Manipulating serotonin function in depression

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The effects of acute tryptophan depletion on heart rate variability in remitted depressed patients

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Abstract

Low heart rate variability (HRV) has been found to be associated with depressive disorder. Recent findings indicate that experimentally lowering serotonin function leads to reduced HRV and increased impulsivity in remitted depressed patients with a history of suicidal ideation (Booij et al., 2006b). Also, symptom effects of ATD correlated with low HRV at baseline. These findings suggest that low HRV may be mediated by serotonin function in a specific group of depression vulnerable patients. Fourteen remitted depressed patients received high-dose and a low-dose ATD in a randomized, double-blind, within-subjects crossover design. Mood was assessed before and after administration of the depletion drink. Five hours after administration, attentional bias was measured. HRV was measured during rest and during the attentional test. ATD led to the expected decreases in plasma tryptophan levels. High-dose ATD increased depressive and anxiety symptoms in half of the patients. High-dose ATD increased heart rate compared to baseline, but no statistically significant effects on HRV were found. At baseline, patients with low HRV had higher attentional bias scores for threat-related stimuli compared to patients with high HRV. Also, mood response to ATD was higher in patients who showed a decrease in HRV following ATD. Unfortunately, a replication of the differential effects in patients with and without a history of suicidal ideation could not be performed, due to unequal sample sizes. The direction of effects of ATD on HRV in patients with a history of suicidal ideation was the same as in a previous study. More research is needed on the role serotonin plays in HRV in depressive disorder. Especially the specific role of impulsivity and suicidal ideation should be further investigated. Our findings do suggest that low HRV may be related to mood and poor affective processing through changes in serotonin function.

Introduction

Heart rate variability (HRV) is a measure of autonomic regulation of the heart (Krantz & McCeney, 2002). HRV reflects the capacity of the autonomic nervous system to vary the intervals between consecutive heart beats (Grippo & Johnson, 2002). Reductions in HRV are related to depression (Agelink et al., 2002; Rechlin et al., 1994), however, negative results have also been found (Gehi et al., 2005). Low HRV is also associated with generalized anxiety disorder (Thayer & Lane, 2000), impulse control disorders such as ADHD (Beauchaine et al., 2001), and alcoholism (Ingjaldsson et al., 2003).

Depression has been found to be an independent risk factor for cardiovascular disease (CVD) (see for a review Rugulies, 2002). Cardiovascular disease is the leading cause of death in the United States (American Heart Association, 2006). Depression after a myocardial infarction also predicts mortality (Anda et al., 1993). Decreased HRV is a risk factor for CVD (Stein & Kleiger, 1999) and has also been associated with depression and may thus underlie the increased risk of cardiovascular disease in depression (Gorman & Sloan, 2000; Grippo & Johnson, 2002; Musselman et al., 1998).

Serotonin dysfunction is suggested to play an etiological role in both depression and cardiac dysfunction (Grippo & Johnson, 2002), and may thus underlie the association between HRV and depression. This has been supported by several studies. Kellett et al. showed changes in cardiac function following serotonin depletion in rats (2005). In a study with depressed patients, resting heart rate and systolic blood pressure decreased following 21 days of treatment with the selective serotonin reuptake inhibitor (SSRI) nefazodone, but no effects on HRV were found (Agelink et al., 2001). Glassman et al. concluded that treatment with the SSRIs paroxetine, fluoxetine and sertraline either had no effects or was beneficial for depressed patients with heart disease

(Glassman et al., 1998). SSRIs may also have a beneficial effect on cardiac function and improve HRV in patients with panic disorder (Gorman & Sloan, 2000). The evidence described above indicates that HRV is a risk factor for cardiovascular disease. Depression is linked to cardiovascular disease and is associated with lowered HRV. Serotonin is implicated in depression and may also play a role in HRV. Therefore, manipulating serotonin function (through SSRI administration or by another means) may affect HRV.

