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Differences in FKBP51 Regulation Following Chronic Social Defeat Stress Correlate with Individual Stress Sensitivity: Influence of Paroxetine Treatment

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Various clinical studies have identified FK506-binding protein 51 (FKBP51) as a target gene involved in the development of psychiatric disorders such as depression. Furthermore, FKBP51 has been shown to affect glucocorticoid receptor signaling by sensitivity modulation and it is implicated in stress reactivity as well as in molecular mechanisms of stress vulnerability and resilience. We investigated the physiological, behavioral, and neuroendocrine parameters in an established chronic stress model both directly after stress and after a recovery period of 3 weeks and also studied the efficacy of paroxetine in this model. We then examined FKBP51 mRNA levels in the dorsal and ventral part of the hippocampus and correlated the expression to behavioral and endocrine parameters. We show robust chronic stress effects in physiological, behavioral, and neuroendocrine parameters, which were only slightly affected by paroxetine treatment. On the contrary, paroxetine led to a disruption of the neuroendocrine system. FKBP51 expression was significantly increased directly after the stress period and correlated with behavioral and neuroendocrine parameters. Taken together, we were able to further elucidate the role of FKBP51 in the mechanisms of stress resilience and vulnerability, especially with respect to behavioral and neuroendocrine parameters. These findings strongly support the concept of FKBP51 as a marker for glucocorticoid receptor sensitivity and its involvement in the development of psychiatric disorders.

INTRODUCTION

Chronic social stress is widely regarded as a risk factor for the development of psychiatric pathologies such as depression and anxiety disorders (Chrousos, 2009; McEwen, 2004). Social stress and economic pressure are most common in western societies and largely increase the risk of psycho-pathologies (Tennant, 2001). Animal models of social stress, including chronic social defeat stress (CSDS), are widely used to model this situation in rodents and are accepted models for key clinical symptoms of depression (Savignac *et al*, 2011).

Current treatment strategies for depressed patients focus mostly on the increase of monoamines in the synaptic cleft, for example, via the use of selective serotonin reuptake inhibitors (SSRIs). However, these approaches suffer from the late onset of therapeutic effects, relatively poor response, and high relapse rates (Rush *et al*, 2006). Treatment efficacy can be increased by combinations of different drugs, but convincing success rates are yet to be reached (Thase, 2006). These data highlight the need to continue the search for novel targets in depression research that may lead to more potent yet well-tolerated drugs for the treatment of affective disorders (Berton and Nestler, 2006).

A malfunction of the hypothalamus–pituitary–adrenal (HPA) axis has been strongly implied in the development of mood disorders (de Kloet *et al*, 2005). Chronic HPA axis activation may lead to a disruption of the feedback process, which results in an overshooting stress response and promotes the risk for developing psychiatric diseases. The glucocorticoid receptor (GR) plays a crucial role in these feedback circuits and therefore in the termination of the stress response (Ulrich-Lai and Herman, 2009). In line with these findings, many depressed patients show altered GR signaling (Pariante and Miller, 2001).

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A chaperone-receptor heterocomplex consisting of heat shock protein 90 and, among others, FK506-binding protein 51 (FKBP51) regulates GR signaling by modulating the activation and trafficking of the receptor as well as its gene transcription properties (Pratt et al, 2006). It has been shown that FKBP51 alters ligand binding sensitivity of the GR, reducing nuclear translocation of the GR-complex, and therefore modulating HPA axis feedback sensitivity (Binder, 2009; Wochnik et al, 2005). In a study conducted by Binder et al (2004), significant associations between FKBP51 polymorphisms and depressive episodes as well as anti-depressant responses were shown. In recent years, a growing body of evidence suggests an important role of genetic variants of FKBP51 in stress susceptibility and occurrence of
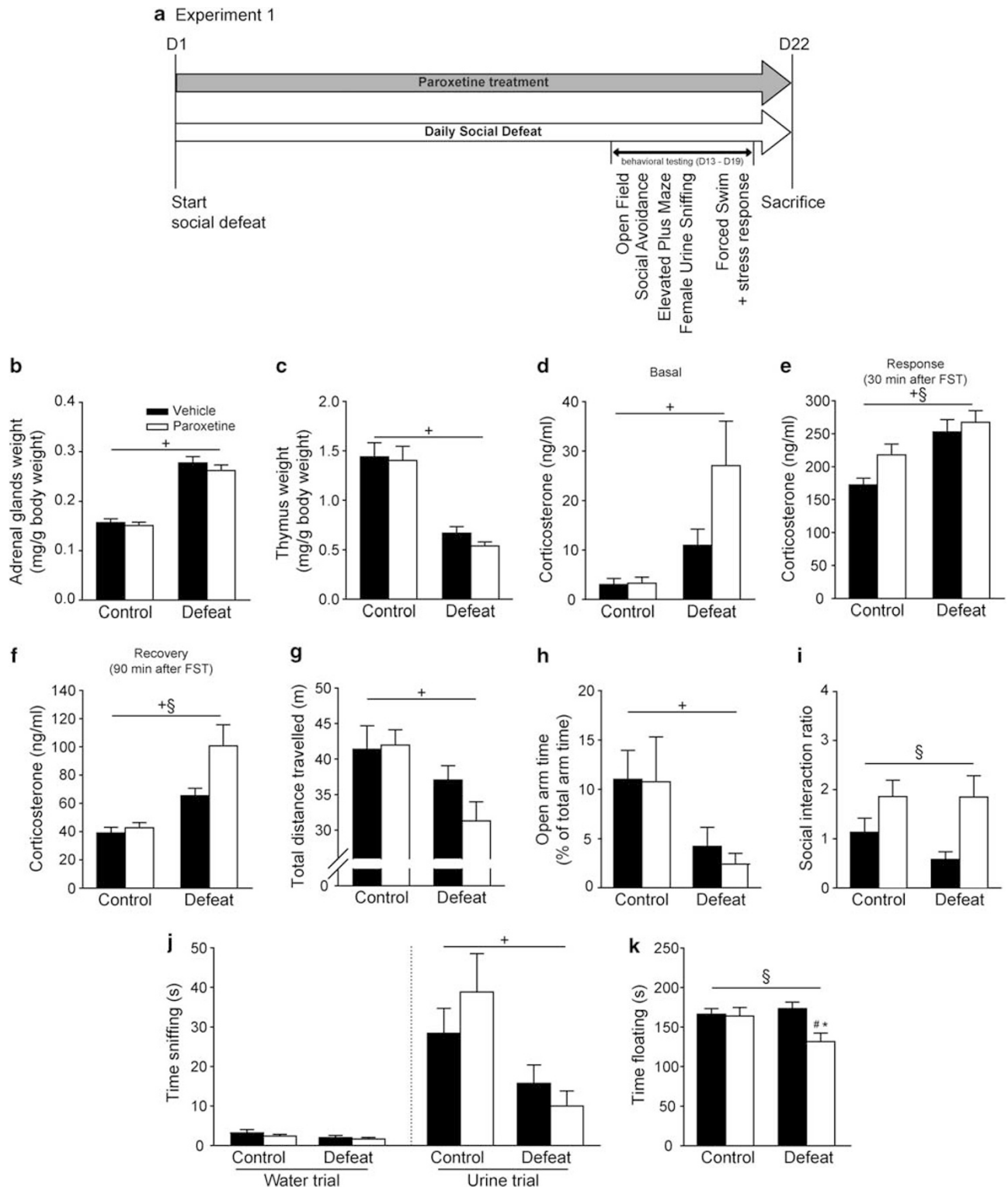
major depression (Ising *et al*, 2008; Zimmermann *et al*, 2011). Other studies could show the involvement of FKBP51 in suicide events (Roy *et al*, 2012) and posttraumatic stress disorder (Sarapas *et al*, 2011).

