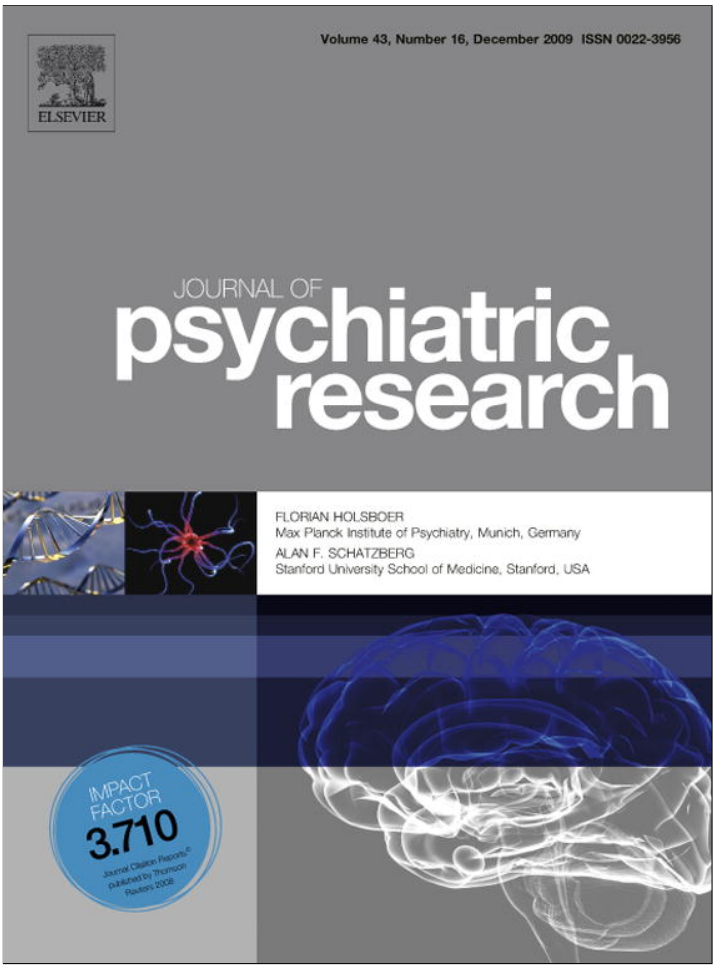

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Pain sensitivity in major depression and its relationship to central serotoninergic function as reflected by the neuroendocrine response to clomipramine

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ABSTRACT

Several studies reported a decreased pain sensitivity in patients with depression, but the underlying neurobiological mechanisms of this phenomenon are unclear. While there is extensive evidence that the serotoninergic system plays a key role in pain modulation, especially in pain inhibitory mechanisms via descending pathways, as well as in the pathophysiology of depression, no study so far has examined its potential relevance in mediating the alteration of pain processing. The present study addresses the question of whether indices of serotoninergic dysfunction, as investigated by a neuroendrocine challenge paradigm, are related to pain sensitivity. Nineteen drug-free inpatients with unipolar major depression underwent a neuroendocrine challenge test by measuring cortisol and prolactin in response to intravenously administered clomipramine (12.5 mg). Heat/cold pain thresholds, warmth/cold detection thresholds, measures of current pain complaints and mood were assessed the day before and three day after challenge procedure. When patients were classified in subgroups based on a median split of their cortisol response values, the low-responsive group showed significantly elevated heat pain thresholds and nearly significantly elevated cold pain thresholds compared to the high-responsive group. No such group differences were found with regard to somatosensory thresholds, measures of pain complaints and mood. Subgrouping on the basis of prolactin responsiveness did not reveal significant differences in any parameter. In summary, a decreased pain sensitivity was demonstrated in patients characterized by a reduced neuroendocrine responsiveness to clomipramine, suggesting an involvement of serotoninergic dysfunction underlying altered pain perception in depression.

1. Introduction

Former research has shown a close association between depression and pain (Von Korff and Simon, 1996). Almost half of the patients suffering from a depressive disorder also experience chronic pain (Simon et al., 1999). Furthermore, the severity of pain complaints in depression was found to predict a longer time to remission (Karp et al., 2005). While most studies in this field have focused on spontaneous pain (clinical pain complaints), only a few studies examined pain sensitivity in depression. Surprisingly, the majority of these studies revealed that depressive patients are less sensitive to experimental painful stimuli compared to healthy controls (see for a meta-analysis Dickens et al., 2003). Evidence for this hypoalgesia seems to depend on specific stimulus characteristics. A decreased sensitivity to noxious stimuli in depressive patients was frequently documented when using phasic cutaneous pain stimuli (e.g. Davis et al., 1979; Adler and Gattaz, 1993; Marazziti et al., 1998; Lautenbacher et al., 1994a,b; Bär et al., 2003, 2005), although an opposite finding was recently reported by Strigo and colleagues (2008). An increased pain responsiveness was preferentially found when using tonic deep somatic stimuli, as it was shown for ischemic muscle pain (e.g. Piñerua-Shuhaibar et al., 1999; Bär et al., 2005). Interestingly, a few studies have shown that pain sensitivity to phasic cutaneous stimuli normalized after clinical recovery (e.g. von Knorring, 1974) or was even susceptible to short-term effects of antidepressive treatments like sleep deprivation (Kundermann et al., 2008), which let the sensitivity to phasic pain appear as a state-like characteristic of depression.

