The effects of tryptophan depletion on impulsivity and mood in healthy men and women

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2007
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These studies are based on a conversation Andres Magnusson and I had while sitting on the grass and enjoying the sun at Ullevål University Hospital in the summer of 2000. Andres being a psychiatrist, and I a psychologist with emphasis on biology, we bridged the gap between psychology and medicine. The tryptophan depletion method used in these studies has its background in medicine, while the variables used to detect changes and interpret their meaning are based on psychological research. Nils Inge Landrø quickly became a key player, and the three of us made a fantastic team. When Andres moved to Iceland, Nils Inge was my main supervisor. At the time the third paper was accepted, Alexander Neumeister wrote: “Lucky you for having Nils Inge as your mentor.” I find this statement accurate, and I am grateful for the times I have had with Nils Inge and Andres.

I would also like to thank Natalie Johnston for acquiring and managing the behavioural data, Dag Erik Eilertsen for statistical expertise, Helen S. Mayberg for giving me the idea to collect samples for genotyping, and Lynn Freligh for revision of the manuscript.

In addition, the participants deserve a special praise. All of you fasted on the day before the experiment, drank the horrible mixture, and some experienced nausea and discomfort during a long day of experiments. Without you this study would have been truly impossible.

The Department of Psychology, University of Oslo, made the research possible by providing me with a salary, a great office with all conveniences and access to all the information a scientist could dream of. The administrative and technical staff has come to my aid a great number of times, always being helpful and professional. I would also like to thank my fellow PhD candidates and colleagues at the Unit of Cognitive and Neuropsychology for sharing and taking part of the ups and downs of research, and for a lot of good laughs. There will be more champagne for us in the future.

The projects have also been supported by grants from the Research Council of Norway, Division for Science; the Department of Psychology, University of Oslo; Johanne and Einar Eilertsens Research Foundation; and Thordis and Johannes Gahrs Research Foundation.
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ABBREVIATIONS

5-HIAA: 5-hydroxyindoleacetic Acid
5-HT: 5-hydroxytryptamine (Serotonin)
5-HTTP: 5-hydroxytryptophan
5-HTTLPR: 5-hydroxytryptamine Transporter Length Polymorphic
AD/HD: Attention-deficit/hyperactivity disorder
ANOVA: Analysis of Variance
ATD: Acute Tryptophan Depletion
Beta (β): Response style
CNS: Central Nervous System
CPT: Continuous Performance Test
CPT-IP: Continuous Performance Test – Identical Pairs
CSF: Cerebrospinal Fluid
D prime (d’): Stimuli sensitivity
IMT: Immediate Memory Task
LNAA: Large Neutral Amino Acids
MAO: Monoamine Oxidase
PCR: Polymerase Chain Reaction
POMS: Profile of Mood States
SNP: Single Nucleotide Polymorphism
SSRI: Selective Serotonin Reuptake Inhibitors
TCA: Tricyclic Antidepressants
TMD: Total Mood Disturbance
TRP: Tryptophan
1. INTRODUCTION

1.1 Serotonin

The neuron is the basic unit of the brain. When a neural impulse travels down the axon and arrives at the synaptic terminals, it triggers the release of a neurotransmitter. The neurotransmitter travels across the synaptic gap and stimulates the next neuron, thereby carrying the impulse from one neuron to the next. A neurotransmitter is a substance that is synthesised and stored in the nerve ending of the sending neuron, and when released may trigger an electrical impulse in the receiving neuron. In addition to neurotransmitters, there are also a number of neuromodulators that modulate the action of a transmitter rather than having a direct physiological effect on their own. Neuromodulators are not responsible for the direct transfer of nerve impulses from one neuron to another, but alter the action of neurotransmitters by enhancing, reducing, or prolonging their effectiveness on the receiving neuron. There are about 50 different known neurotransmitters. The neurotransmitter serotonin is a member of the idoleamine (or monoamine) transmitters, a family that is slightly more complicated chemically than the amino acid transmitters. Serotonin is occasionally a neuromodulator, depending on the nature of the receptors in the receiving neuron.

Serotonergic neurons have been found to have a slow, tonic pattern of firing (approximately one to two spikes per second). The maintenance of rhythmic firing under a wide variety of conditions has suggested that serotonergic neurons possess intrinsic tonic pacemaker mechanisms. The highly regulated pacemaker activity of serotonergic neurons suggests that the serotonin system serves an important homeostatic function. Through its effects on neuronal excitability in diverse regions of the brain and spinal cord, the serotonergic system strategically coordinates complex sensory and motor patterns during various behavioural states. It can be hypothesized that the function of the serotonin system, by its coordinated fluctuations in activity, is to promote a given behavioural state.

Serotonin, or 5-hydroxytryptamine (5-HT), has been implicated in almost every conceivable physiological or behavioural function: affect, aggression, cognition, appetite, nausea, endocrine function, gastrointestinal function, motor function, perception, sensory
function, sex, sleep, and vascular function (Bloom 1995). A great many drugs currently used for the treatment of psychiatric disorders such as depression, mania, schizophrenia, autism, obsessive-compulsive disorder, anxiety disorders are thought to act, at least partially, through serotonergic mechanisms. Serotonin is involved in such a vast range of functions partially because it has a widespread distribution in the brain, spinal cord and peripheral nervous system; partially because of the molecular diversity of the many serotonergic receptor subtypes; and partially because serotonin probably modulates the actions of other neurotransmitters, rather than having a direct physiological effect of its own.

1.2 The origin and fate of serotonin in the brain

Serotonin is closely related to the amino acid tryptophan. Amino acids are organic compounds found in high concentration in all cells in the body. The various types of amino acids combine to form protein. Proteins are the building blocks of the body, and when ingested, the body’s main source of amino acids. Amino acids are also neurotransmitters, and they may be metabolized to form more complex neurotransmitters, such as the monamine transmitter serotonin. The pathway for serotonin synthesis is shown in Figure 1 below:

Figure 1: The synthesis of Serotonin

<table>
<thead>
<tr>
<th>L – Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrahydrobiopterin ↓ Tryptophan hydroxylase</td>
</tr>
<tr>
<td>L – 5 – Hydroxytryptophan (5 – HTP)</td>
</tr>
<tr>
<td>Pyridoxal phosphate ↓ Aromatic L – amino acid decarboxylase</td>
</tr>
<tr>
<td>Serotonin (5 – Hydroxytryptamine; 5 – HT)</td>
</tr>
</tbody>
</table>
The first step in the process of serotonin synthesis involves hydroxylation of L-tryptophan to form 5-hydroxytryptophan (5-HP). This reaction is catalysed by the enzyme tryptophan-5-monooxygenase, commonly known as tryptophan hydroxylase. The synthesis of serotonin is to a large degree dependent on the presence of this enzyme, and in the nervous system, it is localised in the serotonergic neurons. Tryptophan hydroxylase is made in the serotonergic nerve cell bodies and transported to the nerve terminals where most serotonin synthesis occurs, resulting in a high concentration of this enzyme in the serotonergic nerve endings. The product of 5-HP undergoes decarboxylation to form serotonin. This step of the process is rapid, and only low concentrations of 5-HP are present in the brain.

The breakdown, or metabolism, of serotonin in the central nervous system occurs mainly through monoamine oxidase (MAO): see Figure 2 and 3. The final product of this breakdown process, 5-hydroxyindoleacetic acid (5-HIAA), is normally the measure of the amount of serotonin available for neurotransmission, and is assumed to be related to the degree of serotonergic firing in the brain. Logically, this is approximately the same as measuring the exhaust fumes from a car in order to determine how fast the car is going.

**Figure 2: The breakdown of serotonin**

<table>
<thead>
<tr>
<th>Serotonin</th>
<th>(5 – Hydroxytryptamine; 5 – HT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ Monoamine Oxidase (MAO)</td>
<td></td>
</tr>
<tr>
<td>5 – hydroxyindoleacetaldehyde</td>
<td></td>
</tr>
<tr>
<td>↓ Aldehyde dehydrogenase</td>
<td></td>
</tr>
<tr>
<td>5 – hydroxyindoleacetic acid (5 – HIAA)</td>
<td></td>
</tr>
</tbody>
</table>

There are a number of factors regulating the level of serotonin in the central nervous system. Retracing the synthesis and metabolism of serotonin, one can reason that
tryptophan, the enzyme synthesising tryptophan into serotonin, and MAO which breaks serotonin down to other compounds, are involved in modulating the brain levels of serotonin. Figure 3 gives an overview of the synthesis and metabolism of serotonin, and takes into account where the processes occur. Take special note of the transportation of tryptophan from the blood, across the blood-brain barrier and into the cerebrospinal fluid (CSF), and the box representing a serotonergic neuron.