Further support for an involvement of FKBP51 in stress system regulation comes from animal models. FKBP51 mRNA was upregulated in stress-related brain regions such as the hippocampus in response to acute stressors or a glucocorticoid challenge (Scharf *et al*, 2011). Additionally, FKBP51 knockout mice were reported to show increased active stress coping behavior in the forced swim test (Touma *et al*, 2011) and a resilient phenotype in response to CSDS (Hartmann *et al*, 2012), suggesting a prominent role of FKBP51 in stress coping behavior.

In our study, we aimed to investigate the interaction between FKBP51 and antidepressant treatment in modulating depression-related parameters in male mice. We therefore applied an established chronic stress model and studied its direct and long-term effects on physiology and behavior, neuroendocrine parameters, as well as GR-sensitivity-related mRNA and protein levels. Additionally, we investigated the interactions of a commonly prescribed antidepressant, paroxetine, with chronic stress and FKBP51 regulation, hypothesizing that FKBP51 regulation may support stress resilience.

MATERIALS AND METHODS

Animals and Animal Housing

For all experiments, male C57Bl/6N mice (Charles River Laboratories, Maastricht, The Netherlands) were used. At the beginning of the experiment, the age of all animals was 12 weeks. The mice were held under standard conditions (12:12 light/dark cycle, lights on at 0800 h, temperature $23 \pm 2\,^{\circ}$C), were single housed, and were acclimated to the room for 2 weeks before the beginning of the experiments. Food (Altromin 1314, Altromin GmbH, Germany) and tap water were available *ad libitum*. Male CD1 mice (16–18 weeks of age) served as resident mice, which were held under the conditions described above. They were allowed to habituate to the social defeat cage for 2 weeks before the experiment. All experiments were carried out in the animal facilities of the Max Planck Institute of Psychiatry in Munich, Germany. The experiments were carried out in

accordance with the European Communities' Council Directive 86/609/EEC. All efforts were made to minimize animal suffering during the experiments. The protocols were approved by the committee for the Care and Use of Laboratory Animals of the Government of Upper Bavaria, Germany.

Experimental Design

Experiment 1. In the first experiment, the direct effects of CSDS on various parameters were investigated. A total of 48 mice were randomly split into 2×2 groups (control vehicle ($n = 13$), control paroxetine ($n = 13$), chronic stress vehicle ($n = 11$), and chronic stress paroxetine ($n = 11$)) and subjected to the chronic stress procedure described below. The paroxetine treatment commenced at the first day of the stress procedure and lasted until the day of killing (Figure 1a). All behavioral tests were performed during the third week of the stress procedure.

Experiment 2. In the second experiment, the same parameters that were investigated in experiment 1 were studied after a 3-week period of recovery following the chronic stress exposure (Figure 3a). A total of 64 mice were divided into two groups (control and chronic stress) and they underwent the chronic stress paradigm described below. After cessation of the stressor, both groups were subdivided into vehicle-treated and paroxetine-treated animals ($n = 16$ each). The treatment phase lasted for 3 weeks and all animals independent of condition were handled twice per week. All behavioral tests of experiment 2 took place during the last week of the paroxetine treatment.

Chronic Stress Procedure and Physiological Parameters

The CSDS paradigm lasted for 21 days and was conducted as described previously (Wagner *et al*, 2011). Briefly, the experimental mice were introduced into the home cage (45 cm × 25 cm) of a dominant resident mouse and defeated shortly after. When the defeat was achieved, the animals were separated by a wire mesh, preventing physical but allowing sensory contact for 24 h. Each day, stressed animals were defeated by another unfamiliar, dominant resident mouse in order to exclude a repeated encounter throughout the experiment. The daily defeat was performed

Figure 1 Chronic social defeat stress (CSDS) strongly affects the physiology, behavior, and neuroendocrine profile of mice. (a) Time course of experiment 1: treatment with paroxetine and the chronic stress procedure are performed simultaneously. The behavioral testing is carried out in the last week of the treatment and stress phase. (b, c) ANOVA showed a condition effect in adrenal gland weight ($F_{1,47} = 129.185$, $p < 0.001$) as well as in thymus weight ($F_{1,47} = 53.734$, $p < 0.001$), with chronic stress increasing adrenal gland size and reducing thymus weight, independent of the treatment. (d) Basal corticosterone levels were increased by exposure to CSDS as shown by an ANOVA condition effect ($F_{1,44} = 11.248$, $p < 0.01$). (e) ANOVA revealed both a condition ($F_{1,47} = 19.921$, $p < 0.001$) and a treatment effect ($F_{1,47} = 5.055$, $p < 0.05$) in circulating corticosterone 30 min after an acute stressor. Paroxetine increased hypothalamus–pituitary–adrenal axis (HPA axis) activity already under control conditions. (f) Corticosterone recovery was disrupted in stressed animals, an effect that appears to be increased in mice treated with paroxetine (ANOVA condition ($F_{1,47} = 24.573$, $p < 0.001$), treatment effect ($F_{1,47} = 5.082$, $p < 0.05$)). In both the OF (g) and the EPM (h), ANOVA revealed a condition effect, with a reduced locomotion in the OF ($F_{1,47} = 7.814$, $p < 0.01$) and a reduced time on the open arms in the EPM ($F_{1,43} = 7.534$, $p < 0.01$). (i) Paroxetine increased social interaction in the social avoidance test with no significant effect of CSDS (ANOVA treatment: $F_{1,41} = 8.647$, $p < 0.01$). (j) Anhedonic behavior was increased in stressed mice (urine: ANOVA condition: $F_{1,41} = 8.859$, $p < 0.01$; water: ANOVA condition: $F_{1,41} = 2.114$, $p = 0.154$) and was not ameliorated by antidepressant treatment. (k) In the FST, ANOVA reported both a treatment effect ($F_{1,47} = 5.229$, $p < 0.05$) and a condition × treatment interaction ($F_{1,47} = 4.208$, $p < 0.05$). Here, paroxetine only exhibited antidepressant effects in the forced swim test when combined with CSDS. *Significantly different from control condition of the same treatment group, $p < 0.05$; #Significantly different from vehicle treatment of the same condition group, $p < 0.05$; +Significant condition effect, $p < 0.05$; §Significant treatment effect, $p < 0.05$.

between 1100 and 1600 h; varying starting times reduced the predictability of the stressor and therefore minimized a potential habituation effect. Experimental mice were always defeated by resident males during the entire stress period. Control mice were housed in their home cages during the course of experiment. Both stress and control animals were handled daily during the stress procedure; body weight was assessed at the beginning of the experiment as well as before killing.

