Homer1/mGluR5 Activity Moderates Vulnerability to Chronic Social Stress

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Stress-induced psychiatric disorders, such as depression, have recently been linked to changes in glutamate transmission in the central nervous system. Glutamate signaling is mediated by a range of receptors, including metabotropic glutamate receptors (mGluRs). In particular, mGluR subtype 5 (mGluR5) is highly implicated in stress-induced psychopathology. The major scaffold protein Homer1 critically interacts with mGluR5 and has also been linked to several psychopathologies. Yet, the specific role of Homer1 in this context remains poorly understood. We used chronic social defeat stress as an established animal model of depression and investigated changes in transcription of *Homer1a* and *Homer1b/c* isoforms and functional coupling of Homer1 to mGluR5. Next, we investigated the consequences of Homer1 deletion, overexpression of Homer1a, and chronic administration of the mGluR5 inverse agonist CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine) on the effects of chronic stress. In mice exposed to chronic stress, *Homer1b/c*, but not *Homer1a*, mRNA was upregulated and, accordingly, Homer1/mGluR5 coupling was disrupted. We found a marked hyperactivity behavior as well as a dysregulated hypothalamic–pituitary–adrenal axis activity in chronically stressed *Homer1* knockout (KO) mice. Chronic administration of the selective and orally bioavailable mGluR5 inverse agonist, CTEP, was able to recover behavioral alterations induced by chronic stress, whereas overexpression of Homer1a in the hippocampus led to an increased vulnerability to chronic stress, reflected in an increased physiological response to stress as well as enhanced depression-like behavior. Overall, our results implicate the glutamatergic system in the emergence of stress-induced psychiatric disorders, and support the Homer1/mGluR5 complex as a target for the development of novel antidepressant agents.

Neuropsychopharmacology (2015) **40**, 1222–1233; doi:10.1038/npp.2014.308; published online 17 December 2014

INTRODUCTION

Individuals are frequently challenged by stressful events that can trigger the activation of hormonal pathways such as the hypothalamic–pituitary–adrenal (HPA) axis (Chrousos, 2009). Prolonged activation of these systems by chronic stress results in persistently elevated cortisol levels that, in turn, can lead to maladaptive consequences in the organism and may ultimately contribute to the development of psychiatric disorders such as depression (de Kloet *et al*,

2005; McEwen, 2004). Animal models of chronic stress exposure are a valuable tool to further our understanding of the molecular underpinnings of stress-induced psychopathology (Savignac *et al*, 2011; Cryan and Holmes, 2005; Joëls and Baram, 2009), as well as providing a paradigm to assess and validate current and novel treatment strategies for depression (Wagner *et al*, 2012; Mutlu *et al*, 2012; Scharf *et al*, 2013).

Most present treatment options for depression are based on the monoamine hypothesis, and aim to increase the availability of monoamines, such as serotonin, in the synaptic cleft (Prins *et al*, 2011; Rush *et al*, 2006). However, the late onset of therapeutic effects as well as unsatisfactory relapse rates and side effects illustrates the need for improved therapeutics (Thase, 2006). Recent studies have provided convincing evidence that dysregulation of glutamate signaling, mainly via its postsynaptic receptors

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Received 4 June 2014; revised 3 November 2014; accepted 4 November 2014; accepted article preview online 20 November 2014

α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), and metabotropic glutamate receptors (mGluRs), contributes to the emergence of psychiatric disorders (Kendell *et al*, 2005; Sanacora *et al*, 2012; Mathews *et al*, 2012; Yim *et al*, 2012; Tronson *et al*, 2010). Modulation of glutamate receptor function has therefore been proposed as a promising target for antidepressant, anxiolytic, and antipsychotic drug development (Popoli *et al*, 2012). Positive and negative modulators of mGluR subtype 5 (mGluR5), in particular, have been suggested as novel agents for the treatment of depression (Palucha *et al*, 2005; Krystal *et al*, 2010; Pilc *et al*, 2008), but the exact molecular mechanisms that mediate their potential therapeutic effects are yet to be fully understood.

In this context, Homer1 has emerged as a potential target protein in depression. Homer1 is a postsynaptic scaffolding protein that links mGluR5 to downstream targets such as inositol triphosphate receptors (Tu *et al*, 1998). Homer1 also acts as a moderator of the NMDA/mGluR5 complex (Tu *et al*, 1999; Bertaso *et al*, 2010) that is highly implicated in stress-induced neuropsychiatric pathologies. Interestingly, the two main splice variants Homer1a and Homer1b/c appear to have opposite molecular effects (Brakeman *et al*, 1997). Homer1b/c links the mGluR5 to the intracellular signaling machinery and mediates ligand-dependent activity of mGluR5. In contrast, the early immediate gene Homer1a is thought to act as dominant negative isoform disrupting mGluR5/Homer1b/c coupling and predominantly modulates ligand-independent mGluR5 signaling (Ango *et al*, 2001). Clinical studies provided first evidence that Homer1 is involved in the development of major depressive disorders (Rietschel *et al*, 2010), whereas preclinical studies describe its importance in anxiety- and depression-related behavior (Szumlinski *et al*, 2005; Lominac *et al*, 2005), memory formation (Lominac *et al*, 2005), fear (Tronson *et al*, 2010), and reward-related behaviors (Jaubert *et al*, 2007; Szumlinski *et al*, 2004). Furthermore, the activity-induced splice variant Homer1a (Brakeman *et al*, 1997) has been shown to be crucially involved in behavioral alterations that are related to depression (Celikel *et al*, 2007; Mahan *et al*, 2012) and anxiety (Lominac *et al*, 2005). Moreover, prenatal stress was shown to alter Homer1a and Homer1b/c expression in several corticolimbic structures, including the hippocampus (Ary *et al*, 2007). However, the impact of Homer1 and its modulatory effects on glutamate signaling, particularly via mGluR5, in chronic stress situations is largely unknown.