**Figure 3: Synthesis and metabolism of serotonin.** From left to right: tryptophan in the blood competes with other large neutral amino acids for access to the transporter system for these compounds across the blood-brain barrier, into the brain. Free tryptophan in the central nervous system is transported into the serotonergic neuron and enzymatically hydroxylated via tryptophan hydroxylase, and decarboxylated via aromatic amino acid decarboxylase into serotonin. The serotonin can then be taken up by synaptic vesicles for storage and release, or (depending on the degree of neuronal activation) be metabolized directly by monoamine oxidase without being released as a neurotransmitter. Serotonin released into the synaptic gap may or may not activate receptors before it is recycled into the serotonergic neuron in an active re-uptake process, or broken down in the synaptic gap by monoamine oxidase. Serotonin metabolized to 5–HIAA diffuses from the cell or synaptic gap into the extracellular fluid space, and into the cerebrospinal fluid where it can be collected with lumbar puncture. (From Williams et al. 1999, p. 1645).
1.3 Acute Tryptophan Depletion (ATD)

ATD is a method in which researchers manipulate serotonin levels. It may be applied in randomized and placebo-controlled experiments; thus intervention studies on the behavioural consequences of lowered brain serotonin in humans may be conducted. ATD reduces the availability of the serotonin precursor tryptophan. This is achieved by having the participants ingest a mixture of amino acids devoid of tryptophan. When the liver senses an abundance of amino acids in the blood, protein synthesis in the liver is enhanced, and the resulting incorporation of tryptophan into protein leads to a rapid (5 hours) and substantial (80% to 90%) lowering of tryptophan in plasma and tissues (Young et al. 1985; Delgado et al. 1990). Moreover, the ingested amino acids reduce the ratio of tryptophan to the other five Large Neutral Amino Acids (LNAAs), leaving the remaining tryptophan at a competitive disadvantage with regard to the common LNAA-carrier over the blood-brain barrier, with a subsequent even greater tryptophan deficiency in the brain (Fernstrom 1977; Asberg-Wistedt et al. 1998). ATD is thought to reduce brain serotonin levels because conversion of tryptophan to 5-hydroxytryptophan (serotonin) is a rate-limiting step in the synthesis of serotonin (Young & Gauthier, 1981). This process is completed locally in the neurons, as serotonin cannot cross the blood-brain barrier. Animal studies of cerebrospinal fluid and brain tissue have confirmed that ATD is able to lower brain levels of tryptophan, serotonin, and 5-HIAA, the major metabolite of serotonin in the cerebrospinal fluid (Biggio et al. 1974; Gessa et al. 1974; Fernstrom 1977). An ATD-like mixture given to rats diminished the serotonergic neurotransmission in the frontal cortex to about 50% of baseline, in a time course similar to human studies (Bel and Artigas, 1996). In humans, similar results were found in measures of brain levels of tryptophan and 5-HIAA (Carpender et al. 1998; Williams et al. 1999), supporting the assumption that ATD reduces central serotonin functioning.
1.4 Mood and serotonin

Serotonin was discovered in the brain over 50 years ago, and almost immediately linked to psychiatric disorders (Woolley & Shaw, 1954). During the 1950s, several observations during clinical testing of drugs which affected serotonin gave rise to the serotonin hypothesis of major depression. This suggests that depression is related to a deficit of serotonin, whereas mania is associated with an excess of serotonin (Schildkraut, 1965). The serotonin hypothesis for depression is supported by the following evidence: Drugs
which decrease serotonin levels, such as reserpine, will induce depression in a number of patients. Drugs that directly or indirectly enhance central serotonergic activity will alleviate depression in a number of patients. Disorders in serotonergic activity may contribute to many of the symptoms of major depression, for example: mood, activity, sleep, appetite, suicide, sexual and cognitive dysfunction (Meltzer HY & Lowy, MT, 1987).

To further investigate the serotonin hypothesis of depression, techniques such as ATD were developed in order to examine the behavioural consequences of such a pharmacological challenge to the serotonergic system. Following ATD, a significant reappearance of depressive symptoms occurred in remitted depressed patients (Neumeister et al 2006, 2004, 2003; Smith et al 1997a; van der Does & Booij 2005), supporting the hypothesis that reduced serotonergic transmission is an important mechanism in the pathophysiology of major depressive disorder. Studies examining at the effect of ATD on mood in healthy participants, on the other hand, have found highly variable results, apparently dependent on individual characteristics. The majority of studies have found little effect of ATD on mood in healthy volunteers with no personal or family history of affective disorder, and without specific genetic risk factors (eg Schmitt et al. 2000; Evers et al. 2005a; 2005b). Healthy volunteers with a family history of affective disorder (eg Benkelfat et al. 1994; Neumeister et al. 2002), patients with remitted depression (eg Booij et al. 2002; Neumeister et al. 2004), and healthy volunteers with specific genetic risk factors, such as carriers of the short allele variant of a polymorphism in the promoter region of the serotonin transporter gene (Neumeister et al. 2002), generally do experience mood-lowering effects of ATD.

The mood-lowering effect of ATD among healthy volunteers has primarily been detected in women (Ellenbogen et al. 1996; Smith et al. 1997b). Although some early studies also found an effect of ATD on mood in healthy men (Young et al. 1985; Smith et al. 1987), subsequent studies have reported that ATD results in few mood effects in men (Bell et al. 2005). In remitted depressive patients, depressive symptoms were found to be significantly greater in women as compared to men during ATD (Moreno et al. 2006). A reanalysis of the data from six ATD studies on depressed patients in remission found that women showed larger mood-lowering effects to ATD than men (Booij et al. 2002). This larger mood-lowering effect of ATD in women was not accounted for by the greater
tryptophan depletion, nor by clinical characteristics. The specific effects of ATD on mood in women were further confirmed in a recent study comparing male and female outpatients of a mood disorder clinic (Booij et al. 2005). Sex-specific differences in how interference with serotonin transmission affect men vs. women have also been implicated in behaviours such as aggression, conduct disorders and attention-deficit/hyperactivity disorder (AD/HD) (Cadoret et al. 2003).

Impaired impulse control is frequently found in patients with major depressive disorders (Sher et al. 2005; Oquendo et al. 2004; Dumais et al. 2005), particularly in those with a history of violent, impulsive suicide attempts (Aasberg et al. 1997; Booij et al 2006). Reduced serotonin transmission may also constitute an important pathophysiological mechanism in the development of impaired impulse control (Doland et al. 2001; Hibbeln et al. 1998; Coccaro et al. 1996; Virkkunen et al. 1994). Therefore, a perturbation in serotonin transmission might be a shared pathway that modulates both the vulnerability to mood disorders and impaired impulse control.

1.5 Impulsivity and serotonin

Impulsiveness, a term borrowed from everyday language, has proven to be a valuable description of a disruptive behaviour seen in several types of psychiatric patients bearing a wide range of diagnostic labels (American Psychiatric Association, 2000). In Medline, impulsiveness is defined as: “An act performed without delay, reflection, voluntary direction or obvious control in response to a stimulus,” while in a clinical setting, impulsivity is defined as the failure to resist an impulse, drive, or temptation which is harmful to oneself or others (Cherkasky and Hollander, 1997). Functional impulsivity expresses willingness and ability to take risks in situations where this behaviour is appropriate and necessary. Dysfunctional impulsivity, on the other hand, expresses a tendency to thoughtlessness and inability to plan and think one’s actions through, leading to negative consequences. The focus in most research and psychiatry is on dysfunctional impulsivity; this may be a characteristic of many psychiatric illnesses, including personality disorders such as borderline and antisocial personality, neurological disorders characterized by disinhibited behaviour, AD/HD, substance and alcohol abuse, bulimia,
and “disorders of impulse control not elsewhere classified,” such as pathological
gambling, kleptomania, pyromania and explosive disorders.

