The effects of acute tryptophan depletion on impulsivity and mood in adolescents engaging in non-suicidal self-injury

Ragnhild Bø & Øyvind Bastnes Lie

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ABSTRACT

**Supervisor:** Nils Inge Landrø  
**Co-supervisor:** Linn Toril Fikke

**Background:** Reduced serotonergic neurotransmission is associated with impulsivity and negative affect in various psychiatric disorders. Research suggests that impulsivity and negative affect plays a prominent role in the etiology and maintenance of non-suicidal self-injury (NSSI). However, the relationship between serotonin and NSSI has currently received little research interest. The primary aim of the present study was to further delineate this relationship, by examining whether reduced serotonergic neurotransmission leads to heightened impulsivity and increased negative affect in adolescents who engage in NSSI.

**Methods:** Data were collected as part of the project “Repetitive non-suicidal self-injury: Impulsivity, executive control functions, and mentalization”. The authors participated in data collection. 32 adolescent females who had engaged in NSSI during the past twelve months participated in this between-subjects, double-blind experimental design. Participants were randomized into two groups. Half of the participants underwent acute tryptophan depletion (ATD), a method used to decrease serotonergic neurotransmission through ingestion of an amino acid mixture devoid of tryptophan – the dietary precursor of serotonin. The remaining participants were given a sham-depletion mixture containing tryptophan. Blood samples were drawn at baseline to measure plasma concentrations of tryptophan, together with administration of the Profile of Mood States (POMS). 4.5 hours after ingestion of the amino acid mixtures, when plasma concentration of tryptophan was expected to be at the lowest point, the second blood sample was drawn. Subsequently, the verbal and spatial subtasks of the Continuous Performance Test – Identical Pairs (CPT-IP) were administered, together with the second administration of POMS.

**Results:** Acute tryptophan depletion led to a 78.9 % fall in total plasma tryptophan levels. The intervention led to a significantly more impulsive response style and increased discriminative ability on verbal subtask of the CPT-IP. The intervention did not affect mood, suggesting that the effects of ATD on impulsivity are separate from effects on mood.

**Conclusion:** The results indicate that acute lowering of tryptophan increases impulsivity and discriminating ability in adolescents who engage in NSSI. The intervention did not affect mood. The findings suggest that NSSI are engaged in for affect regulating purposes.
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INTRODUCTION

Non-suicidal self-injury (NSSI) is a dangerous public health problem, particularly prevalent in adolescence and young adulthood (Janis & Nock, 2009). Large-scale community studies of adolescents and young adults report NSSI prevalence rates between 13.0\% and 23.2\% (Jacobson & Gould, 2007). Self-injurious behaviour in young girls aged 10 – 14 was recently reported at an alarming lifetime prevalence rate of 56\% (Hilt, Cha & Nolen-Hoeksema, 2008).

NSSI refers to the direct and deliberate destruction or alteration of one’s own body tissue without intent to die (Favazza, 1998; Jacobsen & Gould, 2007). Typical acts of NSSI include skin-cutting, carving, burning, needle sticking, bone breaking, self-hitting, and interference with wound healing (Laye-Gindhu & Schonert-Reichl, 2005; Muehlenkamp & Gutierrez, 2004, 2007). The Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV; American Psychiatric Association [APA], 1994) refers to NSSI as a symptom of borderline personality disorder. However, there is diagnostic heterogeneity among those who perform NSSI (Klonsky & Olino, 2008; Nock, Joiner, Gordon, Lloyd-Richardson & Prinstein, 2006; Nock & Mendes, 2008). Furthermore, a considerable proportion of individuals who engage in NSSI do not meet the criteria for any psychopathological condition according to DSM-IV categories (Stanley, Winchel, Mocho, Simeon & Stanley, 1992). Consequently, NSSI has been suggested to represent a separate clinical syndrome (Muehlenkamp, 2005) or diagnosis in the DSM-5 (APA, 2010).

NSSI is closely associated with suicidal behaviours (Jabobson & Gould, 2007; Nock et al., 2006), the leading cause of death among adolescents in Norway today (Statistics Norway, 2010). In a preventive effort, it is therefore crucial to gain a better understanding of the mechanisms leading to and maintaining NSSI.

NSSI behaviours are complex and currently not well understood. It is likely that a combination of unique biological, physiological, contextual and psychological characteristics lead some adolescents to engage in NSSI (Jacobson & Gould, 2007).

According to the emotion regulation hypothesis, adolescents engage in NSSI to regulate and temporarily reduce negative affective states (Laye-Gindhu & Schonert-Reichl, 2005; Nixon,
Cloutier & Aggarwal, 2002; Nock & Prinstein, 2005; Nock, Prinstein & Sterba, 2009). The emotion regulation hypothesis is supported by research showing that acts of NSSI are preceded by negative emotions such as anger, anxiety and sadness, which subside during or following the act of self-injury (for a review, see Jacobson & Gould, 2007).

The role of impulsivity in NSSI

Impulsivity can be defined as a failure to resist an impulse or urge that exerts disproportionate influence on behaviour, despite harmful consequences to oneself or others (Cherkasky and Hollander, 1997). Several lines of research associate NSSI with impulsivity. Clinicians working with self-injuring patients often describe NSSI as being initiated on the spur of the moment, with little conscious intent or forethought (Herpetz, 1995). In a recent study, adolescents in a community sample reported little or no contemplation at all prior to episodes of NSSI (Lloyd-Richardson, Perrine, Dierker & Kelley, 2007). Moreover, repetitive NSSI is commonly regarded as a disorder of impulse control, with the defining feature being the inability to resist or control an impulse, drive, or temptation to harm oneself physically (Favazza, 1998). Supporting this notion, individuals engaging in NSSI often have a history of former or coinciding problems involving reduced impulse control, such as alcohol and drug dependence, kleptomania, sexual promiscuity and eating disorders (Favazza, 1998). Also, a positive correlation has been found between the frequency and severity of NSSI and self-reported trait-impulsivity (Simeon et al., 1992), further suggesting a role for impulsivity in NSSI.

Integrating these research findings, NSSI can be understood as a short-term coping mechanism that is impulsively engaged in and that serve affect-regulating purposes.

Research supporting the role of impulsivity as a clinical correlate of NSSI has mainly relied on self-report measures, which are problematic with respect to the accuracy of introspective reports on thoughts, feelings and behaviour (Janis & Nock, 2009). Studies using objective, performance-based measures of impulsivity are scarce. Furthermore, NSSI and self-injurious behaviour with suicidal intent are only infrequently differentiated in the research literature, complicating interpretation of findings.
In reviewing the studies that have used objective performance-based measures of impulsivity, findings are mixed. A recent study found an impulsive response style on a stop signal task in adolescents engaging in low-severity NSSI (Fikke, Melinder & Landrø, 2010). Another study found risky decision-making in a mixed sample of adolescents engaging in NSSI and self-harm with suicidal intent (Oldershaw et al., 2009). Contrary to these findings, other research findings show that adolescents who engage in NSSI do not differ from healthy control participants in performance on neuropsychological measures of impulsivity (Janis & Nock, 2009; Ohman et al., 2008).

