Rain with chances of a thunderstorm : on the role of anger in depression

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Chapter 4
Acute tryptophan depletion in remitted depressed patients with and without anger regulation problems: effects on symptoms, cortisol and testosterone

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Abstract

Introduction: Symptoms of anger and aggression occur in approximately 50% of patients with major depressive disorder. Both depression and aggression–related symptoms have been associated with serotonergic alterations. The current study tested the hypothesis that remitted depressed patients with anger regulation problems during their depression (MDD+A) are more sensitive to serotonin reductions than remitted depressed patients without anger regulation problems (MDD-A), reflected in greater mood and endocrine responses to acute tryptophan depletion (ATD).

Methods: 10 MDD+A and 16 MDD-A participants received a 102.5-g (high-dose ATD) and a 25.7-g (low-dose ATD) amino acid mixture in a counterbalanced, randomized, double-blind crossover design. Mood and anger-related symptoms were measured before and after intake of the amino acid mixture as were cortisol and testosterone.

Results: High-dose but not low-dose ATD transiently increased depressive symptoms and decreased testosterone levels, 7 hours after ATD. Mood-responses to high-dose ATD were more pronounced in the MDD+A group than in MDD-A group and were associated with ATD-induced decreases in testosterone. ATD did not affect anger or cortisol levels.

Conclusion: Depressed patients with anger regulation problems may have greater serotonergic alterations than patients without anger regulation problems. The findings of the present study also suggest that depression and testosterone are associated through serotonergic mechanisms.

Introduction

Symptoms of anger and aggression are very common in patients with major depressive disorder (MDD), even though these symptoms are not part of the diagnostic criteria (Van Praag, 2001). In the early nineties, Van Praag proposed an angry/anxious subtype of depression which is primarily characterized by anxiety and aggression and a low tolerance for stress (Van Praag, 1992). Since then, a number of studies have investigated related phenotypes. For instance, Fava et al. have characterized a subtype of depression based on the presence of anger attacks (Fava and Rosenbaum, 1998, 1999). Anger attacks are defined as sudden spells of anger associated with a surge of autonomic arousal accompanied by symptoms such as tachycardia, sweating, flushing and a feeling of being out of control (Fava et al., 1990). They are present in about 40% of depressed outpatients (Fava et al., 1997, 1993, Fava and Rosenbaum, 1999). Irritability was present in about half of depressed outpatients in two independent studies (Verhoeven et al., 2011, Fava et al., 2009). Both putative subtypes (with irritability or anger attacks) are distinct from MDD without these problems on a number of clinical characteristics including greater levels of anxiety, somatization and suicidality (Fava et al., 2009, Fava et al., 1993, Verhoeven et al., 2011). The clinical relevance of irritability for development of depression was demonstrated in a longitudinal study in adolescents, which showed that irritability at age 15 was a stronger predictor of depression than of delinquency (both assessed at age 17) and that the link between irritability and depression may be largely mediated by common underlying biological mechanisms (Stringaris. et al., 2012).

Alterations in serotonin (5-HT) neurotransmission are a consistent finding in depression, as indicated by various direct and indirect markers of 5-HT function (Maes and Meltzer, 1995, Belmaker and Agam, 2008). Decreased 5-HT function is also present in MDD patients in remission (Smith et al., 1997, Neumeister et al., 2002, Yatham et al., 2012). More pronounced 5-HT alterations have been observed in currently depressed patients with anger attacks (Fava et al., 2000) compared to depressed patients without anger attacks, as indicated by neuroendocrine challenge procedures. 5-HTergic alterations were also observed in in remitted MDD patients with suicidal ideation or behavior during the previous depressive episode (Booij et al., 2002, 2006), as shown by greater symptom responses to Acute Tryptophan Depletion. Whether 5-HT alterations in MDD patients with anger problems persist beyond a depressive episode and how these alterations are associated with anger symptoms and anger-related cortisol and testosterone responses is not known.

The aim of the current study was to test the hypothesis that remitted depressed patients with anger problems during their depression have greater 5-HT impairments than remitted depressed patients without anger problems. 5-HT functioning was induced by acute tryptophan depletion (ATD), a method that transiently lowers plasma concentrations of the precursor of 5-HT, L-tryptophan (Trp). This allows the investigation of the causal effects of

lowered serotonin function in an experimental design (Booij et al., 2003, Delgado et al., 1990, Moreno et al., 2010, Young et al., 1985).

