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The effects of acute tryptophan depletion on speech and behavioural mimicry in individuals at familial risk for depression

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Koen Hogenelst[1,2], Anastasios Sarampalis[1,2], N Pontus Leander[1], Barbara CN Müller[3], Robert A Schoevers[4] and Marije aan het Rot[1,2]

Abstract

Major depressive disorder (MDD) has been associated with abnormalities in speech and behavioural mimicry. These abnormalities may contribute to the impairments in interpersonal functioning that are often seen in MDD patients. MDD has also been associated with disturbances in the brain serotonin system, but the extent to which serotonin regulates speech and behavioural mimicry remains unclear. In a randomized, double-blind, crossover study, we induced acute tryptophan depletion (ATD) in individuals with or without a family history of MDD. Five hours afterwards, participants engaged in two behavioural-mimicry experiments in which speech and behaviour were recorded. ATD reduced the time participants waited before speaking, which might indicate increased impulsivity. However, ATD did not significantly alter speech otherwise, nor did it affect mimicry. This suggests that a brief lowering of brain serotonin has limited effects on verbal and non-verbal social behaviour. The null findings may be due to low test sensitivity, but they otherwise suggest that low serotonin has little effect on social interaction quality in never-depressed individuals. It remains possible that recovered MDD patients are more strongly affected.

Keywords

Serotonin, verbal behaviour, mimicry, social interaction, interpersonal impulsivity, major depressive disorder, speech

Introduction

Individuals with major depressive disorder (MDD) are often characterized by impairments in social functioning (Hames et al., 2013; Youngren and Lewinsohn, 1980). For example, the interpersonal relationships of depressed individuals tend to be marked by dissatisfaction, low intimacy and rejection (Gotlib and Lee, 1989). This may be explained by findings indicating that depressed individuals are often less pro-social than non-depressed individuals (Hokanson et al., 1980), more irritable and hostile (Perlis et al., 2005) and more likely to seek reassurance excessively (Hames et al., 2013). The risk of developing MDD is higher in individuals with a family history of MDD (Sullivan et al., 2000). In the present work, we sought to study aspects of social interaction in individuals with and without a family history of MDD. We specifically investigated the role of serotonin in regulating speech and mimicry.

The interpersonal difficulties of individuals with MDD may be explained partially by aberrant verbal and non-verbal behavioural patterns during social interaction (Segrin, 2000). Compared to non-depressed individuals, symptomatic individuals with MDD tend to use more and longer pauses, and their speech is relatively monotonous (Alpert et al., 2001; Nilsonne, 1988). These speech characteristics have been positively associated with depression severity (Mundt et al., 2012). Moreover, depressed individuals are considered more self-focused when interacting with others, and tend to use more negative words (Baddeley et al., 2013; Rude et al., 2004). In short, both the prosody and the verbal content of speech appear to be altered during depression.

Concerning non-verbal social behaviour, depressed individuals engage less in eye contact, use fewer gestures (e.g. Kazdin et al., 1985; Troisi and Moles, 1999) and show a less expressive facial muscle activity pattern when asked to imagine happy or sad events (Gehricke and Shapiro, 2000; Schwartz et al., 1976) compared to non-depressed individuals. Moreover, symptomatic patients mimic others less – they do not automatically respond with facial emotions to the expressions of others (Wexler et al., 1994). These findings are consistent with results from a study in which healthy volunteers watched a video with a pen-playing experimenter. Whereas individuals in a positive mood unintentionally mimicked the pen-playing behaviour, those in a negative

[1]University of Groningen, Department of Psychology, Groningen, The Netherlands
[2]University of Groningen, School of Behavioral and Cognitive Neurosciences, Groningen, The Netherlands
[3]Behavioural Science Institute, Radboud University Nijmegen, Department of Social and Cultural Psychology, Nijmegen, The Netherlands
[4]University of Groningen, University Medical Centre Groningen, Department of Psychiatry, Groningen, The Netherlands

Corresponding author:
Koen Hogenelst, Department of Psychology, University of Groningen, Grote Kruisstraat 2/1, 9712 TS Groningen, The Netherlands.
Email: k.hogenelst@rug.nl

mood did not (van Baaren et al., 2006). Unintentional behavioural mimicry refers to the automatic and unconscious imitation of another person's emotional (including facial) expressions, posture and movements (Chartrand and Bargh, 1999). Unobtrusively mimicking someone may facilitate liking and elicit more pro-social behaviour from others (Chartrand and Bargh, 1999; Chartrand and van Baaren, 2009). As such, mimicry is considered a part of good social interactions. However, the study by van Baaren et al. (2006) suggests that negative mood, which includes depression, may interfere with mimicry.

These studies indicate that depressed individuals may display a range of behavioural deficiencies during social interaction. Social skills impairments may hamper the acquisition of positive reinforcement from the social environment (Segrin, 2011). Additionally, while non-depressed people initially may be sympathetic towards people displaying depressive behaviours, they often become frustrated and rejecting (Hames et al., 2013). Low positive reinforcement and interpersonal rejection may be present before the depression and contribute to its development (Hames et al., 2013; Segrin, 2011). When subsequently present during the depression, these social impairments may also interfere with recovery (Hames et al., 2013; Segrin, 2011).

Social impairments in MDD may be explained by alterations in brain serotonin functioning. MDD has often been associated with disturbances in the serotonin system (Mann, 2013). Experimental evidence suggests that reductions in serotonin levels may elicit depression: acute tryptophan depletion (ATD), which temporarily reduces brain serotonin, induces transient depressive symptoms in recovered MDD patients (Young, 2013) and also tends to result in a worsening of mood in never-depressed individuals with a family history of MDD (Benkelfat et al., 1994; Klaassen et al., 1999; however, see Ellenbogen et al., 1999). In contrast, individuals without a personal or family history of MDD are often unaffected by the mood effects of ATD. These findings have been taken to mean that vulnerability to MDD may be explained by abnormal serotonin functioning. As MDD includes social impairments, these may be explained by serotonin abnormalities as well.

In line with this, a small number of studies has assessed the role of serotonin in social behaviour in MDD patients. In one study, 12 weeks of antidepressant treatment with the selective serotonin reuptake inhibitor (SSRI) sertraline reduced pause duration and improved pitch variation during a semi-structured social interaction (Alpert et al., 2001). Further, it has been shown that MDD patients who responded to 4–6 weeks of treatment with sertraline showed significantly increased pitch variability and fewer pauses during structured clinical interviews (Mundt et al., 2007, 2012). Furthermore, Stassen et al. (1998) studied 43 depressed patients receiving antidepressants in their first two weeks of treatment and found increases in pitch variability and decreases in pause duration. These studies suggest that serotonin may regulate verbal social behaviour in individuals with MDD.