Considering the long withstanding assumption of self-injurers being impulsive, the scarcity of neuropsychological findings supporting the association between NSSI and impulsivity is surprising. A possible explanation for the scarcity of results might be that impulsive behavioural tendencies occur under certain conditions only, involving neurobiological mechanisms not accounted for by previous research. Prominent theories of the mechanisms leading to self-injury with suicidal intent suggest that these behaviours may result from dysregulation in the serotonergic neurotransmitter system, along with a tendency for the individual to experience intense psychological distress (Joiner, Brown & Wingate, 2005; Mann, 1999). Phenomenological and biochemical evidence suggests that similar mechanisms may underlie NSSI and self-injurious behaviour with suicidal ideation (Stanley et al., 1992). Research has demonstrated that adolescents who engage in NSSI experience strong negative affect prior to acts of self-injury (Ross & Heath, 2003). Heightened emotional reactivity has been found in the same individuals (Laye-Gindhu & Schonert-Reichelt, 2005). How serotonergic dysregulation relates to impulsivity and emotional aspects of NSSI, however, has currently received little research attention.

**Impulsivity, emotion and the serotonin system**

Serotonin (5-hydroxytryptamine, 5-HT) is regarded as the central biological marker of impulsivity (Linnoila et al., 1983). Research conducted across several diagnostic groups and samples converge on the centrality of serotonin to impulsive behaviour. Low levels of serotonin are associated with impulsive disinhibition in personality disordered subjects (Coccaro & Kavoussi, 1997), depressed patients with a history of violent impulsive suicide attempts (Åsberg, 1997), and in violent male offenders (Dolan, Anderson & Deakin, 2001). Moreover, low levels of plasma serotonin have been found in adolescents who engage in
impulsive suicidal behaviours, together with a significant negative correlation between serotonin level and the severity of suicidal behaviour (Crowell et al., 2005; Tyano et al., 2006).

Although the picture is not completely consistent (for a review, see Robbins & Crockett, 2010), the response inhibition aspect of impulsivity seems particularly closely associated with the activity of the serotonin system (Brunner & Hen, 1997; Evenden, 1999; Winstanley, Dalley, Theobald & Robbins, 2004). Serotonergic neurons come into play whenever behavioural inhibition is required (Soubrié, as cited in Evenden, 1999). Also, research shows that the ability to inhibit responses is suppressed by decreased serotonergic transmission, resulting in impulsive behaviour (Le Marquand, Benkelfat, Pihl & Young, 1999, Walderhaug et al., 2002; Walderhaug, Herman, Magnusson, Morgan & Landrø, 2010).

Inhibition of behaviour is dependent on the integrity of complex neuronal circuits involving portions of the prefrontal cortex (PFC), the basal ganglia, thalamus, cerebellum, as well as cortical areas outside of the frontal lobes (Aron, 2008). The serotonergic interconnections in the right ventral/inferior PFC have most consistently been described as the substrate for inhibition (Arnsten, 2009). The ventral PFC is also important to the regulation of affect (Phillips, Drevets, Rauch & Lane, 2003). Serotonin, through interconnections in the ventral PFC, may therefore be of importance both with respect to the impulsivity aspect and the emotional regulation aspect of NSSI.

To our knowledge, only two groups have investigated serotonergic function in individuals who engage in NSSI. In a sample of personality-disordered adults, administration of fluoxetine reduced NSSI by 97% (Markowitz, Calabrese, Schulz & Meltzer, 1991), indicating the importance of this neurotransmitter to NSSI. Also, a significantly lower level of peripheral serotonin was found in adolescents who recently had engaged in self-injury, compared to controls, further supporting the relevance of serotonin to NSSI behaviours (Crowell et al., 2008).

Research also supports the centrality of serotonin to emotion. In clinical samples, anxiety, aggression and depression are related to low levels of serotonin (Baldwin & Rudge, 1995; Linnoila et al., 1983). Consequentially, emotional experience and the regulation of affective states seem to be associated with the level of functioning in the serotonergic system. Adolescents who engage in NSSI have elevated levels of anxiety, anger and depression.
compared to controls (for a review, see Jacobsen & Gould, 2007), which accordingly may result from dysregulation of the serotonin system.

Together, these findings suggest that serotonergic dysregulation may be associated with increased impulsivity, negative affect and problems with affect regulation, and thus be of importance to the aetiology and maintenance of NSSI behaviours.

**Serotonergic vulnerability**

Serotonergic vulnerability means that the individual has a vulnerability or sensitivity to dysregulations in the serotonergic system, and therefore is at increased risk of developing symptoms and disorders that are related to serotonin dysfunction (Jans, Riedel, Markus & Blokland, 2007). Serotonergic vulnerability is the result of interactions between innate (genetics, gender, personality, prenatal stress) and environmental factors (postnatal stress, drug use). These factors contribute to the degree of vulnerability additively, until a threshold is reached where the system can no longer compensate and pathology occurs.

According to this perspective, adverse circumstances affect individuals differently. When individuals with a preexisting serotonergic vulnerability faces stressful life events, serotonin-related behaviours such as NSSI may be triggered and maintained. In individuals without this preexisting vulnerability, serotonin-related pathology will not occur. However, stressful circumstances may influence serotonergic functioning of these persons in such a way that vulnerability develops over time, possibly leading to NSSI or other serotonin-related behavioural symptoms in reaction to further adversity. 

Serotonergic dysregulation thus constitutes a biological risk or vulnerability factor. In light of the reviewed research, it seems possible that adolescents who engage in NSSI have serotonergic vulnerability, which may help explain why they engage in impulsive NSSI.

Serotonergic vulnerability can be demonstrated by challenging or manipulating the serotonergic system. If the individual reacts to such a manipulation with a mood or behavioural response, such as increases in negative affect or impulsive disinhibition, this could indicate a vulnerability or sensitivity of the serotonergic system (Jans et al., 2007; Markus, 2008).
Acute tryptophan depletion
The advent of acute tryptophan depletion (ATD) has made it possible to study the effects of decreased serotonin transmission on behaviour (Carpenter et al, 1998; Klaasen et al., 1999; Young, Smith, Pihl & Ervin, 1985). To explain how ATD decreases levels of serotonin in the brain, an explanation of how serotonin is synthesized is necessary. Serotonin is synthesized within the brain, as it cannot pass the blood-brain barrier (Azmitia, 2001). The first step of serotonin synthesis is the uptake of the amino acid precursor tryptophan (TRP) from the blood. The human body itself cannot produce TRP, so the only means by which this amino acid is made available is through dietary intake. In serotonergic neurons, tryptophan is converted into 5-hydroxytryptophan (5-HTP) by the enzyme l-tryptophanhydroxylase. Subsequently, 5-HTP is decarboxylated into 5-hydroxytryptamine, or serotonin, by the aromatic l-amino acid decarboxylase. When the brain tryptophan supply is increased, serotonin synthesis and release therefore should be elevated (Fernstrom, 1990).

Figure 1. Serotonin synthesis, adapted from Markus (2008).

