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Mid-term effects of serial sleep deprivation therapy implemented in cognitive-behavioral treatment on the neuroendocrine response to clomipramine in patients with major depression

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ABSTRACT

While data dealing with neurobiological effects of sleep deprivation (SD) are mainly restricted to the acute effects of a single night, only few studies have investigated mid-term effects after repeated SD. We therefore examined the clinical and hormonal characteristics of depressive patients before and after serial SD to determine potential sustained effects, focusing especially on serotonergic functions. One tool to investigate serotonergic dysfunction in depression is the use of serotonergic agents to stimulate hormonal secretion, which is assumed to normalize during a clinically effective therapy. Eighteen drug-free inpatients with unipolar major depression received cognitive-behavioral treatment for three weeks and – according to a randomized control design – additional SD therapy (six nights of total SD within three weeks, separated by nights of recovery sleep) or no SD therapy (control group). Serotonergic function was assessed by measuring cortisol and prolactin in response to intravenously administered clomipramine (12.5 mg) before and after the treatment period. The post-treatment challenge test was performed three days after the last SD night. Apart from of a transient overnight improvement of mood induced by SD, both groups showed a comparable clinical course during the three-week treatment period. Compared to the control group, the SD-treated patients exhibited significantly decreased pre-stimulation cortisol levels and significantly increased cortisol responses to clomipramine, whereas no treatment effects were observed for prolactin. In conclusion, our findings suggest that the mid-term effects of serial SD therapy lead to a normalization of serotonergic dysfunction, although an obvious impact on clinical symptoms was not detected.

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1. Introduction

Among the various antidepressive treatment strategies, sleep deprivation (SD) therapy is one of the most effective short-term treatments for depression, which is well documented in its rapid antidepressive action in 40–60% of the cases (Giedke and Schwarzer, 2002). Although this treatment strategy is assumed to exert its antidepressive action by enhancing central serotonergic neurotransmission and by adaptive changes in serotonergic autoreceptors (Adrien, 2002), only a few studies are available that investigated the impact of SD on serotonergic function. For example, Salomon et al. (1994) showed an increased tryptophan-stimulated prolactin response in female depressive patients after one night of total SD, suggesting a transient normalization of serotonergic dysfunction. However, the lack of a clear relationship

between prolactin responses and mood changes following SD did not seem to support the hypothesis that serotonergic mechanisms promote mood improvement. One further study with healthy individuals revealed that SD even produced a blunted prolactin response to citalopram following one night of SD (Seifritz et al., 1997).

By contrast, in many more studies the effects of SD on the hypothalamic–pituitary–adrenal axis have been assessed. The dexamethasone suppression test (DST) was used in the majority of these studies, with a few reports of a normalization of DST results following SD therapy (Lee and Taylor, 1983; Holsboer-Trachler and Ernst, 1986).

These neurobiological studies, however, focused on short-term actions the day after a single night of SD while the investigation of more mid-term effects has been neglected. This constraint is critical because the antidepressant effect of a single SD night is mainly transient and a relapse into depression occurs in nearly 80% of drug-free responders after daytime naps or nocturnal sleep

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the day after (Wu and Bunney, 1990). A few studies reported sustained clinical effects of SD therapy in depressive patients when SD – in combination with antidepressive pharmacotherapy – is applied serially (Holsboer-Trachsler et al., 1988; Kuhs et al., 1996). On the other hand, the study of Kuhs et al. (1998) demonstrated that the intensification of SD, by comparing a once weekly with a twice weekly administration did not contribute to a better clinical response. Furthermore, there were also negative results, showing that repeated SD – irrespective of its immediate action – failed to produce a cumulative or sustained effect (Holsboer-Trachsler et al., 1994; Wiegand et al., 2001). In summary, research, examining the mid-term effects of repeated SD therapy, is rather limited and the findings are inconsistent. Moreover, no study has answered the question of whether serial SD produces mid-term effects on neurobiological variables, which persist after recovery sleep.

An investigation of serotonergic functions appears especially promising because both, the mechanisms of actions of SD – as stated above – and the pathophysiology of depression, have been related to this neurotransmitter system. Depression is said to be associated with a hypofunction of central serotonergic (5-HT) systems, especially a downregulation of postsynaptic 5-HT_{1A} receptors (Maes and Meltzer, 1995). One well-established approach to reveal indices of an abnormal 5-HT function is the assessment of neuroendocrine responses to serotonergic agents. These neuroendocrine tests are based on the assumption that the release of ACTH, cortisol and prolactin is under excitatory control of the 5-HT system (Yatham and Steiner, 1993). Increased hormonal responses to serotonergic agonists are assumed to reflect hypersensitivity of postsynaptic receptors while decreased responses suggest receptor hyposensitivity. In line with these assumptions, blunted hormonal responses (especially with respect to prolactin) to various serotonergic agents have been observed in depressive patients compared to healthy subjects, as was shown for tryptophan (Heninger et al., 1984), d-fenfluramine (O'Keane and Dinan, 1991), clomipramine (Golden et al., 1992), citalopram (Kapitany et al., 1999) or ipsapirone (Riedel et al., 2002).

However, findings regarding the relationship between neuroendocrine and serotonergic dysfunction and the severity of depression have been inconsistent. There have been studies, which demonstrated an inverse relationship between cortisol responses to serotonergic agents and the severity of depression (e.g. Cleare et al., 1998) and others, which indicated only a weak and insignificant relationship (e.g. O'Keane and Dinan, 1991). Beyond that, Bhagwagar et al. (2002) found that the cortisol response to citalopram was more blunted in the acute stage of the disease than after recovery and compared to that in healthy subjects. In contrast, the blunted prolactin response did not normalize after recovery. A similar result was found by Markianos et al. (2002), who showed a trend towards increasing cortisol responses but persisting blunted prolactin responses after a clinically effective electroconvulsive treatment (ECT). These findings suggest that certain serotonergic indicators reflect pathophysiological mechanisms sensitive to successful treatment, which have to be differentiated from more trait-like vulnerability markers. However, this interpretation remains speculative since the neuroendocrine responsiveness is a very complex physiological process that is influenced by numerous endogenous and exogenous factors.

The main objective of this study was to investigate the mid-term effects of serial SD on the neuroendocrine responses to the serotonin uptake inhibitor clomipramine in drug-free patients with depression in comparison to a clinical control group without SD therapy. The SD treatment applied included a total of six nights of SD separated by two or three nights of undisturbed sleep. SD therapy was implemented in a cognitive-behavioral treatment program lasting three weeks, which was the only therapy for the clin-

ical control group. In order to evaluate sustained neuroendocrine effects of SD, post-treatment evaluation was performed not until after three nights of undisturbed (recovery) sleep following the last SD night. On the basis of previous findings we assumed that patients with depression are characterized by blunted hormonal responses to clomipramine and that especially the cortisol response is affected by antidepressive treatment. According to this, we hypothesized that serial SD induces a mid-term clinical effect, which is accompanied by an increase in the cortisol response to clomipramine.

