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S100B overexpression increases behavioral and neural plasticity in response to the social environment during adolescence

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ABSTRACT

Genetic variants as well as increased serum levels of the neurotrophic factor S100B are associated with different psychiatric disorders. However, elevated S100B levels are also related to a better therapeutic outcome in psychiatric patients. The functional role of elevated S100B in psychiatric disorders is still unclear. Hence, this study was designed in order to elucidate the differential effects of S100B overexpression in interaction with chronic social stress during adolescence on emotional behavior and adult neurogenesis. S100B transgenic and wild-type mice were housed either in socially stable or unstable environments during adolescence, between postnatal days 28 and 77. In adulthood, anxiety-related behavior in the open field, dark–light, and novelty-induced suppression of feeding test as well as survival of proliferating hippocampal progenitor cells were assessed. S100B transgenic mice revealed significantly reduced anxiety-related behavior in the open field compared to wild-types when reared in stable social conditions. In contrast, when transgenic mice grew up in unstable social conditions, their level of anxiety-related behavior was comparable to the levels of wild-type mice. In addition, S100B overexpressing mice from unstable housing conditions displayed higher numbers of surviving newborn cells in the adult hippocampus which developed into mature neurons. In conclusion, elevated S100B levels increase the susceptibility to environmental stimuli during adolescence resulting in more variable behavioral and neural phenotypes in adulthood. In humans, this increased plasticity might lead to both, enhanced risk for psychiatric disorders in negative environments and improved therapeutic outcome in positive environments.

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1. Introduction

The development of psychiatric disorders is strongly influenced by both, environmental risk factors and individual genetic predispositions (Caspi and Moffitt, 2006). Environmental risk factors such as childhood maltreatment, neglect, or chronic stress are linked to psychiatric disorders especially during sensitive periods of life, for example early childhood or adolescence (McEwen, 2003; Schlossberg et al., 2010; Buwalda et al., 2011). During the last decade, a growing number of genetic variants was found to be associated with an increased risk for mental illness (Sullivan et al., 2012). Among others, single nucleotide polymorphisms (SNPs) in the gene encoding the S100B protein are associated with the psychotic subtype of bipolar disorder (Roche et al., 2007). In carriers of these risk variants increased S100B serum levels are found suggesting that elevated S100B expression might contribute to psychopathology (Hohoff et al., 2010; Dagdan et al., 2011). In contrast, other data suggest a beneficial role of elevated S100B serum levels in patients with major depression. Increased levels of S100B at the time of admission to the hospital are correlated with an improved therapeutic response (Arolt et al., 2003; Jang et al., 2008) as well as normalization of visually evoked event-related potentials (Hetzel et al., 2005) and better word memory processing (Zhang et al., 2009). Thus, the exact functional role of elevated S100B levels in psychiatric disorders remains to be elucidated.

The potentially beneficial role of S100B is supported by several *in vitro* studies. S100B, which is mainly produced and released by astrocytes, exerts neuroprotective and neurotrophic functions (Rothermundt et al., 2003). It enhances the survival of neurons, stimulates neurite outgrowth and supports the development of

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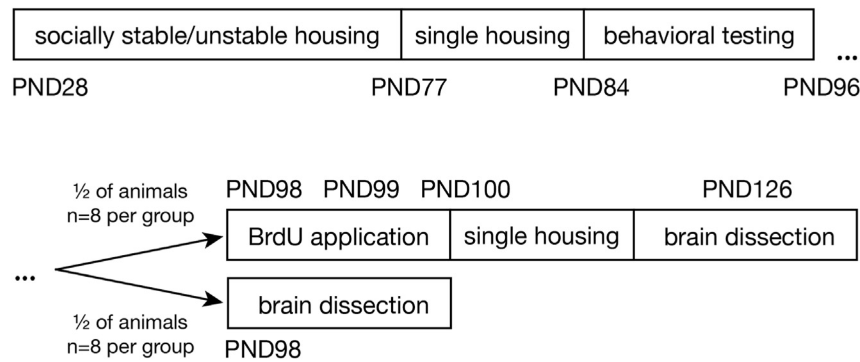

Fig. 1. Time course of the experimental design. PND: postnatal day.

serotoninergic neurons (Alexanian and Bamburg, 1999; Huttunen et al., 2000; Eriksen and Druse, 2001). In an *in vivo* model of traumatic brain injury, intracerebroventricular infusion of S100B improves cognitive recovery and enhances hippocampal neurogenesis (Kleindienst et al., 2004, 2005).

Studies in humans and rodents revealed an association between stress and S100B levels (Gazzolo et al., 2010; Scaccianoce et al., 2004; Diehl et al., 2007). However, the functional role of S100B in interaction with stressful life events for the development of emotional behavior is still elusive. Therefore, the aim of this study was to elucidate the influence of S100B overexpression in a transgenic mouse model in interaction with chronic social stress during adolescence on emotional behavior and adult neurogenesis.

2. Materials and methods

2.1. Animals

For this study transgenic mice of the Tg(huS100B)5Daoh/J strain (The Jackson Laboratory, Bar Harbor, ME, USA) were used in order to reproduce moderately increased S100B levels in psychiatric disorders. These mice carry 8 copies of the human S100B gene which are expressed under control of the endogenous human S100B promotor on a CD-1 genetic background (Friend et al., 1992) resulting in doubled S100B serum levels (see Supplementary material, Fig. S1). Heterozygous male mice were mated with CD-1 wild-type females. A total of 64 male mice of the resulting offspring were analyzed in this study. At postnatal day 21 (PND21), mice were weaned and housed in groups of 4 littermates (2 wild-type and 2 transgenic) in Makrolon type III cages ($38 \times 22 \times 15$ cm^3) with sawdust as bedding material and access to food and water *ad libitum*. Controlled conditions with temperature of 21 °C (±1 °C), humidity of 50% (±10%), and a 12:12 h light–dark cycle (lights on at 6 am) were maintained. The presented work is in accordance with current regulations covering animal experimentation in Germany and the EU (European Communities Council Directive 86/609/EEC). All experiments were approved by the local authority and the "Animal Welfare Officer" of the University of Münster Medical School. All efforts were made to minimize animal suffering as well as to reduce the number of animals used.