Impulsiveness has been correlated to suicide and violent behaviour (Roy and Linnoila
1988; Ho et al. 1998). Asberg et al. (1976) were among the first investigators to relate
impulsive behaviour to dysfunction of the central serotonergic system. They reported that
depressed patients who had made violent, impulsive suicide attempts had reduced levels
of 5-HIAA, the major metabolite of serotonin, in their CSF (Asberg 1997). This finding
has been replicated in some studies, but not by all (Deakin 1989). Subsequent studies of
alcoholics (Fils-Aime et al. 1996), violent offenders (Linnoila et al. 1983; Tiihonen et al.
2001), impulsive arsonists (Virkkunen et al. 1987), patients with personality disorders
(Simeon et al. 1992; Brown et al. 1982) and healthy volunteers with impulsiveness as a
personality trait (Roy et al. 1988) have shown that reduced baseline activity of the central
serotonergic system is associated with aggressive/violent behaviour in general, and
especially with impulsive violent behaviour (Linnoila et al. 1983; Brown and Linnoila
1990). In addition, the serotonin-releasing drug d,l-fenfluramine has been found to
decrease the number of impulsive and aggressive responses in groups of male subjects
with a history of conduct disorders (Cherek and Lane 2000; Cherek and Lane 1999). Thus,
serotonergic functions seem to play a role in the inhibition of behaviour in man (Soubrie
1986).

Most of the studies cited above suffer from several inherent problems which make direct
interpretations problematic: (a) they usually rely upon correlation data; (b) the studies
have often relied on retrospective measures of aggressive or impulsive behaviour; (c) it is
difficult to determine to what extent the observed differences in baseline serotonin are due
to genetic or environmental factors; and (d) a large portion of CSF 5-HIAA is not derived
from brain serotonin neurons (Dougherty et al. 1999c). As a result, it is difficult to
attribute a direct causal relationship between serotonin and impulsive and/or aggressive
behaviours. Stronger evidence can be obtained only by directly manipulating serotonin
levels (e.g. using ATD) and measuring the effect on behaviour.

Animal studies have also found a clear link between impulsiveness and serotonin. Free-
ranging male monkeys with low CSF 5-HIAA concentration spontaneously leaped across
long distances at dangerous heights and exhibited more violent forms of aggression,
indicating more impulsive behaviour (Mehlman et al. 1994). Manipulation of serotonin levels in rats, have resulted in a more impulsive response style in choice serial reaction time tasks (Harrison et al. 1999; Harrison et al. 1997; Carli and Samanin 2000), giving further support to a link between impulsiveness and serotonin.

### 1.6 Impulsivity measured

There seems to be a general agreement between temperament psychologists, psychiatrists and animal experimenters that impulsivity is multifactorial and that these factors are separable and independent of one another (Evenden 1999). From the reports on children with AD/HD, the concept of impulsiveness may include social responsibility, faulty decisions, and speed of response. This is, according to Gerbing et al. (1987), a gross simplification of what they found to be a multidimensional construct. Using a multivariate regression analysis to examine the structure of a pooled set of 378 items taken from the impulsiveness scales and measures of Barrat, Catell, Eysenck, Guilford, Jackson Kagan, and Zuckerman, they found 15 different components of impulsiveness. To make the situation worse: there was a low correlation between the 12 self-report scales (impulsive, thrill seeking, quick decisions, avoid planning, energetic, happy-go-lucky, impulse purchaser, unreflective, distractible, restless, impatient, and avoids complexity) and the 3 objective behavioural scales (simple reaction time, time perception, and a construct made from number of errors and thinking time). Applying a Bonferroni correction for multiple tests to these results would abolish the few significant correlations found between the behavioural and the self-report domains. Low correlations between questionnaire and behavioural measures of impulsiveness have been reported also in other studies (e.g. Barrat & Patterson 1983). There are at least two possible explanations for this: (a) questionnaire measures do not register differences in cognitive preference, but rather social factors (van den Broek et al. 1992); and (b) people are unaware of their own cognitive preferences (Phillips & Rabbitt 1995). Both factors may contribute to the lack of a significant relationship between questionnaire and behavioural measures of impulsiveness.
Self-report scales do not lend themselves easily to pharmacological studies of impulsivity, because they are subjective and measure relatively stable characteristics. Ho et al. (1998) expressed concern for the use of "clinical tools and rating scales" in the measurement of impulsiveness, and emphasized the need for a behavioural definition of impulsiveness that is applicable to animals as well as humans. This is necessary "if progress is to be made towards a thorough understanding of the [biological basis for impulsiveness]" (Ho et al. 1998, p. 69). An inability to inhibit or delay voluntary behaviour is an important characteristic of impulsiveness (Ho et al. 1998), and is frequently measured in ‘delayed response’ paradigms in which the occurrence of premature responses may be taken to signify impulsiveness (McClure & Gordon 1984).

Within the framework of cognitive and neuropsychology, impulse control has been described as an active inhibitory mechanism enabling slower cognitive mechanisms to guide behaviour. Swann et al. (2002) investigated personal and clinical characteristics of impulsivity, and found that the neuropsychological tests which best predicted these measures were of the of the ‘rapid-response impulsivity’ type. The Continuous Performance Test (CPT) is a typical test measuring rapid-response impulsivity, as it requires the individual to make rapid evaluation/discrimination of presented stimuli to decide whether or not to respond. Traditionally, the indices most widely used to assess performance on the CPT have been correct responses to a target stimulus, called ‘hits,’ and responses to nontarget stimuli. There are two types of impulsive responding to nontarget stimuli: a ‘commission error’ is a response to any stimulus other than the target (Sykes et al. 1971), and a ‘false alarm’ is a response to a stimuli that is similar, but not identical, to the target (Dougherty et al. 1999a). Thus, a false alarm is also a commission error, but a commission error is not necessarily a false alarm. False alarms, or impulsive disinhibition, are thought to result from anticipatory or incomplete processing of the stimulus, which leads to a rapid but incorrect response. CPT studies have reported increased false alarm rates in a number of impulsive populations such as children with AD/HD (Halperin et al. 1991a) and disruptive behaviour disorders (Dougherty et al. 2003a), adults with histories of Conduct Disorder (Dougherty et al. 2000a) and Bipolar Disorder (Swann et al. 2001), healthy controls following consumption of alcohol (Dougherty et al. 1999b; Dougherty et al. 2000b), and non-alcoholic participants having a history of driving-while-intoxicated arrests (Koch & Morguet 1985).
More recently, investigators have tended to use signal detection analysis to combine hits and false alarm rates into $d'$ (D-prime) and $\beta$ (beta). The $d'$ is a measure of the subject’s ability to discriminate between stimuli, and involves the observer’s attention and sensory capacity (Davies & Parasuraman 1982; Swets et al. 1961). The $\beta$ is a measure of response style, and individuals with a tendency to over-respond (low $\beta$) are considered risk-taking, while individuals with a tendency to under-respond (high $\beta$) are considered cautious (Rutschmann et al. 1977). Thus, CPTs gives two different impulsivity measures: impulsive response style ($\beta$) and impulsive disinhibition (false alarms). Response style ($\beta$) examines the competition between behavioural suppression and active responding. Response style could be impulsive (Spreen & Strauss 1998; Halperin et al. 1991b; Phillips & Rabbitt 1995; Rutschmann et al. 1977) and associated with AD/HD symptoms (Epstein et al. 2003) on one end of the scale, or cautious (i.e. less impulsive) and associated with depression (Johnson & Magaro 1987) on the other end of the same scale. The effects of ATD on impulsivity have been studied primarily in men (Dougherty et al. 1999c), and to our knowledge no previous study has investigated how the response style of women is affected by ATD. More prominent mood-lowering effects of ATD have been found in women relative to men both for healthy volunteers (Neumeister et al. 2002; Ellenbogen MA, 1996; Smith KA, 1997a) and for depressed patients (Booij et al. 2002; Booij et al. 2005). As a cautious response style is associated with depression (Johnson & Magaro 1987), we expected women to adopt a cautious response style, and men to adopt an impulsive response style, in response to ATD.