In this study, we therefore aimed to investigate the role of Homer1 and mGluR5 in the context of chronic social defeat stress (CSDS) that has been shown to model relevant endophenotypes of depression by us and others (Wang *et al*, 2011; Hartmann *et al*, 2012; Nestler and Hyman, 2010; Berton *et al*, 2006). We hypothesized that stress-induced modulation of Homer1 expression and Homer1/mGluR5 interaction may shape the behavioral and neuroendocrine responses as well as vulnerability to chronic social stress. To test this hypothesis, we used conventional *Homer1* knockout (KO) mice, virus-induced overexpression of the immediate early gene *Homer1a* in the murine hippocampus, as well as a targeted pharmacological approach by treatment with 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine (CTEP), a novel

mGluR5 inverse agonist (Lindemann *et al*, 2011), in combination with CSDS.

MATERIALS AND METHODS

Further detailed methods descriptions can be found in the Supplementary Material.

Animals

For all experiments, male C57Bl/6N mice (Charles River Laboratories, Maastricht, The Netherlands) at the age of 12 weeks were used unless noted otherwise. Conventional *Homer1* KO and wild-type (WT) littermates were bred from heterozygous breeding pairs on a C57Bl/6N background in the animal facilities of the Max Planck Institute of Psychiatry in Munich, Germany. Generation and genotyping of *Homer1* KO mice has been reported previously (Yuan *et al*, 2003) and *Homer1* knockout was verified by PCR. All mice were held under standard conditions (12 h light/12 h dark cycle, lights on at 08:00 h, temperature $23 \pm 2^\circ\text{C}$) and were single-housed and acclimatized to the experimental room for 2 weeks before the beginning of the experiments. Male CD1 mice (16–18 weeks of age) served as resident mice and were held under the conditions described above. They were allowed to habituate to the social defeat cage for 2 weeks before the experiment. Tap water and food (Altromin 1324, Altromin GmbH, Germany) was available *ad libitum*. The experiments were carried out in accordance with the European Communities' Council Directive 2010/63/EU. The protocols were approved by the committee for the Care and Use of Laboratory animals of the Government of Upper Bavaria, Germany.

Experimental Design

For all chronic social defeat experiments, a separate batch of animals was used ($n = 8$ –12 per group). Male *Homer1* WT ($n = 26$) and KO ($n = 22$) animals were randomly distributed across control and stress conditions according to their genotype. For all other experiments including a specific treatment, a total of 48 mice were randomly split into 2×2 groups (control *vs* stress, control *vs* treatment). The CSDS paradigm lasted for 21 days and was conducted as previously described (Wagner *et al*, 2011). See Supplementary Material for details.

Experiment 1. To assess the impact of stress on the Homer1 system, we subjected animals to CSDS and measured the expression of the splice variants *Homer1a* and *Homer1b/c* in the hippocampus in two separate cohorts of mice. We also investigated possible effects of the stress exposure on Homer1 protein turnover and Homer1/mGluR5 coupling.

Experiment 2. We proceeded to investigate the overall role of Homer1—independent of the specific splice variants and the affected brain region—by submitting conventional *Homer1* KO mice to CSDS. We assessed the effects of the stress procedure on physiological, neuroendocrine, and brain gene expression parameters. In addition, the animals

were tested for locomotor activity, social behavior, hedonic behavior, and stress coping.

Experiment 3. Next, we asked whether we could block the effects of chronic stress exposure by pharmacological modulation of the mGluR5 signaling pathway. We therefore applied the novel mGluR5 inverse agonist CTEP (Lindemann *et al*, 2011) during the stress procedure that should counteract the ligand-dependent activity of mGluR5.

Experiment 4. Finally, we explored the possibility that the stress-induced neuroendocrine and behavioral effects could be enhanced by increasing the availability of the splice variant Homer1a in the hippocampus that has previously been shown to trigger ligand-independent mGluR5 activity (Ango *et al*, 2001).