In addition to mood response, we assessed the effects of ATD on two endocrine measures: cortisol and testosterone. Cortisol has been associated with anger problems in MDD (Van Praag, 2002, 1996). Testosterone has frequently been linked to anger and aggression regulation (Archer, 2006, Mehta and Beer, 2009, Persky et al., 1971, Van Honk et al., 1999, Caramaschi et al., 2012). Moreover, both have been shown to interact intensively with the 5-HT system (Cowen, 2002, Strickland et al., 2002, Way and Taylor, 2010, Kuepper et al., 2010, Montoya et al., 2012). Hypothesizing that depressed patients with anger problems are more sensitive to 5-HT alterations than patients without anger problems, we expected that mood and endocrine responses to ATD would be greater in remitted depressed patients with anger problems than in remitted depressed patients without anger problems.

Methods

Participants

Participants were recruited via local mental health institutions and advertisements in local newspapers and university buildings. Inclusion criteria were: age between 18 and 65 years; meeting DSM-IV criteria for past depression; in remission for at least 2 months and a Hamilton Depression Rating Scale (HDRS; Hamilton, M., 1960) score < 10 or Montgomery Åsberg Depression Rating Scale (MADRS;(Montgomery, S. A. and Åsberg, M., 1979) score < 12. Exclusion criteria were: major physical illness; substance abuse and/or dependence within the last 3 months; lifetime psychosis; pregnancy, lactation.

High-dose and low-dose ATD

Participants received a 102.5-g (high-dose ATD) or a 25.7-g (low-dose ATD) amino acid (AA) mixture in a counterbalanced, randomized, double-blind crossover design, as in our previous studies (Booij et al., 2006, Merens et al., 2008). A protein-low lunch was served (Booij et al., 2005a, Riedel et al., 1999) at each test session, approximately 3 hours after the intake of the AA mixture.

Measures during the test sessions

Behavior

Symptoms were assessed with the MADRS (Montgomery and Åsberg, 1979) and the HDRS (Hamilton, 1960). Sleep items were omitted. Anger was assessed using the Anger Expression Inventory (Forgays et al., 1997, Spielberger et al., 1983).

Saliva

Saliva samples were collected using Salicaps (2.0 ml, IBL International) at 4 time-points during each testing day (before ingestion of the AA mixture, + 1.5hrs, + 4hrs, + 7hrs). Before each saliva-collection, participants cleared their mouths with water and then had to refrain from eating and drinking for 30 minutes. Saliva samples were stored at -20°C until assayed at the laboratory of biopsychology at the University of Dresden, Germany. Free cortisol and testosterone concentrations in saliva were measured using a commercially available 'Luminescence Immunoassay for the in-vitro-diagnostic quantitative determination of cortisol in human saliva and serum' and 'Luminescence Immunoassay for the in-vitro-diagnostic quantitative determination of testosterone in human saliva and serum' (IBL, Hamburg, Germany), respectively. The intra and interassay coefficients of variance for cortisol was below 8%. The intra and interassay coefficients of variance for testosterone was below 10%.

Amino acids

Venous blood was obtained (10 ml) from all participants using EDTA tubes to assess total plasma tryptophan and the ratio to its other large neutral amino acids (LNAAs) and processed and analyzed as in our previous studies (Booij et al., 2005a;, see also Fekkes et al., 1995). Since the size of the reductions of both tryptophan levels and the ratio of tryptophan to the other amino acids following adequate intake and tolerance of high and low-dose ATD have been well-documented (at 80-90% and 40-50%, respectively) and have relatively small variability (Booij et al., 2006, 2005a, Merens et al., 2008, Spillmann et al., 2001), these manipulation checks were only determined for participants who had vomited during an ATD session.

Procedure

The study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and all participants gave written informed consent before the start of the intake session.