2.2. Experimental housing conditions

Wild-type (WT) and S100B transgenic (TG) mice were either housed in a stable social environment (stable) or in an unstable social environment (unstable) during adolescence resulting in 4 experimental groups: WT stable, WT unstable, TG stable, and TG unstable ($n = 16$ per group).

In the stable housing condition, the group composition was not changed until PND77. In the unstable housing condition, group compositions were changed twice a week between PND28 and PND77 to avoid the establishment of stable social hierarchies as described by Schmidt et al. (2007). Briefly, four mice from different cages were put together in a clean cage according to a randomized rotation schedule to minimize the likelihood of a repeated encounter of familiar mice throughout the experiment (see Supplementary material, Table S1). Afterwards, all animals were housed individually in Makrolon type II cages ($25 \times 19 \times 13$ cm^3) to avoid any influences of dominance hierarchies on subsequent behavioral testing (Fig. 1).

2.3. Corticosterone metabolites in fecal samples

In the course of the experiment body weight was assessed and fecal samples were collected for analysis of glucocorticoid metabolites as measure of HPA-axis activity (Touma et al., 2004; Touma and Palme, 2005). In each of the 2nd, 4th, and 6th week of the 7-week experimental housing paradigm one sample was taken. One day after changing cages (stable) or changing group composition (unstable), feces were collected from the bedding material of each cage ($n = 4$ per group). Individual samples were collected after one week of individual housing (PND85), also 24 h after changing cages. Samples were stored at −20 °C until homogenization and extraction of 50 mg powdered feces in 1 ml of 80% methanol. Extracts were analyzed for immunoreactive corticosterone metabolites in a 5α-pregnane-3β,11β,21-triol-20-one enzyme-immunoassay as described before (Ambree et al., 2006; Touma et al., 2003). Briefly, standards and samples were incubated together with steroid antibody and biotinylated label overnight at 4 °C. After washing a streptavidin horseradish peroxidase conjugate was added and incubated for 45 min. Following another wash step, 3,3′,5,5′-Tetramethylbenzidine (TMB) was added as substrate and incubated for 45 min at 4 °C before the reaction was stopped by 1 M sulfuric acid. Finally, the optical density at 450 nm was recorded and concentrations were calculated. The intra- and inter-assay coefficients of variation were 9.1% and 14.0%, respectively.

2.4. Behavioral analysis

After 10 days of habituation to individual housing, the open field test was conducted at PND84. Thereafter, the dark–light test was performed at PND91. Testing was completed by the novelty-induced suppression of feeding test at PND96 (Fig. 1). After each test the apparatus was cleaned with 0.1% acetic acid.

2.4.1. Open field test

The open field test was conducted in 40 × 40 × 40 cm^3 boxes. For analysis of anxiety-related behavior, the floor area was divided into a 20 × 20 cm^2 center and the surrounding wall zone. Brightness at the center of the box was 150 lux. At the beginning of a 10 min test session animals were placed into the wall zone. The location of each animal was recorded using automated tracking software (ANY-maze, Stoelting, Wood Dale).

2.4.2. Dark–light test

The dark–light test was performed in a modified Makrolon type III cage, which was subdivided by a dark PVC wall including a sliding door between the light (2/3 of the cage) and the dark zone (1/3 of the cage). The dark zone was covered by a dark lid, while the light zone was brightly illuminated (190 lux). At the beginning of each test session the animal was placed into the dark zone and allowed to habituate for 15 s before the sliding door was opened and the test session started. Then the mouse could freely explore the whole apparatus and the locomotion of the animal was recorded for 5 min using automated tracking software (ANY-maze, Stoelting, Wood Dale).

2.4.3. Novelty-induced suppression of feeding test

All mice were fed pieces of almond in their home cage for two consecutive days before the test started to habituate them to the food. On the second day, the animals consumed the almond pieces within 1 min. In the test session a piece of almond was placed in the center of the arena, a 50 × 39 × 26 cm^3 unfamiliar plastic box which was brightly illuminated (190 lux). At the beginning of the test a mouse was placed into a corner of the box and the latency to feed on the piece of almond was scored for a maximum of 15 min. The location of the test animal was recorded using automated tracking software (ANY-maze, Stoelting, Wood Dale). The maximum time of 15 min was attributed to animals that did not eat. Freeman–Halton extension of the Fisher's exact test revealed no difference in the numbers of non-eaters between the 4 groups ($p = 0.815$).

2.5. BrdU application and staining

As illustrated in Fig. 1, half of the animals ($n = 8$ per group) received intraperitoneal injections of 5-bromo-2-deoxyuridine (BrdU, 50 mg/kg body weight dissolved in 0.9% NaCl) twice a day for 3 consecutive days beginning at PND98. Four weeks later, at PND126 these animals were deeply anesthetized and transcardially perfused with 0.9% NaCl followed by 4% buffered formalin. Hemispheres were cut in the sagittal plane. Tissue was fixed for 24 h in 4% buffered formalin before it was cryoprotected with 30% sucrose dissolved in PBS. Sagittal sections of 40 μm were cut throughout the hippocampus. Sections were stored in 25% ethylene glycol and 25% glycerol in PBS.

To quantify BrdU-positive cells, every 6th section throughout the hippocampus was rinsed with PBS, incubated in 0.6% H_2O_2 for 30 min, rinsed again, then treated with 2 M HCl for 30 min at 37 °C to denature DNA, and neutralized in 0.1 M borax for 10 min. Sections were rinsed in PBS again and blocked with PBS+ containing 0.5% Triton X-100 and 1.5% normal rabbit serum for 60 min, before they were incubated in monoclonal rat anti-BrdU (1:500, OBT-0030, AbDserotec) overnight at 4 °C. The next day sections were rinsed extensively in PBS and incubated for 60 min in biotinylated rabbit anti-rat antibody (1:200, Vector Laboratories). After rinsing in PBS, sections were incubated for 30 min in Vectastain ABC (Vector Laboratories). Sections were rinsed again and peroxidase immunolabeling was done by incubating slices in DAB (Vector Laboratories). BrdU-positive nuclei were counted in the granular cell layer (GCL) and subgranular zone (SGZ) defined as the two-cell layer wide area between the GCL and the hilus using an inverse light microscope (Olympus, IX-81). Counted cells were multiplied by six to estimate the total numbers of BrdU labeled cells in the whole dentate gyrus and by two for both hemispheres.