**1.7 Serotonin transporter gene**

Genetic variations in key serotonergic subsystems result in altered serotonergic tone and neurotransmission (Owens & Nemeroff 1994). A major focus of genetic research on mood disorders has been the serotonergic transporter gene (Roiser et al. 2006). In this work, we have focused on a relatively common polymorphism (5-HTTLPR) in the promoter region of the human serotonergic transporter gene (5-HTT), resulting in two common alleles or variants (Heils et al. 1996); the so-called short (S) and long (L). Both in vivo transfection (Lesch et al. 1996) and in vivo imaging (Heinz et al. 2000) studies have revealed that the 5-HTTLPR has functional effects at the level of serotonergic biology by regulating 5-HTT
expression; specifically, the S allele is associated with a nearly 50% reduction in 5-HTT availability. Although the picture is not completely consistent, there are several reports of associations with the less efficient S allele and risk for affective illness (Lesch & Mossner 1998; Caspi et al. 2003). Subjects carrying the short allele of this polymorphism have also a delayed response to antidepressant treatment; they display heightened amygdala reactivity to fear stimuli (Hariri et al. 2002) as well as increased negative affect in response to tryptophan depletion (Neumeister et al. 2002; 2006; Moreno et al. 2002; Roiser et al. 2006). The 5-HTTLPR polymorphism has been shown to a) be functional in humans (Hu et al. 2005), b) modulate the vulnerability to MDD (Caspi et al 2003) and depression severity (Zalsman et al. 2006), c) modulate vulnerability to heavy drinking with sex-specific differences (Covault et al. 2007), and d) be involved in the regulation of impulsive behaviour (Courtet et al. 2004; Bayle et al. 2003; Steiger et al. 2005; Gorwood et al. 2000). Two variants, a long (L) and a short (S) were originally described, but recently an A>G Single Nucleotide Polymorphism (SNP) within the L allele was identified, suggesting that this polymorphism is tri-allelic. The two L alleles are currently designated as L_G and L_A and S (Hu et al. 2006). The S and L_G alleles are associated with lower expression of the transporter protein relative to the L_A allele (Hu et al 2006). The role of 5-HTTLPR in the effects of ATD on impulse control in healthy men and women has not yet been studied.

1.8 Improvements in design

ATD has been employed to investigate the link between impulsivity and serotonin as suggested by the aforementioned studies, and in a widely cited, influential review by Sourie (1986). The results in healthy volunteers have been generally negative, but this may be due to methodological problems such as order effect (Walderhaug, in press), insufficient ATD (van der Does 2001a), insufficient number of participants to reach a statistically significant result, and the impulsivity measures lacking sensitivity, for example due to ceiling levels of performance in healthy volunteers. The equivocal findings could also be caused by individual differences in impulsivity (Cools et al. 2005), aggression (Dougherty et al. 2000a), and family history (LeMarquand et al. 1999), as well as individual differences in genetic makeup (Neumeister et al. 2002).
ATD studies, as our first study was, are traditionally carried out using a within-subjects, crossover study design in which each participant is given both active depletion and placebo on two separate test days (Reilly et al. 1997). Compared to between-subjects designs in which the participants are randomized into one group given active depletion and another group placebo, crossover study designs are regarded to have more power. This is because the error variance is reduced when the performance of a given participant in the active intervention is compared to his own performance in the placebo condition, rather than to another participant randomized into a different group. An important assumption when using a crossover design is that the order in which the intervention is given must be irrelevant. Conditions in which a previous treatment alters the behaviour observed in subsequent treatments are called ‘carryover effects’ or ‘order effects,’ and constitute a serious threat to the validity of crossover studies.

Carryover effects normally occur because a previous treatment alters the behaviour observed in a subsequent treatment. The previous treatment changes the participant, and those changes “carry over” into the subsequent treatment, in which they affect how the participant performs then. For instance, a crossover experiment in which the participants are given either a cure or placebo can expect a carryover, because the participants given a curative intervention on the first test day will no longer have a condition to be cured on the second test day. As to the psychological aspect, Jan Smedslund (personal communication) notes that a fallacy of crossover experiments is that the participant remember and is changed by the experiences gained the first test day, and cannot be his or her own control for this reason. Doing something for the first time will always be different from subsequent times, no matter how similar the situation.

A number of ATD studies report findings indicating the presence of carryover effects. Murphy et al. (2002) examined the effects of ATD on 12 healthy female volunteers on a visual discrimination task, and found the response times on reversal learning to increase in response to tryptophan depletion, but only when the test was novel to the participants. Participants given active tryptophan depletion on the first test day, and placebo on the second, were found to respond differently compared to the participants given placebo first, and active tryptophan depletion second. The order in which the intervention was given was relevant, because the effect was specific to the first session when the test was novel.
(Murphy et al. 2002). The same effect is seen in other studies manipulating serotonin in healthy humans: Ellenbogen et al. (1996) found that female participants given active ATD responded with a lowering of mood on the Profile of Mood States (POMS) compared to females given placebo, but only the first time they were tryptophan depleted. Park et al. (1994) found that active ATD impaired reversal learning on the two dimensional version of the ID/ED task, but only in the first session in the within-subjects crossover design. This pointed towards carryover effects in ATD studies that used a similar intervention and study design as our first experiment.

Based on experiences from the first study, we made some key changes in the second study. Instead of repeating a crossover design with 24 participants, the second study consisted of 83 participants in a parallel, between-subjects study design. We continued to administer a high dose of ATD to insure sufficient depletion, and introduced genotyping for the serotonin transporter gene (5-HTTLPR). Using sensitive measures for mood and impulsivity, the second study included both men and women. This allowed us to avoid some of the disadvantages of previous studies.
2. RESEARCH QUESTIONS

2.1 Paper I

1. Will reduced serotonergic neurotransmission increase impulsiveness in normal men?

2.2 Paper II

2. Is the effect of reduced serotonergic neurotransmission more pronounced in a novel, rather than in a familiar environment?

2.3 Paper III

3. Are the effects of reduced serotonergic neurotransmission on mood and impulsivity influenced by sex and the serotonin transporter gene (5-HTTLPR)?
3. MATERIALS & METHODS

The papers included in this thesis are based on two separate experiments. Papers I and II are based on the first ATD experiment, using a within-subjects crossover design. Paper III is based on the second ATD experiment using a parallel between-subjects design.

3.1 Participants

Papers I & II
Twenty-four males (aged 21 to 29; mean 25 years) were recruited from medical and psychological students at the University of Oslo. They had an average of 4 years of university education, and all signed a statement that they were drug free, healthy, and without any history of psychiatric problems. All the participants completed the experiment. The study was carried out in accordance with the Helsinki Declaration, it was assessed by the regional ethics committee, and all the participants received extensive information and signed an informed consent.

Paper III
The participants were normal students recruited at the University of Oslo. Following a telephone screening, 86 participants arrived for further evaluation. All the participants provided written informed consent, and underwent a semi-structured interview to rule out those with evidence of major psychiatric diagnoses. Alcohol use was not permitted 24 hours before and during the course of the study. All participants were informed during the recruitment phase that medical illness was an exclusion criterion. Prior to inclusion, health information was collected as part of a questionnaire, and all the participants stated that they did not have a manifest medical or mental illness. All the participants appeared healthy. We acknowledge, however, that we did not perform a physical examination, and did not collect blood samples or ECG, which would have better ensured that only medically healthy people were included. Three participants could not complete the study because they could not tolerate the amino acid beverage, and their data were not included in the statistical analyses. Forty-four women (mean age 24.5 ± 2.4; range 20-32 years) and 39 men (mean age 24.5 ± 3.3; range 20-34 years) completed the study. It was not possible to collect blood samples from one of the women. All the women took part of the study in
the luteal phase of their cycle (10 days to 1 day before menstruation). The 15 women not using contraception were significantly more dysphoric and fatigued on the POMS given after the intervention, and were more (but not significantly) sensitive to tryptophan depletion in terms of impulsivity (higher $\beta$ and lower false alarms & hits) as compared to the 28 women using contraception. Roughly 1/3 of the participants were psychology students, 1/3 medical students, and 1/3 were students with other majors. The study was carried out in accordance with the Helsinki Declaration. The protocol was assessed by the Norwegian Regional Committee for Medical Research Ethics.