During ATD, a reversible lowering of plasma tryptophan is achieved by the ingestion of an amino acid mixture that lacks tryptophan, but contains other large amino acids (LNAAs; van der Does, 2001). The availability of TRP to the brain is not only dependent of plasma TRP, which is decreased during the procedure (Biggio, Fadda, Fanni, Tagliamonte & Gessa, 1974), but also the relative rate of TRP to LNAA’s (Fernstrom & Faller, 1978) and their competition for transport across the blood-brain barrier (Fernstrom & Wurtman, 1972 a, b). Thus, intake
of LNAAs will further reduce entry of TRP to the brain, subsequently reducing serotonin level and function (Hood, Bell & Nutt, 2005).

The ATD procedure does not involve any long-term side effects (for a review, see Moore et al 2000). The only reported side effects of ATD are nausea, vomiting and diarrhoea, found in a small proportion of subjects (Moore et al, 2000; Young et al., 1985). The effects of ATD on serotonin synthesis can be rapidly reversed by consumption of a normal diet. Accordingly, ATD is considered a safe method (Bell, Hood & Nutt, 2005).

Studies of cerebrospinal fluid and brain tissue have confirmed that ATD reduces serotonin function in the periphery of the brain, indicated both by significant decreases in plasma tryptophan concentrations, significant decreases in concentrations of CSF 5-HIAA (Carpenter et al, 1998; Williams, Shoaf, Hommer, Rawlings & Linnoila, 1999) and significant decreases in serotonin synthesis and release (Nishizawa et al 1997). The maximal effect is achieved 5-7 hours after ingestion of the amino acid mixture (Rubia et al, 2005).

**ATD-findings**

The ATD paradigm has been extensively utilized in adult samples. Mood and behavioural effects of tryptophan depletion are particularly found in affected sub-clinical subjects, most likely due to enhanced brain vulnerability to serotonin alteration or manipulation in these individuals (Markus, 2008). In healthy non-affected subjects, ATD generally has little or no effect on mood or emotion-related behavioural and brain changes. Accordingly, ATD might help identify individuals with a specific serotonergic vulnerability (Jans et al., 2007).

To our knowledge, only two research groups have relied on ATD to study the effects of altered serotonergic neurotransmission on emotion and impulsivity in adolescents (LeMarquand et al 1999; Zepf et al., 2008).

In a study of preadolescent boys diagnosed with Attention Deficit Hyperactivity Disorder (ADHD), a condition associated with low serotonin function (Halperin et al, 1994, 1997; Kruesci et al 1990, 1992), subjects that were predefined as highly aggressive became less impulsive as a result of ATD, whereas low-aggressive participants became more impulsive on a task requiring inhibition (Zepf et al., 2008). LeMarquand et al (1998) studied male
adolescents with high trait-aggressiveness, but reported no relation between ATD and impulsive disinhibition. Neither of these research groups found significant changes in self-reported mood after experimental lowering of serotonergic neurotransmission (LeMarquand et al., 1998; Zepf et al., 2009).

The acute tryptophan depletion procedure has not been used with adolescents who engage in NSSI. Given the potential to uncover a neurobiological vulnerability factor of importance to the aetiology of NSSI-behaviours, research using this method in this group of adolescents seems needed.

To summarize, previous research, clinical accounts and current diagnostic schemes emphasize the impulsive nature of NSSI behaviours. At a neurobiological level, disorders of impulse control and impulsive behaviours have consistently been associated with reduced activity of the serotonergic system. Moreover, reduced serotonin levels are associated with negative affect and emotion regulation difficulties. Sensitivity or vulnerability to alterations of the serotonergic system has been suggested in individuals who engage in NSSI. However, no studies to date have sought to delineate the role of serotonergic dysregulation on impulsivity and emotion in individuals who engage in NSSI.

**AIMS OF THE STUDY**

This study will investigate the relationship between serotonin and impulsivity in adolescents who engage in NSSI. By experimentally lowering serotonergic neurotransmission, we hypothesize that adolescents who engage in NSSI will show an impulsive response style compared to their non-depleted counterparts.

As serotonin also has a central role in emotion regulation, we hypothesize that the experimental lowering of serotonin will increase negative affect in adolescents who engage in NSSI.
METHOD

Data were collected as part of a comprehensive laboratory study of NSSI, at the Cognitive Developmental Research Unit (EKUP), Department of Psychology, University of Oslo. The experiment was carried out in accordance with the Helsinki Declaration and approved by the regional ethics committee. The authors participated in data collection and registration.

Participants
Female adolescents with a history of NSSI were recruited from junior high schools in Oslo. A criterion of two or more NSSI behaviours during the past twelve months was applied to be eligible for participation in the study. NSSI was defined as the deliberate and direct destruction or alteration of body tissue without intent to die. Indirect acts of harm to oneself, such as negative self-cognitions or risky behaviours, are excluded from this definition of NSSI.

A total of 40 female adolescents were included for participation. Two participants that were currently using selective serotonin reuptake inhibitors were excluded from further participation. Furthermore, five of the participants could not tolerate the amino acid mixtures, resulting in vomiting after ingestion. One person refused to ingest the mixture. As these six participants failed to receive the experimental manipulation, they were excluded from all data analyses. Thus, 32 participants completed all aspects of the experiment and were included in the data analysis. The age of the participants ranged from 14 to 16 years, with an average age of 15.1 years.

All participants and their parents provided written informed consent prior to participation.

Measures and materials

Amino acid mixtures
Across conditions, the participants were given a liquid mixture of amino acids. The liquid mixture was dosed according to bodyweight, in line with the procedure and recipe previously used with preadolescents and adolescents (Zepf et al., 2007). The mixture contained the following quantities of amino acids per 10 kg: L-phenylalanine 1.32 g, L-leucine 1.32 g, L-
isoleucine 0.84 g, L-methionine 0.50 g, L-valine 0.69 g, L-threonine 0.60 g and L-lysine 0.96 g. In addition, L-tryptophan 0.70 g per 10 kg was added to the mixture in the sham-depletion condition. In preparing the mixtures, amino acid powders were mixed with 200 ml cold water and chilled. 10 ml chocolate syrup and 3 drops of citric essence were added to ease ingestion. The participants were encouraged to swallow the drink quickly, while offered protein-free sweets and juices. The mixtures in the active condition (devoid of L-tryptophan) and the sham-depletion condition (containing L-tryptophan) tasted the same and were visually non-discernable.

Measurement of tryptophan level
Total plasma tryptophan (free and protein-bound) was analyzed using a conventional amino acid analyzer with ion-exchange chromatography by Li-buffers and ninhydrin detection (Perry, Stedman & Hansen, 1968).

Clinical assessment

Functional Assessment of Self-Mutilation (FASM).
The FASM (Lloyd, Kelley & Hope, 1997, as cited in Lloyd-Richardson et al., 2007) is a self-report questionnaire assessing methods, functions and frequency of NSSI. The FASM has demonstrated satisfactory psychometric properties in studies with adolescent samples (Penn, Esposito, Schaeffer, Fritz & Spirito, 2003; Lloyd-Richardson et al., 2007).