In experiment 2, body weight for all mice was assessed at the beginning of the experiment, after the cessation of the stress period, and on the day of killing. Animals that underwent the stress procedure were subsequently single housed in standard cages.

Paroxetine Treatment

Paroxetine was obtained from GlaxoSmithKline (Munich, Germany) as a solution and was diluted in tap water to a final concentration of 0.16 mg/ml. With average water consumption of 5 ml/mouse/day, the daily dose of paroxetine was ~20 mg/kg body weight. Fluid intake was monitored daily and the variation of fluid intake was found to be <10% over the course of the experiment. The chosen dosage has been reported to be effective in chronic stress models (Schmidt et al, 2007) and we confirmed this in a control sample, where paroxetine levels in basal blood plasma were measured (data not shown). In experiment 1, a simultaneous condition × treatment setup was chosen to

provide insights into paroxetine × stress interactions. In experiment 2, paroxetine was administered after the CSDS period to further elucidate the possibly improved recovery from stress.

Behavioral Analysis

The behavioral tests were carried out between 0830 and 1230 h in the same room in which the mice were housed. The testing order was as follows: Open-field (OF), social avoidance (SA), elevated plus-maze (EPM), female urine sniffing test (FUST), forced swim test (FST), and acute stress response. All tests were analyzed using an automated video-tracking system (Anymaze 4.20, Stoelting, Wood Dale, IL). A detailed description of the testing procedures can be found in the Supplementary Information. All animals underwent the same testing battery in the same order of tests. To minimize possible carryover effects of the different behavioral tests, the sequence of tests was arranged from the least stressful to the most stressful (McIlwain et al, 2001).

Sampling Procedure

All animals were killed by decapitation following quick anesthesia by isoflurane at the end of the experiment. Basal trunk blood samples were processed as described above. Brains were removed, snap-frozen in isopentane at $-40\,^\circ$C, and stored at $-80\,^\circ$C for in situ hybridization.
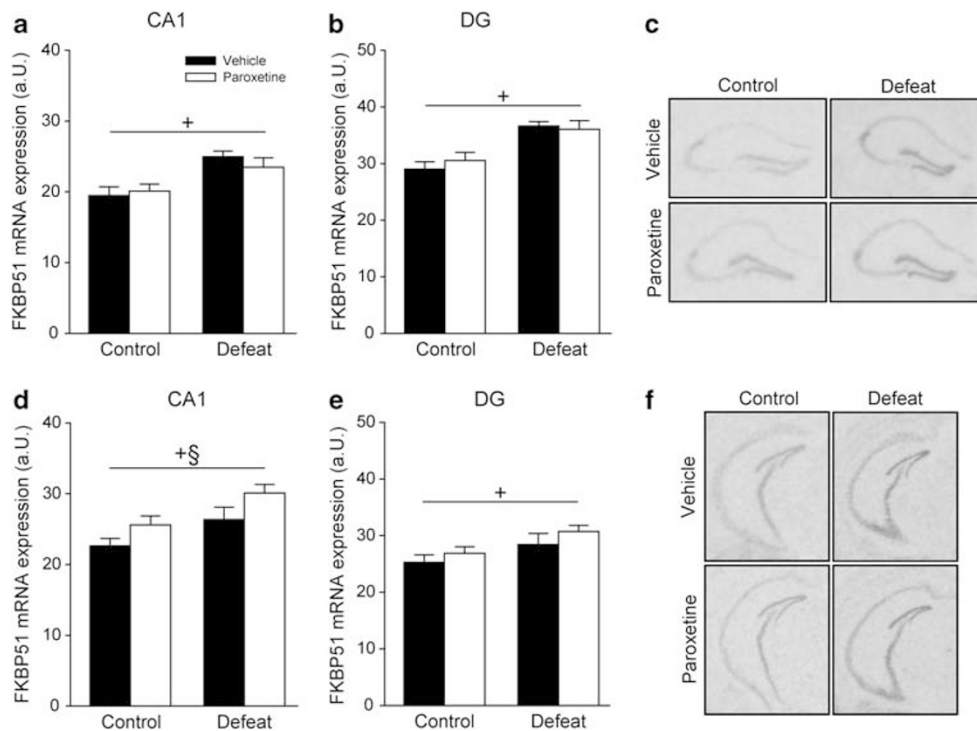
Figure 2 FK506-binding protein 51 (FKBP51) mRNA expression is significantly increased in the hippocampus of stressed animals. (a, b) Both in the CA1 (ANOVA condition: $F_{1,46} = 15.309$, $p < 0.001$) and the dentate gyrus (DG) (ANOVA condition: $F_{1,46} = 24.272$, $p < 0.001$) of the dorsal hippocampus, CSDS increased FKBP51 levels independent of treatment. (c) Representative autoradiographs of FKBP51 mRNA in the dorsal hippocampus. (d, e) Similar to the dorsal hippocampus, FKBP51 expression was increased in the ventral hippocampus of stressed animals (CA1 ANOVA condition: $F_{1,47} = 9888$, $p < 0.01$; DG ANOVA condition: $F_{1,47} = 6.515$, $p < 0.05$), an effect that is slightly more pronounced in animals treated with paroxetine (CA1 ANOVA treatment: $F_{1,47} = 6.524$, $p < 0.05$). (f) Representative autoradiographs of FKBP51 mRNA in the ventral hippocampus. $^+$ Significant condition effect, $p < 0.05$; § Significant treatment effect, $p < 0.05$; other abbreviations as in Figure 1.

Adrenal and thymus glands were removed, dissected from fat, and weighed.

In Situ Hybridization

Frozen brains were sectioned at $-20\,°C$ in a cryostat microtome at $18\,\mu m$, thaw mounted on Super Frost Plus slides, dried, and stored at $-80\,°C$. *In situ* hybridization using ^{35}S UTP-labeled ribonucleotide probes (FKBP51, Metallothionein-1 (MT-1)) was performed as described

previously (Schmidt *et al*, 2007). A detailed protocol can be found in the Supplementary Information section.