After expressing interest in participation, all eligible participants received written information about the study by mail or e-mail. Following a brief telephone interview to verify initial in- and exclusion criteria, patients were invited for an intake session. During this session, the Structured Clinical Interview for DSM-IV (SCID-IV) (First, Spitzer, Gibbon, & Williams, 1995) was conducted to verify past and present psychiatric diagnoses. The MADRS and the HRSD were used to assess depressive symptoms occurring in the past week. Anger status was determined in a clinical interview. This clinical interview consisted of questions regarding anger-related cognitions and behaviors during the participant's depression, based on the Anger Attacks Questionnaire (AAQ) (Fava et al., 1991) and included questions like: 'Did you feel more easily angered or more irritable during your depression?' and 'Did you physically or verbally attack people?'. Participants who reported persistent or repetitive thoughts and feelings of anger during their depressive episodes and who reported at least one example of angry behavior (such as yelling, throwing things or physically attacking others) were assigned to the MDD+A group. Those who denied such cognitions and behaviors were assigned to the MDD-A group. Inter-method reliability of the group classification was confirmed by its association with self-report measures of anger completed during the intake session, including the State Trait Anger Expression Inventory (STAXI; Forgays et al., 1997, Spielberger et al., 1983) and the Buss-Perry Aggression Inventory (Buss and Perry, 1992) (see also: Table 1.). The intake-session was administered as closely to the first ATD session as possible, preferably within two weeks.

For the ATD sessions, participants arrived at the lab in the hospital at 9 AM after an overnight fast. Upon arrival, a blood sample was taken as well as a saliva sample. The MADRS was administered to measure baseline depression symptoms. Patients filled in the STAXI state questionnaire to measure their current anger symptoms. Next, participants ingested the AA-mixture within half an hour. For the next 4.5 hours (+4.5 hours) patients remained in a private research room. Saliva samples were taken 1.5, 4 and 7 hours after the intake of the AA mixture (+1.5 hours, +4 hours, +7 hours, respectively). A cognitive test battery was completed between +4.5 hours and +6 hours (data not described here). Symptoms were re-assessed using the MADRS at +6.5 hours followed by a blood sample. At the end of the session, participants received a snack to facilitate normalization of AA-levels. This procedure was repeated at least one week later; those who had received the high-dose ATD in the first session received low-dose ATD in the second session and vice versa. Each participant received €180 for participation. Both the participant and the researcher were blind for the order of the high- and low-dose ATD. The AA mixture was prepared by the hospital pharmacology department.

Statistical Analyses

Group differences in demographic and clinical characteristics were investigated by means of chi-square tests and univariate analysis of variance (ANOVA) using general linear models (GLMs).

Separate repeated-measures multivariate analyses of variances were conducted to investigate the effects of the two different doses of ATD on mood, anger and psychophysiology in the two different groups. For mood and state anger, intervention (low dose vs. high dose) and time (before ingestion vs. + 7 hours) were the within-subjects factors. Anger status in depression was used as a between-subjects factor. A similar analysis was conducted for the endocrine measures, using the values before ingestion and +7 hours (when Trp levels are expected to be at their minimum) as time factor.

More detailed analyses for the endocrine measures were performed by calculating the area under the curve with respect to the ground (AUCg) as well as the area under the curve with respect to the increase (AUCi). The AUCg and AUCi will provide an indication of hormonal secretion over the day versus the change over time throughout the day, respectively (Pruessner, J. C. et al., 2003). These AUCs were analyzed using ANOVAs to compare the AUCs with anger status as a between subjects factor. Delta-scores for psychophysiology measures and mood at +7 hours vs. before ingestion of ATD mixture (Δ cortisol and Δ testosterone) were also calculated to examine intercorrelations. Testosterone and cortisol values were log10 transformed to achieve normality of the data.

Mood response to high dose ATD was defined as the MADRS score 6 hours after high dose ATD minus the MADRS score just before high dose ATD.

Table 1

Demographic and clinical characteristics of remitted depressed patients with (MDD+A) and without (MDD-A) anger problems during depression

	MDD + A (n = 10)	MDD – A (n = 16)	p	
Age (mean±SD)	26.9±8.1	25.9±9.3	.79	
Sex (male/female)	2/8	6/10	.42	¶
Smoker (yes/no)	2/8	4/12	> .99	¶
Antidepressant use (yes/no)	5/5	4/12	.19	*
Nr. of previous episodes				
single/recurrent	2/8	4/11^	.70	¶
Suicidality (ever; yes/no)	7/3	13/3	.51	¶
MADRS intake (mean±SD)	3.4±4.3	2.8±2.7	.64	
STAXI (mean±SD)				
Trait	22.0±5.5	15.5±4.0	.002	

*Pearson's chi-square test for antidepressant use (yes/no) vs. anger in depression (yes/no)

¶(Fisher's Exact Test)

^ 1 missing value in MDD-A group

Results

Data screening

For an overview of participant enrollment see Figure 1.

