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Longitudinal hypothalamic—pituitary—adrenal axis trait and state effects in recurrent depression

Anja Lok [a,*,1], Roel J.T. Mocking [a,1], Henricus G. Ruhé [a], Ieke Visser [a], Maarten W.J. Koeter [a], Johanna Assies [a], Claudi L.H. Bockting [b], Miranda Olff [c], Aart H. Schene [a]

[a] *Program for Mood Disorders, Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*
[b] *Department of Clinical and Experimental Psychology, University of Groningen, Groningen, The Netherlands*
[c] *Center for Psychological Trauma, Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*

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Case—control studies;
Randomized controlled trial;
Cognitive therapy

Summary
Background: Hypothalamic—pituitary—adrenal (HPA)-axis hyperactivity has been observed in (recurrent) major depressive disorder (MDD), although inconsistently and mainly cross-sectional. Longitudinal studies clarifying state-trait issues are lacking. We aimed to determine whether HPA-axis (hyper)activity in recurrent MDD is: (I) reflecting a persistent trait; (II) influenced by depressive state; (III) associated with stress or previous episodes; (IV) associated with recurrence; and (V) influenced by cognitive therapy.
Methods: We included 187 remitted highly recurrent MDD-patients (mean number of previous episodes: 6.3), participating in a randomized-controlled-trial investigating the preventive effect of additional cognitive therapy on recurrence. In an add-on two-staged patient-control and prospective-cohort design, we first cross-sectionally compared patients' salivary morning and evening cortisol concentrations with 72 age- and sex-matched controls, and subsequently longitudinally followed-up the patients with repeated measures after three months and two years.
Results: Patients had higher cortisol concentrations than controls ($p < .001$), which did not change by MDD-episodes during follow-up. HPA-axis activity had no relation with daily hassles or childhood life events. Cortisol concentrations were lower in patients with more previous episodes ($p = .047$), but not associated with recurrence(s) during follow-up. Finally, randomly assigned cognitive therapy at study-entry enhanced cortisol declines over the day throughout the two-year follow-up ($p = .052$).

* Corresponding author at: Department of Psychiatry, Academic Medical Center, Meibergdreef 5, Amsterdam 1105 AZ, The Netherlands. Tel.: +31 205669111.
E-mail address: a.lok@amc.uva.nl (A. Lok).
[1] Both these authors contributed equally.

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Conclusions: Our results indicate that remitted recurrent MDD-patients have a persistent trait of increased cortisol concentrations, irrespective of stress. In combination with our finding that patients' cortisol concentrations do not change during new MDD-episodes (and thus not represent epiphenomenal or state-effects), our results support that hypercortisolemia fulfills the state-independence criterion for an endophenotype for recurrent depression.

1. Introduction

Hypothalamic–pituitary–adrenal (HPA)-axis research in major depressive disorder (MDD) has been predominantly cross-sectional in nature, comparing patients in a depressed state with healthy controls (Gold et al., 1986). Observations of hypersecretion of corticotrophin releasing hormone (Holsboer, 2000) and cortisol (Vreeburg et al., 2009a; Knorr et al., 2010), reduced feedback from glucocorticoids (Pariante and Miller, 2001), and enlarged endocrine glands (Rubin et al., 1995), resulted in a general consensus of an HPA-axis overdrive in patients with more severe forms of MDD (Stetler and Miller, 2011). However there were also conflicting data; (I) some studies observed no elevated or even lowered cortisol concentrations in MDD-patients compared with controls (Strickland et al., 2002; Ahrens et al., 2008; Carpenter et al., 2009); (II) glucocorticoid treatment and diseases with higher (e.g. Cushing's), but also lower cortisol concentrations (e.g. Addison's; posttraumatic stress disorder) are both associated with MDD (Oquendo et al., 2003; Starckmann, 2003; Wolkowitz et al., 2009); and (III) treatments with glucocorticoid receptor agonists as well as antagonists have both been proposed anti-depressogenic (Pariante, 2009).

In addition, it has been increasingly recognized that MDD is a chronic recurrent disorder. Indicatively, at least 80% of clinically recovered MDD-patients will experience a recurrence during 25-years follow-up (Bhagwagar and Cowen, 2008). With, on average, five subsequent major depressive episodes (MDEs), the recurrent nature of MDD is a severe burden to patients, families and societies (Greden, 2001; Bockting et al., 2006; Bhagwagar and Cowen, 2008). The predominant cross-sectional studies could not address the association of HPA-axis disturbances with this recurrent course of MDD. More recently, longitudinal studies investigated HPA-axis activity preceding, and subsequent to, the depressed state. For example, HPA-axis hyperactivity was also observed in remitted MDD-patients (Bhagwagar et al., 2003; Vreeburg et al., 2009a), although others found no differences or even hypoactivity (Van Den Eede et al., 2006; Ahrens et al., 2008). In addition, during transition from an acute depressive state to remission, sustained HPA-axis hyperactivity predicted recurrence during follow-up (Zobel et al., 2001; Appelhof et al., 2006). Likewise, higher cortisol concentrations in adolescents prospectively determined MDD onset during follow-up (Goodyer et al., 2000).