2. Materials and methods

2.1. Subjects

Twenty inpatients with a current major depressive disorder (MDD) with either a single or a recurrent episode according to DSM-IV (American Psychiatric Association, 1994) participated in the study. They were recruited from consecutively admitted patients for hospital treatment. Each patient was interviewed by an experienced psychiatrist, who used the German version of the Structured Clinical Interview for DSM-IV (Wittchen et al., 1997). Any patient with a comorbid axis-I or axis-II (personality-) disorder was excluded from study participation. Furthermore, suicidal tendencies before or during the study period as well as any change of the diagnosis during inpatient treatment led to exclusion. Further criteria for exclusion were endocrine disorders, pregnancy and shift work within three months or transmeridian travel within one month prior to the study. All patients were studied drug-free for the whole observation period. In case of prior medication, there was a minimum of a 6-day wash-out period, the exact duration of which was at least three times the half-life of the respective drug and its active metabolite. One patient had to be excluded from participation in the neuroendocrine challenge test because of difficulties in obtaining blood samples due to small veins. Another patient withdrew from the study after one week and was also excluded from the statistical analyses. The major clinical characteristics of the remaining 18 patients are shown in Table 1. The protocol was approved by the ethics committee of the medical faculty of the University of Marburg, Germany; all patients gave written, informed consent.

2.2. Study design

All patients underwent a treatment period of three weeks, in which they were basically treated with cognitive-behavioral therapy. According to randomization, the patients received either an additional treatment consisting of serial SD nights or no SD therapy (control group). Because of ethical reasons we could not leave the clinical control group without any form of antidepressant treatment and provide the experimental group merely with SD therapy. Each patient of the SD group underwent two nights of total SD within one week (on day 1 to day 2 and on day 4 to day 5; see Fig. 1). Accordingly, treatment nights were separated by intervals of two to three days with normal (recovery) night sleep in-between. This sequence of SD nights was repeated during the second and third treatment week. Patients of the control group had regular bedtimes for the whole study period. There were no differences between the two groups regarding their clinical characteristics (see Table 1).

Neuroendocrine assessments were performed three days prior to treatment (baseline) and at the end of the three-week treatment period. In the SD group, post-treatment neuroendocrine assessment was performed after three nights of undisturbed (recovery) sleep after the last SD. In order to evaluate acute effects of SD, current mood was assessed the evening before and the morning after

Table 1
Patients' characteristics

Variable		Whole sample	Sleep deprivation group	Control group	Statistical comparison
Number of patients	N	18	8	10	
Age	M	36.8	36.1	37.4	$t = -0.239$; $df = 16$; $p = 0.746$
	SEM	1.9	2.8	2.6	
Gender	N	11	4	7	$\chi^2 = 0.748$; $df = 1$; $p = 0.630$
	Female	N	7	4	
Type of MDD	N	13	6	7	$\chi^2 = 0.055$; $df = 1$; $p = 1.000$
	Recurrent episode	N	5	2	
Severity of depression at baseline as assessed by Hamilton depression rating scale	M	25.9	26.0	25.8	$t = 0.097$; $df = 16$; $p = 0.924$
	SEM	1.0	2.1	0.8	
Beck depression inventory	M	30.3	28.8	31.6	$t = -0.897$; $df = 16$; $p = 0.415$
	SEM	1.7	1.9	2.6	

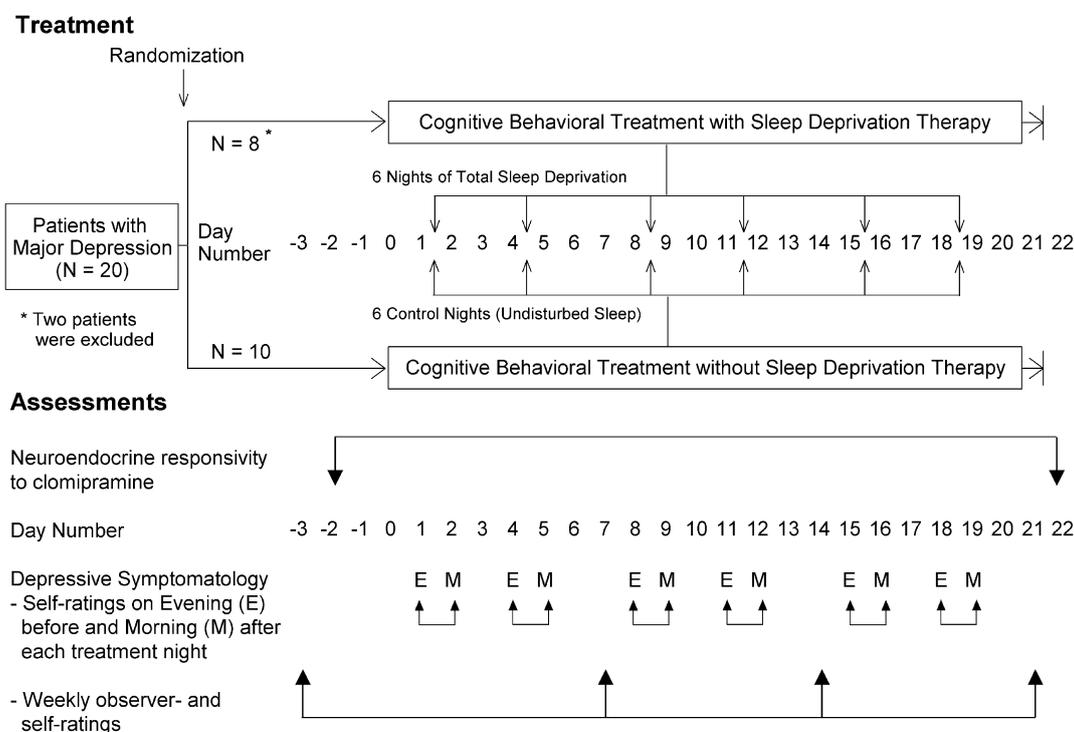


Fig. 1. Experimental design and time schedule.

each treatment night. Furthermore, depressive symptomatology was evaluated at baseline (one day prior to neuroendocrine challenge test) and after each treatment week.¹ The weekly ratings were assessed not earlier than two days after a SD night in order to avoid a confounding impact of short-term clinical effects of SD (see Fig. 1).

2.3. Cognitive-behavioral therapy and experimental treatment conditions

Each study patient received an intensive (five sessions weekly, i.e. on each working day) manual-based treatment (Hautzinger

et al., 2000), consisting of behavioral activation, cognitive restructuring and social skills training.

