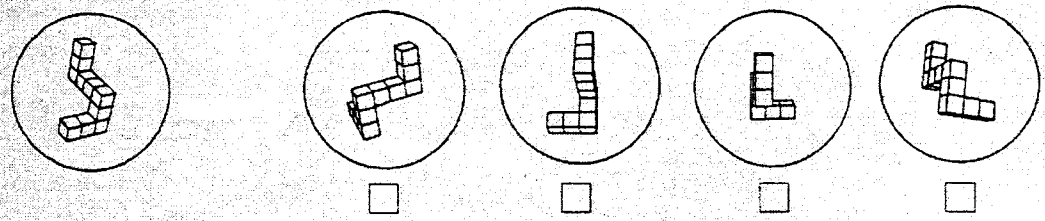
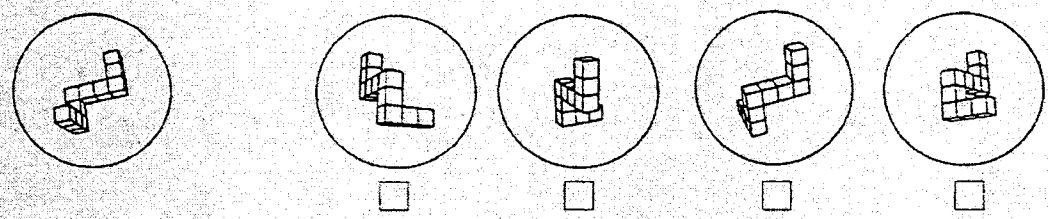
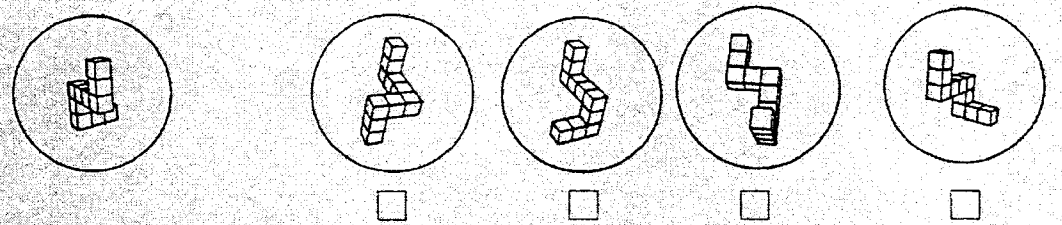
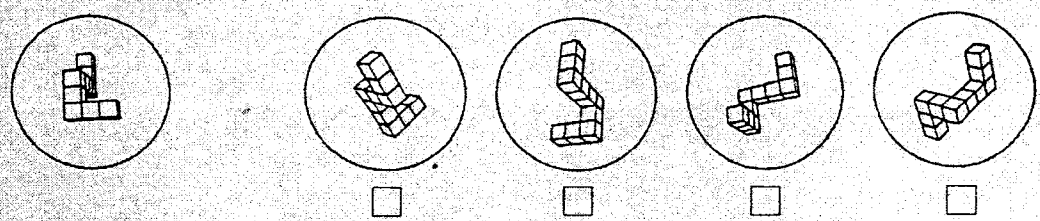
16. 

17. 

18. 

19. 

20. 

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Appendix C

Subject # _____
Date: _____

Session #2 Pre-Administration Questionnaire

A) Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you are currently bothered by each symptom AT THE PRESENT TIME, by placing an X in the corresponding space in the column next to each symptom.

	Not at all	Mildly (I am not bothered much)	Moderately (I feel very unpleasant, but I can handle it)	Severely (I can barely stand it)
1. Numbness or tingling.				
2. Feeling hot.				
3. Wobbliness in legs.				
4. Unable to relax.				
5. Fear of the worst happening.				
6. Dizzy or lightheaded.				
7. Heart pounding or racing.				
8. Unsteady.				
9. Terrified.				
10. Nervous.				
11. Feelings of choking.				
12. Hands trembling.				
13. Shaky.				
14. Fear of losing control.				
15. Difficulty breathing.				
16. Fear of dying.				
17. Scared.				
18. Indigestion or discomfort in abdomen.				
19. Faint.				
20. Face Flushed.				
21. Sweating (due to heat).				