To assess the neuronal differentiation of newborn cells, every 12th section of five randomly chosen animals per group was stained for BrdU and NeuN. In addition to the single staining protocol, sections were incubated with 50% formamide/2× SSC solution for 2 h at 65 °C followed by 2× SSC for 15 min prior to DNA denaturation. Primary antibody mix contained rat anti-BrdU (OBT-0030, AbDserotec, 1:300) and mouse anti-NeuN (MAB377, Millipore, 1:200), secondary antibody mix Alexa Fluor A488 anti-rat and A568 anti-mouse (Life Technologies, 1:300 each). Sections were analyzed using a confocal laser-scanning microscope (Zeiss, LSM 700). For each animal 50 randomly chosen BrdU-positive cells were analyzed for co-localization with NeuN and the percentages of newborn neurons were calculated.

2.6. Gene expression analysis

At the end of the behavioral experiments at PND98, half of the animals ($n = 8$ per group) were anesthetized with isoflurane and immediately decapitated. Brains were rapidly removed, the hippocampi were dissected, immediately snap-frozen in liquid nitrogen, and stored at −80 °C. Isolated hippocampi were homogenized and total RNA was extracted using Trizol Reagent following manufacturer's instructions (life technologies). RNA was cleaned and DNase treated (PureLink RNA Mini Kit, life technologies). Purity of RNA samples was assessed by photometric analysis of A260/A280 ratios.

For TaqMan gene expression assays, cDNA was synthesized from 400 ng RNA of each animal using the High Capacity cDNA RT Kit (life technologies). Assays were run in triplicate with TaqMan Gene Expression Master Mix and inventoried assays (life technologies) for the following genes according to the manufacturer's instructions (life technologies): *Nr3c1* (Mm00433832_m1), *Fkbp5* (Mm00487401_m1), *Bcl2* (Mm00477631_m1), and *Bdnf* (Mm04230607_s1). *Pgk1* (Mm00435617_m1) expression was determined as reference gene for calculation of ΔCt. Fold changes were calculated as $2^{-\Delta\Delta Ct}$ and normalized to the wild-type stable group.

2.7. Statistical analyses

All data sets were checked for normal distribution by one-sample Kolmogorov–Smirnov test. There was no significant deviation from normal distribution and data were analyzed with two by two factorial ANOVA using environment and genotype as between subject factors. When repeated measures were assessed, time was included as within subject factor. To compare only two groups, student's *t*-test was calculated. Statistical tests were computed using SPSS (version 20, IBM). The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Body weight

At the beginning of the experiment (PND28), all animals weighed about 22 g. Weight gain over time (T) was significantly influenced by the environment (E) during adolescence. Animals from unstable housing gained less weight than animals from stable housing (Fig. 2A, T × E: $F_{(2.9,\ 176)} = 4.217$, $p = 0.007$). Weight gain was not significantly influenced by genotype (G) or genotype–environment interaction (T × G: $F_{(2.9,\ 176)} = 1.961$, $p = 0.123$; T × G × E: $F_{(2.9,\ 176)} = 0.615$, $p = 0.603$).