However, the *underlying neurobiological mechanisms for the* associations described are far from being understood. This is – at least in part – due to the fact that only a few studies have both

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assessed systematically and objectively pain in depression and considered those neurobiological variables, which are believed to be involved in the pathophysiology of depression as well as in the modulation of pain.

One recent study (Frew and Drummond, 2008) suggests an overactive opiodergic system underlying the hypoalgesia in depression, but the available data – apart from negative findings (Lautenbacher et al., 1994a,b) – are too sparse to draw confident conclusions concerning a potential role of the opioidergic system. Although the serotoninergic neurotransmission is traditionally regarded to be involved in nociception as well as in the pathophysiology of depression (Delgado, 2004), surprisingly no study so far has systematically assessed the functional status of the serotoninergic system with respect to the alterations in pain sensitivity in patients with depressive disorders. Accordingly, human studies investigating the role of serotonin for pain sensitivity were only conducted in various chronic pain conditions or in healthy controls, preferentially by correlating measures of pain sensitivity with serotonin plasma levels (e.g. Wolfe et al., 1997; Pickering et al., 2003) or binding potential of 5-HT receptors (Martikainen et al., 2007). Furthermore, in a few studies experimental strategies such as (dietary) tryptophan depletion (Abbott et al., 1992) and administration of agents with serotoninergic properties (Ernberg et al., 2000; Enggaard et al., 2001) have been used to study serotoninergic effects on pain sensitivity. Although there is extensive evidence that serotonin – among other neurotransmitters such as noradrenaline and neuromodulators such as opioid peptides – is one of the key neurotransmitters in the descending inhibitory system (Millan, 2002), study results indicating a pronociceptive effect after injection of 5-HT (Ernberg et al., 2000) or a negative correlation of total blood serotonin with experimental pain detection threshold (Pickering et al., 2003) suggest a more complex role of serotonin for pain regulation. Since 5-HT pathways are assumed to be involved in both mood regulation and pain processing, the analysis of serotoninergic functions in depression may be a valuable approach to answer the question of whether disturbances in this neurotransmitter system may account for the altered pain sensitivity.

One well-established approach to reveal abnormal 5-HT function is the assessment of neuroendocrine responses to serotoninergic 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 serotoninergic agonists are assumed to reflect hypersensitivity of postsynaptic receptors while decreased responses suggest receptor hyposensitivity. In line with the assumption that depression is associated with a downregulation of postsynaptic 5-HT_{1A} receptors (Maes and Meltzer, 1995), blunted hormonal responses to various serotoninergic agents have been observed in depressive patients compared to healthy subjects, as was shown for tryptophan (Heninger et al., 1984), d-fenfluramine (ÓKeane and Dinan, 1991), clomipramine (Golden et al., 1992), citalopram (Kapitany et al., 1999; Bhagwagar et al., 2002) or ipsapirone (Riedel et al., 2002).

The main objective of the present study was to investigate pain sensitivity in patients with major depression in relationship to the neuroendocrine (i.e. cortisol and prolactin) responsiveness to a sertoninergic agent (clomipramine). Assuming that – as stated above – hypoalgesia and (affective core symptoms of) depression share a common neurobiological dysfunction, i.e. deficiencies in the serotoninergic system, we hypothesized a close relationship between altered pain sensitivity and neuroendocrine responses to clomipramine. In addition to the measurement of pain sensitivity, somatosensory thresholds, current pain complaints and severity of depressive symptomatology were assessed to determine the specificity and their interrelationships.

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 the DSM-IV criteria (American Psychiatric Association, 1994) participated in the study. They were recruited from consecutively admitted patients for hospital treatment. In order to obtain a reliable diagnosis of a MDD and of other concomitant mental disorders, 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 diagnosis during inpatient treatment led to exclusion. Further criteria for exclusion were endocrine disorders and pregnancy. All patients were studied drug free. In case of prior medications, there was a minimum 6-day wash-out period, the exact duration of which was at least 3 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 and was therefore excluded from statistical analyses.

The mean age of the remaining 19 patients was 36.1 years (standard error of the mean (SEM) = 1.9). The gender ratio within the study sample was roughly balanced (12 males vs. 7 females). With regard to the subtype of depression, 14 patients had a single episode of depression; the rest ($N = 5$) had recurrent depression. The mean severity of depression at baseline, as determined by the Hamilton Depression Rating Scale ($M = 25.8$, SEM = 0.9) and the Beck Depression Inventory ($M = 30.2$; SEM = 1.6), indicated a moderate to severe level of depressive symptoms.