Total SD was performed according to a standard protocol. A staff member monitored the patient and ensured that the patient stayed awake from 8:00 p.m. to 7:00 a.m. by engaging him/her in standardized activities (including conversation, watching television, going for a walk, or playing games). The subject's behavior was recorded each hour. The surveillance ended at 7:00 a.m. and subsequently the patients received breakfast. On the other (non-treatment) nights, the patients of the SD group patients were allowed to have an undisturbed time in bed. The lights were turned off between 10:00 p.m. and 11:00 p.m. to enable sleep. At 7:00 a.m., the patients were wakened and breakfast was served. The patients of the control condition had regular bedtimes throughout the study period. In order to control for sufficient night sleep in the control group, a sleep questionnaire was administered on the

¹ These results concerning the effects of sleep deprivation on mood have already been published elsewhere (Kundermann et al., 2008).

six mornings corresponding to the times of the SD nights, i.e. on the morning of day 2, 5, 9, 12, 16 and 19. Each patient of the control group had to answer questions concerning sleep duration, sleep latency and frequency as well as the duration of awakenings during the preceding night. Furthermore, several subjective aspects of night sleep (calmness, depth and restfulness) were evaluated by ratings on bipolar five-point Likert scales (e.g. depth of sleep: +2 = “very deep” to –2 = “very superficial”). The data of the questionnaire revealed that our experimental control was successful because the patients of the control group reported sufficient and qualitatively undisturbed night sleep during the six nights.

2.4. Clomipramine challenge test

According to the protocol of Golden et al. (1992), each patient underwent a standard low monoamine and caffeine-controlled diet (Mussettola et al., 1977) beginning three days prior to the pre- and post-treatment tests. In addition, the patients had to fast (with the exception of the consumption of water) for 8 h prior to the test.

On the day of testing, the patients were awakened at 7:00 a.m. A physician accompanied them to the nearby sleep laboratory of our hospital, in which the challenge session took place. Then patients had to stay in supine position until the procedure was completed. At 8:30 a.m. an intravenous cannula was inserted into a forearm vein for blood sampling. The first three (pre-stimulation) blood samples were obtained 30, 45 and 60 min after venous puncture. Immediately after the third sample, all patients received an infusion over a period of 15 min containing 12.5 mg clomipramine diluted in 100 cc of saline solution. Post-stimulation blood samples were obtained 30, 45, 60, 90 and 150 min after the administration of clomipramine. Blood was collected in a serum separator and heparinized collection tubes. Each sample was immediately centrifuged and stored at –80 °C for later analysis. Patients were observed continuously for potential side effects.

2.5. Hormone assays

Plasma cortisol and prolactin concentrations were determined using ELISA commercial radioimmunoassay kits (DRG Cortisol EIA-1887, DRG Prolactin EIA-1291, DRG Diagnostics, Germany). To avoid inter-assay variability, the samples of each individual patient were assayed in the same run, using one kit. Cortisol and prolactin were measured in ng/ml. The detection limit for cortisol was 2.5 ng/ml and the intra- and inter-assay coefficients were, respectively, 5% and 8%. Prolactin had a detection limit of 2 ng/ml and the intra- and inter-assay coefficients were 4% and 7%, respectively.

2.6. Assessment of severity of depressive symptoms

In order to evaluate acute effects of SD on mood, the Depression Scale (DS) of von Zerssen (1976) was administered at each evening and morning session. The DS is a 16 item self-rating scale to evaluate the severity of depressive symptoms and is particularly designed to assess short-term variations of mood states. The higher the score, the worse the patient's condition is at the time of evaluation. For the weekly assessment of depressive symptoms, the Beck Depression Inventory (BDI) (Hautzinger et al., 1995) and the 21-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) were applied. A psychiatrist, who was not involved in the therapy of the patients, performed the observer ratings on the HDRS. The two scales were first used before treatment as baseline measures and then after each treatment week. The patients were classified as treatment responders if their HDRS scores showed a decrease of $\geq 50\%$ after the three-week treatment period.

2.7. Statistical analysis

Data were statically analyzed using SPSS version 11.0 for Windows. Results are presented as mean and standard error of the mean (SEM). Exploratory analyses of the data included descriptive statistics, tests for normality distribution and homogeneity of variance and were run for verification of qualification of parametric statistics.

The DS self-ratings of mood on the evening before and the morning after each treatment night were analyzed with an analysis of variance (ANOVA) using one between-subject factor (“treatment”) and two within-subject factors to evaluate “short-term” (from evening to morning of each treatment night) and “long-term” (over the three weeks of treatment) effects. An acute (“overnight”) effect of SD should be indicated by an interaction between the factors “treatment” and “short-term”. The weekly obtained parameters of depressive symptoms (HDRS, BDI) were analyzed by an ANOVA with one between-subject factor (“treatment”) and one within-subject factor (“time”), which evaluated the treatment and time related changes from the baseline week over the following three weeks. In all analyses, the factor “treatment” refers to comparisons between the SD group and the control group. Mauchly's test of sphericity was performed and – if significant – adjusted degrees of freedom according to Greenhouse–Geisser (GG) were used.

The statistical analysis of the neuroendocrine data was based on pre-stimulation values and response values. The pre-stimulation value was the last (of three) assessed hormone sample just prior to clomipramine-stimulation. The response value was defined as the net area under the curve (AUC), which was calculated as the difference between the sum of post-stimulation hormone samples multiplied by the corresponding time intervals (in minutes) and the pre-stimulation value multiplied with the whole post-stimulation time (150 min). AUC was expressed in ng/ml \times minutes for cortisol and prolactin, respectively.

Treatment effects on the neuroendocrine parameters were evaluated using a two-factorial ANOVA, with “treatment” as a between-subjects factor and “time” as a two-leveled (pre- vs. post-treatment) within-subject factor. The same model was used in order to detect potential differential neuroendocrine characteristics of treatment responders. The between-subjects factor “responding” was based on the post-hoc classification, as described above. Furthermore, treatment responders and non-responders were compared with respect to their baseline characteristics including psychopathological and neuroendocrine variables. Since differences between responders and non-responders were observed for age, this variable was included as a covariate into the repeated-measures ANOVA.

Post-hoc pair-wise comparisons were performed using two-tailed *t*-tests for dependent samples to detect differences across time. Questions about the relationships between categorical data (“treatment”, “responding”, sex) were analyzed using χ^2 analyses. To determine whether there was any relationship between the treatment induced changes in cortisol or prolactin, difference scores (i.e. post-treatment–pre-treatment values) of the baseline (pre-stimulation) and the response (AUC) values were calculated and entered in a correlation analysis (Pearson's *r*). The significance level was set at $\alpha < 0.05$.

3. Results

3.1. Severity of depression and responding to treatment

Based upon the evening and morning assessments of current mood, a substantial effect of SD, as indicated by significant ANOVA interaction between “treatment” (SD therapy vs. control condition)

and the within-factor “short-term” ($F(1,16) = 5.972$; $p = 0.027$), was found for the DS. As shown in Fig. 2, patients who underwent SD developed an overnight amelioration of depressive mood in comparison to patients with undisturbed night sleep. A marked improvement in depression assessed by weekly ratings was observed for all subjects from the baseline week to the final assessment after the third week of treatment (see Fig. 3). This finding was independent from the fact whether the patients received SD treatment or not. Correspondingly, ANOVAs revealed a significant main effect for “time” in HDRS ($F(3,48) = 26.112$; $p < 0.001$) and BDI (GG corrected $F(1.787,28.6) = 7.697$; $p = 0.003$) but no main effect for “treatment” ($F(1,16) = 0.021$; $p = 0.887$ for HDRS and $F(1,16) = 0.339$; $p = 0.569$ for BDI, respectively).