Western Blot

An additional cohort of animals (control vehicle *vs* CSDS vehicle, $n = 8$ each) underwent the same CSDS paradigm as in experiment 1 except for the behavioral tests. At 24 h after the last defeat session, animals were deeply anesthetized and quickly decapitated. Hippocampal tissue was extracted
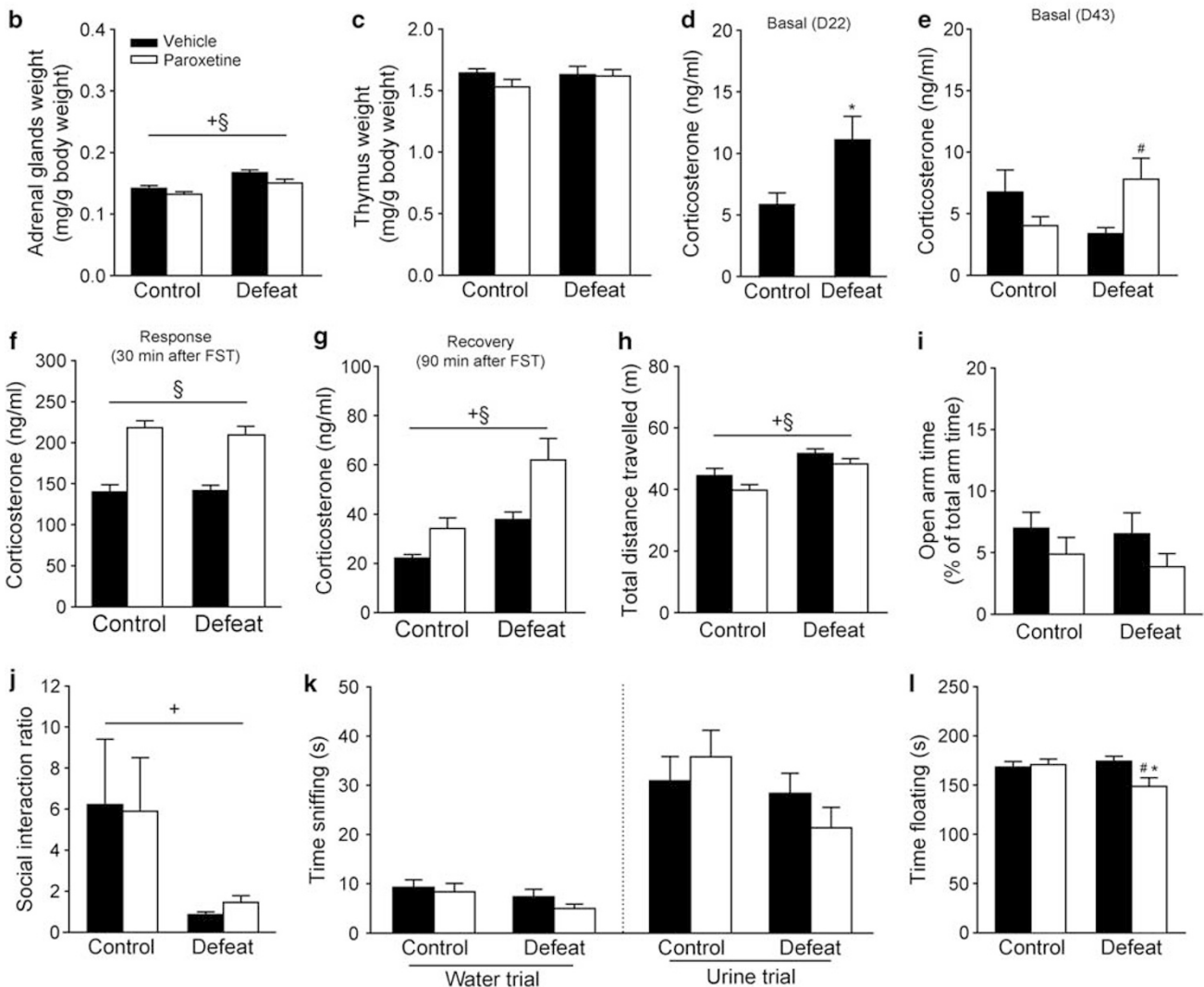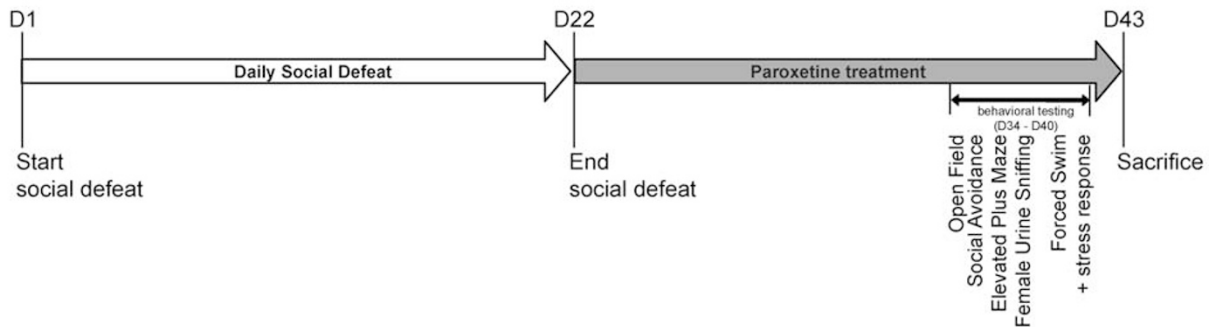
and subcellular fractions (Cytosol, nucleus) were purified using a commercially available kit (Calbiochem ProteoExtract, Merck Millipore). Western blots were then performed as previously described (Wang *et al*, 2011). A detailed description of the protocol is found in the Supplementary Information.

Statistical Analysis

The data presented are shown as means ± SEM, analyzed by the commercially available software SPSS 16.0. Student's *t*-test was employed for comparison of two independent groups. Two-factorial (condition and treatment) ANOVA was employed for all other parameters. Correlations between behavioral parameters and mRNA expression were analyzed with the Pearson product-moment test. A nominal level of significance $P < 0.05$ was accepted and adjusted according to Bonferroni correction by all posteriori tests (univariate F-tests, test of simple effects, or contrasts).

RESULTS

Experiment 1

In the first experiment, we investigated the immediate effects of CSDS and paroxetine by killing the animals 24 h after the last defeat session (Figure 1a).

Physiology. At the beginning of the experiment, no differences in body weight were apparent. Also, there was no effect in body weight gain at the end of the experiment between either groups (control vehicle: 2.17 ± 0.26 g, control paroxetine: 2.78 ± 0.25 g, CSDS vehicle: 2.07 ± 0.35 g, CSDS paroxetine: 2.43 ± 0.26 g). Adrenal glands size was increased and thymus glands size decreased after CSDS, independent of treatment (Figure 1b and c).