Drug Treatment

Oral administration of the inverse mGluR5 agonist CTEP (F. Hoffmann-La Roche, Basel, Switzerland) commenced 7 days before the start of the CSDS paradigm to establish stable baseline receptor occupancy and was continued until the end of the experiment. Treatment by CTEP was performed as described previously (Lindemann *et al*, 2011; Michalon *et al*, 2012). CTEP was formulated as a micro-suspension in vehicle (0.9% NaCl, 0.3% Tween-80). Chronic treatment consisted of one dose per 48 h of 2 mg/kg body weight per os (p.o.) in a volume of 10 ml/kg body weight. Gavage took place immediately before the daily defeat or handling procedure to minimize confounding effects of oral drug administration.

Viral Overexpression of Homer1a

Viral overexpression was performed as previously described (Wagner *et al*, 2013). A detailed description of the procedure can be found in the Supplementary Materials section. Successful targeting and quantification of *Homer1a* overexpression was achieved by *in situ* hybridization using the riboprobe described below (Supplementary Figure S1). Animals that were not infected bilaterally in both the CA1 and DG regions were excluded from the analysis ($n = 3$).

Behavioral Testing

The following behavioral tests were performed between 0800 and 1200 h in the same room where the animals were housed: open field test (OF), social avoidance test (SA), female urine sniffing test (FUST), and forced swim test (FST). All tests were described and validated previously (Wagner *et al*, 2011; Wagner *et al*, 2012; Malkesman *et al*, 2010). Tests were recorded and analyzed using the video tracking software ANY-maze (ANY-maze 4.20, Stoelting, IL). A detailed description of all behavioral tests performed can be found in the Supplementary Material.

In Situ Hybridization

Frozen brains were sectioned at -20°C in a cryostat microtome at 18 or 20 μm (for fixated brains), thaw mounted on Super Frost Plus slides, dried, and stored at -80°C .

In situ hybridization using ^{35}S UTP = labeled ribonucleotide probes (Homer1a, Homer1b/c, Homer2a/b, mGluR5, and CRH) was performed as described previously (Wagner *et al*, 2013; Schmidt *et al*, 2007). A more detailed description of the procedure can be found in the Supplementary Materials.

RNA Processing

RNA was isolated from whole hippocampi using the TRIZOL reagent (Invitrogen) as previously described (Schmidt *et al*, 2010). The quality of the RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The concentration and purity of total RNA was also assessed by 260 nm UV absorption and by 260/280 ratios, respectively (Nanophotometer, Implen, Munich, Germany). All samples had an RNA integrity number ≥ 7 (range 7.0–8.9; mean $8.0 \pm \text{SD } 0.4$).

Quantitative Reverse Transcriptase-PCR

RNA samples were transcribed into cDNA applying a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) following the manufacturer's protocol. The qPCR of 100 ng cDNA per sample was performed using the Quantifast SYBR Green PCR Kit (Qiagen) and the Lightcycler 2.0 (Roche) according to the standard protocols given in the manufacturers' manuals. All samples were normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Co-Immunoprecipitation

For co-immunoprecipitation (Co-IP), hippocampal tissues from two animals of the same experimental group were pooled. Preparation of membrane fractions and Co-IP analysis was performed as described previously (Wagner *et al*, 2013; Wagner *et al*, 2012). In short, membrane fractions were isolated using the Calbiochem Proteoextract kit (EMD Biosciences), protein concentration was determined, and 1.2 mg of lysate was incubated with 2.5 μg mGluR5 antibody (Millipore) overnight at 4°C . Then, 20 μl of BSA-blocked Protein G Dynabeads (Invitrogen, 100-03D) was added to the lysate-antibody mix followed by 3 h of incubation at 4°C . The beads were washed 3 times with PBS and protein-antibody complexes were eluted with 100 $\mu\text{g}/\text{ml}$ mGluR5-peptide solution (Millipore) in Co-IP buffer for 30 min at 4°C . Then, 15 μg of the cell lysates and 10 μl of the immunoprecipitates were further processed by western blot analysis (Wang *et al*, 2011). Antibodies used were rabbit anti-Homer1 (1:1000, Synaptic Systems), rabbit-anti mGluR5, and goat anti-actin (1:2000, Santa Cruz Biotechnology) for primary as well as horseradish peroxidase-conjugated secondary antibodies (1:2000, DAKO).

Quantitative Protein Turnover Analysis

Protein turnover was analyzed as described previously (Webhofer *et al*, 2013). For details, see Supplementary Methods.

Statistical Analysis

All data presented are shown as means + SEM, and analyzed by SPSS 18.0. Two-tailed Student's *t*-test was employed for comparison of two independent groups (control vs CSDS). Two-factorial ANOVA was employed when appropriate. Significant interaction effects were followed by Fisher's LSD *post hoc* analysis when appropriate. As nominal level of significance, $P < 0.05$ was accepted.