Post-hoc analyses for HDRS by using pooled data from both groups (SD and control group) showed a substantial decrease in depressive symptoms from week 0 (baseline) to the end of treatment week 1 ($t = 4.632$; $df = 17$; $p < 0.001$) and slighter but contin-

uous reductions over the further course of treatment (week 1 vs. week 2: $t = 1.943$; $df = 17$; $p = 0.069$; week 2 vs. week 3: $t = 2.232$; $df = 17$; $p = 0.039$). Nine of the eighteen patients were classified as treatment responders. Responders were older than non-responders (40.7 ± 1.8 years vs. 33.0 ± 2.8 years; $t = 2.289$; $p = 0.036$), but did not differ with regard to severity of depression at baseline (HDRS: 26.0 ± 1.7 vs. 25.8 ± 1.1 ; $t = 0.109$; $p = 0.915$), sex (females/males: 4/5 in responders vs. 3/6 in non-responders; $\chi^2 = 0.234$; $df = 1$; $p = 1.0$) and type of MDD (single/recurrent episode: 6/3 in responders vs. 7/2 in non-responders; $\chi^2 = 0.277$; $df = 1$; $p = 1.0$).

A significant reduction of the BDI scores was only detected from baseline to the end of the first treatment week ($t = 4.720$; $df = 17$; $p < 0.001$) by post-hoc analyses, whereas the subsequent week-to-week differences remained insignificant. Since there was no interaction between “treatment” and “time” (HDRS: $F(3,48) = 0.386$; $p = 0.764$; BDI: GG corrected $F(1.787,28.6) = 0.829$; $p = 0.435$), an additional benefit of SD therapy could not be verified. In accordance to these analyses, there was no significant difference in the number of responders between the SD group ($N = 3$) and the control group ($N = 6$) as revealed by the chi-square test ($\chi^2 = 0.900$; $df = 1$; $p = 0.637$).

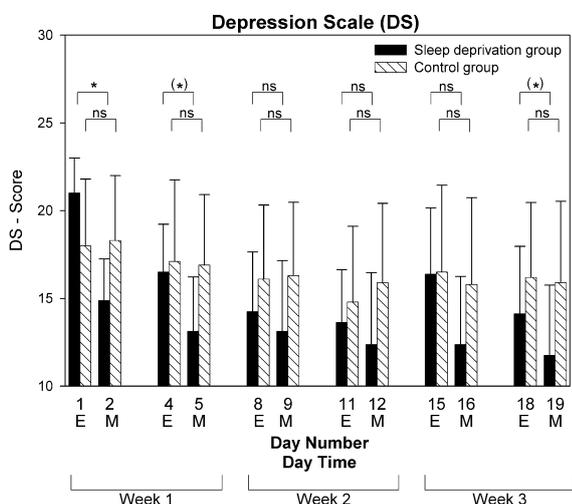


Fig. 2. Self-ratings of mood (DS; mean \pm SEM) on the evening (E) before and the morning (M) after sleep deprivation or control night. Statistical significance, as tested by post-hoc t -tests for pairwise (evening-to-morning) comparisons for the sleep deprivation group ($N = 8$) and the control group ($N = 10$) separately, is denoted by * $p < 0.05$, (*) $p < 0.1$ and ns, non-significant.

3.2. Clomipramine challenge test

The infusion of clomipramine was generally well tolerated. Four patients, two in each treatment group, reported transient mild or moderate nausea or dizziness, one of them both during baseline and post-treatment testing.

3.2.1. Comparison between pre- and post-treatment results in the clomipramine challenge test

No significant changes from baseline to post-treatment were observed for the pre-stimulation cortisol and prolactin levels in all subjects (independent of additional SD treatment; see Fig. 4). While the unstimulated cortisol level showed a nearly significant decline (main effect “time”: $F(1,16) = 4.384$; $p = 0.053$), the prolactin values remained nearly unchanged (main effect “time”: $F(1,16) = 0.645$; $p = 0.434$). Increases in cortisol and prolactin plasma levels in response to the stimulation by clomipramine were observed both at baseline and after treatment leading to positive AUC values (see Fig. 4). The cortisol responses to clomipramine were apparently increased at the end of the treatment period, but this

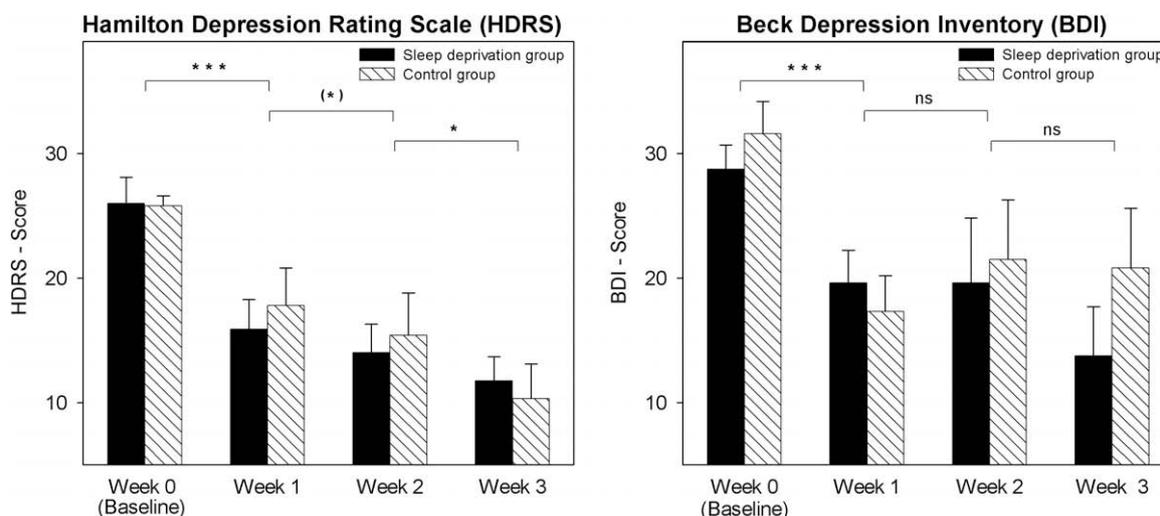


Fig. 3. Weekly ratings of depression (HDRS, BDI; mean \pm SEM) before, during and after a cognitive-behavioral treatment with or without sleep deprivation therapy. Statistical significance, as tested by post-hoc t -tests for pairwise (week-to-week) comparisons of the whole sample ($N = 18$), is denoted by *** $p < 0.001$, * $p < 0.05$, (*) $p < 0.1$ and ns, non-significant.