Taken together these findings raise the question whether abnormal HPA-axis activity in MDD-patients reflects a state only during MDEs, and/or represents a persistent trait. This question is not merely of academic importance. For example, if HPA-axis abnormalities show to be state-dependent, these abnormalities could mediate some of the MDE-symptoms and scarring effects. Proven true, treatment during a MDE directed at normalizing HPA-axis activity could reduce these symptoms and prevent scarring. On the other hand, if HPA-axis abnormalities show to be a trait, they could be involved in the pathogenesis of a new or recurrent MDE. If so, preventive treatment directed at normalizing HPA-axis activity could be indicated (Pariante, 2009). For example, cognitive therapy could be a promising candidate as it was shown to protect against recurrences (Bockting et al., 2005) and to normalize HPA-axis activity (Hsiao et al., 2011).

Besides these clinical aspects of the state-trait discussion, it also poses pathogenetic issues. If HPA-axis abnormalities show to be state-dependent, they might be the consequence of epiphenomenal effects of depressive symptoms or accompanying daily hassles. On the contrary, if HPA-axis abnormalities show to be a trait, they might be the consequence of traumatizing childhood life-events (CLEs) (Heim et al., 2001), scarring-effects of previous MDEs (Kendler et al., 2000) and/or perinatal programming (Matthews, 2002), but could also be genetically regulated (endophenotype) (Hasler et al., 2004). This latter hypothesis is strengthened by previous research showing evidence that fulfilled the following endophenotype criteria: familial association (Mannie et al., 2007), cosegregation (Holsboer et al., 1995), and heritability (Bartels et al., 2003). However, the endophenotype state-independence criterion, i.e. "manifests in an individual whether or not illness is active" (Gottesman and Gould, 2003), has, to our knowledge, not yet been addressed for HPA-axis activity in MDD.

To further clarify these state-trait issues, we performed a longitudinal study to assess HPA-axis activity over time in highly recurrent MDD-patients. At study entry all patients were in remission, and compared with a matched control group. Subsequently, the patients were followed-up prospectively at three months and two years, while MDD-recurrence and HPA-axis activity were monitored.

We hypothesized that in patients with recurrent MDD HPA-axis hyperactivity: (I) reflects a trait, i.e. remitted patients exhibit higher cortisol concentrations compared with controls; (II) is additionally influenced by depressive state (i.e. more outspoken HPA-axis abnormalities during a recurrent MDE at follow-up); (III) is associated with (a) current daily hassles, (b) CLEs, and (c) number of previous MDEs. Furthermore, we hypothesized that: (IV) cortisol concentrations are higher in patients who experience recurrence(s), compared with patients who remain in remission during the entire follow-up period; and finally that (V) preventive cognitive therapy normalizes heightened HPA-axis activity.

2. Methods and materials

2.1. Design

The patient sample used in this study was recruited at psychiatric centers and through media announcements to

participate in a randomized controlled trial assessing recurrence-preventing effects of cognitive therapy (CT) in recurrent MDD (Bockting et al., 2005). We used an add-on two-staged case—control and prospective-cohort design. First, we cross-sectionally compared patients with controls at study-entry (T0) in the case—control stage. Subsequently we longitudinally followed-up the patients with repeated measures at three months (T1; after the CT-intervention period) and two years (T2) in the prospective-cohort stage, to assess short- and long-term (I) stability of patients' characteristics and (II) effects of CT.

We allocated eligible patients to treatment as usual or to an additional preventive CT-module (Bockting et al., 2005). This module consisted of eight weekly group sessions, focusing on dysfunctional attitude identification and change. Treatment as usual involved 'naturalistic' care, ranging from continuous antidepressant use to no treatment at all. The study was approved by the ethics committee of the Academic Medical Center of the University of Amsterdam. All subjects provided written informed consent.

2.2. Study sample

2.2.1. Patients

We included remitted MDD-patients (18—65 years), who had experienced $\geq$2 MDEs in the last five years, according to the DSM-IV and assessed by trained evaluators using the Structured Clinical Interview for *DSM-IV* disorders (SCID) (First et al., 1996). Patients had to have reached remission status $>$10 weeks and $<$2 years ago. We defined remission according to DSM-IV criteria and a score $\leq$9 on the 17-item Hamilton Depression Rating Scale ($HDRS_{17}$) (Hamilton, 1960). We excluded subjects with: (a history of) bipolar spectrum disorder; (a history of) any psychotic disorder; organic brain damage; alcohol and/or drug abuse and/or dependency; or predominant anxiety disorder, all assessed using the SCID. Furthermore, current steroid use was also an exclusion criterion.

2.2.2. Controls

We recruited age- and sex-matched controls by advertisements in a diversity of newspapers and magazines. Controls had to have no current or past (personal and/or family) history of psychiatric axis-I disorders according to the DSM-IV (assessed with the SCID). Furthermore, current steroid use was an exclusion criterion for the controls as well.

2.3. Study measurements

2.3.1. Depression characteristics and covariates

For the case—control stage of our study, at T0, we determined educational level (low, middle, and high), anthropometric measures (body mass index, waist and hip circumference), smoking behavior and medication use (including contraceptives) for both patients and controls.