RESULTS

Hippocampal Homer1 Is Regulated by Chronic Stress

We measured *Homer1b/c* mRNA levels in response to CSDS by *in situ* hybridization and found an increase in both the CA1 ($T_{18} = -3.275$, $p < 0.01$) and CA3 ($T_{18} = -4.556$, $p < 0.001$), but not the dentate gyrus (DG) ($T_{18} = -1.791$, $p = 0.090$), regions of the dorsal hippocampus (Figure 1a and c). On the other hand, *Homer1a* was not significantly regulated in any of the investigated regions of the hippocampus (Figure 1b and c), and this is in line with the idea that this splice variant is an immediate early gene predominantly activated after an acute challenge (Ango et al, 2001). The *Homer1b/c* upregulation was replicated in an independent batch of animals (Supplementary Figure S2A). The physiological and behavioral parameters of these experiments have been reported before (Wang et al, 2011; Wagner et al, 2011). To further validate these transcriptional alterations, we performed RT-PCR in a third batch of animals that underwent the same CSDS paradigm,

and confirmed our findings (Supplementary Figure S2B). Next, we also investigated the protein turnover rate of Homer1 in the synaptosomal fraction of the hippocampus that could indirectly affect Homer1 protein availability. Although ~30% of Homer1 protein was metabolized over the course of 7 days (Supplementary Figure S2C), no difference in protein turnover between CSDS and control animals was found. Finally, we tested whether CSDS might affect physical interaction of Homer1 with mGluR5. Interestingly, we observed that chronic stress reduced coupling of Homer1 to mGluR5, without altering absolute protein levels (Figure 1d).

Deletion of Homer1 Leads to Disturbed HPA Axis Function and Behavioral Hyperactivity

To further elucidate the role of Homer1 in chronic stress-induced behavioral and neuroendocrine alterations, we exposed *Homer1* KO animals to the CSDS paradigm. We identified a large increase in the corticosterone response to a novel acute stressor (FST) in KO compared with WT animals, which was apparent in both stressed and non-stressed mice (Figure 2a). This was further reflected by significantly enlarged adrenal glands in these animals (Figure 2b), but not in basal circulating corticosterone levels (Supplementary Figure S3A and B). Interestingly, this neuroendocrine phenotype was accompanied by gene transcription alterations in the paraventricular nucleus (PVN) of the hypothalamus. Here, CSDS induced an increase in *CRH* mRNA in the PVN irrespective of the genotype (Figure 2c).

Figure 1 Hippocampal *Homer1* mRNA is regulated by chronic stress. (a) Chronic social defeat stress (CSDS) led to increased *Homer1b/c* mRNA levels in the CA1 and CA3 regions of the dorsal hippocampus. (b) The mRNA levels of the immediate early gene *Homer1a* were not significantly altered at the time of killing 24 h after the last defeat session of the CSDS paradigm. (c) Representative radiograph pictures of *Homer1b/c* and *Homer1a* expression. (d) Binding of Homer1 to its primary interaction partner mGluR5 is reduced in stressed animals ($T_9 = 9.429$, $p < 0.001$). *Significant from control, $p < 0.05$.

Figure 2 Neuroendocrine and behavioral profile of *Homer1* KO exposed to CSDS. (a) The corticosterone response to a novel acute stressor was increased in mice that were exposed to CSDS. At the same time, KO mice showed a strongly enhanced response to the stressor irrespective of the condition (ANOVA main effects: condition: $F_{1,44} = 43.232$, $p < 0.001$; genotype: $F_{1,44} = 15.796$, $p < 0.001$). (b) Analogous to the corticosterone response, CSDS induced an increase in adrenal glands weight in both genotypes (main condition effect: $F_{1,45} = 82.163$, $p < 0.001$). However, KO mice already possessed enlarged adrenal glands under basal condition and this effect was also present in the stressed group (main genotype effect: $F_{1,45} = 81.040$, $p < 0.001$). (c) In the paraventricular nucleus of the hypothalamus (PVN), *CRH* expression was increased in response to CSDS (main condition effect: $F_{1,43} = 11.264$, $p < 0.01$). At the same time, KO animals showed significantly lower *CRH* levels (main genotype effect: $F_{1,43} = 10.744$, $p < 0.01$). See representative radiograph pictures in the panel below. (d) Although CSDS resulted in a reduction of locomotion in both groups (main condition effect: $F_{1,43} = 13.129$), a marked hyperactivity was detected in KO mice under basal and stress conditions (main genotype effect: $F_{1,43} = 12.630$, $p < 0.01$). (e) In the social avoidance (SA) test, CSDS resulted in a reduction of interaction in WT but not in KO mice (ANOVA genotype main effect: $F_{1,37} = 5.337$, $p < 0.05$; interaction effect: $F_{1,37} = 4.644$, $p < 0.05$), showing a significantly increased interaction ratio compared with their stressed WT littermates. (f) In the water trial of the female urine sniffing test (FUST), animals with a *Homer1* deletion showed a reduced interest in the presented cotton tip (main genotype effect: $F_{1,45} = 9.269$, $p < 0.01$). In the urine trial, CSDS had a strong effect on sniffing time (main condition effect: $F_{1,45} = 9.185$, $p < 0.01$) where sniffing time was reduced irrespective of the genotype. (g) Independent of CSDS, KO mice exhibited their hyperactive phenotype in the forced swim test (FST) as indicated by reduced floating times (main genotype effect: $F_{1,45} = 32.662$, $p < 0.001$). *Significant main condition effect, $p < 0.05$; #significant main genotype effect, $p < 0.05$; + significant from control of same genotype, $p < 0.05$; §significant from WT of same condition.