Kiddie-Sads - Present and Lifetime Version (K-SADS-PL).
The K-SADS-PL (Kaufman et al., 1997) is a semi-structured diagnostic interview, assessing current and lifetime history of psychopathology in children and adolescents, according to DSM-IV criteria (APA, 1994). The presence of NSSI behaviours were examined in the section covering depressive disorders. Also, the following diagnoses were considered particularly relevant and were examined: major depressive disorder, generalized anxiety, obsessive compulsive disorder, panic disorder, social phobia, posttraumatic stress disorder, anorexia nervosa, bulimia nervosa, eating disorder NOS, attention deficit hyperactivity disorder, alcohol abuse and substance abuse.
Neuropsychological assessment

**Wechsler Abbreviated Scale of Intelligence (WASI).**

The WASI (Psychological Corporation, 1999) is an abbreviated version of the Wechsler Adult Intelligence Scale, consisting of four subtasks: block design, matrix reasoning, similarities and vocabulary. The WASI provides a brief estimate of intelligence. The total intelligence quotient score (IQ), and IQ sum scores on the verbal and non-verbal tests respectively, were examined.

**The Continuous Performance Test – Identical Pairs (CPT-IP).**

The CPT-IP (Cornblatt, Risch, Faris, Freidman & Erlenmeyer-Kimling, 1988) involves verbal (four-digit numbers) and spatial (nonsense shapes) stimulus pairs being presented successively on a computer monitor. The participants are instructed to respond as rapidly as possible whenever two identical numbers or shapes appear in succession in a row. A response is made by lifting the index finger from the computer mouse held in the dominant hand.

In administering the task, verbal and spatial stimuli were presented in separate sequences. The two sequences consisted of 150 stimuli each. Each stimulus was flashed on the screen for a duration of 50 ms, at a constant rate of one stimulus per second. 20% of stimuli in each sequence were target trials requiring a response, while a further 20% were catch trials wherein similar but not entirely identical stimuli were presented successively. The remaining trials in each sequence consisted of numbers or shapes that were clearly dissimilar from the preceding number or shape. Responses to target trials were considered correct detections or “hits”. Responses to catch trials were considered errors of commission, referred to as “false alarms”. Responses to the remainder of the trials were considered random errors (Cornblatt et al., 1988).

The order in which the numbers and shapes sequences were presented was randomized across participants. Using illustrated cards, the participants were taught the principles of the test prior to initiation. Moreover, a practice session of 25 trials was completed before data recording started.
<table>
<thead>
<tr>
<th>First stimulus</th>
<th>Second stimulus</th>
<th>Response</th>
<th>Response classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1381</td>
<td>1381</td>
<td>Yes</td>
<td>Hit (target trial)</td>
</tr>
<tr>
<td>1381</td>
<td>1388</td>
<td>Yes</td>
<td>False alarm (catch trial)</td>
</tr>
<tr>
<td>1381</td>
<td>4216</td>
<td>Yes</td>
<td>Random error</td>
</tr>
</tbody>
</table>

**Figure 2.** Illustration of CPT-IP stimuli (numbers sequence) and classification of responses.

Performance on the CPT-IP was assessed using the rate of false alarms as a dependent measure. False alarms indicate that accuracy of execution is compromised for the sake of speed (Sergeant & Scholten, 1985). Furthermore, on tasks that require careful checking of stimuli, false alarms are thought to reflect an inability to conform to the contextual demands of the task (Evenden, 1999). Consequently, false alarms have commonly been used as a measure of the response inhibition aspect of impulsivity (Dougherty, Bjork, Marsh & Moeller, 2000; Halperin, Wolf, Greenblatt & Young, 1991).

To further determine CPT-IP performance, two signal detection indices were calculated from the proportion of hits and false alarms, the d’ (d-prime) and β (beta). The d’ is a measure of attentional capacity and the ability to discriminate stimuli. The β reflects each participant’s response style - the tendency to impulsively respond or to inhibit behaviour (Cornblatt, Lenzenweger & Erlenmeyer-Kimling, 1989; Walderhaug et al., 2007). No response bias is indicated if \( \beta = 1 \), as this equals an optimal ratio of responses to target and catch trials. A \( \beta \) value higher than 1.0 indicates a cautious response style characterized by a low number of both false alarms and hits. A \( \beta \) value lower than 1.0 indicates an active style of responding to both target and catch trials, associated with impulsive populations (Epstein et al., 2003).
Using the signal detection indices, disparate aspects of functioning that influences performance on the CPT-IP can be teased apart. This allows for a clearer interpretation of the relationship between reduced serotonergic neurotransmission and impulsivity, as measured by the $\beta$, separating out any effects of serotonin lowering on attention and sensory capacity, as measured by the $d'$. A further advantage of the signal detection indices is the control of unengaged or randomized responding (Walderhaug et al., 2010).

**Mood assessment**

*The Profile of Mood States (POMS).*

The POMS (McNair, Lorr & Droppleman, 1971) was administered to assess whether ATD increased negative affect. The POMS is a self-report inventory that measures immediate and fluctuating mood states on several subscales. The POMS consists of 65 adjectives (e.g. tense, lonely, sad) each referring to a mood state. In completing the task, participants were asked to rate each item on a five-point likert scale ranging from “not at all” (score 0) to “extremely” (score 4), according to how well the adjective corresponded to their mood at the very moment. A sum score was then calculated for the following subscales: anger-hostility, confusion-bewilderment, depression-dejection, fatigue-inertia, tension-anxiety and vigor-activity. Furthermore, a total mood disturbance score was obtained by summing the scores on all subscales. The POMS has been used extensively in applied clinical research to measure mood changes (Boyle, 1987), and is commonly used to assess mood changes in ATD studies (Walderhaug et al., 2007). The internal consistency reliability of the POMS’ subscales has been reported to be good, at .84 or greater (Spielberger, 1970).

**Procedure**

*Recruitment session*

After complete description of the study by a researcher, written informed consent was obtained from both potential participants and their parents. Each potential participant then completed FASM in a classroom setting. On the basis of their responses to the FASM, adolescents meeting the inclusion criteria were identified. A total of 327 adolescents were screened for participation, of which 62 met the inclusion criteria. Based on convenience sampling, 40 female adolescents were included for participation.
**Preliminary assessment session**

The participants met at the Institute of Psychology. A clinical psychologist administered the sections covering the selected psychiatric diagnoses in K-SADS-PL. The main objective of the interview was to obtain the diagnostic characteristics of the sample. Furthermore, interview data were used while referring participants in need of mental health assistance to the appropriate professionals. Subsequently, the WASI was administered to obtain basic psychometric properties of the sample. The preliminary assessment session was conducted 0.6 to 32.9 weeks (mean 15.8) prior to participation in the acute tryptophan depletion session.

**Acute tryptophan depletion session**

In an attempt to standardize dietary intake ahead of the experimental manipulation, participants were told to abstain from high protein food the day before participation. Also, they were told to fast from 8.00 p.m. onwards.