Studies in never-depressed individuals have also assessed the role of serotonin in non-verbal social behaviour. ATD has previously been found to decrease cooperative behaviour during the prisoner's dilemma game (Wood et al., 2006) and increase antagonistic responses and impulsive choices in the ultimatum game (Crockett et al., 2010). Conversely, administration of citalopram for two weeks increased cooperative behaviour and stimulated a more dominant pattern of eye contact during speech (Tse and Bond, 2002). The idea that serotonin may also help regulate non-verbal social behaviour is in line with a larger body of research suggesting that serotonin modulates social behaviour in animals other than humans (Kiser et al., 2012; Young, 2013).

In the present study, we examined the effects of ATD on multiple measures of verbal and non-verbal social behaviour in never-depressed individuals with and without a family history of depression (FH+ vs FH-). We studied FH+ individuals because they are at increased risk of developing MDD (Sullivan et al., 2000) and have been found to exhibit subtle impairments in the processing of emotional stimuli (Mannie et al., 2007, 2011) that may help explain their elevated MDD risk. Previous studies have indicated that the emotional processing impairments of FH+ individuals may be exacerbated by ATD (Feder et al., 2011; Firk and Markus, 2008). FH+ individuals have also shown more pronounced abnormalities than FH- individuals in responding to negative facial emotion expressions and other negative affective information after ATD (van der Veen et al., 2007). Thus, ATD might differentially influence responses to affective stimuli, including socially relevant stimuli, in FH+ vs FH- individuals.

With respect to verbal social behaviour, we hypothesized that ATD would increase the number and duration of pauses and decrease pitch variability while speaking. This would be in line with previous studies showing that antidepressant treatment has the opposite effects on speech (e.g. Alpert et al., 2001; Mundt et al. 2007, 2012). We also explored whether, similar to symptomatic MDD patients (e.g. Alpert et al., 2001; Mundt et al., 2007, 2012) participants would use more self-references, more negative words and fewer positive words after ATD. In line with the previously observed effects of ATD on socio-affective processing (Feder et al., 2011; van der Veen et al., 2007), we expected the effects of ATD on verbal social behaviour to be stronger in FH+ individuals than in FH- individuals.

With respect to non-verbal social behaviour, we hypothesized that ATD would lower behavioural mimicry. This hypothesis follows from previous studies showing that ATD may decrease prosocial responses to others (Wood et al., 2006). Behavioural mimicry is considered a form of prosocial behaviour (Chartrand and van Baaren, 2009). Moreover, individuals mimic others less when their mood is worse (van Baaren et al., 2006), and low mood after ATD is more common in FH+ than in FH- individuals (Benkelfat et al., 1994; Klaassen et al., 1999). Therefore, ATD may particularly decrease mimicry in FH+ individuals.

Methods and materials

Participants

The study was approved by the Medical Ethics Committee of the University Medical Centre, Groningen. Participants provided informed consent after an extensive study explanation, and were reimbursed for their time.

Participants were men and women with (FH+) or without (FH-) a family history of MDD. FH+ individuals were recruited via their diagnosed family members (i.e. probands). Probands heard about the study via their health care provider or via advertisements. Upon contacting the laboratory, they were provided with some study information and asked whether they had any never-depressed, first-degree family members (parents, siblings or children) who might be interested in the study. If so, we asked

probands for consent to contact their health care provider to verify the MDD diagnosis. Psychiatric co-morbidity was permitted, with the exception of current or past (hypo)mania. Once the MDD diagnosis of the proband was confirmed, we recruited interested family members. FH+ individuals were eligible for study participation if they met the following inclusion criteria: at least one first-degree relative with a lifetime DSM-IV diagnosis of MDD, age 18–65 years, no current or past mood disorder, no other current DSM-IV Axis-I disorder, no current major medical illness and no use of psychotropic medications. Screening was performed with the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) (First et al., 2002) and the family history method described by Andreasen et al. (1977).

FH- was defined as having no first- or second-degree relatives with a possible (history of) mood disorder. FH- individuals were recruited using advertisements on campus and via a paid participant pool of the University of Groningen. With the exception of the family history criterion, inclusion criteria were identical to those for FH+ individuals. The two groups were matched for gender, age and education.

The details of the screening phase can be found in the Supplemental Information. Forty-four participants started the study. Four participants dropped out (three vomited within the first hour of amino acid mixture ingestion on the first test day and one participant felt sick after the first test day). The results are described for ten men and ten women in the FH+ group (all children or siblings of MDD patients, including one pair of brothers and two pairs of sisters) and 10 men and 10 women in the FH- group (all from different families). There were no significant group differences in terms of participant characteristics (Table 1).

This study is part of a more comprehensive project assessing the role of serotonin in social behaviour; an overview of all included measures can be found at Clinical Trials.gov (NCT020051530). Eleven FH+ individuals also participated in a related project (Hogenelst et al., 2015; NCT02051569).

Table 1. Participant characteristics.

	Group	
	FH- (n=20)	FH+ (n=20)
Mean age in years, (SD in brackets)	21.9 (2.2)	22.2 (3.3)
Occupation (% student)	90	95
Mean body mass index (kg/m^2) (SD in brackets)	23.0 (2.1)	22.2 (2.8)
Smoking status (% yes)	30	25

There were no significant group differences by t-test (p>0.25 for all variables).

Study design and overview

On one of the test days, to induce ATD, participants ingested an amino acid mixture that did not include tryptophan. On the other test day, as a control, participants ingested a nutritionally balanced mixture that included tryptophan and was otherwise identical. The control mixture weighed 103 g for men (including 2.3 g tryptophan; (Young et al., 1985)) and 86 gram for women (including 1.9 g tryptophan (Ellenbogen et al., 1996)).

The treatments were administered under double-blind conditions. Treatment order was randomized by group (FH+, FH-) and gender (male, female) in blocks of four. Just prior to ingestion, water, chocolate syrup or orange juice and sodium cyclamate were added. In the FH+ group, 11 participants received ATD first and nine received the control mixture first. In the FH- group, nine participants received ATD first and 11 received the control mixture first.