Concurrently, KO animals showed significantly reduced *CRH* mRNA levels compared with their WT littermates. We also checked for potential compensatory changes in the *Homer1* KO mice on the level of *mGluR5* and *Homer2a/b*

expression in the hippocampus. For *mGluR5* (Supplementary Figure S3C), CSDS led to a mild increase in expression in the CA1 region in WT, but not in KO animals. However, there was no main effect of genotype in any of the hippo-

campal regions, arguing against a compensatory effect due to the lack of *Homer1* expression. Regarding the *Homer2a/b* expression (Supplementary Figure S3D) we detected a modest decrease in *Homer1* KO mice that was restricted to the CA1 region. In addition, chronic stress exposure tended to reduce *Homer2a/b* expression, an effect that was significant in the CA3 region.

Deletion of *Homer1* also resulted in considerable changes in the animals' behavior. In the OF test, CSDS reduced total locomotion in both genotypes, whereas KO animals displayed greatly increased locomotor activity irrespective of the stress condition (Figure 2d). In the SA test, WT animals displayed a reduced social interaction ratio when under the effects of CSDS ($p < 0.05$), whereas interaction ratios of stressed KO mice were not significantly reduced compared with control conditions ($p = 0.256$; Figure 2e). A strong genotype difference was also apparent in the FUST, where KO mice spent significantly less time with the presented cotton tip in the water trial (Figure 2f). In the urine trial, we detected a reduction in sniffing time of stressed mice compared with their nonstressed littermates, irrespective of the genotype. The above-mentioned hyperactive phenotype was also visible in the FST, where KO mice spent significantly less time floating (Figure 2g).

Chronic Decrease of mGluR5 Activity Reverses Behavioral But Not Neuroendocrine Consequences of Stress and Normalizes Homer1 Expression Levels

Next, we assessed whether decrease of mGluR5 signaling could be protective to the depression-like phenotype of chronically stressed mice. We detected robust CSDS effects, both in basal circulating corticosterone levels (Supplementary Figure S4A) and in the corticosterone response to a novel stressor (Figure 3a). Treatment with the selective mGluR5 inverse agonist CTEP did not affect these parameters under either basal or stress conditions. Recovery from the novel stressor was also disturbed in animals that underwent CSDS, but this was not influenced by CTEP treatment (Supplementary Figure S4B). Accordingly, on the physiological level, CSDS resulted in increased adrenal gland weight with no effect of CTEP treatment (Figure 3b). We observed increased *CRH* mRNA levels in the PVN in response to CSDS, irrespective of treatment condition (Figure 3c). In this experiment we also investigated expression changes of the two *Homer1* isoforms in the hippocampus. Interestingly, the stress-induced elevation of *Homer1b/c* mRNA in the CA1 region of the dorsal hippocampus was reversed by CTEP treatment (Supplementary Figure S4C). Full expression data of *Homer1* isoforms in the hippocampus can be found in Supplementary Table S1.

On the behavioral level, a stress-induced reduction of locomotion in the OF test was reversed by chronic CTEP treatment (Figure 3d), without affecting basal locomotion in the control groups. We did not detect significant effects in the SA test (Figure 3e), but found that CTEP treatment was able to reverse the anhedonic phenotype induced by CSDS in the FUST (Figure 3f). However, CTEP-treated animals also expressed less interest in the urine-dipped cotton tip under control conditions. In the FST, chronic treatment of CTEP did not exert beneficial effects on the animals (Figure 3g).

Overexpression of Homer1a in the Hippocampus Promotes Vulnerability to Stress

Finally, we tested whether overexpression of the *Homer1a* splice variant, which inhibits mGluR5/*Homer1b/c* interaction and promotes ligand-independent mGluR5 signaling, would alter stress susceptibility. The injection site as well as qualitative and quantitative analyses of the viral overexpression can be found in Supplementary Figure S1. Basal corticosterone levels were elevated in response to CSDS, but overexpression of *Homer1a* in the hippocampus did not have an effect (Supplementary Figure S5A). After a novel acute stressor, *Homer1a* OE animals of the nonstressed condition showed an increased response compared with Empty animals, whereas CSDS enhanced circulating corticosterone equally in both treatment groups (Figure 4a). After 90 min of recovery, both *Homer1a* OE and Empty animals showed a disturbed HPA axis recovery indicated by significantly increased corticosterone levels in the CSDS groups (Supplementary Figure S5B). *Homer1a* OE mice had significantly bigger adrenal glands when exposed to CSDS compared with Empty mice that were stressed, indicating an increased HPA axis activity over the course of the stress period (Figure 4b). These HPA axis alterations were not accompanied by gene expression differences of *CRH* in the PVN (Figure 4c).