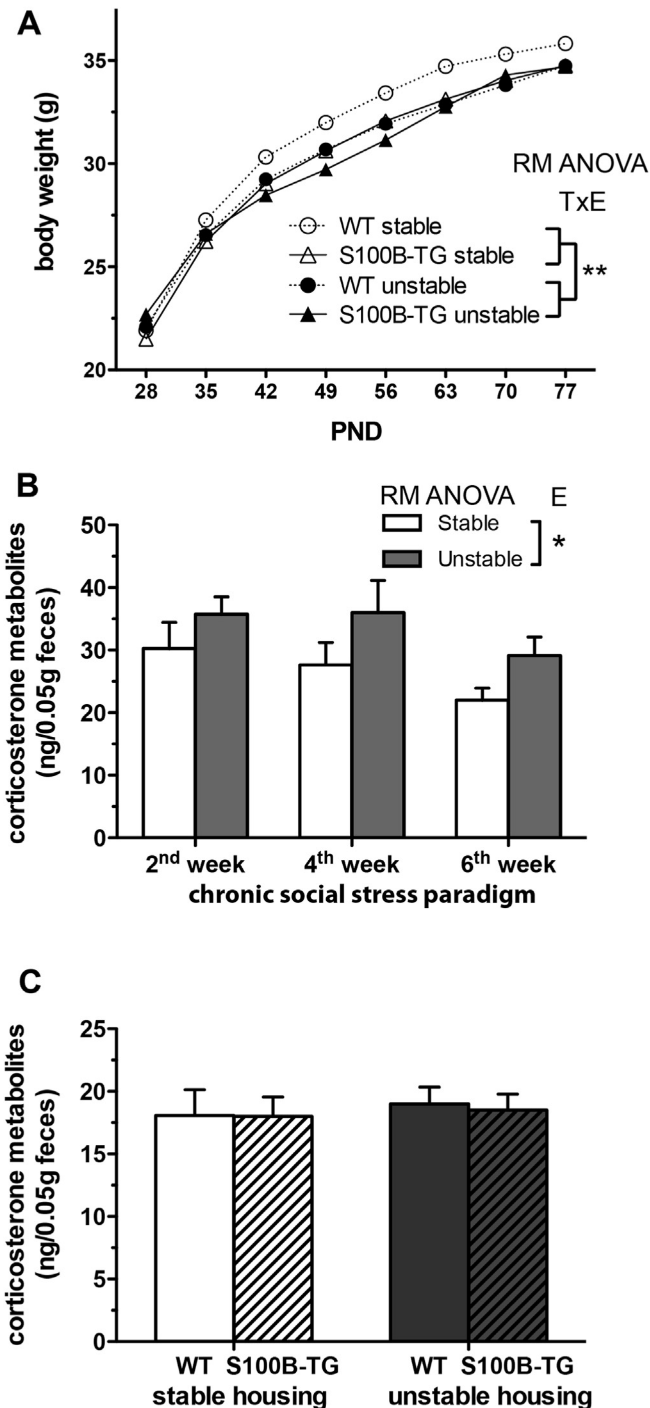

Fig. 2. Physiological correlates of the stress response. (A) Body weight gain during the chronic social stress paradigm. Weight gain was significantly influenced by the environment. (B) Fecal corticosterone metabolite concentrations after 2, 4, and 6 weeks of the chronic social stress paradigm revealed significantly increased levels in mice from unstable housing. (C) Fecal corticosterone metabolites measured one week after cessation of the stress paradigm did not differ between the groups. WT: wild-type mice, S100B-TG: S100B transgenic mice, PND: postnatal day, E: environmental effect, T × E: time–environment interaction, *: $p < 0.05$, **: $p < 0.01$.

3.2. Corticosterone metabolites

Mice from unstable housing had significantly higher corticosterone metabolite concentrations compared to mice from stable conditions during adolescence (Fig. 2B, E: $F_{(1, 42)} = 5.701$, $p = 0.022$). Concerning the course of the stress paradigm, there was a trend for a variation in fecal corticosterone metabolites over time ($F_{(2, 42)} = 2.477$, $p = 0.096$), but there was no significant interaction of time course and environment (T × E: $F_{(2, 42)} = 0.081$, $p = 0.923$). Individual corticosterone metabolite concentrations in adulthood did not differ (Fig. 2C, E: $F_{(1, 60)} = 0.203$, $p = 0.654$; G: $F_{(1, 60)} = 0.031$, $p = 0.861$; G × E: $F_{(1, 60)} = 0.019$, $p = 0.891$).

3.3. Locomotor behavior

In the open field test there were no differences regarding the total distance traveled during the 10 min test period (Fig. 3A; E: $F_{(1, 60)} = 1.729$, $p = 0.194$; G: $F_{(1, 60)} = 0.181$, $p = 0.672$; G × E: $F_{(1, 60)} = 1.201$, $p = 0.278$). Analyzing the distance in 1 min intervals (Fig. 3B), there was a significantly altered course of locomotor activity over time between the environments (T × E: $F_{(4.5, 269)} = 4.712$, $p < 0.001$). In particular, social instability significantly reduced locomotor activity in the first 60 s for both genotypes (Fig. 3C; E: $F_{(1, 60)} = 9.726$, $p = 0.003$) while there were no differences concerning genotype or a gene–environment interaction (G: $F_{(1, 60)} = 0.619$, $p = 0.434$; G × E: $F_{(1, 60)} = 0.002$, $p = 0.963$).

A similar effect could be observed in the novelty-induced suppression of feeding test (Fig. 3D). Mice from unstable housing showed significantly less locomotor activity in the first minute of the test regardless of their genotype (E: $F_{(1, 60)} = 8.792$, $p = 0.004$, G: $F_{(1, 60)} = 0.021$, $p = 0.884$, G × E: $F_{(1, 60)} = 0.448$, $p = 0.506$).

3.4. Anxiety-related behavior

There was no effect of genotype on the center time in the open field test (Fig. 4A, G: $F_{(1, 60)} = 0.982$, $p = 0.326$), but a strong trend that mice from unstable housing spent less time in the center than mice from stable housing (E: $F_{(1, 60)} = 3.865$, $p = 0.054$). Moreover, there was a significant gene–environment interaction (G × E: $F_{(1, 60)} = 6.480$, $p = 0.013$), showing that S100B transgenic mice from stable housing spent significantly more time in the center than wild-type mice from the same housing condition ($t = -2.521$, $p = 0.017$) or S100B transgenic mice from unstable housing ($t = 3.263$, $p = 0.003$). While the center entries showed a similar pattern (Fig. 4B), they did not differ statistically (G: $F_{(1, 60)} = 0.166$, $p = 0.685$, E: $F_{(1, 60)} = 1.019$, $p = 0.317$, G × E: $F_{(1, 60)} = 2.057$, $p = 0.157$).

In the dark–light test the time in the light zone revealed again a similar, but non-significant pattern (Fig. 4C, G: $F_{(1, 60)} = 0.570$, $p = 0.453$, E: $F_{(1, 60)} = 0.379$, $p = 0.541$, G × E: $F_{(1, 60)} = 0.205$, $p = 0.652$). The number of light zone entries did not differ between the groups, either (Fig. 4D, G: $F_{(1, 60)} = 0.580$, $p = 0.449$, E: $F_{(1, 60)} = 1.960$, $p = 0.167$, G × E: $F_{(1, 60)} = 0.005$, $p = 0.942$). Animals from unstable housing showed a tendency for increased latencies until the first entry into the light zone compared to stable housing (Fig. 4E, E: $F_{(1, 60)} = 2.810$, $p = 0.099$). There were no differences with regard to genotypes or a gene–environment interaction (G: $F_{(1, 60)} = 0.873$, $p = 0.354$, G × E: $F_{(1, 60)} = 0.616$, $p = 0.436$). In the novelty-induced suppression of feeding test the latency to feed did also not differ between the groups (Fig. 4F, G: $F_{(1, 60)} = 0.462$, $p = 0.499$, E: $F_{(1, 60)} = 0.017$, $p = 0.896$, G × E: $F_{(1, 60)} = 1.048$, $p = 0.310$).