Acute tryptophan depletion (ATD) is a useful tool to investigate the effects of lowered serotonin function in humans. ATD leads to a rapid lowering of plasma tryptophan levels. ATD results in a temporary depressive 'relapse' in 50-60 % of recovered depressed patients treated with an SSRI (Van der Does, 2001a). In healthy subjects, mood effects of ATD are only found in subjects with a family history of depression (Klaassen et al., 2002). Cognitive effects of ATD have been found in both healthy subjects and in recovered depressed subjects. ATD selectively impairs learning (Park et al., 1994), memory retrieval and consolidation (Klaassen et al., 2002; Park et al., 1994; Riedel et al., 1999) in healthy volunteers and ATD improves attention in healthy samples (Schmitt et al., 2000) and patients (Booij et al., 2005a; Evers et al., 2006). Also, ATD may increase emotional Stroop interference (Booij et al., 2005a; Evers et al., 2006; Hayward, 1995) in recovered depressed patients.

Besides the link between serotonin and cognitive processing, autonomic nervous system regulation as manifested in cardiac variability may be related to both attentional regulation and affective processing, since decreased HRV is a marker for low parasympathetic activation and prefrontal hypoactivity (Thayer & Brosschot, 2005). HRV in particular has been shown to be related to attentional control and to emotional regulation, low HRV being related to poor affective processing (Thayer & Lane, 2000). Further evidence

indicates that HRV is related to cognitive flexibility (Johnsen et al., 2003). The prefrontal cortex is necessary for many tasks involving executive function. The level of resting HRV seems to be related to executive functioning. Subjects with high HRV were found to perform better on executive tasks and working memory tasks compared to subjects with low HRV (Hansen et al., 2003; Hansen et al., 2004).

Since serotonin may underlie the link between HRV and depression, and HRV may in turn be related to cognitive processing, it would be interesting to investigate the effects of lowered serotonin function on HRV and cognitive performance in depression.

Recently, the effects of ATD on HRV were investigated in a sample of remitted depressed patients (Booij et al., 2006b). Selective effects were found in remitted depressed patients with a history of suicidal ideation; high-dose ATD reduced HRV during rest, increased impulsivity (as measured by the Continuous Performance test) and increased anxiety symptoms in this subgroup of patients. Also, symptom effects of ATD correlated with low HRV at baseline (Booij et al., 2006b). The authors suggested that reduced HRV in depression may thus be limited to patients who are prone to display impulsive or aggressive behaviour.

The first objective of the current study is to replicate these findings using a different test of attention, to investigate whether the effects of ATD are mainly linked to impulsivity or to attention and anxiety. If serotonin is indeed implicated in HRV, lowering serotonin function may affect (: decrease) HRV. It is interesting to investigate whether the effects on HRV will be limited to those patients who experience a mood response following ATD or if the effect will be generalized to all patients. We evaluated the effects of ATD on mood, cardiac function and attentional bias to depressive and threatening

information in medicated remitted depressed patients with and without a history of suicidal ideation. ATD affects attentional bias, as measured with the Stroop task (Booij et al., 2005a; Evers et al., 2006; Hayward et al., 2005; Munafò et al., 2006). However, since the Stroop task is used as an experimental stressor in cardiac activity research (Renaud & Blondin, 1997), we chose another measure of attentional bias; the Dot-probe test (MacLeod et al., 1986) to investigate how mental workload may affect the influence of ATD on HRV. Since mental effort is known to lower HRV measures (Johnsen et al., 2003; Van Roon et al., 2004), the workload task may strengthen the lowering effect of ATD on HRV. We hypothesized that ATD would increase symptoms in a subgroup of patients. Furthermore, we expected low HRV to be related to the symptomatic effect of ATD and to a decreased attentional function following ATD. We hypothesized that the effects of ATD on attentional bias would be strongest in patients with a history of suicidal ideation.

Research has identified several predictors of response to ATD in remitted depressed patients, such as chronicity of depression (Booij et al., 2002) and cognitive reactivity (Booij & Van der Does, 2007). The study of Booij et al. (2006b) showed that low HRV predicted a stronger response to ATD in SSRI treated remitted depressed patients. The second aim of the current study is therefore to find support for the predictive role of low HRV in mood response to ATD.