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 and time schedule

The patients entered the study as soon as possible (2–3 days) after hospital admission, i.e. subsequent to the diagnostic examinations and approval to study participation. After inclusion in the study, a diet (related to the pharmacological challenge test, see below) was administered and the patients underwent a baseline period (i.e. without any treatment) of 6 days with a fixed sequence of measurements (see Fig. 1), beginning on day 2 (at 4.00 p.m.) with *thermal thresholds* testing (i.e. detection thresholds for cold and warmth, *pain thresholds for cold* and heat) and evaluation of current pain complaints (baseline 1). Furthermore, depressive symptomatology was assessed by observer and self ratings within the same baseline testing session. On the morning of the following day, the neuroendocrine challenge paradigm for studying serotoninergic functions (clomipramine challenge test) was performed. The baseline period was completed by reassessment of thermal thresholds, current pain complaints and mood three days after the neuroendocrine challenge test (baseline 2), which enhanced statistical power. After baseline assessment, all patients entered a treatment protocol comparing cognitive-behavioral therapy with vs. without serial sleep deprivation with regard to its effects on mood, pain and neuroendrocrine parameters[1].

[1] These results have already been published elsewhere and are not reported here (Kundermann et al., 2008, 2009).

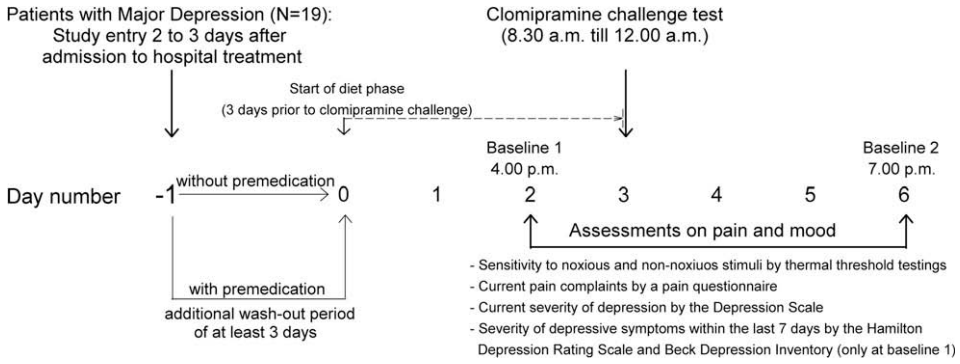

Fig. 1. Study schedule and measurements.

2.3. Thermal thresholds for noxious and non-noxious stimuli

Cold detection threshold (CDT), warmth detection threshold (WDT), cold pain threshold (CPT), and heat pain threshold (HPT) were determined in this order by using the method of limits with ascending stimulus intensities. A Thermal Sensory Analyser (TSA-2001, Medoc Ltd.) was used. The contact thermode (stimulation surface of $3.2 \times 3.2\ cm^2$) was attached to the center of the volar forearm. Beginning at a baseline temperature of 32.0 °C, five stimuli were applied for each of the four thermal thresholds. To avoid tissue damage, the cut-off temperature was 52.0 and 0 °C, respectively. Subjects were instructed to press a button as soon as they felt a change in temperature (CDT and WDT) or the onset of pain sensation (CPT and HPT). In order to minimize artifacts due to reaction time, we used slow rates of temperature change (Yarnitsky, 1997). This rate was 1 °C/s for the two detection thresholds and 1.5 °C/s for the two pain thresholds. Each time the subjects pressed the button, the temperature returned to the baseline temperature, which was held constant until the next trial. CDT, WDT, CPT and HPT were computed by averaging the °C readings of the five successive stimuli designed for each threshold (relative to baseline in the case of the thermal detection thresholds, absolute in the case of the thermal pain thresholds).

2.4. Assessment of current pain complaints

Current pain complaints (number of pain sites, their intensity and unpleasantness) were assessed by a pain questionnaire (PQ), which was used in former studies on patients with depression (Lautenbacher et al., 1999). For the present study, the instruction of the questionnaire was slightly adapted to evaluate exclusively current pain complaints. On two schematic drawings of the front and rear body, the subjects were asked to mark each location in which they currently experienced pain. This allowed for counts of the number of painful sites (PQ-N). Afterwards, subjects were asked to rate the intensity (PQ-I) and unpleasantness (PQ-U) of pain for each site at separate horizontal visual analogue scales (VAS) of 100 mm. To assess overall pain intensity and pain unpleasantness, the VAS ratings of all sites were summed up for each of the two pain dimensions.

2.5. Assessment of severity of depressive symptoms

In order to evaluate current mood, the Depression Scale (DS) of von Zerssen (1976) was administered at both baseline sessions. 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 assessment of the severity of the depressive symptomatology over the previous 7 days, the 21-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) and the Beck Depression Inventory (BDI) (Hautzinger et al., 1995) were only applied in the first baseline session. The HDRS were filled out by a small group of experienced psychiatrists, who were specifically trained in the use of this instrument to achieve high interrater-reliability.

2.6. Clomipramine challenge test

According to the protocol of Golden et al. (1992) for the clomipramine challenge test, each patient underwent a standard low monoamine and caffeine-controlled diet (Muscettola et al., 1977) beginning 3 days prior to the test. In addition, patients had to fast (with the exception of the consumption of water) for 8 h prior to the test.