Six of the 28 participants who completed the experimental procedures vomited on at least one of the testing days. We excluded one participant who had vomited during the high-dose ATD session based on the fact that she had a decrease of plasma Trp of only 42% on that day, whereas a reduction of 75%-90% is expected for high-dose and 40-50% for low-dose ATD (Krahn et al., 1996, Spillmann et al., 2001 , Booij et al., 2005a, Merens et al., 2008,). In the other participants who vomited during high-dose ATD, the decrease in Trp levels was at least 74%. In another participant, baseline depression scores (MADRS: 18) were higher than the inclusion criteria allowed (MADRS: 12), due to a procedural error. This participant completed both sessions but was left out from the analyses because ATD responses in symptomatic depressed patients are highly variable and bimodal (Booij et al., 2005c). Hence, 26 participants, 10 MDD+A and 16 MDD-A, were included in the analyses.

Demographic and clinical measures

No significant differences on demographic or clinical characteristics other than anger and aggression symptoms were found between remitted depressed patients with and without anger problems during depression (Table 1).

Mood

As hypothesized, high-dose ATD led to a greater increase in depressive symptoms than low-dose ATD [$F(1, 24) = 12.351$, $p = .002$] (see Figure 2). Furthermore, the increase in MADRS-scores during the high-dose ATD condition tended to be larger in the A+ group than in the A- group, as shown by a trend level group by intervention by time interaction [$F(1,24) = 3.53$, $p = .072$]. Specific contrast tests between the conditions showed that high-dose ATD induced a significant increase in MADRS scores [$F(1,24) = 8.50$, $p = .008$] in the MDD+A group only. Since previous studies have shown that the use of 5-HTergic antidepressant medication is predictive of mood response to ATD (Booij et al., 2002, Delgado et al., 1990, Delgado et al., 1999) and some of our patients in the A+ group were on 5-HT antidepressants (although not a significantly higher number than patients in the A- group), a hierarchical multiple regression analysis was performed to investigate the unique contribution of anger problems during depression on mood response to ATD, while controlling for the use 5-HTergic antidepressants. Current 5-HTergic antidepressant medication use was entered in the first