B) Please indicate how you feel at this moment by circling the appropriate response; *(1) very slightly or not at all, (2) a little, (3) moderately, (4) quite a bit, (5) extremely.*

	Very Slightly or not at all	A Little	Moderately	Quite a Bit	Extremely
	1	2	3	4	5
Happy	1	2	3	4	5
Joyful	1	2	3	4	5
Content	1	2	3	4	5
At Ease	1	2	3	4	5
Calm	1	2	3	4	5
Sad	1	2	3	4	5
Blue	1	2	3	4	5
Alone	1	2	3	4	5
Downhearted	1	2	3	4	5
Lonely	1	2	3	4	5

C) This scale consists of a number of words and phrases that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate to what extent you currently feel this way. Use the following scale to record your answers:

1 very slightly or not at all	2 a little	3 moderately	4 quite a bit	5 extremely
_____ cheerful	_____ sad	_____ active		_____ angry at self
_____ disgusted	_____ calm	_____ guilty		_____ enthusiastic
_____ attentive	_____ afraid	_____ joyful		_____ downhearted
_____ bashful	_____ tired	_____ nervous		_____ sheepish
_____ sluggish	_____ amazed	_____ lonely		_____ distressed
_____ daring	_____ shaky	_____ sleepy		_____ blameworthy
_____ surprised	_____ happy	_____ excited		_____ determined
_____ strong	_____ timid	_____ hostile		_____ frightened
_____ scornful	_____ alone	_____ proud		_____ astonished
_____ relaxed	_____ alert	_____ jittery		_____ interested
_____ irritable	_____ upset	_____ lively		_____ loathing
_____ delighted	_____ angry	_____ ashamed		_____ confident
_____ inspired	_____ bold	_____ at ease		_____ energetic
_____ fearless	_____ blue	_____ scared		_____ concentrating
_____ disgusted with self	_____ shy	_____ drowsy		_____ dissatisfied with self

Appendix D

Subject # _____
Date: _____

Session #2 Post-Administration Questionnaire

A) Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you are currently bothered by each symptom AT THE PRESENT TIME, by placing an X in the corresponding space in the column next to each symptom.

	Not at all	Mildly (I am not bothered much)	Moderately (I feel very unpleasant, but I can handle it)	Severely (I can barely stand it)
1. Numbness or tingling.				
2. Feeling hot.				
3. Wobbliness in legs.				
4. Unable to relax.				
5. Fear of the worst happening.				
6. Dizzy or lightheaded.				
7. Heart pounding or racing.				
8. Unsteady.				
9. Terrified.				
10. Nervous.				
11. Feelings of choking.				
12. Hands trembling.				
13. Shaky.				
14. Fear of losing control.				
15. Difficulty breathing.				
16. Fear of dying.				
17. Scared.				
18. Indigestion or discomfort in abdomen.				
19. Faint.				
20. Face Flushed.				
21. Sweating (due to heat).				

B) Please indicate how you feel at this moment by circling the appropriate response; *(1) very slightly or not at all, (2) a little, (3) moderately, (4) quite a bit, (5) extremely.*

Very Slightly or not at all	A Little	Moderately	Quite a Bit	Extremely

Happy	1	2	3	4	5
Joyful	1	2	3	4	5
Content	1	2	3	4	5
At Ease	1	2	3	4	5
Calm	1	2	3	4	5
Sad	1	2	3	4	5
Blue	1	2	3	4	5
Alone	1	2	3	4	5
Downhearted	1	2	3	4	5
Lonely	1	2	3	4	5

C) This scale consists of a number of words and phrases that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate to what extent you currently feel this way. Use the following scale to record your answers:

1 very slightly or not at all	2 a little	3 moderately	4 quite a bit	5 extremely
_____ cheerful	_____ sad	_____ active		_____ angry at self
_____ disgusted	_____ calm	_____ guilty		_____ enthusiastic
_____ attentive	_____ afraid	_____ joyful		_____ downhearted
_____ bashful	_____ tired	_____ nervous		_____ sheepish
_____ sluggish	_____ amazed	_____ lonely		_____ distressed
_____ daring	_____ shaky	_____ sleepy		_____ blameworthy
_____ surprised	_____ happy	_____ excited		_____ determined
_____ strong	_____ timid	_____ hostile		_____ frightened
_____ scornful	_____ alone	_____ proud		_____ astonished
_____ relaxed	_____ alert	_____ jittery		_____ interested
_____ irritable	_____ upset	_____ lively		_____ loathing
_____ delighted	_____ angry	_____ ashamed		_____ confident
_____ inspired	_____ bold	_____ at ease		_____ energetic
_____ fearless	_____ blue	_____ scared		_____ concentrating
_____ disgusted with self	_____ shy	_____ drowsy		_____ dissatisfied with self

D) How do you think you did on the mental rotation test? (Circle the appropriate answer)

Very Poorly **Extremely Well**

1 2 3 4 5 6 7

E) What percentage of the items do you think that you answered correctly? _____%

Appendix E

Dear Participant,

Attached you will find two packages of questionnaires. Each package contains eight copies of the hourly mood survey. The hourly mood survey is to be completed eight times over one 24-hour period, preferably at one-hour intervals. Although completing these questionnaires may seem inconvenient at times, we would ask that you adhere as strictly as possible to completing them at one hour intervals. For example, if you begin the hourly mood survey at 8:00 am, we would ask that you try to complete the seven remaining surveys at 9:00 am, 10:00 am, 11:00 am, 12:00 pm, 1:00 pm, 2:00 pm, and lastly at 3:00 pm.

The eighth and final hourly mood questionnaire contains some very brief questions concerning events or activities that may have affected your mood over the course of the day. We ask that you please complete these at the same time as your final hourly mood survey.

In order to facilitate the completion of the questionnaires, we are happy to present several tips which may aid you in establishing a schedule that is conducive to completing the questionnaires. These include such things as setting a watch or timer at one hour intervals, beginning the questionnaires when you get up in the morning, or writing a reminder note in a day planner or on a post-it note.

If for some reason you are unable to complete all of the questionnaires in one day, we have provided a second package of questionnaires that can be completed on a day that is more convenient.

If you have any other questions or concerns, please feel free to contact the experimenter, Meghan Richards (mrichar4@lakeheadu.ca) or the research director, Dr. Kirsten Oinonen 343-8096, (koinonen@lakeheadu.ca).

Thank you for your time,
Sincerely,

Meghan Richards
B.A., B.Sc., M.A. Candidate, Clinical Psychology

Hourly Mood Survey

Date _____
Subject #_____

Time of Day _____

Please indicate how you are feeling at the present time by circling the appropriate response. *(1) very slightly or not at all, (2) a little, (3) moderately, (4) quite a bit, (5) extremely.*

	Very Slightly or not at all	A Little	Moderately	Quite a Bit	Extremely
Attentive	1	2	3	4	5
Interested	1	2	3	4	5
Alert	1	2	3	4	5
Excited	1	2	3	4	5
Enthusiastic	1	2	3	4	5
Inspired	1	2	3	4	5
Proud	1	2	3	4	5
Determined	1	2	3	4	5
Strong	1	2	3	4	5
Active	1	2	3	4	5
Distressed	1	2	3	4	5
Upset	1	2	3	4	5
Guilty	1	2	3	4	5
Scared	1	2	3	4	5
Hostile	1	2	3	4	5
Irritable	1	2	3	4	5
Ashamed	1	2	3	4	5
Nervous	1	2	3	4	5
Jittery	1	2	3	4	5
Afraid	1	2	3	4	5
Happy	1	2	3	4	5
Joyful	1	2	3	4	5
Content	1	2	3	4	5
At Ease	1	2	3	4	5
Calm	1	2	3	4	5
Sad	1	2	3	4	5
Blue	1	2	3	4	5
Alone	1	2	3	4	5
Downhearted	1	2	3	4	5
Lonely	1	2	3	4	5

Appendix F

Hourly Mood Survey

Date _____
Subject #_____

Time of Day _____

Please indicate how you are feeling at the present time by circling the appropriate response. *(1) very slightly or not at all, (2) a little, (3) moderately, (4) quite a bit, (5) extremely.*