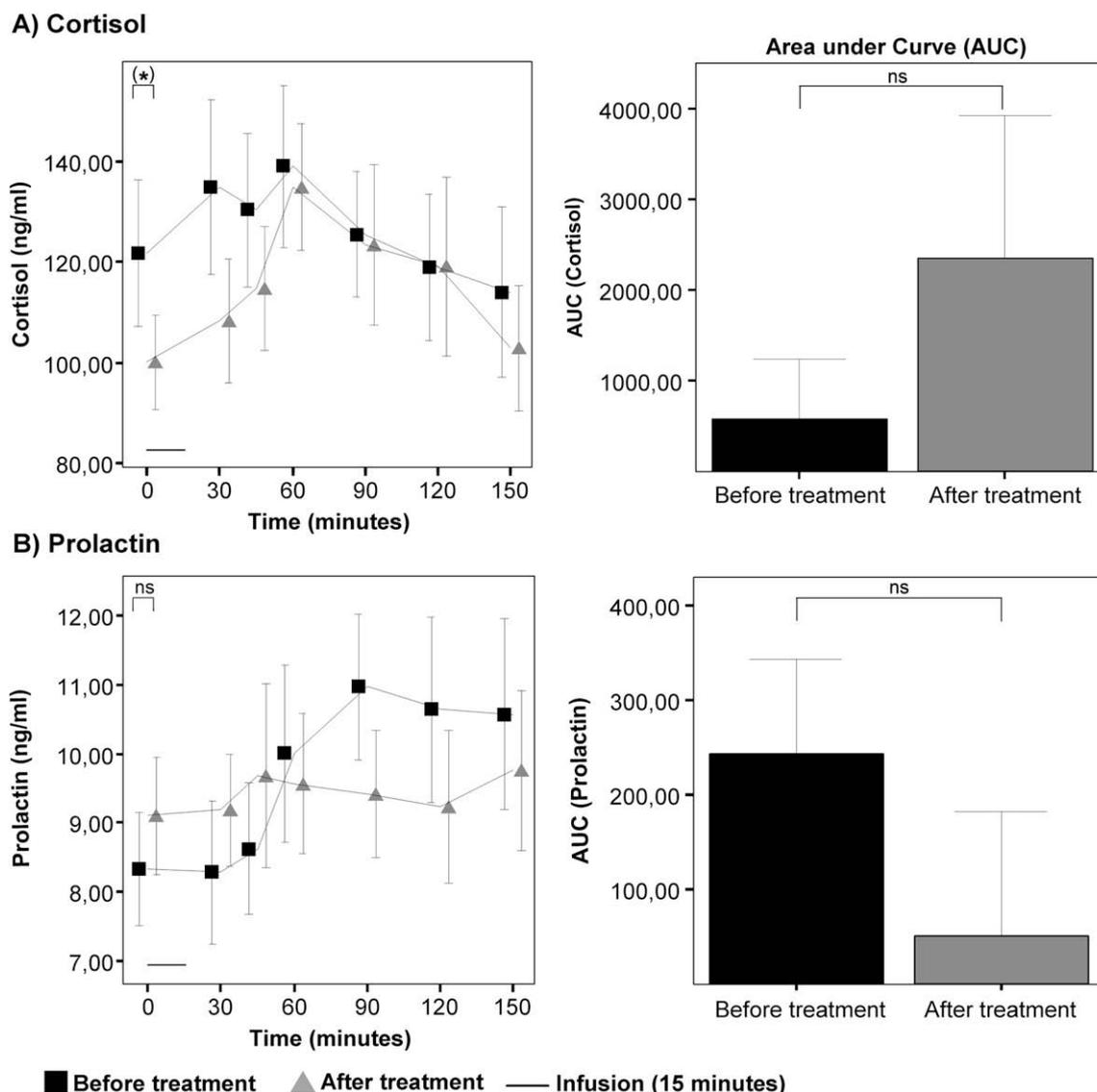


Fig. 4. Plasma cortisol (A) and plasma prolactin (B) during the clomipramine challenge test before and after three weeks of treatment in the whole sample ($N = 18$): raw values and area under the curve (AUC) values (mean \pm SEM). Statistical significance, based on post-hoc paired t -tests by comparing values before vs. after treatment for the unstimulated and the response (AUC) values, is denoted by (*) $p < 0.1$ and ns, non-significant.

change in AUC was not significant ($F(1, 16) = 2.301$; $p = 0.149$). An inverse, but also not significant pattern with slightly higher hormonal responses at baseline was found for prolactin ($F(1, 16) = 1.536$; $p = 0.233$).

3.2.2. Effect of SD therapy on the results of the clomipramine challenge test

For the pre-stimulation values of cortisol, ANOVA revealed a two-way interaction between “treatment” (SD therapy or undisturbed night sleep) and “time” which was found to be significant ($F(1, 16) = 4.644$; $p = 0.047$). As shown in Fig. 5A, the SD group demonstrated a decrease of pre-stimulation cortisol levels from baseline to post-treatment measurement. Post-hoc t -tests for paired samples revealed a nearly significant change in the SD group ($t = 2.162$; $df = 7$; $p = 0.067$), whereas the values of the control group remained unchanged ($t = -0.070$; $df = 9$; $p = 0.946$). No such interaction was detected for the pre-stimulation prolactin values ($F(1, 16) = 0.151$; $p = 0.434$).

SD therapy also induced a markedly enhanced cortisol response to clomipramine as was demonstrated by a significant interaction

between “treatment” and “time” for the AUC values ($F(1, 16) = 6.191$; $p = 0.024$). Post-hoc tests for the SD group demonstrated a significant increase in the response value AUC ($t = -2.439$; $df = 7$; $p = 0.045$) at the end of the treatment period, whereas the pre-post comparison for the control group revealed only an insignificant change ($t = 0.798$; $df = 9$; $p = 0.445$). In contrast to the cortisol response, there was no evidence for an effect of SD therapy on the prolactin response to clomipramine (ANOVA interaction “treatment” \times “time”: $F(1, 16) = 0.061$; $p = 0.808$) as is shown in Fig. 5B. It has to be conceded that, despite our randomized assignment of subjects, there was a significant a-priori difference in the prolactin response between the SD group and the control group at baseline ($t = 2.443$; $df = 16$; $p = 0.027$).