Biochemical analysis

Tryptophan. Fifteen minutes before and five hours after ingestion of the amino acid mixtures, blood samples were collected by venipuncture in 10mL vacutainer tubes containing K_2EDTA solution as anticoagulant. Two morning samples could not be obtained. Blood samples were centrifuged at 2500g for 10 min at 4°C within 30 min of collection. Plasma was then transferred to glass tubes and samples were stored at −20°C until analysis. Total plasma tryptophan concentrations were determined using an automated online solid phase extraction-liquid chromatographic-tandem mass spectrometric (XLC-MS/MS) method with deuterated internal standards. Quality control and method validation have been previously described (de Jong et al., 2009).

Test measures

Depression. Baseline depressive symptoms were assessed using a Dutch version of the Quick Inventory of Depressive Symptomatology (QIDS-SR) (Rush et al., 2003).

Mood state. A Dutch version of the Positive Affect and Negative Affect Schedule (PANAS, Peeters et al., 1996) was administered repeatedly during the test days. The PANAS included ten positive affect (PA) and ten negative affect (NA) items that were rated on a five-point Likert scale. The positive and negative item scores were used to calculate PA and NA, respectively.

Behavioural mimicry. Participants completed two tasks that were both videotaped. The first task was adapted from Stel et al. (2008). Participants viewed two short film clips (~1 min) in which a female confederate talked about her daily activities while touching her face 4–6 times. After each clip, participants were asked to summarize what was said in the clip. Two different sets of clips, equal in length and the amount of face-touching, were presented in random order across the two test days. After the study, two raters coded how often participants touched their face and other upper body parts while viewing each clip. Analogous to van Baaren and colleagues (2006), only touches that occurred within ten seconds of the confederate touching her face were coded as mimicry. The inter-rater reliability (r) was 0.90.

In the second task, participants described four pictures during a short interview. Analogous to Chartrand and Bargh (1999), they were seated on chairs arranged at a 90-degree angle vis-à-vis the experimenter with a white labcoat and clipboard (KH). The experimenter asked what participants saw in each picture and what associations or feelings it elicited. When the participant described the pictures, the experimenter was either constantly shaking his foot or bouncing his knee. The order of the two conditions was randomly distributed over the participants. After the study, two raters coded the videotapes. The outcome variable was

the percentage of time the participant mimicked the experimenter's behaviour – that is, foot-shaking when the experimenter was shaking his foot and knee-bouncing when the experimenter was bouncing his knee (see also Lakin and Chartrand, 2003). The inter-rater reliability (r) was 0.92. All raters worked independently and were blind to mixture and group.

Speech. During both mimicry tasks, speech was recorded. Speech during the first, more structured task, was analysed for pauses and pitch. Because the use of pauses may be influenced by the time participants take before they start speaking, two raters first determined the time between the end of each video clip and the start of speech. This 'preparation time' was expressed as a percentage of the total time the participant took to describe the clip, including pauses. Second, analogous to Stassen et al. (1998), raters determined the number of pauses per second (only pauses longer than 250 ms were included), the mean pause duration (in ms) and speech time (excluding pauses and expressed as a percentage of the total recording). The inter-rater reliability for pause detection was 0.97. After removing the pauses, pitch characteristics were determined using the software Praat (Boersma and Weenink, 2010). Each sound file was organized into 20 frames per second and the mean and standard deviation of pitch were calculated using a freely available script (retrieved from www.fon.hum.uva.nl/praat/manual on March 1, 2014).

Speech during the second, more unstructured, mimicry task was analysed for content. Transcripts made by one rater were checked by another rater. Using the Dutch dictionary of the software Linguistic Word Inquire Count (Zijlstra et al., 2004) we determined the percentage of self-references (e.g. 'I'), positive words (e.g. 'nice'), negative words (e.g. 'ugly') and social words (e.g. 'together') as a function of the total number of words.

Procedures

Test days were separated by at least three days (mean 7.8 days). As in previous studies (e.g. Ellenbogen et al., 1999), women were tested in the follicular phase of the menstrual cycle. Except for toilet visits, participants remained in a room including a desk, lounge chair and TV with DVD player. On both test days, to control light exposure during the day, window blinds and curtains were closed and standard room lighting turned on. The experimenter was present in an adjacent room. Before each test day, participants maintained a 24-hour low-protein diet. They arrived in the laboratory at 08:30 h the next day, following an overnight fast. Mobile devices were switched off. Urine was tested for recent illicit drug use (Triage™ Panel for Drugs of Abuse, Biosite Incorporated®, San Diego, USA) and, in women, for pregnancy (QuickVue hCG urine test, Quidel, San Diego, USA). Baseline depressive symptoms and mood state were then assessed. Approximately 15 min after arrival, a first blood sample was drawn. Subsequently, participants were given up to 30 min to ingest the amino acid mixture. Afterwards (t0), participants could study, read or watch a limited set of movies, but not sleep. No other food or beverages were allowed until the end of the testing seven hours after mixture ingestion, except tap water was available ad lib. Mood state was reassessed at five, six and seven hours after mixture ingestion (t5, t6 and t7, respectively). Between t5 and t6 a second blood draw took place, and behavioural mimicry and speech were assessed. Between t6 and t7, we administered a cognitive empathy task (data not presented here). After the final mood assessment, participants were given a sandwich and two capsules with 500 mg tryptophan (Elvitaal™, Lunteren, The Netherlands) before returning home. Participants received follow-up telephone calls in the evening and the next day.

Data analysis

Baseline differences between the FH groups were examined using t-tests (Table 1). Morning baseline differences were investigated using general linear models with Group (FH+, FH-) as between-subjects factor and Mixture (ATD, Control) as within-subjects factor. All models included Mixture, Group and their interactions. T-tests and general linear models were performed in SPSS 17.0.

The main outcome variables were pauses per second, mean pause duration, speech time, preparation time, pitch, variation in pitch and mimicry in tasks 1 and 2. We also examined word use in terms of the positive words, negative words, social words and self-references. We analysed the data using multilevel models, with maximum likelihood estimation to calculate the denominator degrees of freedom following Satterthwaite's approximation for the fixed effects tests. Multilevel models were analysed using R v3.0.2 (www.r-project.org).