	Very Slightly or not at all	A Little	Moderately	Quite a Bit	Extremely
Attentive	1	2	3	4	5
Interested	1	2	3	4	5
Alert	1	2	3	4	5
Excited	1	2	3	4	5
Enthusiastic	1	2	3	4	5
Inspired	1	2	3	4	5
Proud	1	2	3	4	5
Determined	1	2	3	4	5
Strong	1	2	3	4	5
Active	1	2	3	4	5
Distressed	1	2	3	4	5
Upset	1	2	3	4	5
Guilty	1	2	3	4	5
Scared	1	2	3	4	5
Hostile	1	2	3	4	5
Irritable	1	2	3	4	5
Ashamed	1	2	3	4	5
Nervous	1	2	3	4	5
Jittery	1	2	3	4	5
Afraid	1	2	3	4	5
Happy	1	2	3	4	5
Joyful	1	2	3	4	5
Content	1	2	3	4	5
At Ease	1	2	3	4	5
Calm	1	2	3	4	5
Sad	1	2	3	4	5
Blue	1	2	3	4	5
Alone	1	2	3	4	5
Downhearted	1	2	3	4	5
Lonely	1	2	3	4	5

Has anything happened during the course of the day that may have affected your mood (either negatively or positively)? Please list all of the events that may apply.

_____

_____

_____

_____

How many hours of sleep did you get last night?

_____

Did you exercise today? Yes No
If yes, what did you do?

_____

Have you taken any drugs/medications today? If so list the amount and the brand.

_____

_____

Did you consume any caffeine today? Yes No
If yes, How much did you drink?

_____

Did you consume any alcohol today? Yes No
If yes, How much did you drink?

_____

Appendix G

CONSENT FORM A

This study is being conducted by Dr. Kirsten Oinonen of the Department of Psychology at Lakehead University. Portions of this project will be used as Master's theses for Ms. Jessica Bird, and Ms. Meghan Richards. The purpose of the study is to examine genetic factors in women's health. You will receive one bonus point towards your Introductory Psychology mark for completing this screening questionnaire. The questionnaire will be used to select subjects for one of two studies. Individuals who participate in the subsequent studies will receive an additional one or two bonus points (depending on which study they are selected for) towards their final mark in Introductory Psychology. Please complete the attached bonus point form if you are in Introductory Psychology to ensure that you receive the bonus point.

Your participation in the screening will involve the completion of a questionnaire that will take approximately 40 minutes. The questionnaire includes personal questions about topics such as: demographic information, health information, medical information, reproductive history, relationship information, personalities and mood.

Participation in this experiment is voluntary and you may withdraw at any time without explanation and without penalty. All records of your participation will be kept in strict confidence and any reports of the study will not identify you as a participant. As per university requirements, all data will be stored for seven years by Dr. K. Oinonen at Lakehead University and remain anonymous and confidential. Individuals who meet specific criteria will be asked to participate in the studies. Therefore, we have asked for your name and telephone number on this form (please do not detatch the form). Once we have determined who will be asked to participate in the next phase, this sheet will be removed from your questionnaire and your information will remain both anonymous and confidential. There will be no way that your name can be connected to your responses. There are no known physical or psychological risks associated with participating in this study. If you have any questions or concerns regarding this study please contact Dr. Kirsten Oinonen (343-8096).

I have read and understood the consent form, and I agree to participate in this study under these conditions.

Name (Please Print): _____ Phone Number:_____

Signature: _____ Date: _____

Appendix H

DEBRIEFING FORM A

Thank you for participating in the screening phase of our study. The study is being conducted by Dr. Oinonen, Ms. Richards, and Ms. Bird. Portions of this research constitute Master's theses by Ms. Richards and Ms. Bird. If you are selected to participate in the second part of the study, you will be contacted by one of the researchers in the next three weeks. Participants in the next phases of the study will receive either one or two additional points towards their final mark (if they are Psychology 1100 students). If you are chosen for one of the next phases, you will be asked to provide a DNA sample (oral swab) and complete additional questionnaires.