3.5. Newborn cell survival and differentiation in the dentate gyrus

Mice from unstable housing showed a tendency for more BrdU-positive cells in the granular cell layer (GCL) plus the subgranular zone (SGZ) (Fig. 5H, E: $F_{(1, 27)} = 3.057$, $p = 0.092$). There were neither genotype differences (G: $F_{(1, 27)} = 0.353$, $p = 0.557$) nor a gene–environment interaction (G × E: $F_{(1, 27)} = 0.548$, $p = 0.466$). The difference between the housing environments was due to a significantly increased number of BrdU-positive cells in the GCL in

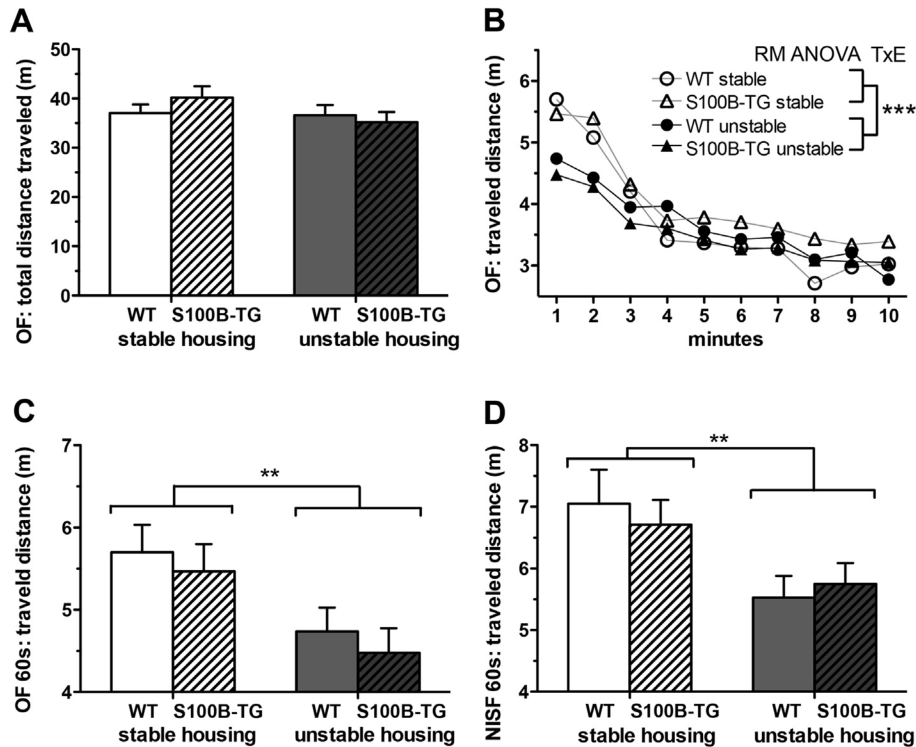

Fig. 3. Locomotor behavior. (A) Total distance traveled in the open field during the 10 min test period did not differ between the groups. (B) Course of the traveled distance in the open field in 1 min intervals was significantly different in mice from the two housing conditions. (C) Distance traveled during the first minute in the open field test revealed significantly less locomotion in mice from the unstable environment. (D) Mice from unstable housing showed significantly decreased distance traveled during the first minute of the novelty-induced suppression of feeding test. OF: open field test, NISF: novelty-induced suppression of feeding test, WT: wild-type mice, S100B-TG: S100B transgenic mice, T × E: time–environment interaction, **: $p < 0.01$, ***: $p < 0.001$.

mice from unstable housing (Fig. 5I, E: $F_{(1, 27)} = 5.730$, $p = 0.024$). The number of BrdU-positive cells in the GCL was markedly higher in S100B transgenic mice than in wild-types from unstable housing reflected by a trend for a gene–environment interaction (G × E: $F_{(1, 27)} = 3.198$, $p = 0.085$). *Post-hoc* analysis revealed a significantly increased number of surviving newborn cells in S100B transgenic mice from unstable housing compared to S100B transgenic mice from stable housing ($t = -2.268$, $p = 0.049$), whereas there was no difference between the wild-types ($t = -0.908$, $p = 0.379$). The genotypes did not differ (G: $F_{(1, 27)} = 1.355$, $p = 0.255$). Regarding the number of BrdU-positive cells in the SGZ, there were no significant differences (G: $F_{(1, 27)} = 0.054$, $p = 0.818$, E: $F_{(1, 27)} = 0.071$, $p = 0.792$, G × E: $F_{(1, 27)} = 0.360$, $p = 0.554$). With regard to the differentiation of the newborn cells, in all groups about 80% of BrdU-positive cells were double labeled with NeuN, a marker of mature neurons (Fig. 5E–G and J). There were no differences between the groups in the percentage of neuronal differentiation (G: $F_{(1, 16)} = 1.659$, $p = 0.216$, E: $F_{(1, 16)} = 0.091$, $p = 0.767$, G × E: $F_{(1, 16)} = 0.333$, $p = 0.572$).

3.6. Hippocampal gene expression

Four genes that are involved in HPA-axis regulation (*Nr3c1*, *Fkbp5*) and neurotrophic function (*Bcl2*, *Bdnf*) were analyzed by TaqMan assays (Fig. 6). A strong trend for increased *Fkbp5* levels in S100B transgenic mice was found (G: $F_{(1, 24)} = 3.894$, $p = 0.060$), while there were no differences between the housing conditions (E: $F_{(1, 24)} = 0.177$, $p = 0.678$) or a gene–environment interaction (G × E: $F_{(1, 24)} = 2.169$, $p = 0.154$). With regard to the expression of the other candidate genes, there were no significant differences (Table 1).

4. Discussion

The aim of this study was to analyze the effects of moderately elevated S100B expression on emotional behavior and adult neurogenesis in mice that were reared either in stable or unstable housing conditions during adolescence. The main findings were: (1) in S100B transgenic mice, stable housing reduced anxiety-related behavior in adulthood whereas unstable housing conditions contrarily increased anxiety-related behavior; (2) unstable housing during adolescence enhanced the number of surviving newborn cells in the granular cell layer of the adult hippocampus in S100B transgenic mice.