### 3.2 Tryptophan depletion procedure

The procedure for Acute Tryptophan Depletion (ATD) has been described in the introduction and, by for instance, Young et al. (1985), Reilly et al. (1997), and Bjork et al. (2000). The active ATD and sham depletion mixture mimics the amino acid composition of breast milk, and was based on the 100 g recipe previously used for men (Young et al. 1985). In Paper III, the amino acid mixture was modified for women to adjust for differences in body mass (Murphy et al. 2002). Based on the lower average body weight in women compared to men, the weight of the mixture was reduced by approximately 17% to 85.9 g of active tryptophan-free mixture. The TRP-free mixture for women was composed of the following amino acids: L-alanine 4.6 g, L-arginine 4.1, L-cysteine 2.3 g, glycine 2.7 g, L-histidine 2.7 g, L-isoleucine 6.7 g, L-leucine 11.3 g, L-lysine monohydrochloride 9.2 g, L-methionine 2.5, L-phenylalanine 4.8 g, L-proline 10.2 g, L-serine 5.8 g, L-threonine 5.8 g, L-tyrosine 5.8 g, L-valine 7.4 g. The placebo mixture was identical in composition, but also contained 1.9 g tryptophan. The mixture of amino acids was ingested by the participants. This stimulated protein synthesis by the liver and the pre-existing available tryptophan was incorporated into the newly synthesised proteins. This has lead to a 75%-90% reduction of serum tryptophan within 5 hours in studies using a similar recipe to ours (van der Does 2001a). The three most foul-tasting amino acids (L-arginine, L-cysteine, and L-methionine) are ingested in capsules. In placebo or sham depletion, tryptophan capsules were ingested; these capsules contained lactose during active depletion. Both the participants and the researchers were blind to the content of these similar- looking and tasteless capsules. The participants were unable to guess if they
had been in the active or placebo condition (more than 2/3 guessed ‘placebo’ when this information was formally collected for Paper III).

We made the following modifications to ease the ingestion: The amino acid were mixed with a total of 25 gram chocolate and caramel syrup, a few drops aromatic oils (2 drops of peppermint, 3 drops of citrus) and mixed with cold water to a total of 2 dl, and chilled. The participants were encouraged to drink the mixture quickly and were observed during the intake. The participants could also ingest protein-free sweets and juices during the ingestion and the following hour.

3.3 Experimental schedule

The participants received written information, and were reminded again the day before the experiment not to drink alcohol, to eat low protein food approximately 18 h before the start of the experiment, and to fast from midnight. The sessions started at approximately 8:30 am with blood sampling and ingestion of the amino acids. During the ensuing 5 h period, the participants were encouraged to read or watch emotionally neutral videos. They were observed during the whole period and could not leave the site. The experimental paradigm was administered following the second blood sample, approximately 6 h after the ingestion of the mixture, when plasma concentration of tryptophan was expected to be at its lowest (Carpender et al. 1998). The participants were tested individually in separate rooms. All subjects were compensated EUR 60 each for their participation.

3.4 Behavioural measures

Paper I & II

Impulsivity assessment: Continuous Performance Test (CPT-IP)
The Continuous Performance Test – Identical pairs (CPT-IP) is a transient attention, sustained response preparation task (Smid et al. 2006). The CPT-IP generated visual stimuli on a computer monitor and recorded responses. The participant's task was to
quickly respond whenever two identical stimuli were presented in a row. The CPT-IP was
divided into two modes: the first produced successively 150 four-digit numbers (the
number mode) and the second presented a string of 150 nonsense shapes (the shapes
mode). Each stimulus was presented at a constant rate of one per second, with a duration
of 50 ms. Thirty of the trials (20%) in each mode were target trials and required a
response.

A correct identification of a matching set is termed ‘hit.’ Each condition also included a
number of catch trials in which the stimulus presented was very similar, but not identical
to, the preceding stimulus. Responses to catch trials were considered a specific type of
commission error, referred to as ‘false alarms’ (see Figure 5). Signal detection analysis
combines hits and false alarms into d’ (D prime) and β (beta). These two signal detection
parameters are increasingly used for human information processing tasks, providing a
valuable tool for summarizing the data in a format that represents participants’ sensitivity
to stimuli (d’) and participants’ response style (β). This allows controlling for false
positive or negative data in cases where the participants are unengaged in the task and
provide responses in a purely randomized manner. The d’ value is a measure of the
subject’s ability to discriminate between stimuli, and involves the observer’s attention and
sensory capacity. The β value is a measure of response style, an indicator of the
competition between behavioural suppression and active responding.

**Paper III**

**Impulsivity assessment: Continuous Performance Tests (CPT-IP and IMT)**

To measure impulsivity, the Continuous Performance Test–Identical pairs (CPT-IP)
(Cornblatt et al. 1989) and the Immediate Memory Task (IMT) (Dougherty et al. 2003b;
Dougherty et al. 2004) were administered. In the IMT, subjects are shown five-digit
numbers for 0.5 seconds, at 0.5-second intervals, while the CPT-IP presented four-digit
numbers in one trial, and nonsense shapes in another. The CPT-IP stimulus appeared for
50 ms, presented at a constant rate of one per second. The participants are instructed to
quickly respond whenever two identical stimuli are presented in a row. To obtain a more
robust measure of β, we combined the CPT-IP (numbers and shapes) and IMT.
Mood Assessment: Profile Of Mood States (POMS)

We administered the POMS (McNair et al. 1992) at baseline and 6 hours after ATD to examine the effects of ATD and sham depletion on mood. A Total Mood Disturbance (TMD) score was obtained by summing the scores on all primary mood factors (depression/dejection, tension/anxiety, anger/hostility, fatigue/inertia, confusion/bewilderment and vigor/activity), weighting vigor/activity negatively. POMS is commonly used to estimate mood changes in ATD-studies (Richell et al. 2005; van der Does et al. 2001b), and is considered to be a valid and sensitive measure with high internal consistency and reliability (Knapp et al. 1998).
3.5 Genotyping

The procedure for genotyping the triallelic HTTLPR polymorphism has been described in detail elsewhere (Stein et al. 2006; Gelernter et al. 1997). Briefly, for detection of the A>G SNP that occurs within the L allele, the polymerase chain reaction (PCR)-amplified fragment containing the Short/Long length polymorphism (Gelernter et al. 1997) was digested with MspI (New England Biolabs, Beverly, MA) (Stein et al. 2006). The PCR fragment contains two obligatory MspI sites (Gelernter et al. 1997). The A>G substitution creates an additional MspI site. Thus, a single PCR reaction and restriction digest followed by size-fractioning on a gel provides classification of the S, LA and LG alleles (Stein et al. 2006; Gelernter et al. 1997). The triallelic classification was then reclassified into a biallelic model, based on level of transporter expression as follows: LG/S, LG/LG and S/S subjects were classified as S/S. LA/S and LA/LG subjects were classified as L/S, and LA/LA were classified as L/L (Hu et al. 2006; Neumeister et al. 2006).

3.6 Assessment of plasma tryptophan concentration

Two blood samples were drawn each test day, the first immediately prior to the ingestion of the amino acids and the second 5 hours after the ingestion of the amino acids. A total of 10 ml were drawn on each occasion: 5 ml were mixed with sulfosalicylic acid so that both free and total tryptophan could be measured from plasma. The samples were drawn at approximately 9 a.m. and 2:30 p.m.

Briefly, the analysis of total and free tryptophan was performed using a LC-MS/MS system which includes a Liquid Chromatograph, Waters 2795, and a double Mass Spectrometer Micromass Qattro Micro (Waters Corporation, Milford, USA). Total tryptophan in plasma was analyzed after deproteinization by acetonitril. Free tryptophan was analyzed after ultrafiltration of plasma through an "Amicon Centrifree device" (2000*g for 30 min.). Tryptophan was eluted with a gradient (10mM ammoniumacetat/ acetonitril) on a reversed phase C18 column ACE-3AQ (Advanced Chromatograph Technologies). The Mass Spectrometer was operated in the positive electrospray mode, and the tryptophan was detected by the mass transition 204.9 >145.9.
3.7 Statistical analyses

**Paper I**
Comparisons between the active and sham depletions were conducted using a paired sample t-test. The level of significance was set at p<0.05, two-tailed.