Neuroendocrinology. Three weeks of chronic defeat stress increased circulating corticosterone under basal conditions independent of treatment (Figure 1d). In response to a novel stressor, defeated animals showed a significantly increased response to an acute stressor compared with control animals (Figure 1e). Also, paroxetine treatment resulted in an increased corticosterone response compared with vehicle treatment. At 90 min after onset of the acute stressor,

defeated animals showed a significantly diminished ability to recover from the acute stressor (Figure 1f). This effect was largely increased in paroxetine-treated animals.

Behavior. Chronic defeat stress markedly altered the animals' behavior in various tests. Reduced locomotion in the OF and increased anxiety-related behavior in the EPM, depicted by reduced open arm time, were induced by CSDS (Figure 1g and h). Paroxetine did not have any alleviating effect in these tests. In the SA test, a treatment effect was revealed, showing a significant increase in the interaction ratio of paroxetine-treated animals compared with vehicle-treated animals (Figure 1i). A stress-related effect could not be found in this test. Defeated animals showed increased anhedonic behavior, as depicted by reduced sniffing time in the FUST urine trial but not in the water trial (Figure 1j). In the FST, defeated mice that received paroxetine displayed a significantly decreased time in immobile posture compared with both vehicle-treated stress animals and paroxetine-treated control animals (Figure 1k), indicating a more active stress coping behavior.

Gene expression analysis. Investigation of FKBP51 mRNA expression revealed a significant increase in the CA1 and the DG of the dorsal hippocampus in defeated animals compared with controls independent of treatment (Figure 2a–c). In the ventral hippocampus, we found FKBP51 mRNA expression to be upregulated in the CA1 and the DG. Animals treated with paroxetine also showed a slight increase in FKBP51 mRNA levels compared with vehicle-treated animals in the CA1.

Levels of MT-1 mRNA, a known GR-responsive gene, were not regulated by CSDS or paroxetine treatment in the investigated hippocampal regions CA1 and DG (Supplementary Figure S1).

Experiment 2

In the second experiment, we investigated the effects of 3 weeks of recovery from CSDS combined with paroxetine treatment (Figure 3a).

Physiology. Although the initial body weight was not different between control and defeated animals, after 3 weeks, chronically stressed animals showed a significantly

Figure 3 Physiological and neuroendocrine effects of CSDS were still present after a recovery period of 3 weeks but behavioral alterations were mostly restored. (a) Time course of experiment 2: treatment with paroxetine commences after the stress procedure. The behavioral testing is performed in the last week of the treatment phase. (b) Investigation of the adrenal glands' weight revealed a condition ($F_{1,63} = 18.999$, $p < 0.001$) and a treatment effect ($F_{1,63} = 7.000$, $p < 0.01$). Adrenal glands were enlarged in stressed animals, but paroxetine diminished the stress effect. (c) Thymus weight was equal throughout all experimental groups. (d, e) Basal corticosterone levels directly after stress were increased ($T_{62} = -2.488$, $p < 0.05$) and subsequent paroxetine treatment disrupted HPA axis recovery to normal levels (ANOVA condition × treatment interaction ($F_{1,62} = 7.261$, $p < 0.01$)). (f) After challenging the animals with a novel acute stressor, paroxetine-treated mice showed an increased corticosterone response independent of condition in the response (ANOVA treatment: $F_{1,63} = 69.884$, $p < 0.001$). (g) At 2 weeks after cessation, CSDS led to an impaired ability to recover from an acute stressor, an effect that was strongly enhanced by paroxetine (ANOVA condition ($F_{1,63} = 17.708$, $p < 0.001$) and treatment effect ($F_{1,63} = 12.243$, $p < 0.001$)). (h) Stressed animals showed a hyperactive phenotype in the open field (ANOVA condition: $F_{1,63} = 17.028$, $p < 0.001$), whereas paroxetine treatment resulted in less activity (ANOVA treatment: $F_{1,63} = 4.543$, $p < 0.05$). (i, j) Although there was no effect on anxiety-related behavior, social interaction was still disrupted in mice that underwent the CSDS paradigm (ANOVA condition: $F_{1,59} = 5.186$, $p < 0.05$). (k) In the female urine sniffing test, no significant anhedonic effect could be found after recovery from the CSDS. (l) As in experiment 1, paroxetine exerted its antidepressant effects only in conjunction with CSDS as shown in reduced floating time in the forced swim test (ANOVA condition × treatment interaction: $F_{1,63} = 4.568$, $p < 0.05$). *Significantly different from control condition of the same treatment group, $p < 0.05$; [#]Significantly different from vehicle treatment of the same condition group, $p < 0.05$; [+]Significant condition effect, $p < 0.05$; [§]Significant treatment effect, $p < 0.05$; other abbreviations as in Figure 1.

increased body weight gain ($T_{62} = -3.096$, $p < 0.01$, control: 1.80 ± 0.20 g, CSDS: 2.58 ± 0.24 g). On the day of killing, ANOVA revealed a treatment effect ($F_{1,63} = 19.222$, $p < 0.001$) as well as a condition × treatment interaction ($F_{1,63} = 8.227$, $p < 0.01$), with paroxetine-treated mice showing increased body weight gain and vehicle-treated mice that underwent the stress paradigm being heavier than their control littermates (control vehicle: 2.42 ± 0.27 g, control paroxetine: 4.63 ± 0.42 g, CSDS vehicle: 3.88 ± 0.21 g, CSDS paroxetine: 4.34 ± 0.28 g). Stressed animals still showed increased adrenal gland size, with paroxetine-treated animals having a reduced overall adrenal gland weight (Figure 3b). The size of the thymus glands was not significantly altered at the end of the experiment (Figure 3c).

Neuroendocrinology. Basal corticosterone levels were significantly increased directly after the cessation of the stressor on day 22 (Figure 3d). At D43, paroxetine increased circulating corticosterone levels when mice were previously exposed to the chronic defeat paradigm (Figure 3e). In response to a novel acute stressor, paroxetine also largely increased the corticosterone response (Figure 3f), independent of the condition. At 90 min after the acute stressor, defeated animals recovered worse from the acute challenge, depicted in prolonged increased corticosterone levels (Figure 3g). Also, paroxetine-treated animals showed higher corticosterone levels than their vehicle-treated littermates.

Behavior. In the third week of the treatment phase, stressed animals showed increased locomotion in the OF test, with paroxetine animals being less active than their vehicle-treated littermates (Figure 3h). In the EPM, neither a condition nor a treatment effect could be detected (Figure 3i), whereas a preceding CSDS significantly decreased social interaction in the SA test (Figure 3j). Although mice showed increased interest in the urine-dipped cotton swab compared with the water-dipped swab in the FUST, no condition or treatment effect could be revealed in the urine trial (Figure 3k). In the FST, paroxetine-treated mice floated less when previously exposed to the chronic stress paradigm (Figure 3l).