The primary analyses aimed to assess the effects of ATD on speech and mimicry. For all speech and mimicry variables, the initial models considered Mixture, Group, and the Mixture-by-Group interaction. We had no a priori hypotheses about Gender or Mixture-by-Gender effects on speech and mimicry. However, as women may be more susceptible to the mood effects of ATD (Booij et al., 2003), we added Gender as a covariate to the analyses described above. Subsequent models considered Mixture, Group, the Mixture-by-Group interaction, Gender and the Mixture-by-Gender interaction.

Secondary analyses considered the effects of ATD on plasma tryptophan and mood state. For plasma tryptophan levels, the models considered Mixture, Group, Sample (morning, afternoon) and their interactions. For PA and NA the models considered Mixture, Group, Time (four levels: t0, t5, t6 and t7) and their interactions.

Significance was set at 0.05. Significant interaction terms in Type III tests were analysed post-hoc using simple contrasts using Tukey–Kramer corrections for multiple comparisons. Findings are reported using estimated least-squares means and standard errors of the mean (SEM).

Results

Baseline measurements

For morning QIDS scores there were no significant effects for Mixture (p=0.74) or Group (p=0.09). The Mixture-by-Group interaction was significant (F(1,37)=4.55, p=0.04), but none of the post-hoc simple contrasts were (all ps >0.06), indicating QIDS scores were similar across the test days for both FH groups. All participants scored <8 on the QIDS.

Verbal behaviour

Pauses. The mean number of pauses per second was 0.26 (SEM=0.007). There were no significant effects for Mixture,

Group or Mixture-by-Group (see Table 2). The effect for Gender was significant ($F(1,40)=21.19$, $p<0.001$, $d=1.46$). Men ($M=0.30$, SEM=0.013) had more pauses than women ($M=0.22$, SEM=0.013). Secondary analyses showed no significant Mixture-by-Gender interaction ($p=0.62$).

The mean pause duration was 816 ms (SEM=19). There were no significant effects for Mixture or for the Mixture-by-Group interaction (Table 2). The main effect for Group was significant ($F(1,38)=4.54$, $p=0.04$, $d=0.68$). The mean pause duration was longer in FH+ individuals (867 ms (SEM=35.5)) than FH- individuals (749 ms (SEM=35.6)). The effect for Gender was also significant ($F(1,39)=7.66$, $p=0.009$, $d=0.89$). Mean pause duration was longer for men ($M=882$ ms, SEM=35.5) than for women ($M=749$ ms, SEM=35.6). Secondary analyses showed no significant effect for the Mixture-by-Gender interaction ($p=0.38$).

As the effect for Group was significant in the primary analyses, we repeated the secondary analyses with the Group-by-Gender interaction added to the analysis. This interaction was not significant ($p=0.59$).

Speech time. There were no significant effects for Mixture, Group or the Mixture-by-Group interaction (Table 2). The effect for Gender was significant ($F(1,40)=21.32$, $p<0.001$, $d=1.46$). The mean speech time was shorter for men ($M=64.71\%$, SEM=1.37%) than for women ($M=73.31\%$, SEM=1.37%). In secondary analyses the Mixture-by-Gender interaction was not significant ($p=0.09$).

Preparation time. The main effect for Mixture was significant ($F(1,118)=4.18$, $p=0.04$, $d=0.37$). The preparation time was shorter after ATD (2682 ms (SEM=292)) than after the control mixture (3309 ms (SEM=289)) (Figure 1). There were no significant effects for Group or for the Mixture-by-Group interaction (Table 2). The effect for Gender was not significant ($p=0.46$). In the secondary analyses, the Gender-by-Mixture interaction was not significant ($p=0.11$).

Pitch characteristics. For mean pitch there were no significant effects for Mixture or Group (Table 2). The Mixture-by-Group interaction was significant, but none of the post-hoc simple comparisons were (all ps >0.13). The effect for Gender was significant ($F(1,40)=291.83$, $p<0.001$, $d=1.46$). As expected, mean pitch was lower for men (M=126.55 (SEM=3.21) than for women (M=201.05 (SEM=3.20)). Secondary analyses showed no significant Mixture-by-Gender interaction ($p=0.15$).

There were no significant effects for Mixture, Group or the Mixture-by-Group interaction for variation in pitch (Table 2). There was also no significant effect for Gender. Secondary analyses showed no significant Mixture-by-Gender interaction ($p=0.77$).

Word use. There were no significant effects for Mixture, Group or the Mixture-by-Group interaction on the use of self-references, positive words, negative words or social words (Table 2). There was no significant effect of gender for any of the variables (Table 2). Secondary analyses showed no significant Mixture-by-Gender interactions (all ps >0.44).

Summary. FH+ and FH- participants took less time to start talking (as indicated by a shorter preparation time) after ATD compared to after the control mixture. Otherwise, ATD did not

Table 2. Means (SEM) for men and women and for the two FH groups on both test days and F-values for the effects of Mixture, Group and the Mixture-by-Group interaction on speech and mimicry.

	Men	Women	FH- Control	FH- ATD	FH+ Control	FH+ ATD	Effect of Gender	Effect of Mixture	Effect of Group	Effect of Mixture by Group
Pauses per second	0.30 (0.01)	0.22 (0.01)	0.27 (0.01)	0.26 (0.01)	0.25 (0.01)	0.26 (0.01)	21.19[d]	0.001	0.649	0.91
Pause duration (ms)	882 (35.5)	749 (35.6)	737.01 (41.11)	791.26 (41.99)	877.66 (41.83)	856.19 (42.07)	7.66[c]	0.284	4.543[b]	1.515
Speech time (s)	64.71 (1.37)	73.31 (1.37)	70.04 (1.50)	71.34 (1.52)	66.57 (1.50)	68.09 (1.50)	21.32[d]	2.701	3.248	0.016
Preparation time (ms)	3114.45 (348.83)	2876.86 (346.19)	3357.63 (408.38)	2402.77 (417.30)	3261.00 (408.38)	2961.25 (408.38)	0.56	4.18[b]	1.40	0.01
Mean pitch	126.55 (3.21)	201.05 (3.20)	160.65 (3.37)	165.34 (3.40)	165.26 (3.37)	163.95 (3.37)	291.83[d]	1.278	0.137	4.025[b]
Pitch Variation	4.97 (0.39)	5.36 (0.38)	5.78 (0.42)	5.48 (0.42)	4.67 (0.42)	4.71 (0.42)	0.56	0.312	3.249	0.588
Self-references[a]	4.41 (0.28)	4.04 (0.28)	4.28 (0.33)	4.10 (0.33)	4.65 (0.33)	3.90 (0.33)	0.97	3.296	0.05	1.224
Positive words[a]	1.37 (0.15)	1.25 (0.15)	1.32 (0.20)	1.23 (0.20)	1.25 (0.20)	1.45 (0.20)	0.36	0.078	0.121	0.575
Negative words[1]	0.50 (0.09)	0.66 (0.09)	0.61 (0.11)	0.58 (0.11)	0.46 (0.11)	0.67 (0.11)	1.59	0.816	0.069	1.552
Social words[a]	5.87 (0.26)	6.09 (0.26)	5.62 (0.34)	6.30 (0.34)	6.03 (0.34)	5.98 (0.34)	0.39	1.094	0.014	1.551
Mimicry task 1	0.64 (0.14)	0.51 (0.14)	0.49 (0.16)	0.78 (0.16)	0.51 (0.16)	0.52 (0.16)	0.48	1.46	0.47	1.30
Mimicry task 2	13.89 (2.86)	15.76 (2.83)	16.28 (3.94)	15.65 (3.94)	14.55 (4.17)	12.84 (4.01)	0.24	0.09	0.35	0.02