**Paper II**
To test if individuals exposed to ATD during the first test day (when the situation is novel) are more sensitive to the serotonin lowering effect of ATD than individuals exposed during the second test day (when the situation is familiar), the participants were divided according to the order in which they were given the active interventions. A paired sample t-test was used to examine the differences between active tryptophan depletion and placebo in each group. The data were further analyzed for ordering effects using repeated-measures analysis of variance (ANOVA) with group (active depletion first and sham depletion second, or sham depletion first and active depletion second) as the between-subjects factor, and treatment (active or sham depletion) as the within-subjects factor. Any interaction indicates that the participants started the second day of the experiment in a dissimilar state; this will make it necessary to discard the data collected the second test day, and conduct an independent samples t-test on the first session data only (Hills & Armitage 1979).

**Paper III**
Mood change was assessed as the difference between the TMD scores from the POMS at 6 hours after intake of the amino acids and baseline. The signal detection parameters $\beta$ (response style) and $d'$ (stimuli sensitivity) were collected from both CPT-IP and IMT and averaged to total scores. Behavioural measures were examined using a 3-way ANOVA with intervention (ATD vs. sham depletion), sex and genotype (L/L, L/S, and S/S) as between-subject factors. Statistically significant main effects and interactions were further examined with t-tests to determine the direction of change in behavioural and biochemical variables. The possible association between $\beta$ and $d'$ was investigated with a Pearson correlation which demonstrate the independence of these variables. Significance was interpreted at p<0.05, 2-tailed.
4. RESULTS

4.1 Paper I

Tryptophan depletion
ATD significantly decreased plasma concentrations of free and total tryptophan by 85% and 83% respectively, whereas sham depletion significantly increased tryptophan concentrations by 230% and 227% respectively. These changes were similar to those previously reported in healthy control subjects (Young et al. 1985; Ellenbogen et al. 1996). The large neutral amino acids (LNAA) that compete with tryptophan for the carriers over the blood-brain barrier are; isoleucine, leucine, phenylalanine, tyrosine and valine. The ratio of free and total tryptophan vs. the other LNAA fell 92% and 91% during active depletion, but increased 124% and 117% during sham depletion, respectively.

Continuous Performance Test
In the Number mode, subjects responded more often, both to correct stimuli and catch trials when depleted; that is, they became more impulsive. This was reflected in a lower β score $t(23)=2.56$, $p<0.05$ when depleted. In the Shapes mode, there was an effect on the number of hits $t(23)=3.06$, $p<0.01$ in the direction of fewer hits when depleted, and a smaller $d'$ $t(23)=2.33$, $p<0.05$ indicating a reduction in the ability to discriminate between visuo-spatial stimuli.

4.2 Paper II

Tryptophan depletion
Papers I and II are based on the same ATD experiment. See Paper I above.

ATD on the first or the second test day
The 12 participants who had experienced active depletion first and sham depletion second were compared with those 12 who experienced sham depletion first and active depletion second. There were no differences in age, years of education, degree of tryptophan depletion or washout period. The participants given the active intervention on the first test day, with placebo on the second, were more impulsive (disinhibited) when tryptophan was
depleted, as compared to the placebo intervention. This was reflected in a higher rate of false alarms in the number mode \( t(11)=3.2, \ p<0.01 \) and the shape mode \( t(11)=2.7, \ p<0.05 \). In addition, a higher response rate to both correct and incorrect stimuli in the number mode occurred, resulting in a decreased \( \beta \) signal detection variable \( t(11)=4.7, \ p<0.001 \) when the participants were depleted. Furthermore, the group given the active intervention first had reduced \( d' \) \( t(11)=7.0, \ p<0.001 \) and reduced number of hits \( t(11)=3.0, \ p<0.05 \) in the shape mode when depleted. There were no significant differences between the active and placebo condition on these or any other variables in the group of participants given placebo on the first test day and active tryptophan depletion on the second. This indicates that the group given placebo on the first test day did not have any statistically significant learning effects on the CPT-IP.

**CPT: order effects on False alarms and spatial d'**

Significant interactions between the order of treatment and intervention emerged on the false alarms measure, both for the number mode \( F(1,22)=9.6, \ p<0.01 \) and the shape mode \( F(1,22)=11.7, \ p<0.01 \). There was also a significant interaction for \( d' \) in the shape mode \( F(1,22)=20.4, \ p<0.01 \), demonstrating an ordering effect for these variables. These interactions were predominantly due to more extreme scores when the task was novel, i.e. were evidenced predominantly in participants who experienced active depletion on the first test day.

**CPT: first session data**

Due to the order effect on the spatial mode \( d' \) and both false alarm variables, it was necessary to discard the data collected the second test day and re-analyze these three variables using an independent samples t-test on the first session data only (Hills & Armitage 1979). The subjects given active intervention committed more false alarms \( t(23)=2.7, \ p<0.05 \) compared to the subjects given placebo in the number mode, but not in the shape mode. The depleted subjects showed a smaller \( d' \) \( t(23)=2.2, \ p<0.05 \) in the shape mode, indicating that active intervention reduced their ability to discriminate between visuo-spatial stimuli.
PAPER III

Tryptophan depletion
As predicted, ATD significantly decreased plasma concentrations of total $[t(41)=37, p<0.001]$ and free $[t(39)=23, p<0.001]$ tryptophan. Sham depletion significantly increased the concentrations of total $[t(40)=9, p<0.001]$ and free $[t(40)=9, p<0.001]$ tryptophan. Genotype and sex did not influence the outcome.

Effects of ATD on mood
Analyses of the mood response to ATD in our sample of healthy men and women showed a statistical significant main effect of sex $[F(1,70)=9.8, p=0.003]$, a trend towards statistical significance for intervention $[F(1,70)=3.4, p=0.07]$, a significant sex × intervention interaction $[F(1,70)=5.3, p=0.024]$, and a significant genotype × sex × intervention interaction $[F(2,70)=4.2, p=0.019]$. Further analyses showed that the sex × intervention interaction could be explained by the significant lowering of mood in women $[t(42)=2.5, p=0.016]$, but not in men.

Evaluation of the effects of 5-HTTLPR on the mood response to ATD revealed a significant sex × intervention interaction in the L/L group $[F(1,15)=10.7, p=0.005]$, but not in the S/S $[F(1,18)=2.8, p=0.1]$ and S/L $[F(1,37)=0.6, p=0.5]$ groups. Comparing the same-sex participants within each genotype, we found a statistically significant lowering of mood during ATD relative to sham depletion in the L/L women $[t(9)=5.0, p=0.001]$ and the S/S women $[t(10)=3.5, p=0.005]$, but not for the L/S women or any of the men, irrespective of genotype.

Effects of ATD on response style (impulsive vs. cautious)
Analyses of the impulsivity measure β found the predicted effect of ATD and sex on response style, as indicated by a statistically significant interaction between sex × intervention $[F(1,70)=12.0, p=0.001]$. Men and women showed opposite directions in their responses to ATD. Compared to the same-sex sham depletion control group, men became more impulsive $[t(37)=2.5, p=0.020]$ during ATD, whereas women became more cautious $[t(42)=2.6, p=0.015]$. Thus, women responded significantly differently to ATD compared to men $[t(40)=3.2, p=0.004]$. In contrast, women did not differ from men in their
response style during sham depletion. There were no statistically significant main effects for genotype, or interactions involving genotype (genotype × intervention; genotype × sex).