During the prospective-cohort stage involving the patients, we assessed the number of previous MDEs at T0. As described previously (Bockting et al., 2005), the range of previous MDEs was 2—70 (median = 4, interquartile range = 3), and not normally distributed, which could not be resolved by transformation. To test the effects of previous MDEs on HPA-axis activity, we therefore dichotomized the variable previous MDEs. We chose a cut-off point that created the most equally numbered groups of patients, to maximize conceivable power and/or contrast. The optimal cut-off point was the median (4), with 59.4% of the patients having $<$5 pervious MDEs and 40.6% having $\geq$5 MDEs. In addition, we assessed CLEs before the age of 16 with the 15-item Negative Life Events Questionnaire (which we dichotomized; experienced CLEs yes/no) (Kraaij and de Wilde, 2001). Events may involve the participant or significant others. This questionnaire proved to have a good predictive validity, as the number of negative life events predicted MDD-symptom severity (Kraaij et al., 2003). We measured daily hassles at T0, with the 114-item Everyday Problem Checklist, providing a continuous score (Vingerhoets and van Tilburg, 1994). Finally we assessed MDD-symptoms, in addition to the SCID, with the Beck Depression Inventory (BDI), at T0 (Beck et al., 1979).

During follow-up, we repeated the assessments of daily hassles and MDD-symptoms at T1 and T2, with the Everyday Problem Checklist, BDI and SCID. With these follow-up assessments of the SCID we diagnosed relapses ($<$6 months after a previous MDE) or recurrences during follow-up, both further addressed as 'recurrence' for clarity reasons. Furthermore, during the whole follow-up, from T0 to T2, we monitored antidepressant medication by using the Trimbos/IMTA Self Report Questionnaire for Costs Associated With Psychiatric Illness, every three months (Hakkaart-van Roijen et al., 2002). To make data manageable for analyses, we operationalized antidepressant use as continuous antidepressant use during follow-up (yes/no). The non-continuous group both included patients who took antidepressants intermittently and patients that did not take antidepressants at all (Bockting et al., 2008).

2.3.2. Hormone measures

For the case—control stage of the study, patients and controls collected saliva with neutral cotton salivettes (Sarstedt AG and Co, Nümbrecht, Germany) at home at three sampling moments on two consecutive days (day one: 0800 h and 2200 h; day two: 0800 h). For the prospective-cohort stage, we repeated the T0 measures with follow-up measures at T1 and T2 in patients only. Saliva reliably reflects blood cortisol concentrations, in a relatively stress-free and minimally intrusive way (Kirschbaum and Hellhammer, 1994). We instructed subjects to rinse their mouth with water and not to brush their teeth before sampling. Subjects collected morning samples after an overnight fast, and kept the samples in the refrigerator until they sent them back by mail on day two. We stored samples at $-20\,^{\circ}C$ until analysis by radioimmunoassay (IBL Hamburg; designed for saliva samples). Intra- and interassay variations were 5.1% and 6.5%, respectively.

2.4. Statistical analysis

2.4.1. Data cleaning, imputation

We assigned cortisol concentrations that exceeded four standard deviations from the mean as missing, because this suggests blood contamination. To reduce bias potentially introduced by missing values, we used a multiple imputation technique using the package Amelia II (Honaker et al., 2010).

Multiple imputation is considered the state-of-the-art way to handle missing values, and results in correctly estimated standard errors and confidence intervals (Donders et al., 2006). We used multiple imputation separately for the cross-sectional comparison between patients and controls and for the longitudinal analysis that only applied to the patients. Imputation resulted in five imputed datasets for the cross-sectional case–control analyses (imputation one) and five imputed data sets for the longitudinal analyses of the patient-cohort (imputation two). After imputation for T0, T1 and T2, we calculated the mean of the two morning cortisol concentrations (day one and two), since variability between morning measures on the two consecutive days was equal for patients and controls. All cortisol values showed normal distributions after log transformations, which we used in all analyses.

2.4.2. Subject characteristics and propensity scores

We compared patients' and controls' baseline characteristics using χ^2 and Student's t-test statistics. In further analyses we adjusted for confounders using propensity scores, representing the predicted probability for a case to belong to a certain group (e.g. patient or control), calculated in a binary logistic model with the chosen confounders as predictors (Rosenbaum and Rubin, 1983). This way, we could correct for multiple confounders in one score at the same time without substantial loss of power. We calculated a propensity score for comparisons between patients and controls that corrects for common confounders (Vreeburg et al., 2009b): sex, age, educational level, contraceptive use, steroid use in the month before assessment (Hakkaart-van Roijen et al., 2002), smoking, weight and waist and hip circumference (PS_1). We created PS_2 to adjust the effect estimates in the longitudinal analyses, which, in addition to the confounders in PS_1, also corrects for the potential confounders: follow-up alcohol and drug use (yes/no), benzodiazepine therapy (yes/no), receiving CT treatment (yes/no) and continuous antidepressant use (yes/no).

2.4.3. Models to distinguish trait and state-effects

To assess whether HPA-axis disturbances are a trait in MDD, we used linear mixed models (Gueorguieva and Krystal, 2004), with cortisol as the dependent variable and sampling moment (morning/evening), group (patient/control) and the moment × group-interaction as independent variables. We used linear mixed models to incorporate correlations between repeated measurements in the same subject, thereby boosting power, and to achieve flexibility to model time effects (Gueorguieva and Krystal, 2004). In case the moment × group-interaction was non-significant, we removed this term and used the remaining more parsimonious model. We adjusted for confounders by adding PS_1 to the final model.