On the day of testing, patients were awakened at 07.00 a.m. and allowed to micturate but remained in bed until the procedure was completed. At 8.30, an intravenous cannula was inserted by a physician into a forearm vein for blood sampling. The first three (pre-stimulation) blood samples were obtained 30, 45 and 60 min after insertion of the intravenous cannula. Immediately after the third probe, 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 administration of clomipramine. Blood was collected in serum separator and heparinized collection tubes. Each sample was immediately centrifuged and stored at −80 °C for later analysis. Patients were observed continuously and potential side effects recorded by the physician. The infusion of clomipramine was generally well tolerated. Four patients reported transient mild or moderate nausea or dizziness.

2.7. Hormone assays

Plasma cortisol and prolactin concentrations were determined by 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 5% and 8%, respectively. Prolactin had a detection limit of 2 ng/ml and the intra- and inter-assay coefficients were 4% and 7%, respectively.

2.8. Statistical analysis

Data were statically analyzed by using SPSS version 11.0 for Windows. Exploratory analyses of the data included descriptive

statistics as well as tests on normal distribution and homogeneity of variance and were run to verify whether the data qualify for parametric statistics. Results are presented as mean and standard error of the mean (SEM). The inference statistical testing was mainly based on a comparison of "low" and "high responders" resulting from a median split with respect to the hormonal response to clomipramine. This approach of dichotomizing the neuroendocrine data was chosen because of distribution characteristics (i.e. violation of the assumption of normal distribution), especially regarding the cortisol-responses. The response value was defined as net area under the curve (AUC), which was calculated as the difference between the sum of post-stimulation hormone samples multiplied with the corresponding time intervals (in minutes) and the pre-stimulation value prior to clomipramine stimulation multiplied with the whole post-stimulation time (150 min). AUC was expressed in ng/ml × min for each hormone. On this basis, patients with AUC values ⩽ median (Md) were classified as "low responders", whereas patients with an AUC above the median were assigned to the "high responder" group. This approach was conducted for cortisol for as well as for prolactin separately.

For those variables, which were assessed once at baseline, differences between the two subgroups were analyzed statistically with Student's *t*-test for dimensional data (e.g. HDRS and BDI) and with the χ^2-test for categorical data (e.g. gender, type of MDD). In order to enhance statistical power, those variables, which were assessed twice before and after the clomipramine test (i.e. pain sensitivity and pain complaints together with current mood), were entered in an analysis of variance (ANOVA) with one between-subject factor ("responsiveness" = "low" vs. "high") and one within-subject factor ("time"). The factor "time" evaluated changes between the first and second assessment. If main effects or interactions were significant, post-hoc *t*-tests were performed to isolate specific differences between groups or across time. The significance level was set at $\alpha < 0.05$.

3. Results

3.1. Classification of patients into subgroups on the basis of their neuroendrocrine response to clomipramine

According to a calculated median of 1356.65 ng/ml × min for the AUC values for cortisol and 188.83 ng/ml × min for prolactin, the patients were classified in subgroups of "low" vs. "high" responders for each hormone separately. Among the nine patients, who were categorized into the "high" cortisol responsiveness group, only four patients could be assigned to the "high responders" regarding prolactin. Accordingly, there was no relationship between the responsiveness of cortisol and prolactin to clomipramine, neither for the categorical data after subgrouping the patients ($\chi^2 = .059$; $p = 1.0$) nor for the dimensional AUC values ($r = -0.025$; $p = 0.920$), providing further plausibility for our strategy to analyze each hormonal response parameter separately.

3.2. Comparison between patients with "low" and "high" neuroendocrine responsiveness with regard to clinical variables

Patients characterized by a "low" cortisol or prolactin response to clomipramine did not significantly differ from those patients with a "high" response in age, sex, type of MDD and various measures of depressive symptomatology (Hamilton Depression Rating Scale, Beck Depression Rating Scale) including the severity of current depression as assessed by the Depression Scale at both baseline examinations (Table 1). When comparing the first and the second assessment (baseline 1 vs. 2) on this scale for the whole sample by a *t*-test for dependent samples, a nearly significant difference ($t = 1.974$; df = 18; $p = 0.064$) emerged, indicating a tendency towards an amelioration of depressive symptomatology. "Low" and "high" responders did not differ in this respect as

Table 1
Comparison of patients with "low" and. "high" cortisol/prolactin responsiveness to clomipramine with respect to their clinical characteristics. *T*-tests for independent samples were based on df = 17; χ^2-analysis on df = 1.