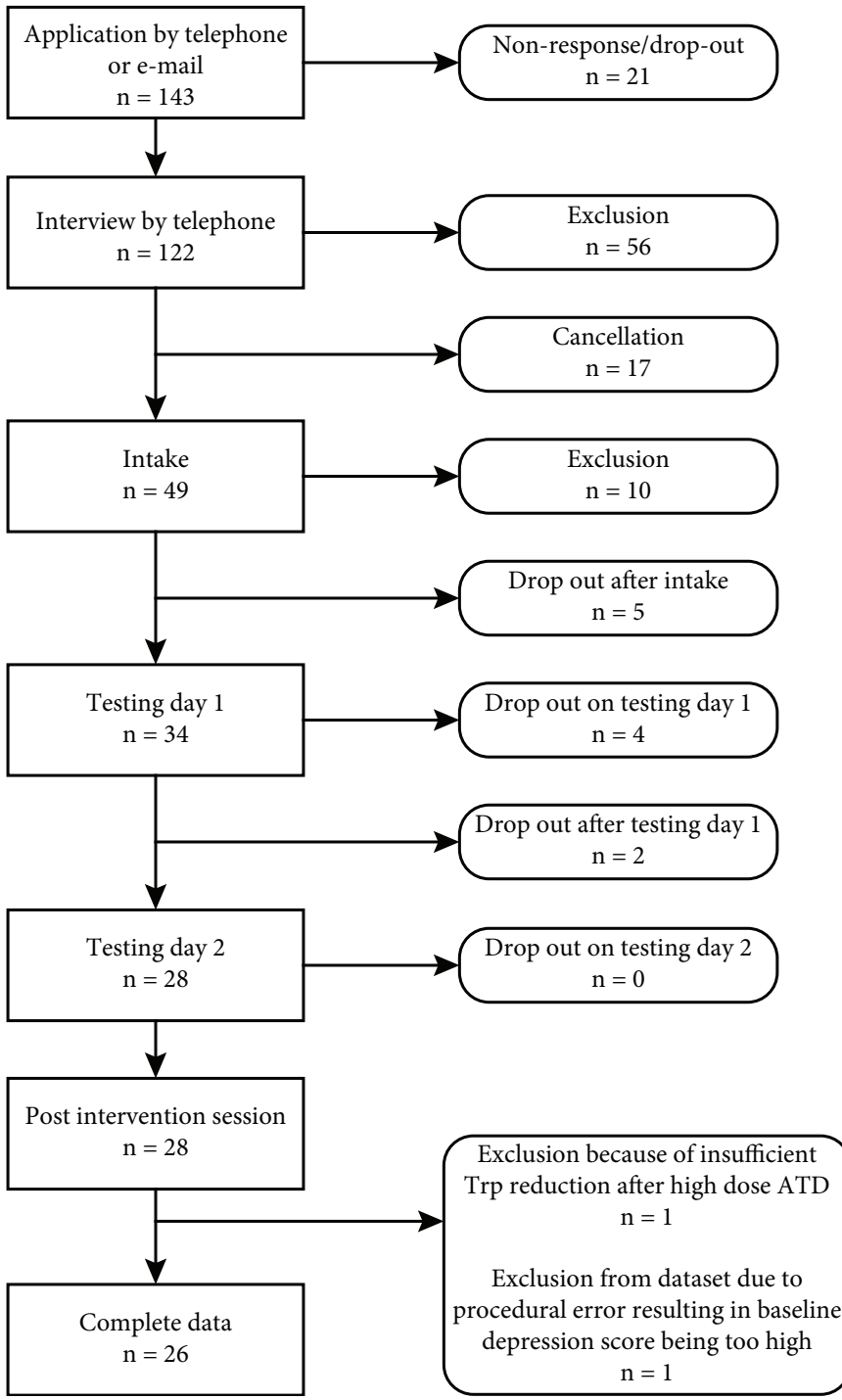

Figure 1
Flow-chart of participant enrollment with drop-out and exclusion rates per phase of the study

block, followed by 'anger problems during depression' in a second block. This analysis showed that anger during depression was associated with mood response to ATD (standardized beta = .455, t = 2.533, p = .019), also when 5-HTergic antidepressant medication was controlled for (standardized beta = .221, t = 1.232, p = .230).

Anger

No effects of intervention, time or intervention by time were found on trait and state anger (STAXI), nor were there any interactions with anger status.

Testosterone

Testosterone levels were significantly lower at +7 hours compared to baseline [$F(1,24) = 17.57$, $p < .001$]. We also found a significant intervention by time interaction. Notably, there was a greater decrease in testosterone levels in the high-dose than in the low-dose condition [$F(1,24) = 6.27$, $p = .019$] (Figure 3). No interaction with anger status was found. No effects of dose, time or dose by time were found on the AUCg or AUCi for testosterone, nor were there any interactions with anger status. The increase in depressive symptoms in the high-dose condition correlated with the decrease of testosterone in the MDD+A group (Pearsons $r = -.72$, $p = .019$). This association was not observed in the MDD-A group (Figure 4a and Figure 4b).

A negative Pearson's r value indicates an association between increases in MADRS scores and decreases in testosterone levels.

Cortisol

Cortisol levels were significantly lower at +7 hours compared to cortisol levels before ATD [$F(1, 24) = 130.98$, $p < .001$]. However, we did not find any interactions with intervention or anger status. No effects of intervention, time or intervention by time were found on the AUCg or AUCi for cortisol, nor were there any interactions with anger status.