To assess the effect of depressive state on cortisol, we modeled depressive state as a time-dependent covariate. We tested a linear mixed model using the longitudinal repeated measures patients' data with cortisol (T0, T1 and T2) as the dependent variable, and follow-up time (T0, T1 and T2), sampling moment (morning/evening), follow-up × moment-interaction, depressive state (indicated by the SCID at T0, T1 and T2) and the state × moment-interaction as independent variables.

2.4.4. Additional analyses

To assess the association of CLEs (yes/no), number of previous MDEs (≥5 previous MDEs yes/no), and the occurrence of recurrence during follow-up (yes/no), with HPA-axis activity, we one by one included these factors in subsequent models with cortisol as dependent variable, and follow-up time (T0, T1 and T2), sampling moment (morning/evening), follow-up × moment-interaction, "factor" (yes/no), "factor" × moment-interaction, and "factor" × follow-up-interaction as independent variables. When a higher order interaction did not contributed significantly to the model, we removed this term and used the resulting more parsimonious model. We determined the effect of daily hassles on HPA-axis activity with a comparable model as the one that was used for the state-effect, where the continuous Everyday Problem Checklist score replaced the state-factor as a time-dependent variable. We adjusted for confounders by incorporating PS_2 to the model.

To determine the effects of CT on HPA-axis activity, we first tested whether the two randomized groups (CT yes/no) were comparable on T0 (before CT). We then assessed the CT-effect on cortisol at T1 and T2 (after CT).

We used PASW statistics 18.0 (SPSS, Inc., 2009, Chicago, IL). For multivariate estimates we combined separate significance tests for the five imputed datasets into one pooled test with a SPSS macro from van Ginkel (2006). We considered $p < .05$ statistically significant.

3. Results

3.1. Subject inclusion, hormone data and characteristics (Table 1)

During the inclusion procedure, 1000 subjects (31% recruited at psychiatric centers, and 69% through media announcements) completed telephonic screening, and 321 were invited for diagnostic interviews. Eventually, 187 patients and 72 controls were eligible to participate. This recruitment led to 1683 conceivable cortisol measures for patients and 216 for controls. Of the 187 included patients, 15 dropped out of the study's CT treatment immediately, but we were able to collect HPA-axis data and so they were included in all analyses. Drop-outs were younger than completers, but did not differ on other characteristics ($p > .05$). For the 172 remaining patients, 10.7%, 21.7% and 42.6% measures were missing at T0, T1 and T2 respectively. For the 216 conceivable values of the 72 controls, 10.6% of the measures were missing. Of the 1361 complete measures, seven values were assigned missing because of suggestive blood contamination.

Patients and controls were successfully matched regarding age and sex. The included patient group was characterized by high recurrence rates; the mean number of previous MDEs was 6.3, and 54.5% had a recurrence during the two-year follow-up.

3.2. HPA-axis disturbance as a trait (Fig. 1)

Remitted patients had significantly higher cortisol concentrations than controls (group-effect; $p < .001$; adjusted for potential confounders [using PS_1]). The course over the day was not significantly different between patients and controls and therefore omitted from the model (group × moment-interaction; $p = .376$).

Table 1 Subject characteristics.

Characteristic	Patients (n = 187)	Controls (n = 72)	p Value
Female, %	68.1	72.7	.46
Age, mean (SD), year	44.2 (9.7)	44.9 (9.3)	.61
Educational level[a]			<.001
Low, %	33.2	4.6	
Middle, %	32.6	19.7	
High, %	34.2	72.3	
Smoking, %	29.9	22.9	.28
Weight, mean (SD), kg	78.9 (16.3)	73.8 (13.4)	.04
Waist circumference, mean (SD), cm	89.3 (13.9)	83.7 (12.3)	.01
Hip circumference, mean (SD), cm	105.3 (11.1)	103.1 (7.8)	.13
Oral contraceptive use, %	22.1	17.1	.40
Steroid use[b], %	.6	1.4	.57
Benzodiazepine use, %	8.0	NA	
Continuous AD use during follow-up, %	27.3	NA	
Antidepressant use at study entry, %	42.2	NA	
TCA, %	3.9	NA	
SSRI, %	29.2	NA	
Other, %	9.1	NA	
Received cognitive therapy, %	51.9	NA	
$HDRS_{17}$ score, mean (SD)	3.8 (2.9)	NA	
Number of previous episodes, mean (SD)	6.3 (8.1)	NA	
Five or more previous episodes, %	40.6	NA	
Age of onset first episode, mean (SD), year	28.5 (12.5)	NA	
Relapse during the 2 year follow-up period, %	54.5	NA	
Depressed at T1, %	15.0	NA	
Depressed at T2, %	16.0	NA	
Negative early life events, %	50.3	NA	
Daily hassles score T0, mean (SD)	52.5 (38.9)	NA	
Daily hassles score T1, mean (SD)	41.3 (30.2)	NA	
Daily hassles score T2, mean (SD)	42.2 (35.1)	NA	

Abbreviations—AD: antidepressant; HDRS: Hamilton depression rating scale; SSRI: selective serotonin reuptake inhibitor; T0, T1, T2: study-entry 3 months and 2 years of follow-up respectively; TCA: tricyclic antidepressant.

[a] Educational level is defined as: *low* primary education or preparatory middle-level applied education; *middle* higher general continued education or middle-level applied education; and *high* preparatory scientific education higher applied education or scientific education.