While being exposed to CSDS, mice overexpressing *Homer1a* in the hippocampus showed a significant reduction in locomotion in the OF test (Figure 4d). Similar results were obtained in the SA test, where CSDS led to a reduced interaction ratio (Figure 4e). In the FUST, CSDS induced anhedonic behavior, with no apparent effects of *Homer1a* overexpression (Figure 4f). Overexpression of *Homer1a* in the hippocampus led to an increased behavioral despair and less active stress coping behavior upon CSDS exposure as depicted by increased floating time in the FST (Figure 4g), additionally indicating increased susceptibility to CSDS.

DISCUSSION

In this study, we provide evidence for the involvement of the *Homer1*/mGluR5 complex in mediating the depression-like phenotype induced by chronic stress exposure. First, we showed that hippocampal *Homer1b/c* mRNA is regulated, and *Homer1*/mGluR5 interaction is disrupted, by CSDS. A total knockout of *Homer1* results in strong hyperactivity and stress susceptibility. Conversely, we were able to rescue some stress-induced behavioral alterations by chronic administration of the novel, orally bioavailable mGluR5 inverse agonist CTEP without interfering with HPA axis function. Furthermore, we demonstrate that overexpressing *Homer1a* in the hippocampus, thereby modulating the activity of mGluR5, increases the vulnerability to chronic stress on the physiological, neuroendocrines and behavioral levels. These findings suggest that the *Homer1*/mGluR5 complex may be valuable as a novel target for the treatment of stress-induced psychopathology, such as depression.

In this series of experiments, *Homer1* has been shown to be regulated in response to CSDS on both mRNA levels, whereas the turnover rate was not affected. In a previous study applying a slightly different chronic defeat model,

Figure 3 Treatment with CTEP does not alter HPA axis function but reverses stress-induced behavioral effects. (a) CSDS resulted in a stronger activation of the HPA axis in response to a novel stressor (main condition effect: $F_{1,44} = 30.317$, $p < 0.001$). CTEP did not affect this phenotype. (b) CSDS induced an increase in adrenal size that was not affected by CTEP treatment (main condition effect: $F_{1,44} = 140.316$, $p < 0.001$). (c) In stressed vehicle-treated animals, CRH mRNA levels in the PVN were elevated compared with their respective control group (main condition effect: $F_{1,44} = 8.894$, $p < 0.01$). CTEP-treated animals did not show a significant alteration in CRH levels. See representative radiograph pictures in the panel below. (d) Although vehicle-treated animals displayed a strong decrease in locomotion while being stressed ($p < 0.001$), CTEP treatment was able to counteract this phenotype and significantly enhanced locomotion in the CSDS group ($p < 0.01$) (main condition effect: $F_{1,43} = 32.861$, $p < 0.001$; main treatment effect: $F_{1,43} = 4.981$, $p < 0.05$; interaction effect: $F_{1,43} = 5.971$, $p < 0.05$). (e) There was no effect of CSDS or CTEP in the SA test. (f) In the FUST, the water trial did not reveal any differences between treatment and condition groups, but stressed animals sniffed significantly less on the urine-dipped cotton tip (main condition effect: $F_{1,43} = 8.349$, $p < 0.01$; interaction effect: $F_{1,43} = 17.281$, $p < 0.001$). Further *post hoc* tests indicated that CTEP reduces the interest in female urine under basal conditions ($p < 0.01$). Yet, although vehicle-treated mice that underwent CSDS showed a strong reduction in sniffing time ($p < 0.001$), this effect was reversed by the CTEP treatment in the same condition group ($p < 0.05$), indicating a protective effect of CTEP. (g) Although CSDS led to a decrease in active stress coping behavior in the FST, CTEP did not influence this behavioral parameter (main condition effect: $F_{1,44} = 14.109$, $p < 0.01$). *Significant main condition effect, $p < 0.05$; #significant main treatment effect, $p < 0.05$; §significant to corresponding vehicle group; +significant to corresponding nonstressed control group.

Homer1 expression was also reported to be upregulated in the nucleus accumbens (Berton *et al*, 2006). Furthermore, microarray data from another study in our lab indicated hippocampal *Homer1* to be differentially regulated between stress-resilient and -vulnerable animals (Schmidt *et al*, 2010), further strengthening the evidence of *Homer1* being

involved in stress-induced psychopathology. At least on the mRNA level, the effects of chronic stress were specific to the *Homer1b/c* splice variant, whereas the expression of the immediate early gene *Homer1a* was not affected. This is in contrast to prenatal stress, where in the hippocampus mainly *Homer1a* expression was found to be increased