[a]percentage of total spoken words.
[b]$p<0.05$.
[c]$p<0.01$.
[d]$p<0.001$.

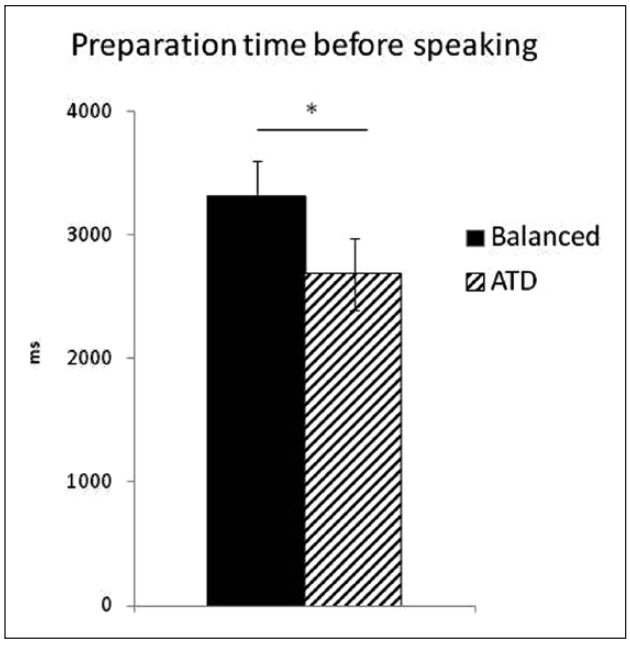

Figure 1. Preparation time (ms) before speaking for the balanced and ATD condition (values are estimated least squares means and standard errors). *p<0.05.

significantly alter speech in either FH group. Independently of whether participants were in the ATD or control condition, FH+ individuals used longer pauses when talking than FH- individuals.

Behavioural mimicry

In the first task, the mean number of face or other upper body touches was 0.58 (SEM=0.07). There were no significant effects for Mixture, Group or the Mixture-by-Group interaction (Table 2). There was no significant effect of Gender *(p=0.49).* In secondary analyses, the Mixture-by-Gender interaction was not significant (p=0.52)

In the second task, the mean percentage of time participants mimicked the experimenter's movements was 14.9% (SEM=1.36). There were no significant effects for Mixture, Group or the Mixture-by-Group interaction (*ps* >0.56). There was no significant effect for Gender *(p=0.63).* In secondary analyses, the Mixture-by-Gender interaction was not significant (F(1,77)=3.31, p=0.07).

In short, ATD had no significant effect on mimicry in either task.

Plasma tryptophan

The Mixture-by-Sample interaction was significant (F(1,118)=443.41, p<0.001). Total tryptophan levels decreased from 61.6 (SEM 2.1) μM to 7.1 (SEM 2.1) μM after ATD, and increased from 61.0 (SEM 2.1) μM to 84.1 (SEM 2.1) μM after the control mixture. The mean ATD-induced decrease in total tryptophan levels was 89% (minimum=77%). There were no significant effects for the Group-by-Sample interaction (p=0.15) or for the Mixture-by-Group-by-Sample interaction (p=0.18), indicating that the ATD-induced reduction in tryptophan levels did not differ across groups.

Mood state

Positive affect (PA). There was a significant main effect for Time (F(3,280)=11.84, p<0.001, d=0.41). PA was generally higher at t0 (M=2.66, SEM=0.10) than at t6 (M=2.51, SEM=0.10) (t(266)=2.81, p=0.03, d=0.34) and t7 (M=2.34, SEM=0.10) (t(266)=5.79, p<0.001, d=0.71), but not t5 (M=2.52, SEM=0.10) (t(266)=2.51, p=0.06, d=0.31). There were no significant effects for the Mixture-by-Time interaction (F(3,280)=0.25, p=0.86) and for the Mixture-by-Group-by-Time interaction (F(3,280)=0.17, p=0.92), indicating that levels of PA did not change differently on the ATD day compared to the control day in either FH group.

There were significant effects for Mixture (F(1,280)=8.56, p<0.01, d=0.35) and for the Mixture-by-Group interaction (F(1,77)=7.04, p<0.01, d=0.32). In FH+ participants, PA was lower throughout the ATD day (M=2.45, SEM=0.14) compared to the control day (M=2.66, SEM=0.14 (t(266)=3.85, p<0.001, d=0.47). In FH- participants, PA did not significantly differ between the ATD day (M=2.46, SEM=0.14) and the control day (M=2.47, SEM=0.14) (t(266)=0.19, p>0.99, d=0.02). Since this differential pattern in the two FH groups was independent of Time, it seems to reflect variation in PA that is unrelated to the experimental manipulation.

Negative affect (NA). There was a significant main effect for Time (F(3,280)=3.74, p=0.01, d=0.23). NA was significantly higher at t0 (M=1.11, SEM=0.02) than at t6 (M=1.05, SEM=0.02) (t(266)=3.22, p=0.008, d=0.39). There were no significant effects for the Mixture-by-Time interaction (F(3,280)=0.85, p=0.47) and for the Mixture-by-Group-by-Time interaction (F(3,280)=0.51, p=0.67).