**Effect of ATD on sensitivity: d’**

There was no significant main effect of ATD on the ability to respond/ not respond when appropriate (d’). None of the interaction effects involving the intervention (intervention × sex, intervention × genotype) were statistically significant. The measures of β and d’ were not correlated, indicating that the effects on response style are not associated with changes in attention.
5. SUMMARY OF PAPERS

5.1 Paper I

*Rationale:* Reduced serotonergic activity has been associated with impulsive behaviour; however, intervention studies have been scarce. *Objectives:* To examine if induced lowering of serotonin levels would increase behavioural measures of impulsivity. *Methods:* 24 healthy young males ingested a mixture of the essential amino acids, excluding tryptophan, in a balanced, randomized, double-blind, placebo controlled, crossover study design. The Continuous Performance Test – IP was administered when the plasma concentration of tryptophan was expected to be at its lowest point. The plasma concentrations of 23 amino acids were measured at baseline and 5 hours after the ingestion of the amino acid mixture. *Results:* The intervention led to a dramatic fall in free and total plasma tryptophan, and the tryptophan/large neutral amino acids ratio. This in turn has been shown to lower the level of serotonin in the central nervous system. The tryptophan depletion resulted in a statistically significant more impulsive response style on the Continuous Performance Test – IP when the subjects were solving verbal tasks. Depleted subjects exposed to spatial stimuli had fewer correct responses and a decreased ability to discriminate between stimuli. *Conclusions:* These results indicate that a rapid lowering of tryptophan increases impulsiveness and decreases discriminating ability in normal individuals. The effect of serotonin depletion on discriminating ability in this study was similar to that previously reported in depressed patients.

5.2 Paper II

Rapid Tryptophan Depletion studies investigate serotonin using amino acid precursor depletion which transiently reduces the brain level of serotonin. This study compares the effects of serotonin reduction given on the first test day (when the situation is novel) with the effects of serotonin reduction given on the second test day (when the environment and test battery are familiar). Twenty-four healthy young males were given either active tryptophan depletion or placebo in this randomized crossover design, while impulsivity was measured by a Continuous Performance Test. The participants showed more
impulsive disinhibition and reduced attention during tryptophan depletion, but only on the first test day when the task was novel. This could be caused by a synergic effect between novel situations and reduced neurotransmission of serotonin.

5.3 Paper III

*Background:* Serotonin (5-HT) plays a central role in mood regulation and impulsivity. We studied whether healthy men and women react differently on mood and impulsivity measures during Acute Tryptophan Depletion (ATD). We also studied the relative contribution of a functional length triallelic polymorphism in the promoter region of the serotonin transporter, designated 5-HTTLPR, to the behavioural responses to ATD.

*Methods:* Thirty-nine men and 44 women participated in a randomized, double-blind, parallel-group ATD study. Behavioural measures of impulsivity and mood were obtained.

*Results:* During ATD, women reported mood reduction and showed a cautious response style which is commonly associated with depression. Men showed an impulsive response style and did not report mood reduction. The 5-HTTLPR influenced the mood response to ATD in women.

*Conclusions:* Healthy men became more impulsive, whereas healthy women showed mood reduction in response to ATD. This suggests that 5-HT could be one of the mechanisms contributing to sex differences in the prevalence of mood and impulsivity disorders. The influence of 5-HTTLPR on mood responses in women further substantiates the relevance of this variant in the pathophysiology of at least a subgroup of patients with depression.
6. DISCUSSION

In both our studies, the ATD robustly reduced serum tryptophan, and consequently the serotonergic functions in the brain (Williams et al. 1999; Nishizawa et al. 1997; Biggio et al. 1974). Tryptophan depleted men adopted an impulsive response style and showed no signs of mood reduction. Tryptophan depleted women adopted a cautious response style that has been associated with depression, and reported a substantial drop in mood on the POMS. The effects of reduced serotonergic neurotransmission on impulsive disinhibition were more pronounced in a novel, rather than familiar environment.

The results indicate increased impulsivity during ATD in men, and were found in two independent samples using two different experimental designs. These findings support the hypothesis of an association between serotonin and impulsivity originally posited by Soubrie (1986), but our results suggest that this hypothesis needs to be differentiated in that the response to serotonin manipulation is sex dependent.

The normal women, but not men, in this study reported a marked mood-lowering response to ATD. Although there are negative findings (Praschak-Rieder et al. 2005; Salomon et al. 1997), comparable studies investigating the effects of ATD on normal women using POMS reach similar conclusions (Ellenbogen et al. 1996; Smith et al. 1997b). In contrast to other studies, ours included both the self-report measure POMS, as well as response style as measured by a computerized neuropsychological test. The tryptophan depleted women in this study also showed a cautious response style, which has been associated with depressive mood (Johnson & Magaro 1987; Miller & Lewis 1977). Further supporting the validity of cautious style (under-responding) as an objective behavioural indication of depressive mood, manic patients have been found to have a significantly more impulsive (over-responding) response style than depressed patients (Corwin et al. 1990). Thus, response style can be viewed as a continuum with impulsivity on one end, cautiousness on the other.

In the first study, reduced serotonergic neurotransmission resulted in increased levels of impulsive disinhibition in a group of participants given active tryptophan depletion on the first test day, placebo on the second. The group receiving placebo on the first test day did
not show such a response when active tryptophan depletion was administered on the second test day. The increased sensitivity to serotonergic depletion under novel circumstances was further demonstrated by significant interactions for both false alarm variables as well as the spatial attention variable (d’). These interactions were caused by increased false alarms and decreased d’ when the participants were depleted, but only when the task was novel. This indicates order effects on impulsive disinhibition (false alarms), but not on impulsive response style (β). This could be caused by a synergic effect between lowered serotonin and novel situations on impulsive disinhibition. Our results, and other ATD studies reporting order effects (see introduction), suggest that the synergic effect between serotonin and novelty might also apply to other aspects of human behaviour.

A few steps down the evolutionary ladder, we find that rats too react differently to novel environments. Male Wistar rats showed reduced immobility in the forced swimming test following the administration of parachloroamphetamine (PCA) which reduced the level of serotonin by 20%, but only in the first (but not the second) swimming session (Harro et al. 2001). This temporary reduction of immobility cannot be interpreted as a learning effect, and as a working hypothesis Harro (2002) suggest that it reflects an increase in “reactive” or “impulsive” behaviour. PCA-treated rats also showed enhanced muricide (mouse killing), provided the exposure to mice is a novel experience. Since previous exposure of mice to rats canceled the increase in muricidal behaviour, Vergnes & Kempf (1982) suggests that decreased serotonergic transmission does not increase aggression per se but rather facilitates the expression of aggressive impulses. Serotonergic neurons might be brought into play when the organism is selecting passive versus active behaviour for an appropriate response in a novel situation (Soubrie 1986), a hypothesis consonant with the proposal that serotonergic transmission is essential in controlling human behaviour in emergency situations (Rydin et al. 1982).

There is general consensus in the field that impulsivity is multifactorial, although there is no agreement as to what these factors are (Evenden 1999). Rapid-response impulsivity measured by CPTs is a good predictor of personal and clinical characteristics of impulsivity (Swann et al. 2002). The CPTs gives two different impulsivity measures: impulsive response style (β) and impulsive disinhibition (false alarms). Previous ATD studies on healthy volunteers generally found little effect on impulsive inhibition (Evers et
ATD did not influence response inhibition on the Stop Signal Task as compared to placebo, and there were no sex differences (Clark et al. 2005). LeMarquand et al. (1998) used the ATD technique to demonstrate that non-alcoholic young men with family histories of alcoholism made significantly more commission errors (similar to false alarms) on a Go/No-Go task when tryptophan depleted, a response which was not found in individuals without a family history of alcoholism (LeMarquand et al. 1999). These findings might appear contradictory to our results, but they reflect different facets of impulsivity and they used crossover designs without taking the order effect into account. Impulsive response style, on the other hand, was found to increase for the tryptophan depleted men in the first study when the stimuli were conceptualized as verbal (the number mode). This was replicated a few years later in our second study, and in a study by Booij et al. (2006). In our second study, we combined the CPT-IP (numbers and shapes) and IMT β to obtain a more robust measure of impulsive response style. To my knowledge, these are the only studies ever conducted on tryptophan depletion and impulsive response style. All three studies found that reduced serotonergic transmission on average increased impulsive response style. Impulsive response style therefore seems to be influenced by serotonin. Impulsive response style is also a stable measure, as opposed to impulsive disinhibition that was found to suffer methodological problems such as order effects.