Figure 4 Overexpression of *Homer1a* in the hippocampus promotes stress vulnerability. (a) Under control conditions, *Homer1a* OE led to a hyperactivation of the HPA axis compared with Empty animals ($p < 0.05$). This effect was not apparent in mice that underwent CSDS, possibly because of a ceiling effect, as CSDS strongly enhanced the corticosterone response to a novel stressor (main condition effect: $F_{1,44} = 61.134$, $p < 0.001$; interaction effect: $F_{1,44} = 4.845$, $p < 0.05$). (b) CSDS and overexpression of *Homer1a* had profound impact on the adrenal gland size of the animals (main condition effect: $F_{1,44} = 61.134$, $p < 0.001$; main AAV treatment effect: $F_{1,44} = 5.365$, $p < 0.05$; interaction effect: $F_{1,44} = 4.845$, $p < 0.05$). The *post hoc* testing confirmed that CSDS increased adrenal gland sizes in both AAV groups (Empty: $p < 0.001$; *Homer1a* OE: $p < 0.001$), but in stressed *Homer1a* OE animals, this increase was significantly bigger compared with stressed Empty animals ($p < 0.05$). (c) The mRNA levels of *CRH* in the PVN were not significantly altered in this experiment. See representative radiograph pictures in the panel below. (d) Although CSDS did not lead to a reduction in locomotion in Empty animals, overexpression of *Homer1a* affected the animals' behavior, indicating a more pronounced susceptibility to CSDS (main condition effect: $F_{1,44} = 6.722$, $p < 0.05$). (e) This effect was also apparent in the SA test, where CSDS led to a reduced interaction ratio that was more pronounced in *Homer1a* OE animals (main condition effect: $F_{1,44} = 5.171$, $p < 0.05$). (f) The FUST revealed a significant stress effect in both the water ($F_{1,45} = 5.863$, $p < 0.05$) and the urine trials ($F_{1,44} = 27.368$, $p < 0.001$), with both AAV groups showing significant reductions in sniffing time when exposed to CSDS compared with the respective control groups. (g) In the FST, ANOVA revealed a condition ($F_{1,43} = 7.045$, $p < 0.05$), an AAV treatment ($F_{1,43} = 6.185$, $p < 0.05$), and a condition $\times$ AAV interaction effect ($F_{1,43} = 5.496$, $p < 0.05$). Following *post hoc* analysis, *Homer1a* OE mice showed significantly increased floating time compared with both their respective controls ($p < 0.001$) and stressed Empty mice ($p < 0.05$). *Significant main condition effect, $p < 0.05$; #significant main AAV treatment effect, $p < 0.05$; + significant from control of same AAV treatment, $p < 0.05$; §significant from Empty AAV of same condition.

(Ary *et al*, 2007). This difference could be because of the developmental time window of stress exposure, and also the time of testing (weanling *vs* adult) or the sex of the animals (females *vs* males). We also observed a significant decrease in Homer1/mGluR5 coupling following chronic

social defeat. This effect cannot be specified to either Homer1 splice variant at the moment because of the lack of specific and reliable antibodies targeting Homer1a (Tronson *et al*, 2010). Further studies will be required to unravel the functional consequence of this effect.

We report a major disturbance of HPA axis activity in mice that are deficient in *Homer1*. This is evidenced on one hand on the physiological level, where *Homer1* KO mice show enlarged adrenal glands, in line with previous reports (Grinevich *et al*, 2011). On the other hand, we also showed that corticosterone release in response to stress is severely altered in these animals. Hyperactive corticosterone responses induced by CSDS are frequently observed in this paradigm (Wang *et al*, 2011; Wagner *et al*, 2011; Hartmann *et al*, 2012) and deletion of *Homer1* further increased this effect, indicating a prominent regulatory role of this glutamatergic pathway in the feedback regulation of the HPA axis. Compensatory effects caused by changes in *mGluR5* or *Homer2a/b* expression (Ary *et al*, 2013) in *Homer1* KO mice seem unlikely, as the observed effects in the hippocampus were rather modest.

We further observed a strong hyperactive phenotype because of *Homer1* deletion that has been previously reported in studies that employed this mouse model (Szumlinski *et al*, 2005; Jaubert *et al*, 2007). These hyperactive behaviors in general led to an apparent reversal of the CSDS-induced phenotype that was mostly visible in locomotive and social behavior. However, we also detected a reduced interest in interacting with novel stimuli in KO animals, such as in the FUST. These behavioral patterns may be ascribed to an attention deficit hyperactivity disorder (ADHD)-like phenotype (Sagvolden *et al*, 2005) that has previously been linked to altered *Homer1* expression profiles in the prefrontal cortex and the hippocampus (Hong *et al*, 2009; Hong *et al*, 2011), although the increased activity of *Homer1* KO mice may also be a confounding factor in some of the behavioral tests. It is important to note that based on the present data, we are not able to discern immediate effects of *Homer1* deletion from developmental effects that originate in earlier stages of the animals' life. Indeed, *Homer1* has been shown to be strongly expressed in developing tissues (Shiraishi-Yamaguchi and Furuichi, 2007) and a total knockout is therefore likely to exert major effects on these animals before the CSDS procedure started. Nonetheless, these findings indicate the importance of *Homer1*-mediated signaling in the response to stress.