Correlation analysis revealed a significant relationship between the difference scores (post-treatment–pre-treatment) of the unstimulated cortisol levels and the response values (AUC) ($r = -0.740$; $p = 0.036$ for the SD group, $r = -0.787$; $p = 0.007$ for the control group and $r = -0.799$; $p < 0.001$ for the whole sample, respectively), indicating that the stronger the decline in pre-stimulated cortisol levels is, the higher is the increase in the cortisol

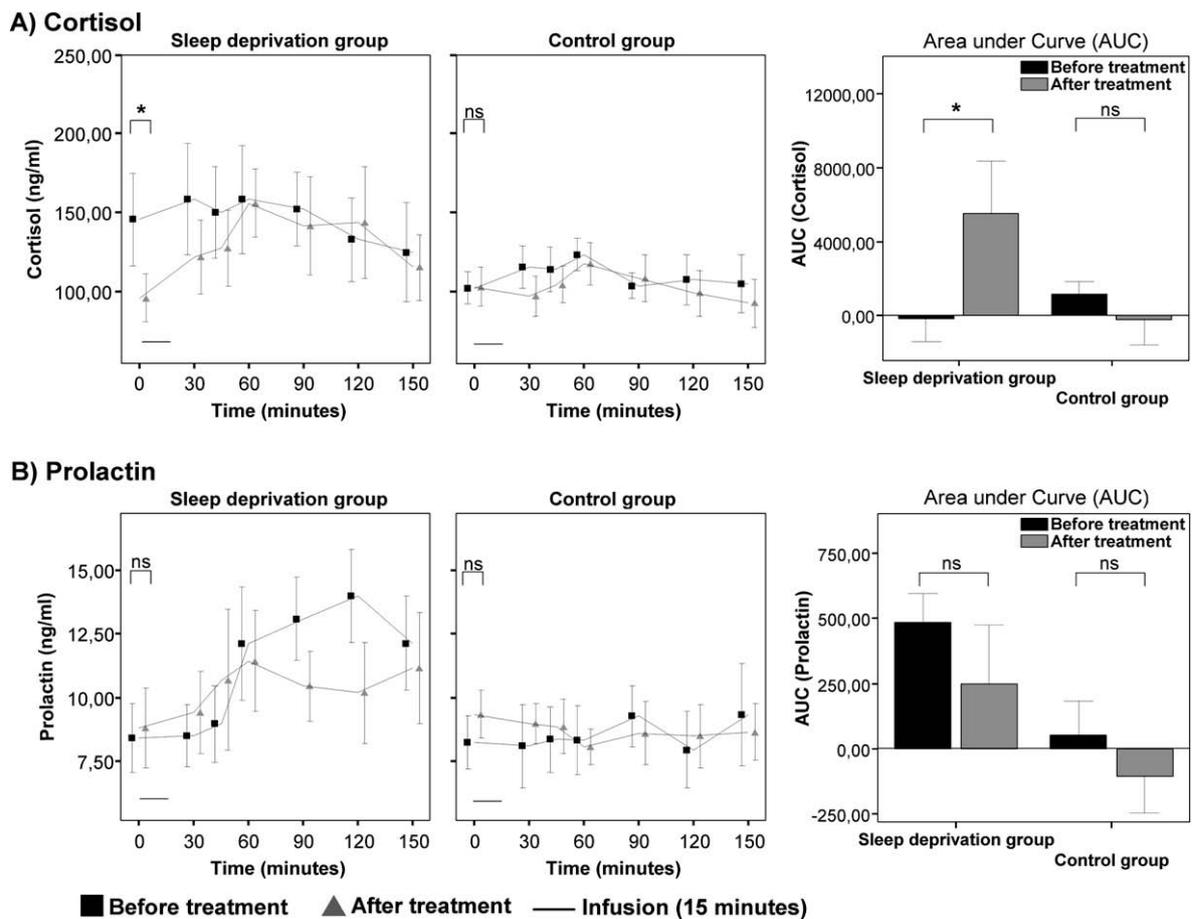


Fig. 5. Plasma cortisol (A) and plasma prolactin (B) during the clomipramine challenge test before and after three weeks of treatment in patients with ($N = 8$) vs. without ($N = 10$) additional sleep deprivation therapy: raw values and area under the curve (AUC) values (mean \pm SEM). Statistical significance, based on post hoc paired t -tests by comparing values before vs. after treatment (each group separately) for the unstimulated and the response (AUC) values, is denoted by $p < 0.05$ and ns, non-significant.

response at the end of the treatment period. A comparable result pattern was observed for prolactin, i.e. significant negative correlations between the change of pre-stimulation and response values ($r = -0.810$; $p = 0.015$ for the SD group, $r = -0.812$; $p = 0.004$ for the control group and $r = -0.770$; $p < 0.001$ for the whole sample, respectively).

3.2.3. Comparison of treatment responders and non-responders in respect to the results of the clomipramine challenge test

At baseline, t -tests for independent samples did not indicate significant pre-treatment differences between responders and non-responders, neither for the pre-stimulation values (cortisol: $t = -0.216$; $df = 17$; $p = 0.831$; prolactin: $t = -0.234$; $df = 17$; $p = 0.818$) nor for the response (AUC) values (cortisol: $t = -0.363$; $df = 16$; $p = 0.721$; prolactin: $t = -1.305$, $df = 16$; $p = 0.210$). Furthermore, there was no evidence for different time courses between responders and non-responders as indicated by non-significant ANOVA interaction effects “responding” \times “time” for the pre-stimulation values (cortisol: $F(1, 16) = 0.479$; $p = 0.499$; prolactin: $F(1, 16) = 0.098$; $p = 0.758$) as well as for the AUC values (cortisol: $F(1, 16) = 0.074$; $p = 0.790$; prolactin: $F(1, 16) = 1.177$; $p = 0.294$) as shown in Fig. 6. When controlling for the differences in age between responders and non-responders by including age as a covariate into the analysis, all these results remained insignificant. Taken these results together, there was no association between the clinical response, and any measure of the clomipramine challenge test.

4. Discussion

The major finding of this on depressive patients is that serial SD (i.e. six nights of SD within a treatment period of three weeks with additional cognitive-behavioral treatment (CBT) resulted in significantly decreased basal (pre-stimulation) levels of cortisol and significantly increased cortisol responses to a stimulation by clomipramine compared to a clinical control group without SD therapy. These neuroendocrine changes occurred although serial SD therapy failed to produce an additional clinical benefit. We obtained the latter result although we demonstrated on the same sample a short-term (“overnight”) improvement of mood after each single SD night, which was however abolished after subsequent recovery sleep (see also Kundermann et al. (2008)).

In contrast to this, the effects on cortisol secretion were mid-term and not due to the acute effects of SD since they persisted over an interval of three days with undisturbed night sleep after the last SD night. This extends the scope of previous reports, which showed neuroendocrine effects of SD mainly concurrent with the short-term phases of mood amelioration. No significant treatment effects were observed for basal and clomipramine-stimulated prolactin secretion.