Discussion

The current study investigated the effects of low- versus high-dose ATD on mood, testosterone and cortisol in remitted depressed patients who had or had not experienced anger problems during their depression. Consistent with previous studies, high-dose ATD increased depressive symptoms and low-dose ATD did not (Booij et al., 2005a, Merens et al., 2008). In line with our hypotheses, the increase was greater in remitted depressed patients who had

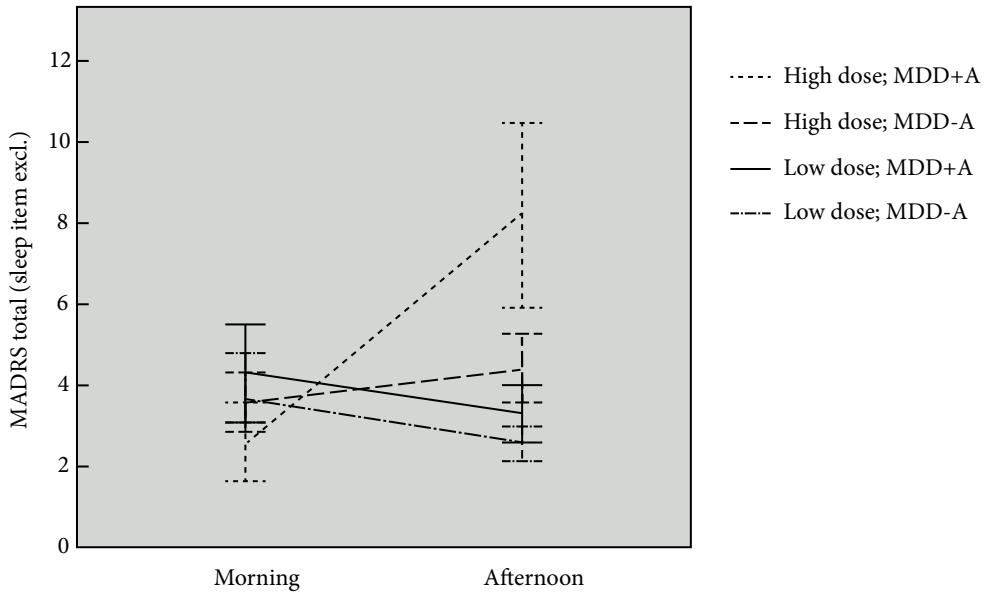

Figure 2
Mood scores before (morning) and after (+6.5h; afternoon) low- and high-dose ATD. Data represent mean-scores ± SE for both MDD+A and MDD-A.

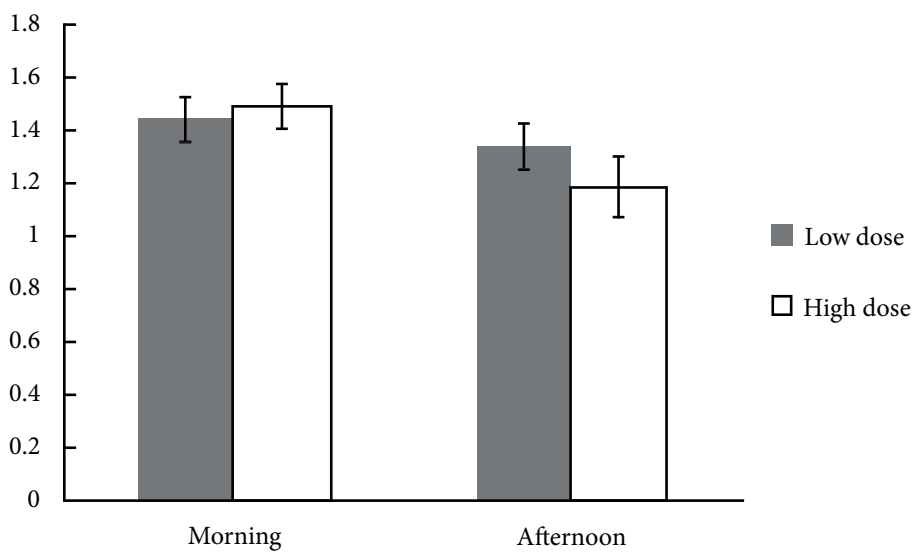

Figure 3
Testosterone levels before (morning) and after (+7h; afternoon) low- and high-dose ATD. Data represent mean-scores ± SE