Fkbp51 gene expression. In both the dorsal and the ventral hippocampus, FKBP51 mRNA expression was not influenced by chronic defeat (Supplementary Figure S2).

FKBP51 Correlation Analyses

Correlation analyses were performed in both experiments and significant effects could be found between FKBP51 expression and behavioral and neuroendocrine parameters in the vehicle-treated stress animals of experiment 1 (Figure 4). FKBP51 mRNA expression in the CA1 region of the dorsal hippocampus correlated both with the time struggling in the FST (Figure 4a) and the total distance traveled in the OF directly after cessation of the CSDS (Figure 4c). In the same experimental subgroup, FKBP51 expression in the CA1 of the dorsal hippocampus also correlated with corticosterone values of the acute stress response test (Figure 4e and g). In the DG, FKBP51 mRNA also correlated with the corticosterone values as well as with

the locomotive behavior in the OF (Supplementary Figure S2). These effects were not present after 3 weeks of recovery (Figure 4, right panels). In paroxetine-treated animals, no significant correlations could be shown.

GR Sensitivity

To test whether varying FKBP51 levels would result in an altered GR sensitivity, we also measured the expression of a known GR target gene, MT-1 (Wang *et al*, 2004). MT-1 mRNA expression in the CA1 of the hippocampus was correlated to both FKBP51 levels in the same region (Figure 5a) and struggling time in the FST (Figure 5b). Again, in all other experimental subgroups of experiment 1, no significant correlations could be shown. To further investigate GR sensitivity in response to CSDS, we measured the relative protein levels of GR in the cytosolic and nucleic fraction of hippocampal tissue. Here, GR levels were shifted to the nuclear fraction when subjected to CSDS, compared with GR levels in control animals (Figure 5c and d). Overall levels of GR protein were not significantly different from control animals.

DISCUSSION

In this study, we show an association between FKBP51 and the behavioral and neuroendocrine response to chronic stress. Our CSDS model generated robust changes in physiology, neuroendocrinology, and behavior, both directly after the stress and after a recovery period of 3 weeks. These effects included increased anxiety-related behavior, a disturbed HPA axis function, as well as increased adrenal gland size. Treatment with the commonly used SSRI paroxetine had only small effects in ameliorating the stress-induced phenotype with regard to behavioral changes and deteriorated the neuroendocrine system independent of the time point of the treatment. FKBP51 mRNA expression was increased by CSDS and the level of induction is significantly correlated to both behavioral and neuroendocrine parameters, suggesting an important role of FKBP51 during HPA axis activity and GR sensitivity in a challenging environment. This is further supported by an increased GR translocation to the nucleus in stressed animals as well as FKBP51-correlated expression levels of a downstream target of GR.

The complex immediate phenotype induced by the CSDS model applied in experiment 1 of this study reproduced previous findings to a large extent (Hartmann *et al*, 2012; Wagner *et al*, 2011; Wang *et al*, 2011). An increase in adrenal gland weight is consistently regarded as a reliable marker for a successful chronic stress paradigm (Schmidt *et al*, 2007), whereas body weight alterations in mice seem to underlie more intricate mechanisms, including type and intensity of the stressor as well as stress duration and social status of the animals involved (Bartolomucci *et al*, 2005). However, a tendency to increased body weight after CSDS is in line with previous observations made with this paradigm. Also, HPA axis function was severely disrupted in experiment 1, with an increase in corticosterone release and diminished feedback recovery after an acute stressor (Bartolomucci *et al*, 2005; Schmidt *et al*, 2010). We were
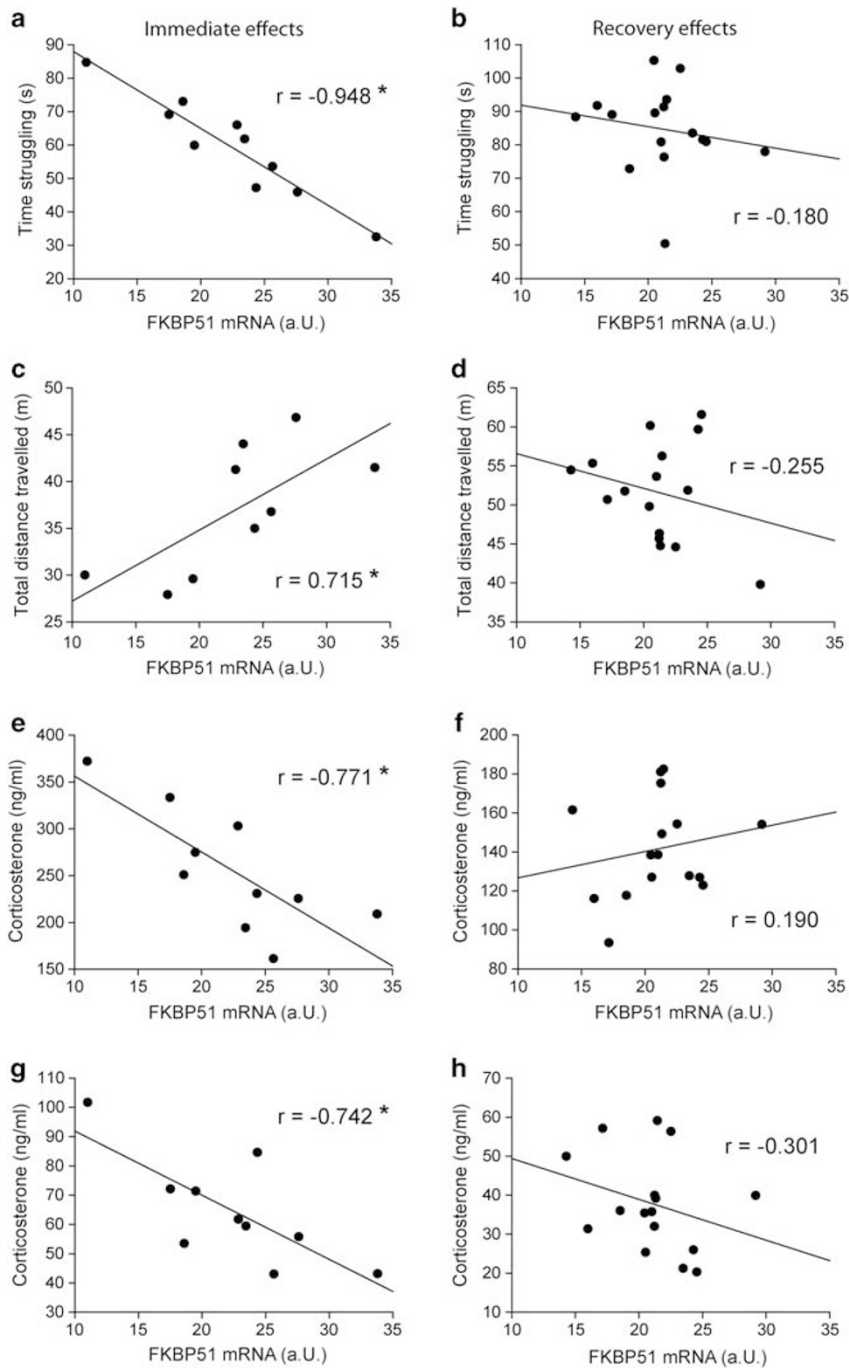
Figure 4 FKBP51 mRNA expression correlates with behavioral and neuroendocrine parameters in stressed animals. (a, b) The stronger the FKBP51 expression levels in the dorsal hippocampus, the lower the time spent with active stress coping in the forced swim test ($r = -0.948$, $p < 0.001$). This effect is only visible in a system activated by CSDS, as there is no significant correlation after the recovery period. (c, d) Although CSDS reduced locomotion in the open field, an enhanced FKBP51 expression counteracts this behavioral phenotype ($r = 0.715$, $p < 0.05$), which is also exclusively visible in an activated system. (e–h) Circulating corticosterone is directly correlated to the increased FKBP51 levels at both the response to an acute stressor and the recovery from it (response: $r = -0.771$, $p < 0.01$; recovery: $r = -0.742$, $p < 0.05$). *Significant correlation, $p < 0.05$; other abbreviations as in Figures 1 and 2.