[b] Steroid use in the month before assessment.

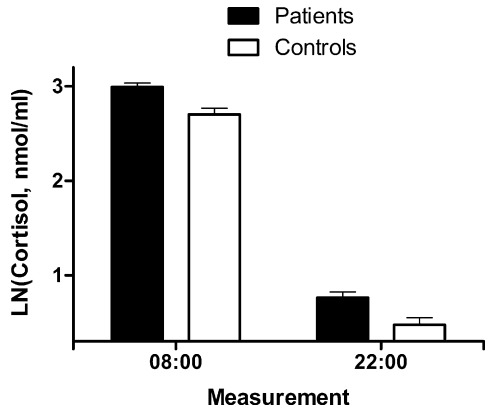

Figure 1 Morning and evening cortisol concentrations for remitted recurrent MDD-patients compared to controls. All results are adjusted for sex, age, educational level, contraceptive and steroid use, smoking, weight and waist and hip circumference. Error bars indicate SE. Mixed model analyses results: remitted patients versus controls $F_{1,656.13} = 14.77$, $p < .001$.

3.3. HPA-axis disturbance as a state (Fig. 2)

The within-subject time-dependent variable modeling depressive state (current MDE during follow-up measurement) had no significant influence on cortisol concentrations (state-effect; $p = .419$), after omission of the non-significant effect on course over the day (state × moment-interaction; $p = .833$).

In addition, the continuous BDI-score was used to assess state-effects as well. This approach also did not reveal any significant state-effects ($p = .467$). Correction for possible confounding by antidepressant use did not change these findings.

3.4. Influence of daily hassles, CLEs and previous episodes (Fig. 3)

The interaction of daily hassle score on a given time point during follow-up (T0, T1 and T2) with moment, was not significant and therefore omitted (hassles × moment-interaction; $p = .249$). This indicates there were no associations

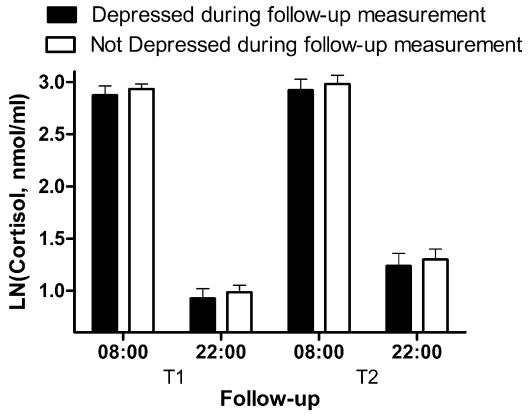

Figure 2 Effect of a current depressive episode at sampling moment after three months (T1) and two years of follow-up (T2), on cortisol concentrations in recurrently depressed patients. Error bars indicate SE. Mixed model analyses results: being depressed according to the SCID at sampling moment (yes/no) $F_{1,65.54} = .66$, $p = .419$. Measures at study entry (T0) were included in the analyses, but because none of the subjects was depressed at that moment (exclusion criterion) T0 is not included in this figure.

between daily hassles and cortisol-course over the day. In the subsequent most parsimonious model there were no associations between overall cortisol concentrations and daily hassle score (hassles-effect; $p = .744$).

For CLEs, interactions with follow-up and moment were not significant, and therefore omitted from the model. In the subsequent most parsimonious model, the main-effect of CLEs also was non-significant ($p = .254$).

Regarding the number of previous episodes, there were no significant differences in cortisol-course over the day (MDEs × moment-interaction; $p = .818$) or follow-up (MDEs × follow-up-interaction; $p = .510$) between patients with <5 previous MDEs compared to patients with ≥5 previous MDEs, so these interactions were omitted from the model. In the most parsimonious model the main-effect of previous MDEs was significant (MDEs; $p = .047$), with lower cortisol concentrations in the patients with ≥5 previous MDEs, compared to the patients with <5 MDEs.