Animals from unstable housing conditions showed elevated corticosterone metabolites and reduced weight gain during adolescence compared to the stably housed group. These parameters represent an increased physiological stress response, as it has been described in response to other chronic stressors (Ibarguen-Vargas et al., 2008), and indicate that social instability during adolescence was an effective chronic stressor. It is of interest to note that even in unstable social conditions some animals might have held a prevailing dominant or subordinate status. Although it is possible that these animals reacted differently to social stress as it has been shown before (Bartolomucci et al., 2005), there were no indications for this in terms of an increased variability in behavioral data of mice from unstable housing. Chronic social stress during adolescence resulted in initially reduced exploratory behavior in the first minute of the open field and the novelty-induced suppression of feeding test, a tendency for reduced time in the center of the open field, as well as an increased latency to enter the light zone in the dark–light test. A similar housing effect was reported for different behavioral and endocrinological measures in wild-

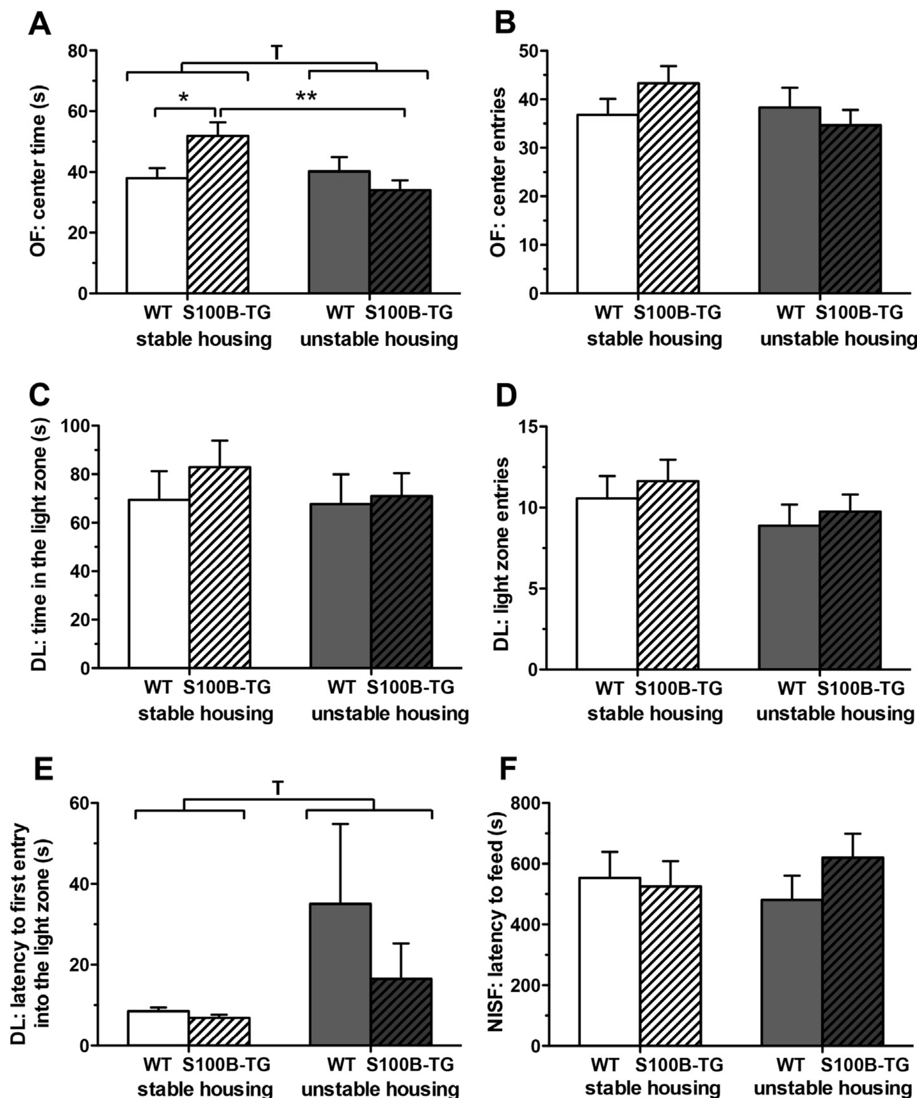

Fig. 4. Anxiety-related behavior. (A) Center time in the open field test. There was a significant gene–environment interaction revealing that S100B transgenic mice from stable housing spent significantly more time in the center zone than wild-type mice from the same housing and S100B transgenic mice reared in unstable housing. (B) Center entries in the open field test. There were no significant differences for the four experimental groups. (C) Time in the light zone in the dark–light test. No significant differences were found between the four different groups. (D) Light zone entries in the dark–light test. All four experimental groups did not differ. (E) Latency to first entry into the light zone in the dark–light test. Mice of both genotypes from unstable housing showed a trend for higher latencies compared to mice from stable housing. (F) Latency to feed in the novelty-induced suppression of feeding test. No differences between the four experimental groups were found. OF: open field test, DL: dark–light test, NISF: novelty-induced suppression of feeding test, WT: wild-type mice, S100B-TG: S100B transgenic mice, Trend (T): $0.05 < T < 0.1$, *: $p < 0.05$, **: $p < 0.01$.

type mice in previous studies (Schmidt et al., 2007; Sterlemann et al., 2008).

S100B overexpression induced distinct effects dependent on the presence of social stress during adolescence. S100B transgenic mice from stable housing showed reduced levels of anxiety-related behavior compared to all other groups. Although this effect was significant in only one measure of anxiety-related behavior, similar non-significant tendencies can possibly be attributed to an increased variance due to repeated testing experience which was shown to result in reduced exploratory activity and emotionality (Voikar et al., 2004). In contrast to stable housing, S100B transgenics from unstable housing showed comparable levels of anxiety-related behavior to wild-type mice. Altered behavioral profiles after the presence or absence of chronic social stress during adolescence might be based on an increased developmental plasticity in the programming of adult emotional behavior (Brown and Spencer, 2013; Macri, 2012) and represent an adaptive response to face current challenges in different social environments (Sachser et al., 2011).