Methods

Participants

Participants were outpatients of the Mood Disorders clinic of a psychiatric hospital. Inclusion criteria were age between 18 to 65 years; primary DSM-IV diagnosis of major depressive disorder; no longer fulfilling DSM-IV criteria for

depression; Hamilton-17 scores below 16; ongoing treatment with an SSRI or selective serotonergic and noradrenergic reuptake inhibitor (SSNRI) for at least four weeks; no psychotic disorder (lifetime); no substance abuse in the past 3 months (DSM-IV criteria); BMI above 18 kg/m^2; free of neuro-endocrine or neurological disease; no pregnancy or lactation.

Design

The study was conducted according to a double-blind, randomized crossover design.

Composition of the amino-acid mixtures

The high-dose amino-acid (AA) mixture consisted of fifteen amino acids (102.5 g) (Young et al., 1985). The low-dose mixture consisted of the same amino acids but at one quarter strength (25.7 g) (Krahn et al., 1996). The amino acids were given in drink form by mixing the amino acid powders with water to a final volume of 300 ml. To compensate for the unpleasant taste of the mixture, chocolate syrup was added and the mixtures were served chilled.

Instruments

Symptoms

The Hamilton depression rating Scale (HDRS) (Hamilton, 1967) and the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979) were administered at screening. Symptoms were assessed during the test sessions using the Comprehensive Psychopathological Rating Scale (CPRS) (Goekoop et al., 1991), an observer-rating scale that includes the MADRS and the Brief Anxiety Scale (BAS) (Tyrer et al., 1984). Because

changes in weight or sleep do not occur during the course of an ATD session, these items were omitted.

Attentional bias

The Dot-probe task measures attentional bias (MacLeod et al., 1986). Word pairs were presented one by one on a computer screen for 500 ms, one in the upper part of the screen and one below. Following the termination of that display, a dot appeared on the location of either word. Participants had to indicate at which location the dot was placed by pressing a key. The duration of the presentation of the dot on the screen was variable since the dot disappeared and a new screen appeared as soon as a response was given. Each trial started with a white fixation cross for 500 ms. Subjects were first run through six practice trials. The main experiment consisted of trials of neutral words paired with threat-related words and trials of depression-related words paired with positive words. Only median latencies for correct responses were included in the analyses to reduce the influence of possible outliers. Attentional bias was associated with faster reaction times if the dot replaces the negative word compared to trials where the dot replaces the neutral or positive word. Attentional bias is calculated as the mean reaction time for depressive (or threat related) words minus the mean reaction time for positive (or neutral) words.

Biochemical analyses

To determine total plasma tryptophan concentrations and the ratio total tryptophan/ large neutral amino acids (LNAA), venous blood was obtained (10ml) in EDTA tubes. After sampling, the blood was centrifuged for 20 minutes at $2650g_{max}$. Plasma was stored at – 65°C until quantitative amino acid

analysis by high-performance liquid chromatography took place (as described by Fekkes et al., 1995).

Cardiac activity

Heart rate and HRV were measured by the VU-AMS device (version 4.6. TD-FPP, Vrije Universiteit, Amsterdam, the Netherlands). This device has been used extensively and details of its characteristics have been published elsewhere (AMS; Klaver et al., 1994). In the present study the electrocardiogram signal was recorded using disposable pre-gelled Ag-AgCL electrodes (ConMed, New York, USA) that were placed at the jugular notch of the sternum, 4 cm under the left nipple and at the lateral right side. The device detects the R-wave of the electrocardiogram and records the time in milliseconds (with one millisecond resolution). Using this three electrode configuration inter-beat intervals were generated from which HRV was computed using software from the Leiden University Medical Center (Bootsma et al., 1994). Apart from heart rate (HR), different time domain measures of HRV were taken: the standard deviation of the inter-beat interval (SD), the coefficient of variation, CVr (SD/ inter-beat interval), the square root of the mean squared differences of successive inter-beat intervals (RMSSD) and the percentage of successive normal interval differences greater than 50 ms (PNN50). Frequency domain measures included low frequency power (LF: 0.07-0.14 Hz) which is associated with both parasympathetic and sympathetic modulation and high frequency power (HF: 0.14-0.40 Hz) which is a measure of centrally mediated cardiac vagal control (Berntson et al., 1997). HF variations are variations in HR related to the respiratory frequency (also called respiratory sinus arrhythmia: RSA). Also, the LF/HF ratio was calculated, which is thought to reflect the relative balance of sympathovagal influences on the heart (Bootsma et al., 1994). We chose to