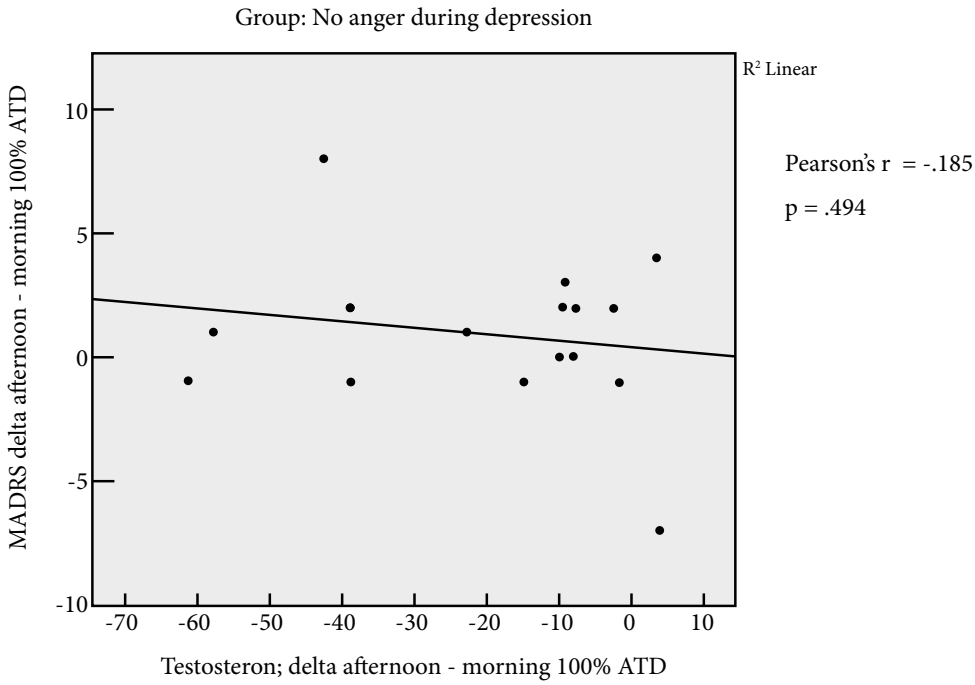

Figure 4a

The correlation between mood response and change in testosterone-levels (Δ testosterone = +7h testosterone levels – morning testosterone levels) in the MDD-A group.

A negative Pearson's r value indicates an association between increases in MADRS scores and decreases in testosterone levels

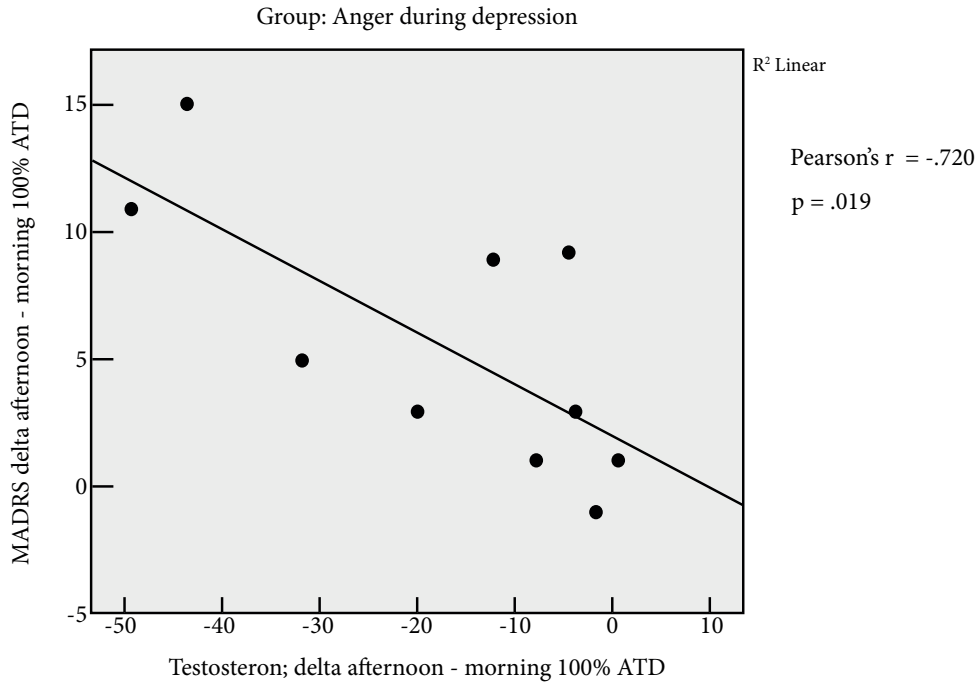

Figure 4b

The correlation between mood response and change in testosterone-levels (Δ testosterone = +7h testosterone levels – morning testosterone levels) in the MDD+A group.