Lastly, in response to a reviewer's suggestions, we also present the analyses for PA and NA using repeated measures ANOVA, with Time (two levels: baseline; the average of t5, t6 and t7 as 'afternoon') as the within-subjects factor and Mixture (ATD; control) and Group (FH+; FH-) as the between-subjects factor (Table 3). For PA, the effect of Time was significant – PA was higher at baseline than in the afternoon. For NA, the effect of Time was also significant – NA was higher at baseline than in the afternoon. However, similar to the analyses reported using multilevel modelling, for both PA and NA, the effects of Mixture-by-Time and Mixture-by-Group-by-Time were not significant, indicating that the effect of Time did not differ significantly according to mixture.

In sum, mood state varied somewhat over the course of the test days. However, the time pattern did not differ by mixture. In other words, ATD did not influence mood.

Discussion

Effect of ATD on speech

ATD, which temporarily reduces brain serotonin, mostly did not significantly affect speech. This is at odds with previous studies suggesting a role of serotonin in the regulation of speech. For example, treatment with a selective serotonin reuptake inhibitor

Table 3. Means (SD) for positive affect (PA) and negative affect (NA) and F-values for the effects of Mixture, Group, Time and their interactions on PA and NA.

		PA baseline	PA afternoon[a]	NA baseline	NA afternoon[a]
Mean (SD)	Men	2.79 (0.67)	2.63 (0.61)	1.11 (0.21)	1.07 (0.09)
	Women	2.54 (0.60)	2.31 (0.70)	1.11 (0.14)	1.07 (0.09)
	FH- control	2.65 (0.59)	2.46 (0.63)	1.08 (0.11)	1.05 (0.07)
	FH- ATD	2.65 (0.63)	2.44 (0.74)	1.07 (0.13)	1.05 (0.05)
	FH+ control	2.77 (0.70)	2.60 (0.66)	1.17 (0.18)	1.09 (0.12)
	FH+ ATD	2.61 (0.70)	2.38 (0.66)	1.13 (0.25)	1.09 (0.10)
F-values	**Effect**	**PA**		**NA**	
	Mixture	0.48		0.21	
	Group	0.08		5.21[b]	
	Mixture by Group	0.42		0.02	
	Time	21.1[c]		5.67[b]	
	Mixture by Time	0.22		0.58	
	Effect of Group by Time	0.00		0.82	
	Effect of Mixture by Group by Time	0.05		0.13	

[a]average of t5, t6, and t7 scores. Note: in our repeated-measures ANOVA, the relevant interaction terms for testing the effect of ATD on mood are the Mixture-by-Time interaction and the Mixture-by-Group-by-Time interaction.
[b]$p<0.05$.
[c]$p<0.001$.

(SSRI) has been shown to reduce the number of pauses in speech and pause duration, and increase variation in pitch in symptomatic MDD patients (Alpert et al., 2001, Mundt et al., 2007, 2012). Speech alterations may be concomitants of MDD and improve with reductions in depressive symptoms. Mundt and colleagues (2012) demonstrated that MDD patients who showed an antidepressant response to SSRI treatment also improved significantly in terms of their speech, while treatment non-responders did not. Participants in the present study had no depressive symptoms and mood did not change after ATD. In short, our findings suggest that an acute reduction in serotonin has few, if any, effects on speech in never-depressed individuals.

Though most speech characteristics were unaffected, unexpectedly we found that preparation time before speech was significantly shorter after ATD than after the control mixture. We speculate that this may reflect increased interpersonal impulsivity. This would be in line with studies showing increased impulsive behaviour after ATD in social settings (Cleare and Bond, 1995; Crockett et al., 2010) as well as in non-social settings (LeMarquand et al., 1999; Walderhaug et al., 2002). It has been suggested that impulsiveness associated with low serotonin may undermine social functioning (Wood et al., 2006). Moreover, impulsiveness during social interactions has been associated with increased quarrelsomeness and, in more impulsive individuals, with decreased agreeableness (aan het Rot et al., 2015). Nevertheless, the extent to which a decrease in preparation time before speech may influence social interactions remains to be determined.

No significant effects of ATD on mimicry

Previous studies in healthy, never-depressed individuals indicate that ATD can reduce cooperative behaviour (Wood et al., 2006) and increase antagonistic responses to others (Crockett et al., 2008). Conversely, administration of citalopram for two weeks increased cooperative behaviour and stimulated a dominant pattern of eye contact during speech (Tse and Bond, 2002). The findings of these studies suggest that serotonin may modulate pro-social behaviour. Though we hypothesized that this may include behavioural mimicry, in the present study ATD did not significantly affect mimicry. On the one hand, this suggests that serotonin does not play a role in the regulation of mimicking behaviour. On the other hand, a floor effect may have prevented ATD from lowering mimicry. In the first mimicry task, the number of face touches was zero or one for most of the participants, whereas in previous studies using a similar paradigm the average was around five face touches (e.g. Lakin and Chartrand, 2003). In the second mimicry task, participants mimicked the experimenter's movements about 15% of the time, while in another study using a similar procedure this was around 35% (Lakin and Chartrand, 2003). Thus, the level of mimicry observed in the present study was relatively low, which may have prevented ATD from reducing mimicry further.

Differences between the FH groups

Mean pause duration was generally longer in FH+ individuals than in FH- individuals. This is interesting, given similar findings in MDD patients compared to controls (Alpert et al., 2001; Mundt et al., 2012). Moreover, a longer pause duration has been associated with higher MDD severity, and can be normalized with SSRI treatment (Alpert et al., 2001; Mundt et al., 2012). However, it is unclear if the longer pause duration in FH+ individuals has an impact on their everyday lives. In the present study, baseline QIDS scores were low, did not differ between the FH groups and were unrelated to mean pause duration (results not shown).

Limitations in the study design

The family history method we used provides insight in inter-individual variability in familial MDD load (Andreasen et al., 1977). However, it does not provide MDD diagnoses in relatives. FH+ individuals with multiple family members diagnosed with MDD may respond more strongly to ATD than FH+ individuals with a single affected family member. We were unable to formally test this.