At the day of attendance, starting 8.30 a.m., blood sampling of baseline tryptophan level were carried out. Following blood sampling, the participants completed the POMS. At 9.00 a.m. the participants ingested the amino acid mixtures. Random assignment to the active depletion or sham-depletion condition had been done beforehand by a fellow not affiliated with the study. Participants in the active depletion condition received a mixture devoid of tryptophan, while participants in the sham-depletion condition received an amino acid mixture containing tryptophan. This procedure maximizes group differences in tryptophan level and subsequent serotonin synthesis and release in the brain (Hood et al., 2005).

In the ensuing 4.5 hours, participants were free to read magazines, socialize or watch emotionally neutral cartoons under the supervision of a researcher. To prevent a lack of the tryptophan-derived vitamin nicotine-adenine dinucleotide (NAD) in response to ATD (Zepf et al., 2008), participants were given a vitamin b supplement two hours after amino acid ingestion. A tryptophan-free fruit was served after 2-3 hours, but otherwise participants were not allowed any food. They were not allowed to sleep during this waiting period. At 1.30 p.m. the second blood sample was drawn, measuring plasma tryptophan level. The CPT-IP was then administered within the time interval of predicted peak tryptophan depletion effects (Carpenter et al., 1998; Young et al., 1985). Finally, the participants completed the POMS a second time.
Each participant was tested individually in separate rooms. Following debriefing, all participants received a protein-rich meal. A compensation of 500 NOK was given for their participation in the study. The session ended at 4.00 p.m.

The experiment was designed as a between subjects, double-blind study. Neither the experimenters nor the participants were aware of group affiliation. Participants’ guesses of whether they had been in the active depletion condition (74.2% of guesses) or sham-depletion condition (25.8% of guesses) were no better than chance.

**Statistical analysis**

SPSS 16.0 for Windows (SPSS Inc., USA) was used to register and analyze data. Comparisons of results on each CPT-IP variable for the active and sham-depletion conditions were performed using independent samples t-tests. The dependent variables of the CPT-IP (hits, false alarms, d’ and β) were analyzed separately for the numbers and shapes sequences, as previous research indicates lateralized and independent processing of spatial from verbal stimuli (Keilp, Herrera, Stritzke & Cornblatt, 1997; Walderhaug et al., 2002).

Mood change was assessed as the difference between the score of each POMS subscale at 6 hours after amino acid intake and baseline. Changes in scores of each POMS subscale were then compared between conditions using independent samples t-tests. The criterion of statistical significance was set at \( p < 0.05 \).
RESULTS

Acute tryptophan depletion
The effect of the acute tryptophan depletion procedure on total plasma tryptophan level is shown in table 1. Participants in the experimental condition, receiving an amino acid mixture devoid of tryptophan, had an average decrease in tryptophan level of 78.9%. Participants in the placebo condition, receiving an amino acid mixture containing tryptophan, showed an average increase in tryptophan level of 276.6%.

Table 1. Total plasma tryptophan (TRP) level before and 4.5 hours after ingestion of the amino acid mixture

<table>
<thead>
<tr>
<th></th>
<th>TRP before</th>
<th>TRP after (+4.5 h)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active depletion condition</td>
<td>40.4</td>
<td>8.5</td>
<td>- 78.9</td>
</tr>
<tr>
<td>N = 17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-depletion condition</td>
<td>40.8</td>
<td>153.6</td>
<td>+ 276.6</td>
</tr>
<tr>
<td>N = 15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TRP, tryptophan.
Unit of measurement: µmol/L.

Clinical and psychometric characteristics
In the total sample, 18.8% met the diagnostic criteria for a current major depressive disorder, 9.4% met the diagnostic criteria for a current social phobia, 3.1% met the diagnostic criteria for a current generalized anxiety disorder, 6.3% met the diagnostic criteria for a current eating disorder none otherwise specified, and 3.1% met the diagnostic criteria for an attention deficit hyperactivity disorder. No participants met criteria for any of the other diagnoses. Importantly, there were no significant group differences in frequencies of any of the diagnoses.

WASI scores are displayed in table 2. There were no significant group-differences in the verbal IQ score, the non-verbal IQ score or the total IQ score. All WASI scores were within the normal range.
Table 2. Means and standard deviations on each of the WASI variables in each condition

<table>
<thead>
<tr>
<th></th>
<th>Verbal IQ</th>
<th>Non-verbal IQ</th>
<th>Total IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active depletion condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 17</td>
<td>94.1 (12.7)</td>
<td>100.2 (14.5)</td>
<td>96.9 (13.4)</td>
</tr>
<tr>
<td>Sham-depletion condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 15</td>
<td>92.1 (12.7)</td>
<td>98.8 (12.5)</td>
<td>95.2 (12.1)</td>
</tr>
</tbody>
</table>

IQ, intelligence quotient.
WASI, Wechsler Abbreviated Scale of Intelligence.
Values are given as mean (standard deviation).

The Continuous Performance Test – Identical Pairs
To make sure that participants had understood and responded to the task as instructed, a thorough inspection of the CPT-IP data was performed prior to statistical analysis. A low rate of random errors was found across conditions, suggesting that participants were motivated and paying attention to the task at hand. There were no statistically significant differences in rate of random errors between conditions.

An extreme outlier value was found for the β shapes variable, showing that one participant in the active depletion condition only had responded to the shapes sequence of the CPT-IP intermittently. Accordingly, the data from the shapes sequence of this participant were omitted from statistical analysis. Summary of results on the CPT-IP variables are displayed in table 3.
Table 3. *Means and standard deviation on each of the CPT-IP variables in each condition*

<table>
<thead>
<tr>
<th></th>
<th>Sham-depletion</th>
<th>Active depletion</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 17</td>
<td></td>
</tr>
<tr>
<td>Hits numbers</td>
<td>0.68 (0.14)</td>
<td>0.80 (0.15)</td>
<td>0.027*</td>
</tr>
<tr>
<td>Hits shapes</td>
<td>0.79 (0.17)</td>
<td>0.78 (0.12)</td>
<td>0.794</td>
</tr>
<tr>
<td>False alarms numbers</td>
<td>0.40 (0.16)</td>
<td>0.37 (0.09)</td>
<td>0.543</td>
</tr>
<tr>
<td>False alarms shapes</td>
<td>0.26 (0.11)</td>
<td>0.26 (0.09)</td>
<td>0.902</td>
</tr>
<tr>
<td>$d'$ numbers</td>
<td>0.76 (0.66)</td>
<td>1.30 (0.61)</td>
<td>0.024*</td>
</tr>
<tr>
<td>$d'$ shapes</td>
<td>1.64 (0.81)</td>
<td>1.53 (0.67)</td>
<td>0.681</td>
</tr>
<tr>
<td>$\beta$ numbers</td>
<td>0.94 (0.17)</td>
<td>0.66 (0.32)</td>
<td>0.005*</td>
</tr>
<tr>
<td>$\beta$ shapes</td>
<td>0.85 (0.43)</td>
<td>0.91 (0.44)</td>
<td>0.711</td>
</tr>
</tbody>
</table>

CPT, Continuous Performance Test – Identical Pairs.