Please be assured that once participants have been selected for the study, the consent forms will be removed from the questionnaires and there will be no way to identify your responses. All of your responses will be coded to conceal your identity on the questionnaires and all data will remain anonymous. If you have any questions, please feel free to contact Dr. Oinonen at the contact information below.

Kirsten Oinonen, Ph.D. C. Psych.
Department of Psychology
Lakehead University
955 Oliver Road
Thunder Bay, ON P7B 5E1
(807) 343-8086, koinonen@lakeheadu.ca

Ms. Jessica Bird, MA Candidate
Department of Psychology
Lakehead University
955 Oliver Road
Thunder Bay, ON P7B 5E1
jbird@lakeheadu.ca

Ms. Meghan Richards, MA Candidate
Department of Psychology
Lakehead University
955 Oliver Road
Thunder Bay, ON P7B 5E1
mrichar4@lakeheadu.ca

Appendix I

CONSENT FORM B

I agree to participate in this study that is investigating genetic factors in women's health. I understand that my participation is entirely voluntary: I can leave the experiment at any time and this will have no bearing on any remuneration I will receive, nor will it have any undesirable consequences.

The following points have been explained to me:

1. The purpose of this research is to find out what factors are related to women's health. The benefits I may expect from the study are (a) an appreciation of research on health, (b) an opportunity to contribute to scientific research and (c) course credit (One bonus point for Introductory Psychology Students).
2. The procedure will be as follows: During a single session, researchers will obtain a DNA sample (via an oral swab) and my body measurements (e.g. height, weight, hand measurements) will be taken, I will then be required to complete a total of three paper and pencil questionnaires.
3. There are no known serious risks involved in participating in this study.
4. All of the data collected as well as my DNA sample will remain strictly confidential. My responses will not be associated with my name. Instead, my data will be associated with a code number when the researchers store the data.
5. The experimenter(s) will answer any other questions about the research either now or during the course of the experiment (other than specific questions about the hypotheses). If I have any other questions or concerns, I can address them to the experimenter(s) Meghan Richards (mrichar4@lakeheadu.ca) or Jessica Bird (jbird@lakeheadu.ca) or to the research director, Dr. Kirsten Oinonen 343-8096, (koinonen@lakeheadu.ca).
6. Upon completion of my participation, I will receive a more detailed written explanation about the rationale underlying this experiment.
7. I am interested in receiving a summary of the results upon completion of the study: yes ☐ no ☐
   If yes, please indicate your email address: _____

_____ _____

Participant's Printed Name Signature

_____ _____

Date Experimenter Name

Appendix J

DEBRIEFING FORM B

Principal Investigators:

Dr. K. Oinonen, Psychology Department,
Lakehead University,
955 Oliver Road
Thunder Bay, ON P7E 5E1
koinonen@lakeheadu.ca, 343-8096

Ms. Meghan Richards, MA Candidate,
Psychology Department
Lakehead University
955 Oliver Road, Thunder Bay, ON P7E 5E1
mrichar4@lakeheadu.ca

Ms. Jessica Bird, MA Candidate,
Psychology Department
Lakehead University
955 Oliver Road, Thunder Bay, ON P7E 5E1
jbird@lakeheadu.ca

We appreciate your participation in our study, and thank you for spending your time to help us with our research. When you arrived here you were told that the purpose of this study was to investigate genetic factors relating to women's health. One of the factors in which we are interested is how genetic factors are related to mood in young women. In order to examine mood, we attempted to induce a negative mood state by telling you that we would be evaluating your intelligence based on your performance on a mental rotation test. *We want to assure you that this test is not a measure of your intellectual ability.*

We misled you about this test because we expected that people may have responded differently if they had known the nature of our research questions. We apologize, and hope you understand why it was necessary. As you can see, we would not have been able to investigate this research question without misleading you regarding the exact purpose of the test. In case you have any concerns about your mood and would like to see a mental health professional, we have provided you with a list of such resources on the attached sheet.

Given that this study involves some aspects of which you were not fully informed at the start, it is very important that you not discuss your experiences with other students until the end of the term. If participants have prior knowledge of our specific predictions it would influence their results, and the data we collect would be not be useable. Since you will be given a copy of this feedback to take home, please do not make it available to other students. If you do not keep this form, please dispose of it rather than leaving it somewhere that other students might read it. Please feel free to discuss with the experimenter any feelings you have about the study right away. Should you have further questions, do not hesitate to contact Meghan Richards, Jessica Bird, or Dr. Kirsten Oinonen, using the information listed above.