The results of the present study are in accordance with an extensive literature on sex differences between men and women in the serotonergic system. ATD is more likely to have mood-lowering effects (although small and subclinical) in healthy women as compared to men (Ellenbogen et al. 1996; Smith et al. 1997b). Even though some early studies also found an effect of ATD on mood in healthy men (Young et al. 1985; Smith et al. 1987), subsequent studies have reported that ATD results in few mood effects in men (Bell et al. 2005). A PET 6-[11C]methyl-L-tryptophan study suggests that ATD leads to greater decreases in brain serotonin synthesis in women than in men (Nishizawa et al. 1997), although this could be due to the lower plasma free tryptophan in women under the experimental conditions (Young et al. 1999). Differences between healthy men and women have also been observed in CSF concentrations of 5-HIAA (higher in women) (Agren et al. 1986), neuroendocrine responses to serotonin challenges (larger in women) (Goodwinn et al. 1994) and 5-HT2 receptor binding in several parts of the central nervous system (CNS) (lower in women) (Biver et al. 1996). Evidence for sex differences in
Peripheral and central serotonin metabolism are also reflected in the animal literature. Preclinical data suggest that female rats have a higher activity of serotonin synthesizing enzymes, a greater storage capacity for serotonin in brain neurons, a more pronounced serotonin behavioural syndrome in response to serotonin agonists, and higher CNS levels of tryptophan, serotonin, and 5-HIAA as compared to males (Carlsson et al. 1985). On the basis of these studies, it can be assumed that there are sex differences in serotonin metabolism in the CNS and in the periphery.

Returning to humans, the sex differences in mood response to ATD is of special interest considering that fertile women may have a higher response rate to the selective serotonin reuptake inhibitors (SSRI) sertraline than men, whereas men may respond more favourably to the tricyclic antidepressant (TCA) imipramine, which is not serotonin specific (Kornstein et al. 2000; Khan et al. 2005). It should be noted that Parker et al. (2003) failed to find preferential response to SSRI in women and TCA in men. Old age, however, was associated with a superior TCA response and younger age with a superior SSRI response (Parker et al. 2003). Hormonal factors may play a role in sex differences in serotonin brain function, in addition to risk and treatment outcome of depression. Studies have found that estrogen hormones facilitate serotonin receptor sensitivity (Joffe & Cohen 1998). The sex difference in impulsivity and mood response to ATD found in the present study extends previous serotonin challenge studies in healthy samples. However, our findings of normal men and women adopting opposite response styles in response to reduced serotonergic neurotransmission is novel, and a full explanation for that cannot be found in previous studies.

There is solid evidence for sex differences in the prevalence of the major psychiatric disorders (American Psychiatric Association 2000; Holden 2005). Women show a higher prevalence of mood and anxiety disorders compared to men (Kessler et al. 2005; American Psychiatric Association 2000). This sex difference in prevalence occurs across treatment centres and cultures (Gater et al. 1998). Men in turn show a higher prevalence of impulse control and substance use disorders (Kessler et al. 2005), in addition to antisocial personality disorder (American Psychiatric Association 2000) and completed suicide (Schwartz et al. 2006). It is difficult to account for the higher rates of suicides in men compared to women given that depression is a major risk factor for suicide. It is possible that a sex difference in how men and women react to disrupted serotonergic transmission
is the most parsimonious explanation of this discrepancy. A particular sub-type of depression has been delineated, in which the clinical picture is predominantly characterized by lowered stress tolerance, disturbed impulse control and low CSF 5-HIAA (van Praag 1996). The clinical presentation of depressive symptoms may differ between men and women, and a “male depressive syndrome” has been proposed (Rihmer et al. 1998). However, a study by Moller-Leimkuhler et al. (2004) did not find that men scored higher on the Gotland scale for male depression in a large sample of unipolar depressed inpatients. Using a factor analysis, they did find support for the hypothesis of a male depression syndrome by demonstrating a gender-related difference in the symptom patterns: atypical symptoms like irritability, aggressiveness and antisocial behaviour were strongly intercorrelated in male, but not in female, depressed patients (Moller-Leimkuhler et al. 2004). A link between serotonin and a subtype of depression characterized by anger attack has been suggested (Painuly et al. 2005), and anger attacks were eliminated in 71% of patients with comorbid unipolar depression treated with the SSRI fluoxetine (Fava et al. 1993). Serotonin could be one of the mechanisms explaining the sex difference in the prevalence and clinical presentation of some key psychiatric disorders and symptoms, such as depression.

The triallelic 5-HTTLPR polymorphism did influence mood response to ATD in women on the POMS, but did not influence the impulsivity measures during ATD or sham depletion. The mood-lowering response to ATD in women was especially marked for the L/L and the S/S polymorphisms. The combination of the L/L or the S/S polymorphisms in addition to female sex gives an interactive effect, suggesting that women with these genotypes are more vulnerable to adverse mood effects following a disturbance of the serotonin neurotransmission. It is equally true to say that the S/L phenotype is a protective factor moderating the mood reduction women experience in response to reduced serotonin transmission. This unexpected finding needs to be confirmed in independent cohorts, considering that earlier in vitro studies suggest that the S and L alleles are of equal importance, but opposite in function (Hu et al. 2006). The increased response to ATD in female L/L carriers was unexpected, but a similar result was reported in depressed patients (Neumeister et al. 2006). Lowering mood response to ATD in the S/S group is in accordance with Neumeister et al. (2002). They found that the S/S genotype was associated with increased risk of developing depressive symptoms during ATD in healthy women. It is difficult to explain why the sham depleted male L/L carriers reported
substantial mood reduction: it should be noted that this group consisted of only four participants. The large response of ATD in the S/S women is in line with Sjöberg et al. (2006). They found that S/S women, but not men, develop depressive symptoms in response to environmental stress factors. “One possible explanation for this finding is that adolescent males develop other kinds of pathological behaviours, which might be regarded as a male variant of depression, but which produces inverted scores on the depression scale used.” (Sjöberg et al. 2006, p. 448).

In the second study, we designed a double-blind, placebo-controlled, between-subjects parallel design to avoid the order effects reported in traditional ATD studies using crossover designs (see introduction). It was especially designed to uncover sex differences, and this was documented with a self-report measure as well as with an objective neuropsychological paradigm. Other specific features of our study design was that we did not use a “reduced” dose ATD as some researchers have done, but instead used a “full” ATD dose to ensure that the reduction in plasma tryptophan exceeded the threshold necessary to obtain an effect of ATD (van der Does 2001a). Additionally, our study consisted of a larger number of participants compared to other ATD studies. In relation to genes, Paper III still has a problem with statistical power. When the data are broken down by the intervention, sex and three different genotypes, the cell size becomes small, increasing the chance for Type I and II errors. Another limitation to be considered is that we did not systematically collect family histories of mood or impulsivity disorders, and cannot rule out that some of the participants have genetic vulnerabilities related to serotonin. The findings presented here should be confirmed in independent cohorts using the same design as in Paper III. Future studies examining genetic variables should use a much higher number of participants, or pre-screen the participants’ genotype to insure an increased cell size.

We conclude that impulsive response style is a behaviour sensitive to manipulation of the serotonergic system in the brain. This confirms that serotonergic neurotransmission is involved in a competition between behavioural suppression and active responding. When the serotonergic neurotransmission is reduced, men will on average increase active responding, while women increase behavioural suppression. This finding is unique in that men and women on average react in opposite manner to the same intervention. This implies that the serotonergic system might be “wired” differently for men and women, a
proposition strengthened by the sex-specific responses on the mood questionnaire. Serotonin could therefore be one of the mechanisms explaining the sex difference in the prevalence and clinical presentation of mood and impulsivity disorders. The sex of a given participant or patient should therefore be taken into consideration when conducting research, prescribing treatment or diagnosing conditions associated with serotonin.
7. CONCLUSION

7.1 Paper I

1. Reduced serotonergic transmission increased impulsive response style in normal men.

7.2 Paper II

2. The effects of reduced serotonergic neurotransmission on impulsive disinhibition are more pronounced in a novel, rather than familiar environment.

7.3 Paper IIII

3. The effects of reduced serotonergic neurotransmission on mood and impulsivity are different for men and women. Tryptophan depleted men adopted an impulsive response style and showed no signs of mood reduction. Tryptophan depleted women adopted a cautious response style associated with depression, and reported a substantial drop in mood. The effect of tryptophan depletion on mood in women was influenced by the triallelic 5-HTTLPR polymorphism.
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