Mimicry was assessed in a room in the psychiatry department and in response to a person in a video, as well as in response to the experimenter who was wearing a white lab coat. Previous research by Dalton et al. (2010) and Leander et al. (2012) suggests that mimicry is inappropriate in more formal or business-like contexts. It is conceivable that the norms of the situation did not invite mimicry. Perhaps our participants did not feel close to the person in the video, or the clinical setting prevented the expression of interpersonal warmth. Mimicry effects tend to backfire or disappear altogether in non-affiliative contexts (though there is evidence of people trying to mimic high-power others; e.g. Cheng and Chartrand, 2003). Further, mimicry of foot-shaking and knee-bouncing may not have been relevant for our participants.

While the experimenter was blind to the mixtures, he was not blind to family history. Nevertheless, we think it is unlikely that this caused the longer pause duration in the speech of FH+ individuals, as other aspects of verbal behaviour did not differ between the FH groups.

Suggestions for future studies

Individuals recovered from MDD are more sensitive to the mood-lowering effect of ATD than never-depressed individuals (Young, 2013). Given that individuals with more negative mood mimic others less (van Baaren et al., 2006), future studies may assess whether ATD lowers mimicry in recovered MDD patients. This may be combined with using a mimicry procedure that more frequently elicits mimicry than the procedure used in the present study. This could be accomplished by asking study participants to interact with a spouse or friend rather than with the experimenter.

Ethical and practical reasons preclude the possibility of studying the effects of repeated ATD on verbal behaviour during everyday social interactions. In contrast, interventions aimed at increasing brain levels of serotonin can be combined with assessment of everyday social interactions (e.g. Hogenelst et al., 2015). Further, the interpersonal difficulties of depressed individuals often emerge due to repeating, everyday patterns (Hames et al., 2013), for example, in the context of intimate relationships (Baddeley et al., 2013; Segrin and Flora, 2000). For example, depressed people have been found to use more negative emotion words around romantic partners, especially in reference to the self (Baddeley et al., 2013). Future studies may assess the extent to which experimental increases in serotonin may alter speech, including in everyday life.

Conclusion

Overall, an acute lowering of brain serotonin may not have any pronounced effects on speech and behavioural mimicry in never-depressed individuals (regardless of their familial risk for MDD). Nevertheless, our study suggests that low levels of serotonin can induce impulsive responding in a social context, as indicated by a reduced preparation time before speaking. It remains possible that alterations in brain serotonin alter speech and mimicry in MDD patients.

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References

aan het Rot M, Moskowitz DS and Young SN (2015) Impulsive behaviour in interpersonal encounters: associations with quarrelsomeness and agreeableness. *Br J Psychol* 106: 152–161.

Alpert M, Pouget ER and Silva RR (2001) Reflections of depression in acoustic measures of the patient's speech. *J Affect Disorders* 66: 59–69.

Andreasen NC, Endicott J, Spitzer RL, et al. (1977) The family history method using diagnostic criteria. Reliability and validity. *Arch Gen Psychiat* 34: 1229–1235.

Baddeley JL, Pennebaker JW and Beevers CG (2013) Everyday social behavior during a major depressive episode. *Soc Psychol Personal Sci* 4: 445–452.

Benkelfat C, Ellenbogen MA, Dean P, et al. (1994) Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiat* 51: 687–697.

Boersma P and Weenink D (2010) Praat: doing phonetics by computer. Version 5.2. Available at: http://www.praat.org (accessed 29 October 2010).

Booij L, Van der Does AJ and Riedel WJ (2003) Monoamine depletion in psychiatric and healthy populations: review. *Mol Psychiat*r 8: 951–973.

Chartrand TL and Bargh JA (1999) The chameleon effect: the perception-behavior link and social interaction. *J Personality Soc Psychol* 76: 893–910.

Chartrand TL and van Baaren R (2009) *Human mimicry* (Zanna MP (ed.)). San Diego, CA: Elsevier Academic Press, pp. 219–274.

Cheng CM and Chartrand TL (2003) Self-monitoring without awareness: Using mimicry as a nonconscious affiliation strategy. *J Personality Soc Psychol* 85: 1170–1179.

Cleare AJ and Bond AJ (1995) The effect of tryptophan depletion and enhancement on subjective and behavioural aggression in normal male subjects. *Psychopharmacology* 118: 72–81.

Crockett MJ, Clark L, Lieberman MD, et al. (2010) Impulsive choice and altruistic punishment are correlated and increase in tandem with serotonin depletion. *Emotion* 10: 855–862.

Crockett MJ, Clark L, Tabibnia G, et al. (2008) Serotonin modulates behavioral reactions to unfairness. *Science* 320: 1739–1739.

Dalton AN, Chartrand TL and Finkel EJ (2010) The schema-driven chameleon: how mimicry affects executive and self-regulatory resources. *J Personality Soc Psychol* 98: 605–617.

de Jong WH, Smit R, Bakker SJ, et al. (2009) Plasma tryptophan, kynurenine and 3-hydroxykynurenine measurement using automated on-line solid-phase extraction HPLC-tandem mass spectrometry. *J Chromatogr B* 877: 603–609.

Ellenbogen MA, Young SN, Dean P, et al. (1999) Acute tryptophan depletion in healthy young women with a family history of major affective disorder. *Psychol Med* 29: 35–46.

Ellenbogen MA, Young SN, Dean P, et al. (1996) Mood response to acute tryptophan depletion in healthy volunteers: sex differences and temporal stability. *Neuropsychopharmacology* 15: 465–474.

Feder A, Skipper J, Blair JR, et al. (2011) Tryptophan depletion and emotional processing in healthy volunteers at high risk for depression. *Biol Psychiat* 69: 804–807.

Firk C and Markus CR (2008) Effects of acute tryptophan depletion on affective processing in first-degree relatives of depressive patients and controls after exposure to uncontrollable stress. *Psychopharmacology* 199: 151–160.

First MB, Spitzer RL, Gibbon M, et al. (2002) *Structured clinical interview for DSM-IV-TR Axis I disorders, Research version, Patient edition. (SCID-I/P)*. New York: Biometrics Research, New York State Psychiatric Institute.

Gehricke J and Shapiro D (2000) Reduced facial expression and social context in major depression: discrepancies between facial muscle activity and self-reported emotion. *Psych Res* 95: 157–167.

Gotlib IH and Lee CM (1989) The social functioning of depressed patients: A longitudinal assessment. *J Soc Clin Psychol* 8: 223–237.

Hames JL, Hagan CR and Joiner TE (2013) Interpersonal processes in depression. *Ann Rev Clin Psychol* 9: 355–377.