include both time and frequency domain measures of HRV since studies on depression and HRV usually investigate both (e.g. Rechlin et al., 1994; Van der Kooy et al., 2006), although studies on the effect of workload or mental stress on HRV focus mostly on frequency measures (Hjortskov et al., 2004; Van Roon et al., 2004).

Procedure

After showing interest in taking part, all volunteers were given oral and written information about the study. Participants who seemed to meet inclusion criteria were invited to a screening session during which informed consent was obtained. During the screening session, the SCID-IV interview (First et al., 1995), HDRS and MADRS were administered. A clinical psychologist or a trained research assistant conducted the interviews. At screening the cognitive tasks were done for the first time. The day before each ATD session, participants kept a low-tryptophan diet (Booij et al., 2005a).

The scheme of the test sessions is presented in Table 1. Participants arrived at 9.00 am at the laboratory after fasting overnight. They were instructed not to use alcohol 24 hours prior to the sessions and to arrive well rested. After arrival (t-1), blood pressure was assessed; baseline mood measures were taken, followed by a blood sample. Next, participants ingested the AA mixture within half an hour (t0). Participants remained in the research room until 5.00 pm. Neutral music and magazines were available. Participants were not allowed to sleep. Water, (de)caffeinated coffee and (herb) tea were allowed in standard amounts. Three hours after ingestion of the AA mixture, participants were served a protein-poor lunch (Booij et al., 2005a; Riedel et al., 1999). During the hour preceding the cognitive and cardiac measures, patients were not allowed to smoke or to drink caffeinated coffee or tea. At around

2.15 pm, the electrodes were placed and the AMS device was connected to the computer. Also, blood-pressure was assessed. Patients performed a neutral distraction task for 5 minutes ('resting' period): they were given a Swedish text and were instructed to cross each letter *a*. It was explained that this was intended as a simple baseline task and that there were no demands concerning time or accuracy. At 2.30 pm (t4.5) the cognitive tasks were performed which lasted about 50 minutes. Thereafter, the electrodes were removed, a blood sample was obtained and the symptom interview and questionnaires were administered (t6.5). Before the participants went home, again the blood-pressure was assessed and they received a snack to speed up normalization of tryptophan levels. The procedure was repeated approximately seven days later; participants who had first received the 100% strength mixture now received the 25% strength mixture and vice versa. The morning after each session, patients returned to the lab where symptoms were assessed as well as blood pressure and a blood sample was obtained. The day after the second ATD session, cardiac activity was assessed while participants again performed the cognitive tests (post-intervention session). This study was approved by an independent medical ethics committee (METIGG, Utrecht). Participants were tested individually and were paid € 115,-.

Table 1. Scheme of the sessions

Time	Assessment	
9.00 am	Arrival, CPRS, blood pressure	(t-1)
9.50	Blood sample	
10.00	Ingestion AA mixture	(t0)
1.00 pm	Lunch	
2.30	Cardiac assessment, blood pressure & cognitive tests	(t4,5)
4.00	Blood sample	
4.30	CPRS, blood pressure	(t6,5)
5.00	End of session	

AA = amino acid; CPRS = Comprehensive Psychopathological Rating Scale

Statistical analysis:

The effects of tryptophan depletion on symptoms and Dot-probe performance were analyzed separately using General Linear Models (GLM) repeated measures analyses. Symptom ratings were analyzed with Intervention (low-dose vs. high-dose) and Time (pre-depletion vs. post-depletion vs. the next day) as within-subject factors. Dot-probe performance was analyzed with Intervention (baseline vs. low-dose vs. high-dose) as within-subject factor. Baseline cognitive performance was calculated by taking the mean score of the screening session and the post-intervention session (the day after the second depletion session). This procedure is in lieu of a baseline measure at the morning of each ATD session and corrects for any learning effects that may occur over the course of four assessments (Booij et al., 2005a). The post-intervention HRV assessment was taken as a baseline for cardiac activity. Cardiac activity measures were analyzed separately using Intervention (baseline,

low-dose, high-dose ATD) and Period (resting vs. Dot-probe) as within-subject factors. Since cardiac activity may be influenced by age, gender and smoking, the effect of these variables were checked separately. The effect of order of administration on cardiac activity and Dot-probe performance was also analyzed. In those instances in which the assumption of homogeneity of covariance in repeated measured analysis was violated, as assessed with Mauchly's Test of Sphericity, Huynh-Feldt corrected p-values were used (Field, 2005). Bonferroni corrections were used to adjust for multiple comparisons.

Results

Data screening

All data were screened for accuracy of data-entry, missing values and the assumptions of multivariate data-analysis. The MADRS and BAS data were not normally distributed; hence a logarithmic transformation was performed. PNN50, LF, HF and LF/HF ratio were $\log^{10}$ transformed because of skewed distributions. All transformations were successful.

Participants

Twenty patients were included. One patient withdrew after the screening session due to family problems. One patient dropped out after the first day (high-dose depletion) due to physical and mood complaints following that session. These complaints had disappeared the day after, however she decided to withdraw participation. One patient was excluded from analyses due to a possible lack of sinus rhythm. Data were missing for one patient at the post-intervention session following the second day, due to an emotional reaction to the high-dose depletion drink. After having contacted the patient by telephone the next morning, she reported to be less emotional but she was still very tired

and did not want to come to the lab. For two patients cardiac data were missing due to technical problems. Analyses were therefore performed on fourteen patients. Four patients vomited after high-dose ATD; one patient also vomited after low-dose ATD. One patient could only finish 75% of the drink on both days. All these cases were retained. Analyses with and without those patients did not show any differences in results. Clinical and demographical characteristics of the subjects are shown in Table 2.

Table 2. Characteristics of the sample (n = 14)

	Mean (SD)
Age	42.9 (12.0)
Males/Females	1/ 13
Number of smokers	8
Type of medication: SSRI/SSNRI	9 / 5
Number of previous episodes	5.4 (4.7)
Duration of remission (months)	15.3 (25.7)
Single/recurrent episodes	2 / 12
Partial/ full remission	5 / 9
SI+/ SI-	11 / 3
MADRS	9.1 (5.8)
HDRS	7.4 (3.8)

SSRI: selective serotonin reuptake inhibitor, SSNRI: selective serotonin noradrenalin reuptake inhibitor, SI: history of suicidal ideation, MADRS = Montgomery-Asberg Depression Rating Scale, HDRS = Hamilton Depression Rating Scale

Effects on amino-acid levels

In the low-dose condition, mean plasma tryptophan concentrations decreased by 58.3% from 43.0 ± 5.1 μmol/l to 18.1 ± 5.7 μmol/l. In the high-dose condition plasma tryptophan levels decreased by 82.2% from 41.4 ± 6.9 μmol/l to 7.3 ± 4.7 μmol/l. The plasma tryptophan/LNAA ratio decreased by

56.2% in the low-dose condition (from 10.2 ± 1.5 to 4.4 ± 1.4) and by 89.9% in the high-dose condition (from 9.9 ± 1.6 to 1.0 ± 0.9). Significant effects of Intervention ($F(1,13) = 30.8$, $p < .001$), Time ($F(2,26) = 225.9$, $p < .001$) and Time x Intervention ($F(2,26) = 34.0$, $p < .001$) were found for the tryptophan/LNAA ratio. Both interventions resulted in a significant decrease in plasma tryptophan and the tryptophan/LNAA ratio and the decrease was larger after high-dose than after low-dose ATD.