Variable	Cortisol responsiveness			Prolactin responsiveness		
	Low	High	t/χ^2-value p (2-tailed)	Low	High	t/χ^2-value p (2-tailed)
Number of patients						
N	10	9		10	9	
Age						
M	33.4	39.1	$t = -1.546$	37.8	34.2	$t = 0.929$
SEM	2.2	3.0	$p = 0.141$	2.4	3.1	$p = 0.366$
Gender: male/female						
N	5/5	7/2	$\chi^2 = 1.571$ $p = 0.350$	7/3	5/4	$\chi^2 = 0.425$ $p = 0.650$
Type of MDD: single/recurrent episode						
N			$\chi^2 = 0.148$ $p = 1.0$	9/1	5/4	$\chi^2 = 2.898$ $p = 0.141$
Severity of depressive symptoms over the previous 7 days						
Hamilton depression rating scale						
M	25.8	25.9	$t = -0.046$	24.8	27.0	$t = -1.185$
SEM	1.0	1.7	$p = 0.964$	1.4	1.2	$p = 0.252$
Beck depression inventory						
M	28.8	31.7	$t = -0.892$	30.7	29.6	$t = 0.349$
SEM	1.7	2.8	$p = 0.385$	1.7	2.9	$p = 0.731$
Current severity of depressive symptoms						
Depression Scale at day 1 (baseline 1)						
M	23.8	24.6	$t = -0.182$	23.9	24.4	$t = -0.131$
SEM	2.3	3.6	$p = 0.858$	3.0	2.8	$p = 0.897$
Depression Scale at day 5 (baseline 2)						
M	18.9	21.3	$t = -0.528$	19.5	20.7	$t = -0.252$
SEM	3.1	3.4	$p = 0.604$	2.8	3.7	$p = 0.804$

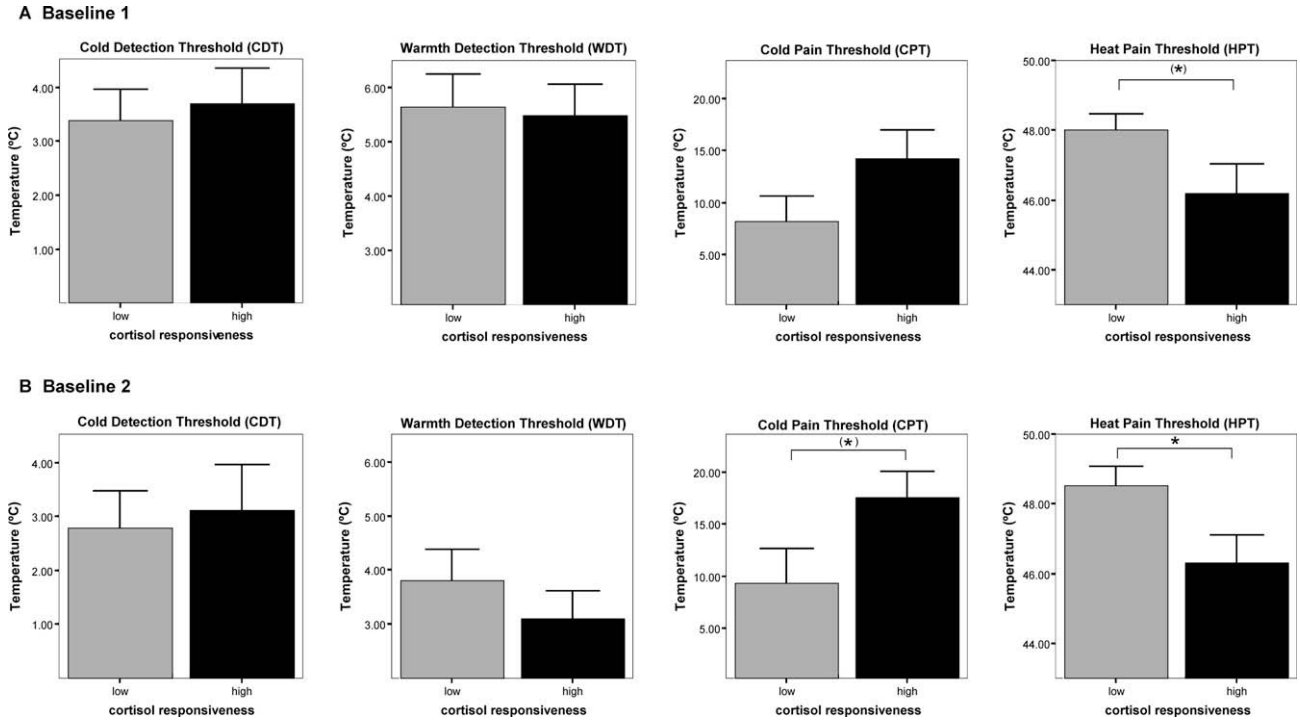

Fig. 2. Comparison of patients characterized by "low" and "high" cortisol responsiveness with regard to measures of thermal threshold testings on baseline 1 (day 2) and baseline 2 (day 6). Statistical significance, based on post-hoc *t*-tests for independent samples, is denoted by $* = p < 0.05$ and $(*) = p < 0.1$.[2]

suggested by the lack of significant interaction effects (cortisol: $F(1,17) = 0.155$; $p = 0.699$; prolactin: $F(1,17) = 0.021$; $p = 0.886$).

3.3. Comparison between patients with "low" and "high" neuroendocrine responsiveness with regard to pain and somatosensory sensitivity

3.3.1. Comparisons according to cortisol responses

In support to our hypothesis, the classification on the basis of the cortisol response into subgroups of "low" versus "high" responsive patients to clomipramine yielded substantial group differences with regard to the measures of thermal pain sensitivity.