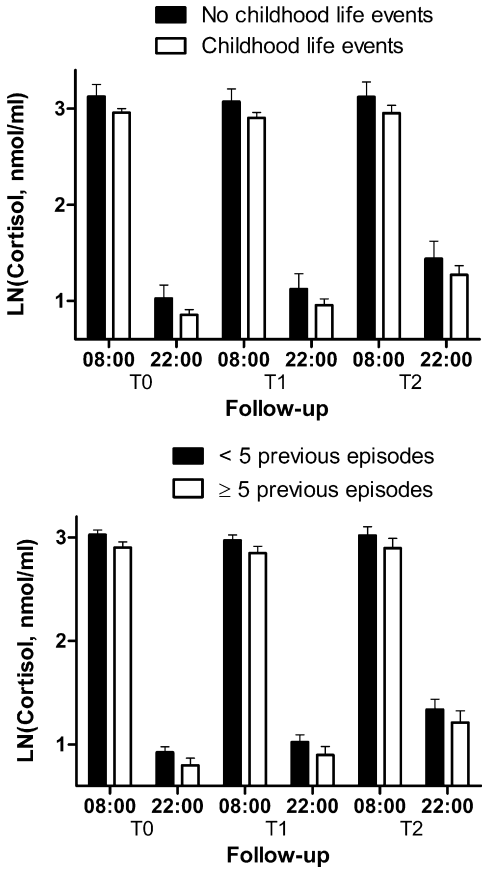

Figure 3 Cortisol concentrations in relation to the experience of childhood life events and the number of previous episodes (<5/≥5) in recurrently depressed patients during a two-year follow-up (T0, T1 and T2). All results are adjusted for sex, age, educational level, contraceptive and steroid use, smoking, weight and waist and hip circumference, alcohol and drug use (yes/no), benzodiazepine therapy (yes/no), receiving CT treatment (yes/no) and using continuous antidepressants (yes/no). Mixed model analyses results for the effect of CLEs: CLEs (yes/no) $F_{1,12.14} = 1.435$, $p = .254$. Mixed model analyses results for previous episodes: ≥5 previous episodes (yes/no) $F_{1,46.15} = 4.152$, $p = .047$.

3.5. Association with recurrence (Fig. 4)

There were no differences in cortisol-courses over the day or follow-up between patients who experienced a recurrence compared with those who remained in remission (recurrence × moment-interaction; $p = .707$, recurrence × follow-up-interaction; $p = .957$). In the final, most parsimonious, model the main effect of recurrence was also non-significant (recurrence during follow-up-effect; $p = .513$).

3.6. Effect of cognitive therapy (Fig. 5)

At T0, before CT, cortisol-course over the day was comparable between patients that were randomly assigned to receive CT and patients that were assigned to not receive CT (CT × moment-interaction; $p = .216$), and therefore omitted. The main effect of CT was also non-significant (CT-group-effect $p = .430$), indicating comparable cortisol concentrations in the two randomized groups before randomization to CT.

During follow-up after CT (T1 and T2), when comparing the patients who received CT in the first eight weeks of the study with patients who did not, the 3-way-interaction between follow-up time (T1, T2), sampling moment and CT was nonsignificant (CT × follow-up × moment-interaction; $p = .960$), so this term was omitted. Thereafter, the interaction of CT with follow-up was also nonsignificant (CT × follow-up-interaction; $p = .514$) and omitted. In the subsequent model, the interaction of CT with sampling moment reached borderline significance (CT × moment-interaction; $p = .052$), indicating steeper cortisol declines over the day in patients who received CT.

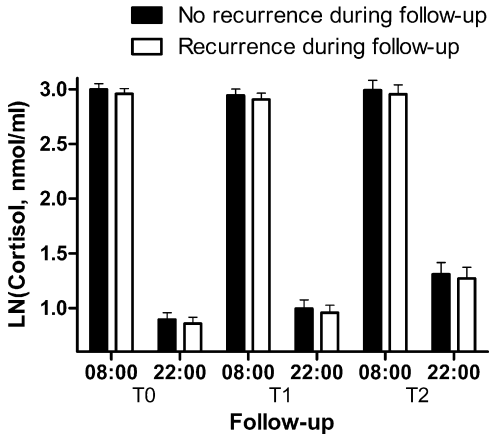

Figure 4 Cortisol concentrations in recurrently depressed patients who remained in remission during a two-year follow-up (T0, T1 and T2) compared to those who did experience at least one recurrence. All results are adjusted for sex, age, educational level, contraceptive and steroid use, smoking, weight and waist and hip circumference, alcohol and drug use (yes/no), benzodiazepine therapy (yes/no), receiving CT treatment (yes/no) and using continuous antidepressants (yes/no). Error bars indicate SE. Mixed model analyses results: experiencing a recurrence during follow-up (yes/no) $F_{1,135.90} = .43$, $p = .513$.

4. Discussion

To our knowledge, this is the first HPA-axis study in remitted patients with recurrent MDD which applied a longitudinal repeated-measures design. With this design, we showed that high cortisol represents a trait in recurrent MDD, while there is no apparent state-effect.

First, we found significantly higher cortisol concentrations in patients compared to controls, suggestive of HPA-axis hyperactivity as a trait in recurrent MDD. *Second*, our data did not show state-effects on HPA-axis activity, because cortisol did not change during MDEs during follow-up. *Third*, HPA-axis hyperactivity was neither associated with (a) daily hassles (epiphenomenal effects), (b) CLEs (early programming), nor (c) number of previous MDEs (i.e. scarring, which on the contrary, was associated with lower cortisol concentrations). *Fourth*, in patients, the hypercortisolemic trait was, unexpectedly, not associated with recurrence during entire follow-up. *Finally*, CT caused long-lasting steeper cortisol declines over the day during the two-year follow-up (borderline significant).

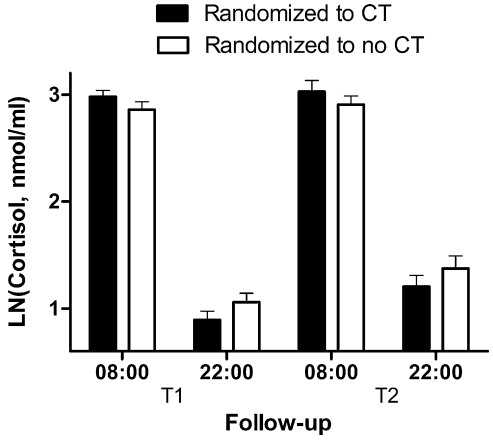

Figure 5 Effect of a randomized 8-week cognitive therapy (CT) module at study entry (T0), on cortisol concentrations after three months (T1) and two years (T2) of follow-up, in recurrently depressed patients. Error bars indicate SE. Mixed model analyses results for CT: main effect of CT (yes/no) $F_{1,68.07} = 3.07$, $p = .085$, and CT × sampling moment (morning/evening) interaction $F_{1,18.85} = 4.30$, $p = .052$.