Since this study was designed to investigate whether indices of a serotonergic dysfunction in depression are related to clinical improvement, it is of interest to discuss first the treatment effects on the depressive symptomatology. The patients showed a significant decline of depressive symptoms as evidenced by the weekly

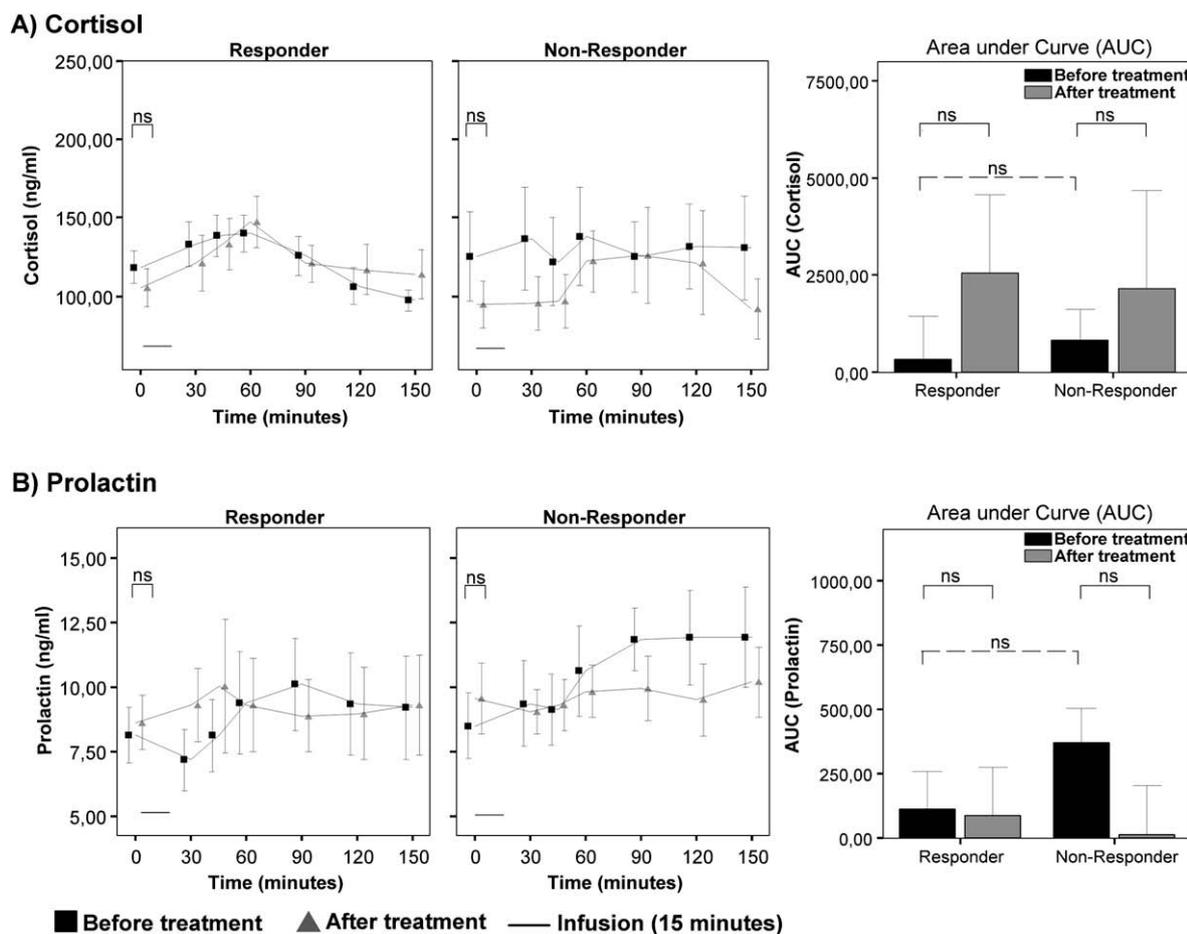


Fig. 6. Plasma cortisol (A) and plasma prolactin (B) during the clomipramine challenge test before and after three weeks of treatment in responder ($N = 9$) vs. non-responder ($N = 9$): raw values and area under the curve (AUC) values (mean \pm SEM). Statistical significance, based on post hoc paired t -tests by comparing values before vs. after treatment (each group separately) for the unstimulated and the response (AUC) values, is denoted by ns, non-significant. Furthermore, results of the pre-treatment comparison between responders and non-responders (marked by brackets with dashed lines) by t -tests for independent samples are indicated for the response (AUC) values.

assessments over the three weeks of treatment, which was independent from belonging to the SD or the control group. Ethical considerations did not allow for including an untreated control group. Therefore, it cannot be excluded that the overall clinical improvement was due to unspecific effects like spontaneous remission or a response mediated by expectancy. Furthermore, it should be noted that mild to moderate severity of depressive symptoms was still present at the end of the study period.

Nevertheless, it is of major interest that serial SD combined with CBT was not superior to cognitive-behavioral monotherapy. One might argue that the failure to observe an additional therapeutic effect of serial SD might be due to the low sample size in this study, especially when comparing a combination treatment with a clinically highly effective treatment such as CBT. On the other hand, the statistical analysis did not even reveal a trend towards a beneficial effect of serial SD. Furthermore, there is no compelling empirical evidence from other studies that serially applied SD alone produces medium-term clinical effects, which persist after recovery sleep. Only a few studies (Holsboer-Trachsler and Ernst, 1986; Kuhs et al., 1996) suggest that sustained clinical effects of SD can be achieved by serial administration.

The reasons for these discrepancies might be explained by methodological differences. The study of Kuhs et al. (1996) demonstrated a better clinical response of patients additionally treated with serial SD (in comparison to amitriptyline monotherapy). However, this effect became obvious only in the observers' ratings, but not in the self-ratings. Holsboer-Trachsler and Ernst (1986) did not assess

the severity of depression after the first night with undisturbed nocturnal sleep subsequent to the last SD night. This issue is of major importance given the high rate of relapses occurring after recovery sleep (Wu and Bunney, 1990; Wiegand et al, 1993).

However, there are studies, which provide good evidence for prolonged clinical effects of SD, but not as monotherapy and only in combination with other treatment strategies such as with lithium, light therapy or sleep phase advance (for a review see Giedke and Schwarzler (2002)).

Although in this study the treatment effects on depressive symptomatology were not different between the treatment conditions, serially applied SD therapy produced significantly different alterations in the hormonal variables, suggesting dissociation between clinical and neuroendocrine changes during SD treatment. Serially applied SD induced a substantially enhanced cortisol response to clomipramine, which was accompanied by decreased basal (pre-stimulation) levels. Since our experimental design did not include a non-clinical control group, one cannot firmly conclude that the increased cortisol response to clomipramine following serial SD reflects a normalization of a decreased responsiveness in the acute stage of depression. This blunting of response was previously reported by Bhagwagar et al. (2002) for acute depression, who used citalopram as a stimulating agent. However, in this study the neuroendocrine changes were paralleled by a clinical improvement, which is in contrast to our observations.

The failure to find a relationship between treatment-induced changes in the severity of depression and in the basal as well as

in the stimulated cortisol levels was also confirmed by the observation that treatment responders did not differ in their hormonal pattern from non-responders. Thus, it is reasonable to assume that the observed effects of serially applied SD on the basal cortisol levels and stimulated cortisol responses are not mediated by an amelioration of depressive symptomatology.