In addition to altered anxiety-related behavior, adult S100B transgenic mice from unstable housing showed increased survival of proliferating cells in the granular cell layer (GCL) of the dentate gyrus. The GCL is especially important as maturing newborn neurons migrate from the subgranular zone into this cell layer to integrate into the existing neural network (Deng et al., 2010). Indeed confocal analysis of double labeled BrdU-positive cells with the neuronal marker NeuN revealed that more than 80% of these cells became mature neurons already four weeks after labeling implicating an increased number of newborn neurons in S100B transgenic mice from unstable housing. At first glance, the increased survival of newborn neurons in the adult hippocampus in S100B transgenic mice from unstable housing seems surprising as stress is usually associated with a decrease in the proliferation of progenitor cells (Dranovsky and Hen, 2006). However, recent

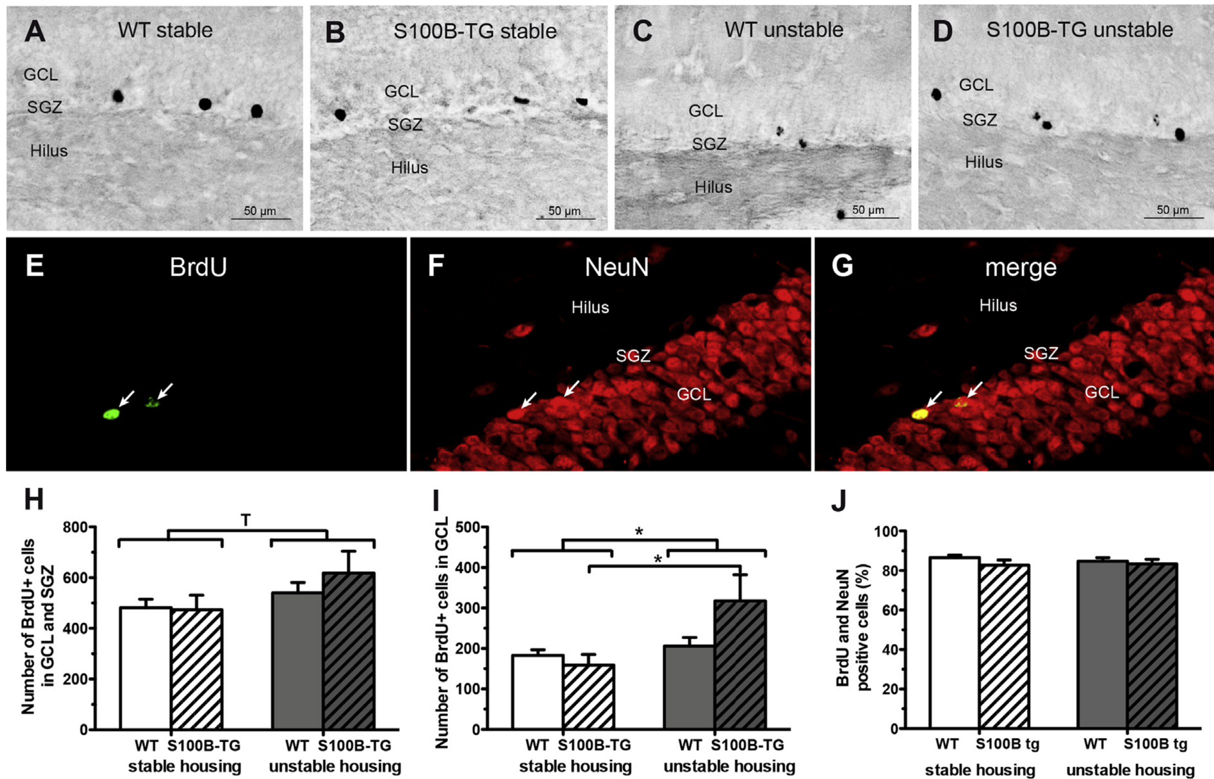

Fig. 5. Adult neurogenesis. (A–D) Representative immunostainings of BrdU-positive cells in the dentate gyrus for each experimental group. (E–G) Confocal image of BrdU (E, green) and NeuN (F, red) staining in the dentate gyrus of the hippocampus. Arrows indicate two double labeled cells which were considered as neurons. Co-localization appears yellow in the merged image (G). (H) The number of BrdU-positive cells in the GCL and SGZ together showed a trend for increased survival of newborn cells in mice from unstable housing. (I) In the GCL the number of BrdU-positive cells was significantly increased in mice from unstable housing. *Post-hoc* analysis revealed significantly higher numbers of newborn cells in S100B transgenic mice from unstable compared to stable housing. (J) Percentage of BrdU and NeuN double labeled cells in the GCL and SGZ did not differ between the groups. All groups showed about 80% double labeled cells. GCL: granular cell layer, SGZ: subgranular zone, WT: wild-type mice, S100B-TG: S100B transgenic mice, Trend (T): $0.05 < T < 0.1$, *: $p < 0.05$.

studies have shown that stress can even enhance adult neurogenesis especially during predictable stress (Lyons et al., 2010; Parihar et al., 2011) or after the cessation of the stressor (Lagace et al., 2010). The latter is in line with our study in which mice were exposed to the social stressor during adolescence while cell proliferation was analyzed in adulthood. Lagace et al. have reported that animals with an increased cell survival show sustained behavioral alterations after stress (Lagace et al., 2010). This is comparable to elevated levels of anxiety-related behavior in adult S100B transgenic mice from unstable housing that was accompanied by a marked increase in hippocampal neurogenesis.

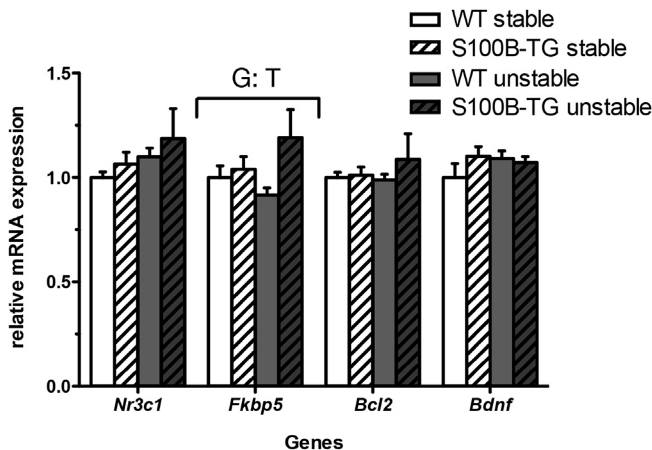

Fig. 6. Hippocampal gene expression. Relative mRNA expression of two genes related to HPA-axis regulation (*Nr3c1* and *Fkbp5*) and two genes involved in neurotrophic functioning (*Bcl2* and *Bdnf*). There was a trend for increased *Fkbp5* levels in S100B transgenic mice while there were no differences with regard to the other analyzed gene expression levels. WT: wild-type mice, S100B-TG: S100B transgenic.