Symptoms

Depressive symptoms increased significantly in the high-dose condition from 4.1 ± 3.5 to 10.0 ± 6.8, but not in the low-dose condition (from 4.4 ± 4.1 to 3.1 ± 4.1). All scores were back to baseline the next morning (Intervention $F(1,13) = 13.9$, $p = .003$; Time $F(2,26) = 4.0$, $p = .030$; Intervention x Time $F(2,26) = 6.7$, $p = .004$). High-dose ATD induced a depressive relapse (defined as an increase in MADRS score of 6 points or more) in seven of fourteen patients. These patients will be referred to as mood-responders. Anxiety symptoms also increased following high-dose ATD but not following low-dose ATD (Time x Intervention: $F(2,26) = 8.1$, $p = .002$). Side-effects of the tryptophan depletion drinks are reported elsewhere (Merens et al., in press).

The effect of ATD on cardiac activity

Analyses with Intervention (low-dose vs. high-dose) and Time (morning (t-1) vs. beginning of the afternoon (t4,5) vs. end of the afternoon (t6,5) vs. next morning (t+24h)) on blood pressure revealed no effects of ATD on blood pressure. Only a Time effect was found for the diastolic blood pressure ($F(3,39) = 4.1$, $p = .012$) indicating a decrease in diastolic BP from morning to

the beginning of the afternoon ($F(1,13) = 14.2$, $p = .002$) during both interventions.

Analyses with Intervention and Period (rest vs. Dot –probe) revealed effects of Intervention ($F(2,26) = 4.3$, $p = .025$) and Period ($F(1,13) = 14.2$, $p = .002$) on heart rate. Heart rate increased following high-dose ATD compared to baseline ($F(1,13) = 7.9$, $p = .015$) and low-dose ($F(1,13) = 7.4$, $p = .018$). Heart rate was higher during rest than during the Dot-probe (M ± SEM: 78.2 ± 2.2 vs. 76.0 ± 2.1). No significant effect of ATD on any of the HRV measures was found.

Eleven patients reported to have suffered from suicidal ideation (SI+) in the past, while three patients did not (SI-). Due to these unequal sample sizes, analyses using suicidal ideation as a between subjects factor could not be performed. Hence, analyses on HR and HRV measures were done for the eleven SI+ patients only. Again significant effects of Intervention ($F(2,20) = 6.9$, $p = .005$) and Period ($F(1,10) = 8.6$, $p = .015$) were found on HR. HR increased following high-dose ATD compared to baseline and was higher during rest than during Dot-probe. Also an interaction effect of Intervention x Period ($F(2,20) = 6,9$, $p = .005$) was found. Post-hoc contrast tests showed that low-dose ATD decreased HR slightly during rest, but increased HR during the Dot-probe ($F(1,10) = 7.9$, $p = .018$). HR increased following high-dose compared to baseline during both periods ($F(1,10) = 6.5$, $p = .029$).

For RMSSD, also an interaction effect of Intervention x Period was found ($F(2,20) = 5.4$, $p = .013$). Post hoc tests indicated that following low-dose ATD, RMSSD decreased during the Dot-probe but increased during rest ($F(1,10) = 6.2$, $p = .032$). RMSSD and PNN50 both decreased following high-

dose ATD compared to baseline; however these changes were not significant. See Table 3 for the cardiac measures per intervention for the SI+ group.

Analyses on HR and HRV were rerun to check for possible influences of smoking, order of administration and age. The effect of gender could not be investigated since only one male was included in the sample. Regarding HR, the Intervention effect was no longer significant when age was included as a covariate, (F(2,24) = 0.1, p = .907), and the effect of Period became a trend (F(1,12) = 4.2, p = .062); however no main effect of age was present (F(1,12) = 0.7, p = 431). Order did not have an effect on HR (F(1,12) = 0.02, p = .904), neither did smoking (F(1,12) = 0.8, p = .388). Also, no effects were found of order or smoking on any of the HRV measures. When age was included as a covariate in the repeated measures of LF, a main effect of age was found (F(1,12) = 9.2, p = .010), indicating that LF was lower for older patients. For RMSSD, an effect of Period (F(1,12) = 5.8, p = .033) and an interaction effect for Period x Age (F(1,12) = 5.9, p = .032) were found when age was included as a covariate; RMSSD was lower during rest than during the Dot-probe. The same effects were found for PNN50 (Period F(1,12) = 5.1, p = .044; Period x Age F(1,12) = 5.1, p = .043); PNN50 was lower during rest than during the Dot-probe when age was included as a covariate.