Values are given as mean (standard deviation).

*Results from the shapes sequence were omitted for one participant.

* Indicates statistical significance at the $p < 0.05$ level.

**Hits**

There was a statistically significant difference between conditions in the mean proportion of hits on the numbers sequence of the CPT-IP ($t(30) = -2.317, p < 0.027$). Participants in the active depletion condition had a higher number of responses to target trials in the numbers sequence of the CPT-IP, as compared to participants in the sham-depletion condition. No statistically significant difference on the shapes sequence was found between the active depletion condition and the sham-depletion condition.

**False alarms**

There were no significant differences between conditions in mean proportion of false alarms, neither for the numbers sequence or shapes sequence. However, a significantly higher number of false alarms was evident for both the active depletion condition ($t(15) = 3.913, p < 0.001$).
and the sham-depletion condition \((t (14) = 3.069, p<0.008)\) in the numbers sequence compared to the shapes sequence.

\(\beta\)

Mean \(\beta\) values are displayed in figure 3. Analysis of the impulsivity measure \(\beta\) revealed a significant difference between conditions for the CPT-IP numbers sequence \((t (25.162) = 3.061, p<0.005)\). Variance was unequal for this t-test, so degrees of freedom and the resulting t-statistic was adjusted accordingly. Participants in the active tryptophan depletion condition had a significantly lower mean \(\beta\) value, as compared with sham-depleted participants. There was no statistically significant difference between the active and the sham-depletion condition on the \(\beta\) shapes variable.

![Figure 3](image)

**Figure 3.** Mean \(\beta\) values on the numbers and shapes sequences respectively, of the CPT-IP, for each condition. Error bars indicate 1 standard error of means. CPT-IP, Continuous performance test – identical pairs.

\(d'\)

The \(d'\) numbers variable differed significantly between conditions \((t (30) = -2.380, p<0.024)\). A higher \(d'\) numbers mean value was found in the active depletion condition, as compared
with the sham-depletion condition. The d’ shapes variable did not differ significantly between the active tryptophan depletion condition and the sham-depletion condition.

The Profile of Mood States
There was no statistically significant difference between the active and sham-depletion condition on any of the POMS subscales, neither at baseline assessment or assessment six hours after amino-acid intake. The total mood disturbance score, a summation of self-reported changes in mood from baseline assessment to post-intervention assessment, was not significantly different between the conditions (active depletion: $M = 6.56$, $SD = 16.27$, sham-depletion: $M = 8.13$, $SD = 14.33$).
DISCUSSION

The main finding of the present study was that decreased serotonergic neurotransmission resulted in increased impulsivity in adolescents with a history of NSSI. Decreased serotonergic neurotransmission did not cause mood change in the participants.

The use of a double blind design, controlling for experimenter bias and demand characteristics, strengthen the internal validity of the findings. Furthermore, the use of a between subjects parallel group-design eliminates the possibility of differential ATD-effects on consecutive test days, associated with crossover designs (Walderhaug, Landrø & Magnusson, 2008). The ATD procedure is a safe and benign method of experimentally inducing dysfunction in cerebral serotonergic neurotransmission (Moore et al., 2000). The intervention constitutes an objective, biological trigger of serotonergic dysregulation. ATD thus surpasses procedures that are reliant on subjective perception and experience of stress, in terms of experimental control. It is currently regarded as the best way of identifying individuals with serotonergic vulnerability (Jans et al., 2007). The effect of the intervention can be reliably measured by a blood sample.

Acute tryptophan depletion caused a significant decrease in total plasma tryptophan, comparable to the level of reduction found in adults (Fusar-Poli et al., 2006; Young et al., 1985). Moreover, the decrease in the experimental condition was above the suggested threshold of a 60 % reduction in plasma tryptophan to elicit behavioural and mood effects (van der Does, 2001). Thus, the magnitude of reduction in tryptophan level demonstrates that the intervention had its intended effect. To our knowledge, the present study is the first to report a significant reduction in plasma tryptophan using this particular ATD-procedure.

The increased impulsivity found in the present study is inconsistent with previous null findings in high- and low-aggressive adolescent males after ATD (LeMarquand et al., 1998). However, the null finding in the above study may have resulted from a ceiling effect, due to impulsive responding at baseline (LeMarquand et al., 1998). Nevertheless, the result of the present study is consistent with findings of increased impulsive responding following ATD in low-aggressive boys with ADHD (Zepf et al., 2008).
The manner in which impulsivity is measured and calculated represents a strength of the present study. Relying on commission errors as the dependent measure of impulsivity, as in the ATD-studies referred to above, would have led us to infer that serotonergic dysregulation was not influencing impulsivity. The calculation of $\beta$ – the main impulsivity index of CPT-IP, takes into account rates of hits and false alarms and is founded on signal detection theory. It therefore provides a more refined understanding of the phenomenon under study, as it separates impulsivity from changes in attention (Cornblatt et al., 1989).

We found a decrease in $\beta$ following ATD, indicating that adolescents who engage in NSSI adopt an impulsive response style when depleted of serotonin. Impulsive behaviours are often considered engaged in at the blink of an eye, without any conscious intent, direction or regard of the consequences (Cherasky & Hollander, 1997). Inspecting the CPT-IP data closely, we found that participants in the active depletion condition actually produced a higher number of hits and a lower number of commission errors compared to the sham depleted participants. Furthermore, the increase in $d'$ suggest that tryptophan-depleted participants became more focused and goal-directed. Thus, the present findings suggest that participants with reduced serotonergic neurotransmission efficiently engaged in the task at hand, in spite of adhering to an impulsive response style. Consequently, the response style of tryptophan-depleted subjects in the present study can be described as one of functional impulsivity (Dickman, 1990), as it is optimal in performing the task.

The increased impulsivity demonstrated in the present study might be a consequence of decreases in the serotonergically mediated inhibitory control of behaviour (Spoont, 1992). ATD might jeopardize frontal cognitive functions in vulnerable subjects, lowering inhibitory control and leaving the neural system susceptible to exogenous influences. Rather than thinking matters through, the individual becomes responsive to associative cues of the moment, thus reacting impulsively to salient or intense emotions (Spoont, 1992).

Accordingly, when serotonergically vulnerable individuals experience emotional distress, the threshold for engagement in impulsive behaviours such as NSSI is lowered.

The increased attentional capacity associated with tryptophan depletion found in the present study was not expected. It contradicts ATD-findings in adult samples performing the CPT-IP (Walderhaug et al., 2002, 2007), which suggests that adolescents who engage in NSSI differ
from healthy adults with respect to the goal-directedness of their response style. In accordance with the findings of the present study, decreased serotonergic neurotransmission has left attention unaffected or even improved selective attention in research employing other tasks than the CPT-IP (for a review, see Mendelsohn, Riedel & Sambeth, 2009). The increase in attentional capacity may result from the effects of ATD on cerebral activity. Tryptophan-depleted subjects show heightened activation in superior and temporal cortices when engaging in tasks that require inhibition (Rubia et al., 2005), possibly reflecting compensatory mechanisms. The temporal region is important for mediating selective attention (Poldrack, Wagner & Yantis, 2008). Thus, the increased activity in temporal regions that may have resulted from ATD could have heightened the attentional capacity of our participants.