able to replicate several behavioral phenotypes that have been frequently described, such as disturbed exploratory and social behavior as well as increased anxiety-related and anhedonic behavior (Choleris *et al*, 2001; Berton *et al*, 2006; Hartmann *et al*, 2012; Malkesman *et al*, 2010).

Regarding the long-lasting effects of our CSDS model, which were investigated in experiment 2, most assessed parameters returned to basal levels. We were not able to

show an anxiety-related or anhedonic phenotype, and locomotion was, contrary to the immediate effects of CSDS, slightly increased. This increased explorative behavior after recovery from CSDS may possibly resemble psychomotor agitation (Gupta, 2009). Additionally, a strong social avoidance was still visible in experiment 2, a finding that is in line with previous reports in which the applied CSDS reliably led to a strong aversion toward social targets
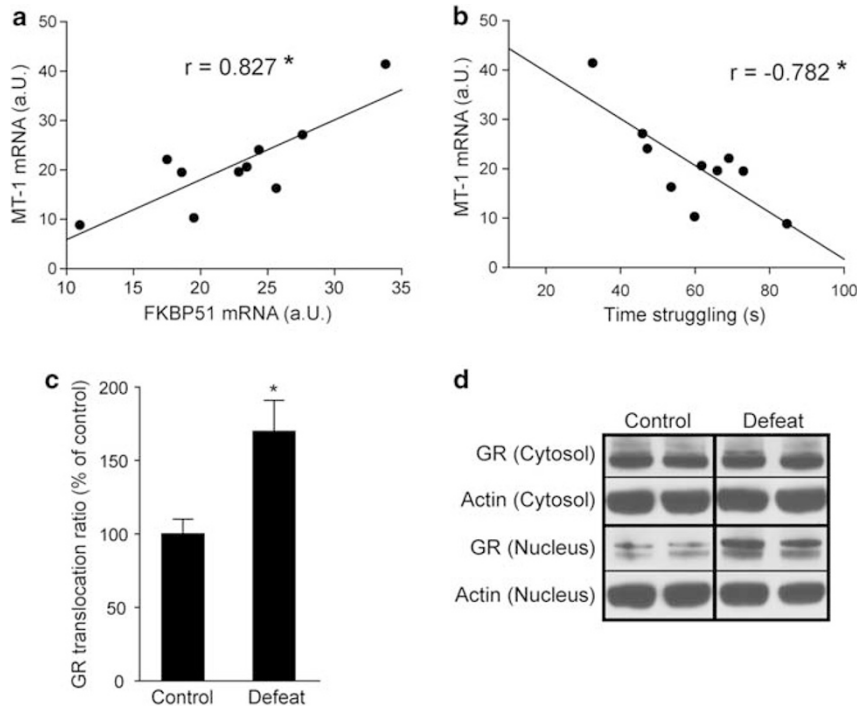
Figure 5 GR sensitivity is increased depending on FKBP51 levels. (a) Metallothionein-1 (MT-1) mRNA levels correlate significantly with FKBP51 levels in the CA1 region of the hippocampus, when animals underwent the CSDS paradigm ($r = 0.827$, $p < 0.01$). As MT-1 is a downstream target of glucocorticoid receptors (GRs), this suggests increased GR sensitivity in response to FKBP51 activation. (b) MT-1 mRNA also correlates to struggling behavior in the FST ($r = -0.782$, $p < 0.01$). (c) Animals that underwent the CSDS paradigm have an increased rate of GR translocation to the nucleus compared with control mice ($T_{14} = -3.113$, $p < 0.01$). (d) Protein bands of GR (94 kD) and actin (42 kD) in the cytosolic and nucleic fraction of hippocampal tissue. *Significant from control, $p < 0.05$; other abbreviations as in Figures 1 and 2.

(Tsankova et al, 2006; Berton et al, 2006). In these studies, important roles of various brain regions in stress resilience, including the nucleus accumbens and the ventral tegmental area, are highlighted, which are likely to play a role in the recovery mechanisms observed in our study (Krishnan and Nestler, 2008). Additionally, although corticosterone levels did not show differences under both basal and challenging conditions, the recovery from an acute stressor was still impaired, suggesting lasting changes in GR feedback mechanisms, possibly in the paraventricular nucleus of the hypothalamus and the prefrontal cortex (Mizoguchi et al, 2003). Taken together, the strong immediate effects of CSDS on physiology, neuroendocrinology, and behavior can mostly be restored by sufficient recovery time, in this case, 21 days. However, some alterations, such as increased social avoidance and diminished HPA axis feedback, are still present and promote the role of CSDS as a risk factor for the development of psychiatric diseases.