For heat pain thresholds (HTP), ANOVA revealed a significant main effect for "group" ($F(1,17) = 5.978$; $p = 0.026$). Post hoc *t*-tests showed a nearly significantly enhanced HPT in the first ($t = 2.000$; df = 17; $p = 0.061$) and a significantly enhanced HPT in the second baseline session ($t = 2.258$; df = 17; $p = 0.029$) in the "low responders" compared with the "high responders" (Fig. 2). The statistical analysis of the cold pain thresholds (CPT) produced a similar pattern of results, i.e. the "low"-responsive group exhibited descriptively increased CPT levels in comparison to the "high"-responsive group. However, evaluation was limited by the fact that only some of the patients ($n = 7$ in each group) consistently developed a sensation of cold pain above the lower temperature cutoff at 0 °C. Nonetheless, ANOVA indicated at least a tendency of group difference ($F(1,12) = 3.904$; $p = 0.072$).

In contrast to the thermal pain thresholds, no differences between the "low" and "high" cortisol responsive group were observed for detection thresholds for cold ($F(1,17) = 0.442$; $p = 0.515$) and warmth ($F(1,17) = 0.214$; $p = 0.650$).

3.3.2. Comparisons according to prolactin responses

When patients were classified according to their prolactin responses into "low" and "high responders" and statistically compared with respect to their thermal thresholds (Fig. 3), nonsignificant group differences were consistently noted: CPT ($F(1,12) = 0.157$; $p = 0.699$), HPT ($F(1,17) = 0.249$; $p = 0.624$), CDT ($F(1,17) = 0.181$; $p = 0.676$) and WDT $F(1,17) = 1.042$; $p = 0.322$).

3.4. Comparison between patients with "low" and "high" neuroendocrine responsiveness with regard to clinical pain complaints

As presented in Table 2, ANOVA did not reveal any significant main effect "responsiveness" (when classifying patients according to their cortisol or prolactin responses) on the clinical pain parameters assessed by questionnaire, i.e. current number of painful sites, VAS ratings of intensity and unpleasantness of pain complaints.

4. Discussion

The major finding of this study is that thermal pain sensitivity in patients with major depression was related to a well-known neuroendocrine marker of serotoninergic function, i.e. the cortisol response to the serotoninergic agent clomipramine. "Low" and "high responders" significantly differed with respect to experimental pain sensitivity, with patients characterized by low *cortisol* responsiveness showed lesser sensitivity to noxious thermal stimuli. For this group, a consistent pattern of enhanced thermal pain thresholds was demonstrated at both times of assessment (i.e. baseline 1 and 2) and for both pain sensitivity parameters (i.e. physical stressors heat and cold). The failure of finding more than a trend of a group difference for cold pain thresholds was probably only due to the lowered statistical power (caused by missing data because cold threshold was not reached within the technically possible temperature limit of 0 °C in some individuals).

[2] Bars representing threshold temperatures are relative in the case of detection thresholds (CDT and WDT), absolute in the case of thermal pain thresholds (CPT and HPT), i.e. decreased heat pain thresholds are indicated by lowered temperature values, whereas decreasing cold pain thresholds are expressed in elevated temperature values.