In addition to examining genetic factors involved in mood, we will also be using your DNA samples to examine specific genes that are involved in oral contraceptive side effects and eating disorder symptomatology. We have included three references on the following page in case you are interested in doing further reading relating to the study topics.

We hope that you have enjoyed participating in our study, and thank you very much for your assistance. As noted on the consent form, you will receive a summary of the results of the study at its completion if you have indicated an interest.

Mental Health Resource Sheet

Sometimes people can feel upset when thinking about their mood. If you feel as though you would like to talk to a mental health practitioner for any reason please consider the resources listed below:

- Lakehead University Health and Counseling Centre: 343-8361
- Family Services Thunder Bay: 626-1880
- Catholic Family Development Centre: 345-7323
- Emergency services are available at the Thunder Bay Health Sciences Centre
- Thunder Bay Crisis Response (24 hours): 346-8282.

If you are interested in doing further reading that is related to this study, here are three relevant journal articles that you might want to obtain.

Ogilvie, A.D., Battersby, S., Bubb, V. J., Fink, G., Harmar, A. J., Goodwin, G. M. &Smith, C. A. D. (1996). Polymorphism in serotonin transporter gene associated with susceptibility to major depression. *Lancet, 347*, 731-733

Oinonen, K., & Mazmanian, D. (2002). To what extent do oral contraceptives influence mood and affect? *Journal of Affective Disorders, 70,* 229-240.

Wade, T.D., Wilkinson, J., & Ben-Tovim, D. (2003). The genetic epidemiology of body attitudes, the attitudinal component of body image in women. *Psychological Medicine 33,* 1395-1405.

Appendix K

CONSENT FORM C

I agree to participate in a study that is investigating genetic factors in women's health. I understand that my participation is entirely voluntary: I can leave the experiment at any time and this will have no bearing on any remuneration I receive, nor will it have any other undesirable consequences.

The following points have been explained to me:

1. The purpose of this research is to find out what genetic factors are related to women's health. The benefits I may expect from the study are: (a) an appreciation of research on health, (b) an opportunity to contribute to scientific research, and (c) one bonus point for this portion of the study (If I am an Introductory Psychology Student).

2. The procedure will be as follows: Over the course of one day within the next week, I will be asked to complete 8 hourly mood surveys using the questionnaires that are provided.

3. There are no known serious risks to me for participating in this study.

4. All of the data collected will remain strictly confidential. My responses will not be associated with my name; instead, my name will be converted to a code number when the researchers store the data.

5. The experimenter will answer any other questions about the research either now or during the course of the experiment (other than questions that may reveal the hypotheses). If I have any other questions or concerns, I can address them to the experimenters, Meghan Richards and Jessica Bird or to the research director, Dr. K. Oinonen.

6. Upon completion of my participation, I will receive a more detailed written explanation about the rationale underlying this experiment.

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_____ _____

Participant's Printed Name Signature

_____ _____

Experimenter Name Date

Appendix L

DEBRIEFING FORM C

Principal Investigators: Dr. K. Oinonen, Psychology Department,
Lakehead University,
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Thunder Bay, ON P7E 5E1
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Ms. Meghan Richards, MA Candidate,
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Lakehead University
955 Oliver Road, Thunder Bay, ON P7E 5E1
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Ms. Jessica Bird, MA Candidate,
Psychology Department
Lakehead University
955 Oliver Road, Thunder Bay, ON P7E 5E1
jbird@lkaeheadu.ca

We appreciate your participation in our study, and thank you for spending the time helping us with our research.

When you agreed to participate in this phase of the study, you were told that the purpose of this study was to investigate genetic factors relating to women's health. In this portion of the study, we are more particularly interested in how genetic factors are related to mood and mood variability in young women. Thus, we will be using your daily mood questionnaires and your DNA sample to determine whether specific genes influence women's mood over the course of the day. In case you have any concerns about your mood and would like to see a mental health professional, we have provided you with a list of such resources on the back of this sheet. We have also included some references to journal articles that are relevant to this study in case you would like to do some further reading.