A direct comparison with other studies, dealing with the effect of SD on neuroendocrine systems, cannot be drawn because of differences in methodology, especially regarding the stimulation agent used, the frequency (single vs. serial) and type (partial vs. total) of SD treatment as well as the time interval of measurement after completion of SD. Nevertheless, previous studies provided evidence that SD treatment in depressive patients normalizes the suppression rate in the DST on the following day (Lee and Taylor, 1983; Holsboer-Trachsler and Ernst, 1986), a finding, which could be interpreted as a dampening impact of SD on the HPA-axis activity in depression. In line with this reasoning, our result of an enhanced cortisol response to a stimulation by clomipramine in connection with the observation of significantly decreased basal (pre-stimulation) cortisol levels following serial SD may indicate a down-regulation of the HPA-axis hyperactivity. Interestingly, we observed a substantial negative correlation between the change in unstimulated cortisol levels and the cortisol response to clomipramine following SD therapy, suggesting that both phenomena were affected by a common neurobiological process. However, since such a relationship was also found within the control group as well as for the hormone prolactin, these results appear more attributable to a general association between basal hormone release and neuroendocrine responsiveness.

Our finding of decreased basal cortisol levels seems to be in contrast with several findings in depressive patients, showing elevated basal cortisol levels the day after SD (Baumgartner et al., 1990; Bouhuys et al., 1990; Voderholzer et al., 2004). However, these findings were not obtained after the recovery night and therefore do not deal with mid-term effects as assessed in this study. Interestingly, one study performed on healthy volunteers measured cortisol during the post-deprivation night and also found decreased levels, which were attributed to an inhibiting effect of increased slow wave sleep during recovery sleep on the HPA-axis activity (Vgontzas et al., 1999). Since we did not perform sleep EEG-recordings in this study, we cannot confirm whether an increase in slow wave sleep following serial SD was still present after the three recovery nights, i.e. immediately prior to the post clomipramine-test, and, if so, whether this effect was related to the observed indices of decreased HPA-activity.

However, the underlying mechanisms responsible for the observed neuroendocrine effects of serial SD remain unclear. One can speculate that the normalization of HPA-dysregulation in depression is one key target for different successful treatment modalities including strategies, which affect the central monoaminergic transmission (Holsboer, 2001). Considering that SD and pharmacological treatment with antidepressants were described as producing similar effects on the level of 5-HT neurotransmission (Adrien, 2002), it appears reasonable to assume that serial SD influences HPA-activity via an enhanced serotonergic neurotransmission. In accordance with this reasoning, the observed mid-term effects (i.e. after three weeks) of a down-regulated HPA-activity by additional SD therapy could indicate a first step to an antidepressive action on a neurobiological level, which precedes a more enduring long-term clinical response.

With regard to plasma prolactin, no treatment effects were observed in this study. This finding was not surprising because previous studies had shown that the prolactin response following serotonergic stimulation remains unchanged despite clinical amelioration (Bhagwagar et al., 2002; Markianos et al., 2002; Golden et al., 2002). The prolactin response appears to be less sensitive

to treatment-induced changes than the cortisol response. There are exceptions. Salomon et al. (1994) reported an enhanced prolactin response to tryptophan-stimulation in depressive patients after SD. However, this finding does not apply to our situation because it again reflects only the acute effects of a single SD night.

Although the major aim of this study was not to isolate neuroendocrine predictors for the clinical outcome, this possibility was indirectly tested by a comparison between responders and non-responders. According to this, none of the neuroendocrine variables studied differed significantly between the two groups, indicating no predictive value for treatment outcome. Neurobiological predictors of the outcome of psychotherapy in patients with depression have yet been rarely identified. There are a few exceptions, such as measures of hypothalamic–pituitary–adrenocortical activity (Thase et al., 1996) and EEG sleep variables (Thase et al., 1997).

A limitation of this study was that the neuroendocrine challenge tests were performed without a placebo stimulation procedure in order to control for unspecific neuroendocrine reactions, especially related to stress due to the blood sampling procedure. Thus, one could argue that changes in the hormonal levels and responses during the treatment period were merely due to habituation to the test procedure resulting in decreased stress responses. We had to omit the placebo condition because of the enormous efforts required to study patients in our design of serial SD. Furthermore, it is most likely that these confounders were balanced between the two treatment groups so that the major result of a neuroendocrine pattern influenced by serial SD treatment cannot be easily explained as a methodological artefact. This argument is also true for the potential impact of unwanted side effects caused by clomipramine. In accordance to other neuroendocrine challenge studies using this agent (e.g. Golden et al., 1992; Anderson et al., 1992), we only observed a low frequency of side effects, which was equally distributed over the two treatment conditions. Furthermore, a study using the serotonergic agonist citalopram failed to demonstrate a relationship between the severity of nausea and the neuroendocrine response parameters (Kapitany et al., 1999).

A further limitation of this study is the use of clomipramine as a challenging agent for estimating the functional status of the central serotonergic system. Although clomipramine is well documented in its properties to increase 5-HT transmission by inhibiting 5-HT uptake, its validity as a specific serotonergic probe is questionable since other neurotransmitter systems, especially the central noradrenergic system, are also affected. However, at the beginning of the study, citalopram as a 5-HT probe with a high specificity for 5-HT reuptake and a favorable side-effect profile (e.g. Seifritz et al., 1996) has not yet been approved in an intravenous application form by the German Federal Institute for Drugs and Medical Devices (BfArM).

Finally, one could argue that a wash-out period of 6 days was still too short given that an abrupt termination of treatment with antidepressants can result in withdrawal reactions (e.g. dizziness, fatigue and insomnia) that persist over a longer period. Therefore, it cannot be excluded that such symptoms and its underlying physiological mechanisms (e.g. a rapid decrease in serotonin availability) interfere with our measurements, especially at baseline. On the other hand, such reactions typically characterized by a rapid onset within the first seven days after termination of treatment with serotonergic antidepressants (Shelton, 2006) were not observed.

In summary, this study provided evidence that serially applied SD exerts an inhibiting effect on the HPA-activity, as was indicated by a decreased basal (pre-stimulation) cortisol secretion and an increased cortisol response to a stimulation with clomipramine. This effect did not appear to be mediated by apparent clinical changes in the depressive psychopathology and was mid-term in duration

because it persisted over three days after the last SD. One can conclude that serial SD therapy promotes the normalization of serotonergic function on a sub-clinical level. Future studies are needed to clarify whether these sub-clinical, yet functional changes in consequence of serial SD lead to a more favorable course of a depressive episode.

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Contributors

Stefan Lautenbacher and Jürgen-Christian Krieg designed the study and wrote the protocol. Peter Strate, Julia Hemmeyer-Spernal and Martin Tobias Huber performed the psychiatric examinations and conducted the neuroendocrine challenge tests (including the primary data processing). Bernd Kundermann undertook the statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest statement

None declared.

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