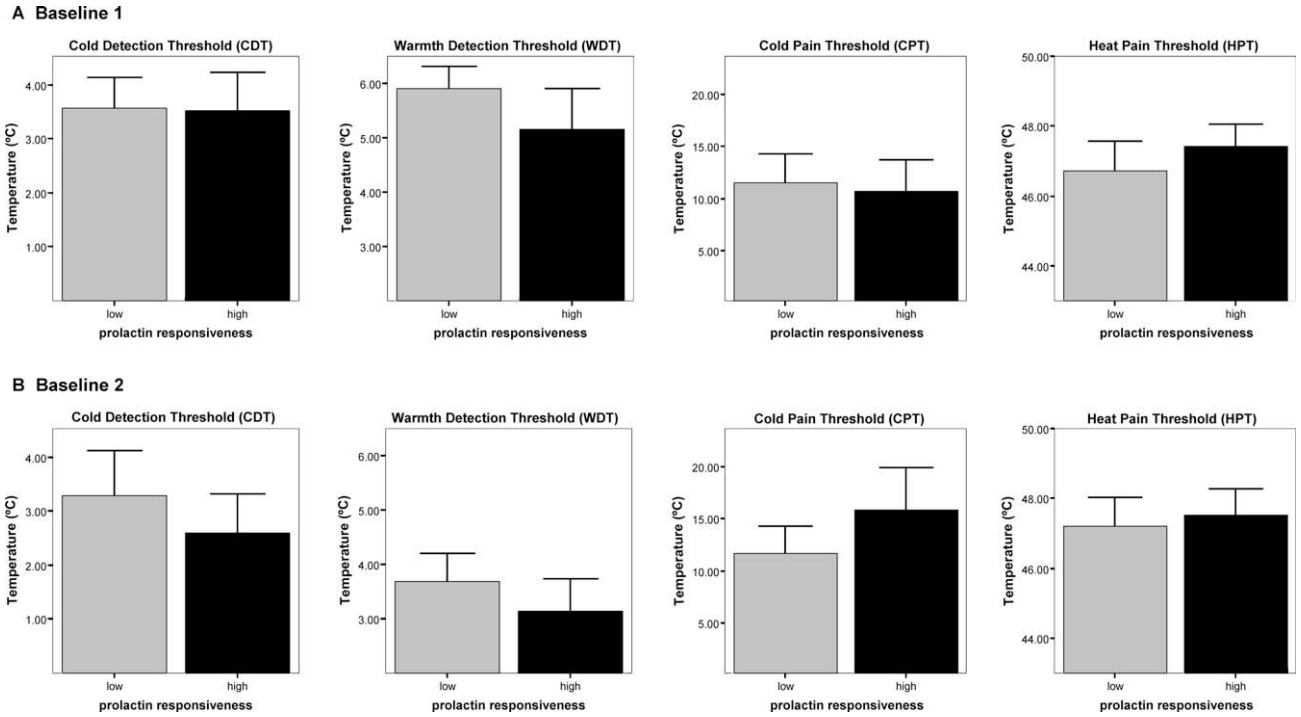

Fig. 3. Comparison of patients characterized by "low" and "high" prolactin responsiveness with regard to measures of thermal threshold testings on baseline 1 (day 2) and baseline 2 (day 6).

Table 2
Comparison of patients with "low" and "high" cortisol/prolactin responsiveness to clomipramine with respect to current pain complaints. ANOVA main effect analyses of between factor "group" were based on df = 1,17.

Variable of the pain questionnaire (PQ)	Cortisol responsiveness			Prolactin responsiveness		
	Low	High	ANOVA: main effect "group"	Low	High	ANOVA: main effect "group"
Number of painful sites (PQ-N)						
Baseline 1 (day 1)						
M	1.0	1.7		0.8	1.9	
SEM	0.5	0.5	$F = 0.599$	0.5	0.5	$F = 1.389$
Baseline 2 (day 5)						
M	0.7	0.9	$p = 0.447$	0.4	1.2	$p = 0.253$
SEM	0.4	0.4		0.3	0.4	
Intensity (PQ-I) (VAS rating in mm)						
Baseline 1 (day 1)						
M	40.5	81.3		35.7	86.7	
SEM	20.8	26.2	$F = 0.715$	23.2	22.1	$F = 1.395$
Baseline 2 (day 5)						
M	29.0	30.9	$p = 0.409$	16.4	44.9	$p = 0.254$
SEM	18.4	12.5		11.8	18.7	
Unpleasantness (PQ-U) (VAS rating in mm)						
Baseline 1 (day 1)						
M	52.0	84.2		39.8	97.8	
SEM	25.1	28.4	$F = 0.340$	26.3	24.0	$F = 0.852$
Baseline 2 (day 5)						
M	38.2	41.4	$p = 0.567$	24.4	56.8	$p = 0.369$
SEM	21.6	18.3		17.4	21.8	

With regard to the measures of detection thresholds for warmth and cold, no such group differences were observed. Therefore, the observed differences in the sensitivity to noxious thermal stimuli among the subgroups appear to be specific to pain and not part of more general changes in somatosensory sensitivity.

In accordance with other studies, failing to demonstrate a relationship between the severity of depressive symptoms and the magnitude of the cortisol response to serotoninergic agents (e.g. O'Keane and Dinan, 1991; Kapitany et al., 1999), our subgroups, defined by the magnitude of cortisol responsiveness, did not show any significant differences regarding subtype and severity of major depression as assessed by well established clinical rating scales. Therefore, we can exclude the possibility that these potentially confounding variables contributed significantly to the differences observed in pain sensitivity between "low" and "high cortisol responders". Furthermore, previous studies failed to show significant relationships between severity of depression and pain sensitivity (e.g. Lautenbacher et al., 1994a,b; Bär et al., 2005).

There was a higher portion of men in the group with increased cortisol responsiveness and pain sensitivity but this difference did

not reach significance. Since men have been repeatedly found to be less sensitive to experimentally induced pain (Riley et al., 1998), the increased pain sensitivity in that group with a slight preponderance of males is not likely due to the sex of the subjects.