Furthermore, increased attentional capacity following ATD may relate to the ability of serotonin to modulate and potentiate other systems of neurotransmitters (De Simoni, Dal Toso, Fodritto, Sokola & Algeri, 1987). Serotonin serves an inhibitory mechanism in the human neurobiology (Soubrié, as cited in Evenden, 1999). A slight decrease in serotonergic activity might therefore lead to increased activity in dopaminergic, noradrenergic and acetylcholinergic systems (Robbins, 1997). Increased activity in these systems could be advantageous for higher attentional functions (Robbins, 1997).

Contrary to our expectations, ATD did not affect mood in the present study. Female gender and a history of self-injurious behaviour are both independent predictors of mood responses to ATD (Booij et al., 2002). Furthermore, mood effects following ATD is primarily found in subclinical samples with a vulnerability towards the development of affective, impulsivity or alcohol disorders (van der Does, 2001; Booij, van der Does & Riedel, 2003). Considering the presence of affective and anxiety diagnoses in our sample of adolescent females, the null finding is intriguing.

Several studies refer to the independence of mood and impulsivity following ATD (Mendelsohn et al., 2009, Walderhaug et al., 2002, 2007, 2010; LeMarquand et al., 1999; Zepf et al, 2009). Carver, Johnson & Joorman (2008) has proposed a theory that helps explain how the apparently contradictory tendencies of depression and impulsivity can have the same neurochemical underpinning. The theory is based on the premise that people experience the world through two simultaneous but distinct modes of processing; a reflexive, associationist mode, and a reflective, planful mode. Low serotonergic function is proposed to mark a deficit
in the self-regulatory processes that override or inhibit the inclination towards one of these modes over the other. Hence, at an optimal level, the serotonergic system can override and flexibly counter the reactive tendencies of the individual. If the person has an underlying reflective and planful response tendency, low levels of serotonin will cause the person to withdraw and experience mood lowering. By contrast, low levels of serotonin will lead to impulsive behaviours in individuals with a reflexive and associationist response tendency (Carver et al., 2008). In light of the present findings, it is possible that adolescents who engage in NSSI have an inclination towards reflexive and associationist processing, that become evident when inhibitory capacity is attenuated by serotonergic dysregulation.

The increased impulsivity and absence of mood change presently found are in accordance with findings in healthy, young adult males undergoing ATD (Walderhaug et al., 2002, 2007, 2010). The resemblance of female adolescents who engage in NSSI and adult males, with respect to ATD-effects, is intriguing. Researchers have found sex-specific differences in serotonergic markers, indicating that male and female brains are neurochemically distinct (Cosgrove, Mazure & Staley, 2007). These sex-specific differences represent themselves in higher prevalence rates of affective and impulsivity disorders in women and men, respectively (Gater et al., 1998; Kessler, Berglund, Demler, Jin & Walters, 2005). In line with this, mood lowering effects and a conservative style of responding have been found in healthy adult females undergoing ATD (Walderhaug et al., 2007, 2010).

Taking these findings into account, the adolescent females of the present study display a distinct behavioural pattern compared to what is usually associated with serotonergic dysregulation in female individuals. However, the curvilinear relationship between age and ratio of serotonin to dopamine in the cerebral spinal fluid (Fairbanks et al., 1999) indicate greater vulnerability to serotonergic challenges in adolescents compared to adults. Future studies using the ATD-procedure should investigate the specificity of our findings by comparing individuals engaging in NSSI with healthy individuals across age and sex. This will help us disentangle whether the present effects of ATD on impulsivity and mood are merely age- or sex-dependent, or represent distinctive qualities in adolescents who engage in NSSI.

Relevant to the absence of mood effects in the present study, research demonstrates that adolescents who engage in NSSI are reluctant to express their feelings, thoughts or inner state
to other people (Ross, Heath & Toste, 2009). Furthermore, they display confusion when identifying and recognizing their emotional experience (Ross et al., 2009; Muehlenkamp, Kerr, Bradley & Larsen, 2010). Accordingly, the null finding might be attributed to the distinct way adolescents engaging in NSSI are dealing with emotional material.

The POMS has been extensively used in ATD studies to measure immediate and transitory non-clinical mood states in adults (for a review, see van der Does, 2001). However, whether the POMS measure trait-like, rather than state-like qualities of mood have been questioned (Knapp, Kimble & Dunbar, 1998; Spielberger, 1970). Research points to the possibility of using observer-rating scales, as they result in larger reported changes in depressive mood than self-report questionnaires (Booij, van der Does, Haffmans, Spinhoven & McNally, 2005). Future studies may triangulate methods to overcome the difficulties associated with the self-report format.

The significant increase in impulsivity found following ATD is limited to the numbers sequence of the CPT-IP. The numbers sequence represents the verbal subtask of the CPT-IP, whereas the shapes sequence represents the spatial subtask (Cornblatt et al., 1988). The stimuli of the two sequences are processed independently (Cornblatt et al., 1988). Research indicates that the left hemisphere is relatively more active than the right during performance of the verbal subtask (numbers sequence), whereas the right hemisphere is more active than the left during performance of the spatial task (shapes sequence) (Cornblatt & Keilp, 1994; Mataix-Cols et al., 1997).

Developmental differences in spatial and verbal ability may help explain why the increase in impulsivity is limited to the verbal subtask only (Cornblatt et al., 1988). Attention to spatial stimuli is at its peak in childhood and young adolescence, and it is actually superior to spatial and verbal abilities in adulthood. Thus, adolescents appear to attend to spatial input more efficiently than verbal stimuli. In accordance with this interpretation, adolescents in both conditions had a higher hit-rate, fewer false alarms and a higher degree of discriminability on the non-verbal compared to the verbal subtask.

An impulsive response style on the verbal subtask of the CPT-IP may indicate deficits in language comprehension. In our sample, there was a 6-point gap between verbal IQ (VIQ) and performance IQ (PIQ). Wechsler (as cited in Sattler & Hoge, 2006) has recommended
that a split of 15 or more points (±1 SD) is required to draw interferences on an individual level. Even so, the direction of the discrepancy is opposite to what is typically found in healthy girls at this age (N.I. Landrø, personal communication, September 10, 2010). The VIQ< PIQ pattern of our sample is hence more similar to adolescents with conduct disorder (CD; Isen, 2010). The coinciding pattern in CD and NSSI may be due to the central role verbal skills have in the development of self-regulation (Kopp, 1982) and subsequent affect regulation (Pine, as cited in Greenberg, Kusche & Speltz, 1991). Deficits in affect regulation are central to both CD (Lewis, Granic & Lamm, 2006) and NSSI (Laye-Gindhu & Schonert-Reichl, 2005; Jacobsen & Gould, 2007).