Attentional bias

Attentional bias scores were calculated for depression- and threat-related stimuli. One patient appeared to be an outlier, due to extremely slow responses. Analyses were therefore performed with and without this patient. Using repeated measures with Intervention as within-subject factor, no effects of low-dose and high dose ATD on attentional bias were found (p > .10).

Order of administration (low-dose first vs. high-dose first) did not have an effect on Dot-probe performance.

Relationship between HRV and Dot-probe performance

Patients with low HRV levels during rest at baseline (median split) had higher attentional bias scores for threat-related words at baseline than patients with high HRV. The difference was significant for the ratio LF/HF (-18.1 vs. 7.4 ms; $t(12) = -3.1$, $p = .009$). Statistical trends were found for SD (-14.6 vs. 4.0 ms: $t(12) = -2.0$, $p = .075$), PNN50 (-17.7 vs. 1.6 ms; $t(12) = -1.9$, $p = .078$), and CVr (-14.6 vs. 4.0 ms: $t(12) = -2.0$, $p = .075$).

Relationship between HRV and clinical variables

The change in HRV (Δ) from baseline to high-dose ATD was calculated (for RMSSD, PNN50, SD, CVr, LF, HF, and ratio LF/HF separately). Scores were divided into groups of patients that showed either an increase or a decrease in HRV following high-dose ATD. The degrees of freedom for the different tests may vary because patients whose HRV levels remained unchanged ($\Delta = 0$) following ATD were left out of the analyses. Mood-response was higher in patients who showed a decrease in HRV following ATD. This was significant for LF ($t(12) = 2.2$, $p = .050$) and a trend for RMSSD ($t(11) = -2.1$, $p = .061$). Patients with a decrease in HRV following ATD were older compared to patients showing an increased HRV. This was significant for RMSSD ($t(11) = -2.5$, $p = .030$) and PNN50 ($t(9) = -2.3$, $p = .048$).

Table 3. Means (SD) of the cardiac measures per intervention for patients with a history of suicidal ideation (SI+) (n = 11)

	Baseline	**Low-dose**	**High-dose**	***F***	***p***
HR (beats/min)				6.9	.005**
- rest	73.9 (7.5)	71.9 (6.0)	80.1 (9.6)		
- dot-probe	71.5 (7.9)	72.8(7.4)	76.8 (8.0)		
SD IBI (ms)				1.1	.338
- rest	32.1 (8.7)	35.5 (12.9)	31.4 (7.7)		
- dot-probe	33.3 (11.5)	35.1 (11.6)	31.0 (9.3)		
CVr (%)				0.4	.647
- rest	3.9 (1.1)	4.2 (1.3)	4.2 (1.0)		
- dot-probe	3.9 (1.3)	4.3 (1.5)	4.0 (1.3)		
RMSSD (ms)				1.7	.209
- rest	18.9 (7.5)	20.7 (8.5)	15.4 (4.5)		
- dot-probe	20.4(9.6)	18.2 (8.6)	16.6 (4.7)		
PNN50				2.1	.143
- rest	2.6 (4.2)	3.6 (5.9)	0.8 (1.5)		
- dot-probe	4.0 (5.7)	2.7 (4.3)	1.1 (1.8)		
LF (ms^2)				0.2	.816
- rest	319.6 (181.3)	410.2 (388.9)	409.7 (244.6)		
- dot-probe	347.9 (181.1)	335.4 (257.1)	375.9 (254.2)		
HF (ms^2)				0.2	.829
- rest	288.7 (182.4)	417.9 (374.6)	285.4 (177.1)		
- dot-probe	316.1 (220.0)	307.3 (210.0)	241.3 (162.6)		
LF/HF ratio				0.6	.558
- rest	1.9 (2.5)	1.1 (0.9)	2.0 (2.4)		
- dot-probe	1.7 (2.1)	1.3 (0.6)	1.6 (1.2)		

The original data are reported. Analyses on the PNN50, LF, HF, LF/HF ratio were done using log transformed variables. ** $p < .01$
F = main effect of Intervention in the repeated measures analysis, df = (2,20)

Relationship between baseline HRV and mood response to ATD

A trend was found for the correlation between baseline PNN50 during rest and the change in MADRS following high-dose ATD; $r = .497$, $p = .070$. No other correlations were found between baseline HRV and mood response to ATD. Patients with relatively low (below median) HRV levels did not have a depressive response to ATD more often compared to patients with high HRV levels.