4.1. Trait of increased cortisol

The present study corroborates with previous studies which reported higher cortisol concentrations in remitted MDD-patients (Bhagwagar et al., 2003; Vreeburg et al., 2009a), thereby not supportive of earlier reports of HPA-axis hypoactivity (Ahrens et al., 2008). As distinct from previous cross-sectional studies comparing HPA-axis activity in heterogeneous samples (recurrent and first episode MDD-patients combined) (Bhagwagar et al., 2003; Van Den Eede et al., 2006; Ahrens et al., 2008), our data longitudinally describe the course of the cortisol-abnormalities specifically in highly recurrent MDD-patients. Previous more heterogeneous samples might possibly have underestimated HPA-axis abnormalities (Vreeburg et al., 2009a). Our finding of persistently elevated cortisol concentrations suggests a permanent hypercortisolemic trait in this subpopulation of recurrent MDD-patients. This is further supported by studies reporting sustained higher cortisol concentrations preceding a first MDE (Modell et al., 1998; Goodyer et al., 2000; Mannie et al., 2007).

4.2. Absence of state-effects

The absence of state effects in our two-year follow-up comparing different levels of depressive symptoms within subjects, extends and strengthens previous cross-sectional research which reported no differences in cortisol between currently depressed patients and remitted MDD-patients (Vreeburg et al., 2009a). The absence of state-effects may appear in contrast with previous treatment-studies, which found changes in HPA-axis activity after acute MDE treatment with antidepressants (Schule et al., 2009). Although suggestive of state-dependent changes, this influence of antidepressants could also be explained by an effect of antidepressants *per se* rather than a depressive state-effect (Manthey et al., 2011). This is suggested by a study reporting declines in salivary cortisol concentrations during recovery from the depressive state only in responders to amitriptyline, and not in paroxetine responders (Deuschle et al., 2003). In addition, because our study is the first report of longitudinal HPA-axis activity in recurrent MDD, we hope our finding of state-independency encourages further replication.

4.3. The absence of HPA-axis activating effects of daily hassles, childhood life events and previous episodes

Our data showed no association between the daily hassle scores and cortisol concentrations, rejecting the idea that

increased current stress (e.g. during a MDE) has an epiphenomenal effect on the HPA-axis in recurrent MDD. Also, CLEs did not explain the observed hypercortisolemic trait, in line with previous literature (Heim et al., 2001). However, unexpectedly, having ≥5 previous MDEs was associated with lower cortisol concentrations, compared to patients with <5 previous MDEs, in our sample of recurrent MDD-patients. A possible explanation for this specific association could be that HPA-axis activity could exhaust, or get suppressed, after the experience of multiple MDEs. This could resemble parts of the effects of chronic stress on HPA-axis activity, e.g. as an adaptation to its associated increased allostatic load (Heim et al., 2000; Fries et al., 2005). Therefore, it could be hypothesized that the hypercortisolaemic trait observed in our patients is attenuated by the experience of MDEs. This could imply that the trait would have been more pronounced in our patients if we would have measured them before they experienced their MDEs.

4.4. Hypercortisolemia in recurrent MDD as an endophenotype

The absence of HPA-axis activating effects of exogenous factors in our sample, e.g. daily hassles, CLEs, and scarring effects of previous MDEs, may indicate a more endogenously regulated sustained HPA-axis hyperactivity. In addition, previous research found familial association (Mannie et al., 2007), cosegregation (Holsboer et al., 1995), and heritability (Bartels et al., 2003) of HPA-axis activity (Hasler et al., 2004), all criteria of an endophenotype, thereby suggestive of HPA-axis hyperactivity as an endophenotype. However, the state-independence criterion for an endophenotype, has to our knowledge not yet been addressed. With our observations of a state-independent HPA-axis hyperactivity trait, all criteria for hypercortisolemia as an endophenotype for recurrent MDD could be considered to have been fulfilled. We hope our observations will stimulate future research into the evolutionary nature of hypercortisolemia in MDD (Nesse, 2000; Putman and Roelofs, 2011).

4.5. The association between HPA-axis activity and recurrence

We observed no association between repeatedly measured follow-up cortisol concentrations in our patients and the occurrence of recurrence(s) during this follow-up. This is consistent with studies that found no association between cortisol concentrations measured once at the start of a follow-up in long-term remitted patients and their prospective recurrence(s) (Bouhuys et al., 2006; Aubry et al., 2007).

In contrast, after acute treatment, persistently high cortisol concentrations predicted recurrence during follow-up (Zobel et al., 2001; Appelhof et al., 2006; Hatzinger et al., 2009). This discrepancy could be explained by differences in study populations in the above mentioned two study-types: (I) patients who were in long-term remission (e.g. in our study >10 weeks), versus (II) acute treatment remitters. It could be hypothesized that in the latter population the patients who were not stabilized well by antidepressants had higher cortisol concentrations (e.g. by a placebo-response), and subsequent higher relapse-rates, and that these patients were responsible for the association between cortisol and relapse. This merits further exploration.