On the basis of these findings, we suppose that dysfunctions in the serotoninergic neurotransmission, as indicated by a diminished cortisol response to clomipramine, are involved in mediating a decreased thermal pain sensitivity in depressive patients. In other words, an unresponsiveness of the cortisol system by a serotoninergic agent seems to be associated with a decrease in pain sensitivity in patients with depression. Since central 5-HT often appears to promote pain inhibition (Suzuki et al., 2004), this observed association between a diminished serotoninergic function, as indicated by a blunted cortisol response to clomipramine, and a concurrent decrease of pain sensitivity seems to be paradox at first glance. However, this neuroendocrine pathology marks apparently a profound alteration of neurochemical homeostasis in depression because it subsides during recovery (Bhagwagar et al., 2002; Markianos et al., 2002a,b; Golden et al., 2002). Therefore, it is reasonable to assume that a sub-sample of depressive patients, characterized by this pathology contributes most to the finding of a decreased pain sensitivity. Findings obtained in other psychiatric disorder corroborate this assumption. In patients with borderline personality disorder, which have also been extensively characterized in psychopharmacological challenge studies by such blunted neuroendocrine responses to serotoninergic probes (i.e. predominantly for prolactin, but also for cortisol, Rinne et al., 2000), a reduced pain sensitivity could also be demonstrated (e.g. Schmahl et al., 2008). In accordance to this idea, antidepressive successful treatments as sleep deprivation therapy, which is well-documented in its pro-serotoninergic properties on the level of 5-HT turnover (Asikainen et al., 1997), the firing rate of serotoninergic neurons in the dorsal nucleus raphé (Gardner et al., 1997) and 5-HT receptor functions (Prevot et al., 1996), was found to reverse pain sensitivity towards hyperalgesia in patients with major depression (Kundermann et al., 2008). Interestingly, a recent fMRI study comparing the cerebral responses to thermal painful stimuli between patients with MDD and healthy controls revealed an increased activity in (ventrolateral and dorsolateral) prefrontal areas in the patients, suggesting an inhibitory action of these regions and a hyperactivity in MDD patients (Bär et al., 2007). This may be *a hint for* an involvement of serotoninergic mechanisms given the facts that the prefrontal cortex is critical for pain processing and known for its high density of different 5-HT receptors (Briand et al., 2007), which are alleged to play diverse activation roles in the descending antinociception pathway (Qu et al., 2008). *Taking these considerations* into account, it is reasonable to suggest a more complex role of serotoninergic neurotransmission for pain perception. This notion is supported by agonist studies showing that 5-HT can also produce pronociceptive properties, in which – among the different classes of 5-HT receptors – especially the activation of the 5-HT_{1A} receptor subtype was found to be involved in such actions (Millan, 2002). According to this, one can speculate that serotoninergic dysfunction is directly or indirectly – via interactions with functionally linked neurotransmitter systems – related to reduced pain sensitivity in major depression.

In contrast to our observation that pain sensitivity in patients with MD was significantly related to the cortisol responsiveness to clomipramine, such an association with regard to prolactin responsiveness was not observed in the present study. Apparently, the two measures indicate different aspects of serotoninergic functioning. This view is supported by studies showing an increased (i.e. normalized) cortisol response to serotoninergic agents in the course of recovery (Markianos et al., 2002a,b; Bhagwagar et al., 2002; Kundermann et al., 2009), while the blunted prolactin response did not normalize, probably reflecting a trait-like character or a neurobiological scar.

With regard to clinical pain, a relationship to any of the neuroendocrine measures could not be demonstrated. Therefore our findings regarding pain thresholds were not paralleled by similar results on the side of clinical pain. Although it is tempting to assume a close relationship between experimental pain sensitivity and clinical pain, we have also failed in an earlier study to demonstrate this relationship in patients with depression (Lautenbacher et al., 1999). This does not necessarily mean that there is none but that a cross-sectional analysis is not apt to reveal it. Support for this idea stems from a later longitudinal study, in which increases in pain sensitivity during therapy were accompanied by increases in pain complaints (Kundermann et al., 2008).

A limitation of this study is the failure to include a non-clinical control group. This would have allowed verifying that healthy individuals, when compared with our sample of depressive patients, have an increased neuroendocrine responsiveness to clomipramine, accompanied by decreased thermal thresholds as demonstrated in numerous studies.

A further limitation is the use of clomipramine as a challenging agent for estimating the functional status of the central serotoninergic system, because this agent is known to affect also other neurotransmitter systems which are crucial in modulating pain processing, especially through its pharmacological properties to affect also α-adrenergic receptors and to inhibit – although only to a small extent – the reuptake of noradrenaline (Hyttel, 1994). However, at the beginning of the study, citalopram, described in its usefulness as a 5-HT probe with a high specificity for 5-HT reuptake and a favorable side-effect profile by Seifritz et al. (1996), had not yet been approved in an intravenous application form by the German Federal Institute for Drugs and Medical Devices (BfArM).

Furthermore, the assessment of pain tolerance thresholds in addition to pain thresholds might have allowed collecting data over the whole pain range. However, apart from safety considerations, which did not permit us to test pain sensitivity beyond certain limits that might be still too low for potentially hypoalgesic individuals, numerous studies (e.g. Pickering et al., 2002) showed close relationships between thermal pain thresholds and thermal pain tolerance thresholds. The latter fact makes it unlikely that we missed the essence as regards pain sensitivity, confirming the validity and generalizability *of our results*.

To our knowledge, this is the first study which demonstrates an association between serotoninergic functioning and pain sensitivity in patients with major depression. In summary, we provided evidence that serotoninergic dysfunction, as indicated by a reduced cortisol response to clomipramine, could be involved in a diminished sensitivity to noxious thermal pain to depression.

Contributors

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

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005137; they had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

All authors of the manuscript (B.K., J.H.-S., P.S., S.G., M.T.H., J.-C.K. & S.L.) declare that they have no conflicts of interest.

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