Chronic treatment with the SSRI paroxetine was only partly able to ameliorate the various phenotypes evoked by CSDS. Notably, paroxetine treatment led to elevated HPA axis activity and responsiveness as well as to reduced feedback ability independent of the condition. This is surprising as previous studies reported HPA axis normalization after chronic stress exposure when treated with antidepressants (Reul et al, 1993). Chronic paroxetine treatment had a positive effect on social and anhedonic behavior, but did not influence the anxiety-like phenotype observed in the EPM or reduced locomotion in the OF. Previous studies provide inconsistent results concerning the

behavioral effects of SSRIs, with some reporting reduced anxiety (Burghardt et al, 2004) whereas others showing unchanged or even increased anxiety depending on the duration of treatment (Norcross et al, 2008; Kurt et al, 2000). A recent study by Thoeringer et al (2010) could report anxiolytic action of paroxetine only after acute but not chronic administration. In the current study, paroxetine also led to a significant decrease of floating time in the FST when combined with CSDS and increased social behavior, thereby showing positive chronic treatment effects (Sillaber et al, 2008). We therefore conclude that paroxetine treatment in mice, although showing therapeutic efficacy in some parameters, was not able to fully restore the CSDS-induced phenotype. In line with our findings, it has recently been suggested that the behavioral effects of CSDS models are largely independent of the serotonergic system (Venzala et al, 2012). Regarding our study, it can be speculated that these effects might rather be driven by HPA axis activation and sensitivity.

In recent years, it has been shown that FKBP51 plays a major role in stress reactivity and GR-mediated feedback processes that are crucial for a functional HPA axis. We further contribute to these understandings by reporting a distinct increase in FKBP51 expression in response to chronic stress. Additionally, the level of FKBP51 induction in the hippocampus is significantly correlated to the neuroendocrine and behavioral phenotype in a complex manner. In FKBP51 KO mice, Touma et al (2011) reported an increased active stress coping in the FST, which was only present after a strong stressor. In line with these findings,

we here show that higher FKBP51 levels in response to a challenge, in this case CSDS, were correlated to a reduction in active stress coping. These findings can be attributed to a higher GR sensitivity in the presence of low FKBP51 levels. Interestingly, higher FKBP51 levels following CSDS also correlated with higher locomotion in a novel environment. Accordingly, FKBP51 KO mice that underwent the same CSDS paradigm showed a strong reduction in locomotion that even exceeded the stress-induced effect seen in wild-type animals (Hartmann *et al*, 2012).

A modulation in GR signaling and sensitivity has been found in both *in vitro* and *in vivo* studies and is suggested to be an important cofactor for the development of depression (Pariante and Miller, 2001). In line with these findings, increased FKBP51 induction correlated with reduced corticosterone response and recovery values. It has been proposed that the magnitude of FKBP51 induction is a marker of GR sensitivity. Indeed, this has recently been shown for FKBP51 mRNA induction in peripheral blood in humans (Menke *et al*, 2012). In this study, Menke *et al* (2012) could show that a dexamethasone challenge is rapidly increasing FKBP51 mRNA levels in peripheral blood, suggesting a prominent role of FKBP51 in the intracellular short feedback loop to immediately reduce GR sensitivity in response to a stressor (Vermeer *et al*, 2003). Our findings support this hypothesis by showing that FKBP51 mRNA upregulation is connected to neuroendocrine parameters that resemble increased GR sensitivity (Wulsin *et al*, 2010). We could also show an increased GR translocation to the nucleus in stressed animals compared with control littermates, which indicates increased GR signaling processes. An increase in expression of the GR-sensitive gene MT-1 expression has been shown to be induced by GR activity (Wang *et al*, 2004) and was also directly correlated to FKBP51 mRNA levels in the CA1 region of the hippocampus and to struggling time in the FST. However, during *in vivo* processes it is difficult to disentangle the effects of a strong FKBP51 induction, which would indicate a high GR sensitivity and a consequently high FKBP51 expression that would again decrease GR sensitivity. The dynamics of this ultrashort feedback loop are likely also brain region dependent and may explain why FKBP51 expression can correlate with endocrine and behavioral phenotype in apparently opposite directions. Also, although FKBP51 and MT-1 mRNA strongly correlate with coping styles in the FST, there was no main effect of CSDS in this test. This may suggest that individuals challenged by CSDS resort to different molecular coping mechanisms than animals under basal conditions.

In paroxetine-treated animals, FKBP51 expression and the parameters mentioned above were not correlated. At first glance, this is surprising as FKBP51 mRNA induction was equally present in both treatment groups, but significant effects of antidepressants on GR activity and synthesis have been described (Pariante *et al*, 2004; Carvalho and Pariante, 2008). It is therefore likely that extensive paroxetine treatment manipulates the native feedback system to a large extent, which is overruling any regulative effect that FKBP51 may have on GR signaling. This is also reflected in increased plasma corticosterone responses in paroxetine-treated animals, irrespective of the condition (Linthorst and Reul, 2008).

This study also revealed some findings that are not fully in line with previous literature reports. Most prominent, paroxetine treatment was not able to induce more active coping behavior in the FST in control animals: a treatment effect was only detected in mice that previously underwent CSDS. The low efficacy of paroxetine concerning this parameter may be attributed to the application method, the dosage, or the fact that chronic treatment, when compared with an acute treatment with SSRIs, has been reported to elicit reduced behavioral effects (Thoeringer *et al*, 2010). It has also been suggested that C57Bl/6 mice, in contrast to other strains such as CD1 mice, are not as responsive to SSRI treatment in the FST (Petit-Demouliere *et al*, 2005). Another possible confounding factor may be the extensive testing battery that all animals underwent (Blokland *et al*, 2012). Although the order of the tests was chosen to reduce carryover effects to a minimum (McIlwain *et al*, 2001), it cannot be excluded that there is a test × condition interaction. However, it has also been shown that a combination of stressors and different behavioral tests do not necessarily lead to confounding interactions (Chourbaji *et al*, 2008). Furthermore, it has to be pointed out that the measurements of the behavioral and neuroendocrine phenotypes and the mRNA sampling are temporally separated, and hence it cannot be ruled out that the FKBP51 expression levels observed at the time of killing are not the same as at the time of the test. However, it is likely that the inductive effects of CSDS on FKBP51 mRNA levels have reached a steady state by the time the behavioral testing occurs, and thus the levels at the time point of killing can give a meaningful insight into the mechanisms of the individual's stress response.

In summary, we could provide evidence that FKBP51 expression is strongly involved in adaption to chronic stress on both behavioral and neuroendocrine levels. When the stress system is chronically activated due to external challenges, higher FKBP51 levels are closely correlated to a more passive stress coping strategy, possibly because of rapid changes in the short feedback of GR sensitivity. This is indicated by increased GR translocation in stressed animals as well as a correlational increase in a GR-activated downstream target. In conjunction with previous studies, these findings highlight the important role of FKPB51 in the development of stress-associated psychiatric disorders and especially emphasize FKBP51 as a biomarker for GR sensitivity in response to stressful challenges, thus making it a potential target for future treatment options.

DISCLOSURE

Florian Holsboer is a co-inventor of the following pending patent application: FKBP5: a novel target for antidepressant

therapy (International publication number: WO 2005/05450). The other authors declare no conflict of interest.

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