4.6. Effect of CT

In our intention to treat analysis, CT had a – borderline significant – effect of steeper cortisol declines over the day throughout the 2-year follow-up. Because baseline declines in cortisol did not differ and CT was provided at random, this effect suggests a long-lasting causal influence of CT-treatment on the hypercortisolemic trait. Interestingly, these findings could represent a biological underpinning for the recurrence preventing effect of the CT module (Bockting et al., 2009). This finding corresponds with previous findings of steeper cortisol declines over the day in patients receiving psychotherapy plus antidepressants compared to patients receiving antidepressant monotherapy (Yang et al., 2009; Hsiao et al., 2011). As far as we know, our data for the first time show the long-lastingness of this effect over a two-year follow-up in highly recurrent remitted MDD patients. Depending on the definition used, the effect of CT on the HPA-axis might seem in contradiction with the proposed state-independence of HPA-axis activity (Gottesman and Gould, 2003; Hasler et al., 2004). In our view, if any treatment is aimed directly at an endophenotype, such as the HPA-axis, and would be effective in the treatment of MDD-symptoms through this endophenotypic effect, it could be observed that after treatment, HPA-axis activity is diminished together with a remission of symptoms. However, in this case, it would not be a state-effect of MDD-symptoms on the HPA-axis, but an effect of CT directly at the HPA-axis, coinciding with changes in MDD-symptoms, and thereby in accordance with the state-independence criterion.

The mechanisms underlying the observed effect of CT on the HPA-axis are not elucidated yet. It could be hypothesized that the preventive CT changes coping strategies, e.g. stress perception, management of stress and generation of subsequent stress (Bockting et al., 2006). These effects could possibly mediate its recurrence-preventive effects.

5. Limitations and strengths

Our study has its limitations that need to be addressed. First, because of the longitudinal design and outpatient setting, full compliance to all study protocol measurements and timing could not be attained, resulting in potential bias from missing values. The concern of bias may be reduced by the observation that missing rates did not differ between patients and controls (10.7% vs. 10.6%, respectively). In addition, we aimed to reduce possible bias from missing values by using multiple imputation, also enabling an intention to treat analysis for the effects of CT on cortisol (Donders et al., 2006). Second, we only measured one morning concentration instead of the whole cortisol awakening response, and so based our cortisol course over the day estimation on two measurements. In addition, we asked subjects to provide two 0800 h saliva samples, without assessing their actual awakening times. Therefore, we do not know whether the measured morning value falls into the cortisol awakening response or not. However, all effects were estimated on these values measured systematically using identical methodology,

in patients and controls and for baseline and follow-up. Conceivable consequences of these shortcomings are twofold: it could have caused differences that were actually not present, if patients handled the protocol differently than controls. On the other hand, differences could be diminished due to increases in external variability. Third, we did not include information on sleep quality in our analyses. Disrupted sleep might have influenced morning cortisol concentrations, which could have resulted in an overestimation of the difference between the patients and controls (Vreeburg et al., 2009a). Fourth, we did not follow-up the controls. Although this was not essential to answer our research questions, it would be very interesting to investigate stability and reactivity (e.g. in response to daily hassles) of the HPA-axis in the healthy control subjects in future research. Fifth, we were unable to correct for diet and detailed lifestyle variables, e.g. sedentary life style, employment status, sampling day (weekday vs. weekend), that possibly could have confounded results. Nevertheless, we had the opportunity to correct for smoking, alcohol/drugs use, educational level, weight, waist and hip circumference using propensity scores. These variables are associated with dietary and lifestyle characteristics (Galobardes et al., 2000) and primary mediators of their effects on the HPA-axis, and therefore, the confounding possibly introduced by the lack of information on dietary and more detailed lifestyle factors is expected to be minor. Sixth, we had inadequate power to differentiate the diverse MDD-subtypes, e.g. melancholic, atypical, which could be relevant in the activity of the HPA-axis and therefore an interesting point for further research. Finally, this study was not initially set up as a strictly experimental endocrinological study. This might have resulted in smaller effect sizes. However, the naturalistic setting of our study enables follow-up of this clinical high-risk population for two years.

Our study also had major strengths. First, our inclusion procedure resulted in a well-defined patient sample, characterized by high recurrence rates, reducing the change of heterogeneity of patients and therefore inconsistent findings (Wardenaar et al., 2011). This patient group is thought to (I) represent a more biologically determined MDD-subtype, and (II) contribute largely to the major burden of MDD (Greden, 2001). Second, our longitudinal design enabled us, to our knowledge for the first time, to repeatedly sample cortisol concentrations over a two-year follow-up. This opened the possibility to determine the course of, and unravel state- and trait-effects in, HPA-axis activity.

6. Conclusion

In this longitudinal study on HPA-axis activity, we demonstrated evidence for a trait of hypercortisolemia in remitted patients with recurrent depression. Additionally, we found no state-effects on HPA-axis activity when patients became depressed again. These findings support the state-independence criterion for HPA-axis activity, and together with previous endophenotypical characteristics, the evidence for hypercortisolemia as an endophenotype for recurrent MDD is thereby further strengthened. Finally, our data indicated that preventive CT may improve the decline of cortisol over the day, which might be linked to its therapeutic effects.

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Conflict of interest

None declared.

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