We ask that you not discuss your experiences in the study with any other students who potentially could be in this study until after the end of the term. If people come into the study knowing about our specific predictions, as you can imagine, it would influence their results, and the data we collect would be not be useable. We really appreciate your participation, and hope that this has been an interesting experience for you.

Mental Health Resource Sheet

Sometimes people can feel upset when thinking about their mood. If you feel as though you would like to talk to a mental health practitioner for any reason please consider the resources listed below:

- Lakehead University Health and Counselling Centre: 343-8361
- Family Services Thunder Bay: 626-1880
- Catholic Family Development Centre: 345-7323
- Emergency services are available at the Thunder Bay Health Sciences Centre
- Thunder Bay Crisis Response (24 hours): 346-8282.

If you are interested in doing further reading that is related to this study, here are three relevant journal articles that you might want to obtain.

Ogilvie, A.D., Battersby, S., Bubb, V. J., Fink, G., Harmar, A. J., Goodwin, G. M. Goodwin, G. M., et al. (1996). Polymorphism in serotonin transporter gene associated with susceptibility to major depression. *Lancet, 347*, 731-733

Oinonen, K., & Mazmanian, D. (2002). To what extent do oral contraceptives influence mood and affect? *Journal of Affective Disorders, 70,* 229-240.

Wade, T.D., Wilkinson, J., Ben-Tovim, D. 2003. The genetic epidemiology of body attitudes, the attitudinal component of body image in women. *Psychological Medicine 33,* 1395-1405.

Appendix M

CONSENT FORM D

The study that you have just participated in is being conducted by Dr. Kirsten Oinonen of the Department of Psychology at Lakehead University, and graduate students Ms. Jessica Bird, and Ms. Meghan Richards. The purpose of the study is to examine genetic factors in women's health. Given the valuable time and data that you have contributed to this study, we hope to maximally contribute to scientific knowledge with this data. If our hypotheses are supported in the current study and/or if there appears to be value in conducting a longitudinal follow-up project, we would like to ask for your permission to contact you within the next five years, to ask whether you might be willing to participate in a follow-up study. Any follow-up study would only be conducted if it had received ethical clearance by the Lakehead University Research Ethics Board and any relevant granting agency. Thus, you can be assured that you would only be contacted in such a situation. Furthermore, your signature on this form does not constitute your consent to participate in a follow-up study. Your signature on this form would only allow us the opportunity to attempt to contact you to see if you are interested in participating. If we were to receive ethics approval for such a study, we would open up the sealed envelope that you would be sealing today. This envelope will contain your name, participant number, and contact information (e-mail addresses, telephone numbers, addresses). The envelope would only be opened in such a situation, and will be destroyed if a follow-up study is not planned within the next five years.

Participation in this portion of the study is voluntary and you may withdraw at any time without explanation and without penalty. All records of your participation will be kept in strict confidence and any reports of the study will not identify you as a participant. As per university requirements, all data will be stored for seven years by Dr. K. Oinonen at Lakehead University and remain anonymous and confidential. There are no known physical or psychological risks associated with participating in this study. If you have any questions or concerns regarding this study please contact Dr. Kirsten Oinonen (343-8096).

I have read and understood the consent form, and I agree to participate in this study under these conditions.

Name (Please Print): _____

Signature: _____ Date: _____

FOLLOW-UP STUDY

Please complete the following contact information. This information will only be removed from the sealed envelope in the event that a follow-up study has received ethical clearance. The information will only be used for the purpose of attempting to contact you to determine if you would like to participate in a follow-up study. Given that it can be difficult to know how to contact a person in the future, please feel free to provide any additional information that might be of help to us.

Full Name (Please Print): _____

Participant Number: _____

E-mail addresses: _____

_____

_____

Mailing Addresses: _____ (current)

_____

_____

_____ (home or other)

_____

_____

Telephone Numbers: _____ (current)

_____ (other)

Please fold this paper and place it in the envelope provided and seal the envelope. Please write your participant number on the front of the envelope. Thank you for your participation.