On the molecular level, the expression of genes which are involved in HPA-axis regulation (*Nr3c1*, *Fkbp5*) or neurotrophic functions (*Bcl2*, *Bdnf*) revealed no clear evidence for a contribution of these candidates to the observed behavioral and neural changes. Merely the increased expression of the glucocorticoid receptor co-chaperone FK506-binding protein 51 (encoded by *Fkbp5*) in S100B transgenic mice could indicate an altered glucocorticoid receptor signaling (Binder, 2009; Touma et al., 2011). However, it is likely that further mechanisms contribute to altered anxiety-related behavior and adult neurogenesis in S100B transgenic mice. For instance, the release of S100B could stimulate prolactin secretion

Table 1
Statistics of gene expression analysis.

Gene	Genotype effect (G)		Environment effect (E)		Genotype–environment interaction (G × E)	
	$F_{(1, 24)}$	p	$F_{(1, 24)}$	p	$F_{(1, 24)}$	p
Nr3c1	0.910	0.350	1.971	0.173	0.020	0.888
Fkbp5	3.894	0.060	0.177	0.678	2.169	0.154
Bcl2	0.696	0.412	0.239	0.630	0.432	0.517
Bdnf	0.801	0.380	0.450	0.509	1.720	0.202

(Ishikawa et al., 1983; Lloyd and Mailloux, 1988) resulting in reduced anxiety-related behavior (Torner et al., 2001) and stimulation of adult neurogenesis (Walker et al., 2012; Wang et al., 2013). Alternatively, by inducing serotonin transporter expression, S100B was shown to reduce depression-like behavior which often correlates with anxiety-related phenotypes (Baudry et al., 2010). With regard to increased cell survival, S100B itself has been shown to enhance adult neurogenesis in a model of traumatic brain injury (Kleindienst et al., 2005). S100B as well as the indirect mediators prolactin or the serotonin transporter respond to stress and might account for the observed gene–environment interactions (Gazzolo et al., 2010; Diehl et al., 2007; Lennartsson and Jonsdottir, 2011; Torner and Neumann, 2002; Bartolomucci et al., 2010; Jansen et al., 2010). If one or more of these mechanisms contribute to the observed behavioral and neural alterations has to be studied in further experiments.

Although the role of S100B in psychiatric disorders is still unsolved, our data might help to understand the apparently ambiguous findings in psychiatric patients. Those include on one hand elevated serum levels of S100B in risk allele carriers and on the other hand the association of elevated serum levels with an improved therapeutic response (Hohoff et al., 2010; Dagdan et al., 2011; Arolt et al., 2003; Jang et al., 2008). In the present study, S100B overexpression was associated with a more variable phenotype depending on the environmental experiences during adolescence suggesting an increased sensitivity to both, positive and negative environmental stimuli. This might explain, why individuals carrying SNPs associated with elevated S100B expression have a higher risk to develop a psychiatric disorder if they experience adverse life events (Caspi and Moffitt, 2006). If these individuals receive some supportive environmental stimuli, as during an inpatient treatment, they have a better therapeutic response (Arolt et al., 2003; Jang et al., 2008). Hence, the coexistence of an elevated S100B expression with an increased behavioral and neural plasticity might explain the clinical findings that initially appear contradictory.

In this context, Belsky et al. proposed that some so-called vulnerability genes could be more appropriately conceptualized as plasticity genes. Variants of these genes increase the sensitivity of an individual to both, positive and negative environmental experiences (Belsky et al., 2009; Belsky and Pluess, 2009). This was observed for the monoamine oxidase-A (*MAOA*), the 5-hydroxytryptamine transporter (*SLC6A4*) or the dopamine receptor D4 gene (*DRD4*). Under adverse life events, carriers of plasticity variants of these genes are predisposed to develop psychopathological conditions, whereas in the absence of stressful life experiences, the same individuals gain the most benefit from the environmental condition (Caspi et al., 2002, 2003; Kim-Cohen et al., 2006; Burmeister et al., 2008; Van Ijzendoorn and Bakermans-Kranenburg, 2006). The present study provides strong evidence that S100B overexpression modifies behavioral plasticity dependent on the environmental condition – the absence and presence of chronic stress – suggesting that S100B risk allele carriers associated with a higher S100B expression might also benefit from a higher neuroplasticity under supportive environmental conditions.

5. Conclusion

S100B transgenic mice from stable housing showed significantly reduced anxiety-related behavior in the open field whereas S100B transgenic mice from unstable housing during adolescence showed comparable levels of anxiety-related behavior as wild-types and an increased number of surviving newborn cells in the adult hippocampus which developed into mature neurons. These results suggest a prominent role for the interaction of elevated S100B levels and environmental factors for the developmental programming of emotional behavior and neuroplasticity. In conclusion, there is strong evidence that elevated S100B expression increases the susceptibility to environmental stimuli resulting in more variable phenotypes in adulthood as result of the social environment during adolescence. Thus, negative environmental stimuli lead to higher risk for psychiatric disorders whereas positive environmental stimuli improve therapeutic outcome in psychiatric patients with elevated S100B levels. A better understanding of the function of S100B expression in interaction with various types of environmental factors could provide an appropriate prediction of both, vulnerability and therapeutic responsiveness in these patients.

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Contributors

Jens Buschert, Christa Hohoff, Chadi Touma, and Rupert Palme acquired data. Matthias Rothermund, Volker Arolt, Weiqi Zhang, and Oliver Ambrée designed the study. Jens Buschert, Chadi Touma, and Oliver Ambrée managed the literature searches and analyses. Oliver Ambrée and Jens Buschert 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

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2013.08.001.

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