Hogenelst K, Schoevers RA and aan het Rot M (2015) The effects of tryptophan on everyday interpersonal encounters and social cognitions in individuals with a family history of depression. *Int J Neuropsychopharmacol*. Epub ahead of print 2 March 2015. DOI: 10.1093/ijnp/pyv012.

Hokanson JE, Sacco WP, Blumberg SR, et al. (1980) Interpersonal behavior of depressive individuals in a mixed-motive game. *J Abnormal Psychol* 89: 320–332.

Kazdin AE, Sherick RB, Esveldt-Dawson K, et al. (1985) Nonverbal behavior and childhood depression. *J Am Acad Child Psych* 24: 303–309.

Kiser D, Steemers B, Branchi I, et al. (2012) The reciprocal interaction between serotonin and social behaviour. *Neurosci Biobehav Rev* 36: 786–798.

Klaassen T, Riedel WJ, van Someren A, et al. (1999) Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. *Biol Psychiat* 46: 489–497.

Lakin JL and Chartrand TL (2003) Using nonconscious behavioral mimicry to create affiliation and rapport. *Psychol Sci* 14: 334–339.

Leander NP, Chartrand TL and Bargh JA (2012) You give me the chills: embodied reactions to inappropriate amounts of behavioral mimicry. *Psychol Sci* 23: 772–779.

LeMarquand DG, Benkelfat C, Pihl RO, et al. (1999) Behavioral disinhibition induced by tryptophan depletion in nonalcoholic young men with multigenerational family histories of paternal alcoholism. *Am J Psychiatry* 156: 1771–1779.

Mann JJ (2013) The serotonergic system in mood disorders and suicidal behaviour. *Phil Trans Roy Soc Lond Ser B* 368:. 20120537.

Mannie ZN, Bristow GC, Harmer CJ, et al. (2007) Impaired emotional categorisation in young people at increased familial risk of depression. *Neuropsychologia* 45: 2975–2980.

Mannie ZN, Taylor MJ, Harmer CJ, et al. (2011) Frontolimbic responses to emotional faces in young people at familial risk of depression. *J Affect Dis* 130: 127–132.

Mundt JC, Snyder PJ, Cannizzaro MS, et al. (2007) Voice acoustic measures of depression severity and treatment response collected via interactive voice response (IVR) technology. *J Neurolinguist* 20: 50–64.

Mundt JC, Vogel AP, Feltner DE, et al. (2012) Vocal acoustic biomarkers of depression severity and treatment response. *Biol Psychiat* 72: 580–587.

Nilsonne A (1988) Speech characteristics as indicators of depressive illness. *Acta Psychiatrica Scand* 77: 253–263.

Peeters FPML, Ponds RWHM and Vermeeren MTG (1996) Affectiviteit en zelfbeoordeling van depressie en angst. *Tijdschift Psychiat* 38: 240–250.

Perlis RH, Fraguas R, Fava M, et al. (2005) Prevalence and clinical correlates of irritability in major depressive disorder: a preliminary report from the Sequenced Treatment Alternatives to Relieve Depression study. *J Clin Psychiat* 66: 159–66.

Rude SS, Gortner E and Pennebaker JW (2004) Language use of depressed and depression-vulnerable college students. *Cognition Emotion* 18: 1121–1133.

Rush AJ, Trivedi MH, Ibrahim HM, et al. (2003) The 16-Item Quick Inventory of Depressive Symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression. *Biol Psychiat* 54: 573–583.

Schwartz GE, Fair P, Salt P, et al. (1976) Facial muscle patterning to affective imagery in depressed and nondepressed subjects. *Science* 192: 489–491.

Segrin C (2011) Depressive disorders and interpersonal processes (Horowitz LM and Strack S (eds)). Hoboken, NJ: John Wiley, pp. 425–448.

Segrin C (2000) Social skills deficits associated with depression. *Clin Psychol Rev* 20: 379–403.

Segrin C and Flora J (2000) Poor social skills are a vulnerability factor in the development of psychosocial problems. *Hum Comm Res* 26: 489–514.

Stassen HH, Kuny S and Hell D (1998) The speech analysis approach to determining onset of improvement under antidepressants. *Eur Neuropsychopharmacol* 8: 303–310.

Stel M, Van Baaren RB and Vonk R (2008) Effects of mimicking: Acting prosocially by being emotionally moved. *Eur J Soc Psychol* 38: 965–976.

Sullivan PF, Neale MC and Kendler KS (2000) Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiat* 157: 1552–1562.

Troisi A and Moles A (1999) Gender differences in depression: an ethological study of nonverbal behavior during interviews. *J Psychiat Res* 33: 243–250.

Tse WS and Bond AJ (2002) Serotonergic intervention affects both social dominance and affiliative behaviour. *Psychopharmacology* 161: 324–330.

van Baaren R, Fockenberg DA, Holland RW, et al. (2006) The moody chameleon: the effect of mood on non-conscious mimicry. *Soc Cogn* 24: 426–437.

van der Veen FM, Evers EA, Deutz NE, et al. (2007) Effects of acute tryptophan depletion on mood and facial emotion perception related brain activation and performance in healthy women with and without a family history of depression. *Neuropsychopharmacology* 32: 216–224.

Walderhaug E, Lunde H, Nordvik JE, et al. (2002) Lowering of serotonin by rapid tryptophan depletion increases impulsiveness in normal individuals. *Psychopharmacology* 164: 385–391.

Wexler BE, Levenson L, Warrenburg S, et al. (1994) Decreased perceptual sensitivity to emotion-evoking stimuli in depression. *Psychiat Res* 51: 127–138.

Wood RM, Rilling JK, Sanfey AG, et al. (2006) Effects of tryptophan depletion on the performance of an iterated Prisoner's Dilemma game in healthy adults. *Neuropsychopharmacology* 31: 1075–1084.

Young SN (2013) The effect of raising and lowering tryptophan levels on human mood and social behaviour. *Phil Trans Roy Soc Lond B* 368: 20110375.

Young SN, Smith SE, Pihl RO, et al. (1985) Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology* 87: 173–177.

Youngren MA and Lewinsohn PM (1980) The functional relation between depression and problematic interpersonal behaviour. *J Abnorm Psychol* 89: 333–341.

Zijlstra H, van Meerveld T, van Middendorp H, et al. (2004) Dutch version of the Linguistic Inquiry and Word Count (LIWC), a computerized text analysis programme. *Gedrag Gezond* 32: 271–281 (in Dutch).