A higher non-verbal than verbal IQ may indicate that immediate problem solving is better developed than knowledge acquired through accumulated experience (Sattler & Hoge, 2006). NSSI can be understood as an immediate problem solving strategy that is engaged in impulsively to dampen intolerable affective states, supporting this suggestion. However, post hoc correlational analysis did not support any association between VIQ and impulsive response style on the verbal subtask of the CPT-IP. The discrepancy between VIQ and PIQ could still be germane on group level. The investigation of the co-occurrence of less developed verbal skills and impulsivity in adolescent females engaging in NSSI is required in future studies.

Another possible explanation for the discrepant findings between the shapes and numbers mode is the decline in performance on the shapes subtask found in people suffering from major depressive disorder (MDD, Keilp et al, 1997). The MDD diagnosis is related to deficits in processing of right hemisphere-type tasks (Flor-Henry, 1976), such as the shapes subtask of the CPT-IP. Furthermore, depressed mood is often associated with a conservative response style (Johnson & Magora, 1987; Miller & Lewis, 1977). In ATD studies, low mood and a conservative response style is found to co-occur in female subjects (Walderhaug et al., 2007). The impulsive response style found in adolescents who engage in NSSI contradicts all the mentioned findings attributable to MDD. It therefore suggests that NSSI is separate from, although often comorbid with, MDD. As such, it seems unlikely that the affective diagnoses observed in some of the participants could be the underlying cause of our findings.

The present findings allow us to form hypotheses concerning the relation between serotonin, impulsivity and emotion-regulation aspects of NSSI. Negative emotional states commonly
Serotonin, impulsivity and mood in non-suicidal self-injury

precede acts of NSSI. Emotional states are associated with certain behavioural tendencies – actions that the individual initiate as a result of the emotion. Inhibition is required to control these behavioural tendencies (Nash et al., 2007). Inhibition does not necessarily provide relief from the emotional state, but expression of behavioural emotion tendencies is hindered (Gross & Levenson, 1997). Accordingly, decreased serotonergic neurotransmission may render the individual incapable of inhibiting NSSI in response to intolerable emotional states. Consequently, the individual engages in NSSI in an attempt to alleviate negative affect (Jacobson & Gould, 2007).

The results of the current study indicate that adolescents who engage in NSSI display a form of functional and deliberate impulsivity, which was appropriate to the demands of the CPT-IP. However, real-life is more complex than performance on impulsivity measures like CPT-IP. Long-term engagement in impulsive NSSI can have a fortifying effect on symptoms. NSSI may in fact increase negative feelings about oneself, thus serving to exacerbate symptoms and distress (Jacobsen & Gould, 2007). Moreover, the impulsivity demonstrated in our sample may preclude engagement in more complex emotion regulating strategies (Ross et al., 2009). Interventions aimed at developing alternative emotion regulating capacities in adolescents who engage in NSSI are therefore essential to help them abstain from this potentially fatal behaviour (Gratz, 2007; Klonsky & Muhlenkamp, 2007).

The increased impulsivity in adolescents who engage in NSSI enhances the probability of exposure to stressors (Jang, Wolf & Larstone, as cited in Williams, Suchy & Rau, 2009). Lack of inhibitory control may cause difficulties in overriding dominant emotional tendencies, which in turn predispose to interpersonal difficulties - a primary source of stress (Williams et al., 2009). Impulsivity is also found to increase rapid, marked shifts in affective states (van Reekum, Links & Fedorov, 1994), possibly implying that adolescents who engage in NSSI are more emotionally reactive. Emotional reactivity may lead to abnormalities in affect regulation (Phillips et al., 2003). Furthermore, poor self-regulation in the context of interpersonal relationships may degrade social support, thereby removing a potential buffer against the adverse effects of stress. Supporting this suggestion, individuals engaging in NSSI consider interpersonal relationships as tense, disappointing and of poor quality compared to adolescents who do not engage in NSSI (Ross et al., 2009). Thus, stress caused by inhibitory deficiency may act as a preponderant and maintaining factor of NSSI behaviours (Nock & Mendes, 2008).
The heightened impulsivity evident in response to ATD implies that our participants were unable to compensate for transitory down-regulation of serotonergic neurotransmission. The findings of the present study therefore indicate that adolescents who engage in NSSI are vulnerable to changes in activity of the serotonin system. Consequently, developing prevention programs that focus on factors pertinent to the development of serotonergic vulnerability is central to this field. This work should be conducted in line with the biopsychosocial model of health and disease (Engel, 1977), focusing on biological, psychological and social factors. It is important to note, however, that serotonergic vulnerability not necessarily leads to psychopathology (Jans et al., 2007). Preventive efforts should therefore be initiated towards already vulnerable groups, to avoid negative trajectories.

Different NSSI inclusion criteria have been applied across studies (Jacobsen & Gould, 2007). The present study was based on a community sample of adolescents engaging in NSSI. The inclusion criteria was set at engagement in at least two different types of NSSI over the last twelve months, in spite of research showing that the majority of adolescents in community samples only use one NSSI-method (Muhlenkamp & Guttierez, 2004; Ross & Heath, 2002). The requirement of two different types of NSSI helped exclude adolescents who had only picked at a wound, a behaviour that in isolation is considered non-pathological (Lloyd-Richardson et al., 2007). However, research shows that the same underlying neurobiological dysfunction is evident in severe and less severe forms of self-injury (Stanley et al., 1992). Thus, there are reasons to expect that the findings of the present study might generalize to other samples of individuals engaging in NSSI. Nevertheless, future studies are needed to further explore this.

A challenge when testing this age group is the interactional effects of oestrogen, the primary female sex hormone, and serotonin (Fink, Sumner, McQueen, Wilson & Rosie, 1998; Rubinow, Scmidt & Roca, 1998). As a result of oestrogenic fluctuations, levels of serotonin vary throughout the menstrual cycle. ATD should therefore be conducted when female participants are in the luteal phase. To comply with this, we systematically asked each participant about their menstrual cycles. However, few could report on their cycle. Some of our participants had not reached sexual maturity, some did not have a stable menstrual cycle, and some simply could not remember when they had their last three periods. To restrict testing to the luteal phase of the menstrual cycle was therefore impossible to go through with.
The present study has focused on the inhibition aspect of impulsivity. There is a general agreement in the field that impulsivity represents a multifactorial construct (Ho, Al-Zahrani, Al-Ruwaitea, Bradshaw & Szabadi, 1998; Lane, Cherek, Rhoades, Pietras & Tcheremissine, 2003; Reynolds, Penfold & Patak, 2008). Future studies are therefore needed to establish how other aspects of impulsivity relate to serotonergic function in adolescents who engage in NSSI.

**Concluding remarks**

The present study investigated the relationship between serotonin, impulsivity and mood in adolescents who engage in NSSI - a group assumed to have a tendency towards impulsive behaviour. Using an established method to reduce the level of serotonin in the central nervous system, we found a significant increase in impulsive responding, presumably triggering the typical response style of this group during times of distress. Decreased serotonergic neurotransmission was not associated with mood change. However, a reduced level of serotonin resulted in increased discriminative ability, suggesting that the participants became more focused and determined. The present study contributes to an understanding of NSSI-behaviours being purposefully engaged in to regulate affect.
REFERENCES