Discussion

As reported elsewhere (Merens et al., in press), the current findings confirm that high-dose ATD affects mood in remitted depressed patients, whereas low-dose does not affect mood (Booij et al., 2005a; Spillman et al., 2001). Half of the patients were mood-responders in response to high-dose ATD. The reported decreases in plasma tryptophan levels and the ratio tryptophan/LNAA following ATD are also in line with expectations. Previous research using low-dose and high-dose ATD found similar changes in tryptophan levels (Booij et al., 2005a).

High-dose ATD increased heart rate both during rest and during the Dot-probe test. This is in line with previous findings that also showed an effect of ATD on HR (Booij et al., 2006b). The finding that HR was higher during rest than during the Dot-probe test is in line with the finding that HR decreases during tests that require sustained attention (Swenne et al., 1995), although HR has been found to increase during the Stroop task (Renaud & Blondin, 1997). In that study however, the Stroop task was suggested to act as an experimental stressor.

However, although the effects of ATD on the HRV measures in the SI+ group were in the same direction as in our previous study, none of these

changes was statistically significant. Unfortunately, a replication of the differential effects in patients with and without a history of suicidal ideation was not possible due to a low number of patients without such a history.

At baseline, low HRV was related to a higher attentional bias for threat-related words. This is in line with previous research that found a higher attentional bias for threat-related words in low HRV patients with dental anxiety compared to high HRV patients (Johnsen et al., 2003). Our results also follow the findings that low HRV is related to poor affective information processing (Thayer & Lane, 2000).

In the total group of remitted depressed patients, a decreased HRV following high-dose ATD was related to a stronger mood-response compared to an increased HRV. This suggests that patients who are sensitive to changes in serotonin in the sense that they show a mood response following ATD, may also respond to ATD with a lowered HRV.

In contrast to expectations, low PNN50 at baseline was not related to an increased mood response to ATD (Booij et al., 2006b) but to a decreased mood response. This correlation however did not reach significance.

Limitations and suggestions for future research:

The current findings are limited by the small number of patients. Replication in larger samples is therefore warranted. Also, we did not select for a history of suicidal ideation when recruiting patients for this study.

We assessed HRV during rest and during a Dot-probe test. The choice of the cognitive tests was based on the possible link between serotonin function and attentional bias. Other tests that may be more specifically linked to cardiac function, such as the Stroop test or other tests of executive function, should also be investigated in future studies. A strength of the Dot-probe test

should also be mentioned. The inter-stimulus-intervals of the task were variable between and within individuals due to variable response times (a new stimulus was presented as soon as a response was given). The HF component of HRV is sensitive to task-induced signal repetition, but by using this version of the Dot-probe no task related peak in HF was formed and the HF component of HRV was not influenced (Mulder, 1992).

In addition to the effects of acute tryptophan depletion, it would also be interesting to investigate the effects of other serotonin manipulations on heart rate variability, to clarify the exact mechanisms underlying the link between serotonin and cardiac variability. Previous studies have found effects of SSRI treatment on cardiac activity in depressed patients (Agelink et al., 2001; Bär et al., 2004; Glassman et al., 1998) however not much is known about the acute effects of SSRI administration on HR and HRV in depression vulnerable subjects. Kemp et al. investigated the effects of acute SSRI administration in healthy subjects (Kemp et al., 2004). Compared to placebo, citalopram suppressed differences in heart rate associated with the viewing of pleasant and unpleasant images. Also, the electrophysiological activation to unpleasant images was attenuated following citalopram, while the activation to pleasant images was potentiated.

In summary, the present study could not establish a link between serotonin function, HRV and impulsivity. However our findings do indicate that low HRV may be related to mood and poor affective processing through changes in serotonin function.