A negative Pearson's r value indicates an association between increases in MADRS scores and decreases in testosterone levels

experienced anger problems (MDD+A) compared to those who had not experienced anger problems (MDD-A).

This greater mood response to ATD in patients whose prior depression had been characterized by impulse dysregulation is in line with previous studies (Booij et al., 2002, Booij et al., 2006, Leyton et al., 2000), showing that mood response to ATD was larger in individuals with strong suicidal ideation during their depression. It is reasonable to assume that the crucial distinction is impulsive behavior, but the current sample size was insufficient to make a direct comparison between patients characterized by anger vs. characterized by suicidality during their depressive episode.

With regard to the endocrine responses to ATD, high-dose ATD significantly lowered testosterone levels at the time that Trp levels were lowest, whereas low-dose ATD did not. In the MDD+A group but not in the MDD-A group, the testosterone response to ATD correlated with mood response to high-dose ATD. The finding that ATD lowers testosterone is consistent with diminished testosterone levels commonly observed in depressed patients (Zarrouf et al., 2009, Giltay et al., 2012). Administration of testosterone may have antidepressant effects in some patients such as older depressed males (Zarrouf et al., 2009). We are not aware of other studies that investigated the effects of ATD on testosterone. The findings of the present study suggest that depression and testosterone are associated through serotonergic mechanisms.

Contrary to our hypothesis, ATD did not increase anger symptoms. Moreover, ATD did not induce the endocrine responses that commonly co-occur with anger such as increased testosterone and cortisol (Harris, 1999, Van Bokhoven et al., 2005). The lack of effects on anger measures suggests that the behavioral response to ATD in remitted depressed patients is primarily expressed in the core symptoms of depression such as low mood and anhedonia (Booij et al., 2005b). These core symptoms may obscure or even counteract an anger response to ATD. In other words, although high-dose ATD affects MDD+A patients to a greater extent than MDD-A patients, anger symptoms may be associated with a different biological mechanism of depression which was not addressed by ATD. Alternatively, the questionnaires may not have been sensitive enough or the laboratory environment may have been too 'neutral' to measure the effects of ATD on anger symptomatology. For future ATD studies in MDD+A patients, it would be of interest to include a provocation task to elicit an anger-response.

The pros and cons of the ATD design using low-dose ATD as the control condition instead of the more often used amino acid mixture containing Trp as placebo, have been discussed previously (Booij et al., 2006, 2005a, Merens et al., 2008). Although the sample size was in line with our other ATD studies in remitted depressed patients, the study would have benefited from a larger sample, especially in the MDD+A group, allowing to investigate higher order interactions between ATD response, anger status and other clinical and demographic

characteristics known to predict mood response, including recurrent depression, suicidality and female sex (Booij et al., 2002). Finally, our patient sample was rather heterogeneous in terms of medication status. Although previous studies have found greater effects of ATD in remitted depressed patients using selective serotonin reuptake inhibitors (SSRI) compared to noradrenergic antidepressant medication (Delgado et al., 1990, Delgado et al., 1999), a reanalysis of several ATD studies showed that other factors such as sex and previous suicidal ideation were better predictors of mood reaction to ATD than the use of medication. This was especially the case for those patients who had already been in remission longer (Booij et al., 2002), as is the case in the current study. Moreover, the MDD+A and MDD-A group did not differ in medication status, neither did the results change when use of medication was controlled for, making it less likely that medication has confounded the results. Nevertheless, it would be of interest to redo the study in an unmedicated sample.

The current study is a first to compare 5-HT function and associated symptom and endocrine responses in patients with and without anger symptoms during their depression, using ATD. In previous studies (Verhoeven et al., 2011, Fava et al., 20010, Fava and Rosenbaum, 1998) it was shown that MDD patients with anger or irritability differ on many behavioral and clinical characteristics. In the present study, we found some indications of greater serotonergic vulnerability in patients whose depression is accompanied by anger symptomatology. Longitudinal studies are needed to further investigate the clinical relevance of our findings in terms of relapse rates and treatment response.

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