As the deletion of *Homer1* results in the loss of both *Homer1a* and *Homer1b/c* splice variants, which are hypothesized to have opposing effects (Brakeman *et al*, 1997), it is important to more specifically modulate *mGluR5*/*Homer1* signaling. We therefore administered the novel, bioavailable *mGluR5* inverse agonist CTEP (Lindemann *et al*, 2011) to mice subjected to chronic stress. Under basal conditions, CTEP did not have any detrimental effects on the physiological or neuroendocrine level. In addition, decrease of *mGluR5* activity over the course of the stress exposure did not affect HPA axis function or modulation, as both treatment groups showed similar corticosterone profiles under all measured conditions. Given the results from the KO animals presented above, it is likely that *Homer1* influences HPA axis responsiveness independent from *mGluR5* signaling. Yet, CTEP did have beneficial effects on the behavioral phenotype of stressed animals. Here, stress-induced anhedonia and reduced locomotion was rescued in animals that received CTEP. Thus, although CTEP did not reverse the stress-induced molecular profile, it showed therapeutic value in behavioral parameters. These

results strengthen the idea of combining different antidepressant treatments to maximize therapeutic efficacy (Connolly and Thase, 2011; Palaniyappan *et al*, 2009). Indeed, CTEP may serve as a basis for future antidepressants that specifically target the glutamate system, as its pharmacokinetic properties are significantly improved from previous *mGluR5* antagonists such as MPEP and MTEP (Lindemann *et al*, 2011; Anderson *et al*, 2003; Busse *et al*, 2004).

In contrast to the pharmacological modulation of *mGluR5* activity by CTEP, which was also not hippocampus specific and likely altered *mGluR5* signaling in many different brain regions, a specific increase in the short *Homer1a* isoform is expected to disrupt glutamate-stimulated intracellular calcium signaling (Tu *et al*, 1998; Yuan *et al*, 2003) and activate ligand-independent *mGluR5* pathways (Ango *et al*, 2001). Indeed, a specific overexpression of *Homer1a* in the hippocampus led to changes in HPA axis activity under both basal and CSDS conditions, thereby indicating an increase in vulnerability to both acute and chronic stress. This phenotype is not only present on the neuroendocrine and physiological level but also reflected in different behavioral parameters related to stress coping (FST) and locomotor activity, but not social interaction and hedonic behavior. Interestingly, some of the behavioral alterations induced by *Homer1a* overexpression in the hippocampus, for example in the FST, parallel those previously observed by Lominac *et al* (2005) following overexpression of *Homer1a* in the prefrontal cortex.

Activation of *Homer1a* gene transcription is a rapid and plastic process in response to synaptic activity (de Bartolomeis and Iasevoli, 2003; Brakeman *et al*, 1997; Kato *et al*, 1997). It can be hypothesized that repeated transcriptional activation of this immediate early gene in response to the daily defeat sessions induces counter-regulatory changes in the central stress systems, including the upregulation of *Homer1b/c* (Berton *et al*, 2006). These disturbances, in turn, may contribute to the vulnerable behavioral and neuroendocrine phenotypes that we observed under the influence of CSDS. Prolonged ligand-independent activation of *mGluR5* via abundant *Homer1a* protein levels also severely affects IP₃ receptor activation and subsequent downstream signaling (Ango *et al*, 2001; Kammermeier, 2008). The continuous presence of *Homer1a* may therefore profoundly change neuronal signaling pathways that may in turn render the organism more vulnerable to chronic stress. In addition, it has previously been shown that interactions between NMDA and *mGluR5* receptors are mediated by the PSD95/Shank/*Homer1* complex (Hayashi *et al*, 2009; Bertaso *et al*, 2010), and *Homer1a* was demonstrated to be a key modulator of *mGluR5* coupling to effector targets that produce excitatory postsynaptic currents (Kammermeier and Worley, 2007). Given the increasing body of evidence showing that NMDA receptor targeting agents produce rapid-acting antidepressant effects (Krystal *et al*, 2013; Kavalali and Monteggia, 2012), a *Homer1a*-mediated overactivation of this signaling pathway may profoundly affect antidepressant treatment efficacy. The development of new drugs that target this system, mainly via *mGluR5*, is therefore of great value and importance (Sanacora *et al*, 2012; Krystal *et al*, 2010).

There are some limitations in the presented data sets that need to be considered when interpreting the results.

Figure 5 Schematic model describing the outcome and proposed mechanism of the different manipulations of mGluR5/Homer1 interaction under chronic stress conditions. (a) Under wild-type conditions, chronic stress increases the levels of the long isoform Homer1b/c (H1b/c), thereby strengthening the intracellular link of mGluR5 to calcium stores in the endoplasmic reticulum (ER), but also to NMDA receptor (NMDA-R) signaling. (b) In *Homer1* KO animals, all splice variants of Homer1 are missing, and thereby the mGluR5 ligand-dependent and ligand-independent signaling cascades will be disrupted. (c) When applying the inverse agonist CTEP during chronic stress, ligand-dependent signaling via inositol triphosphate (IP₃) will be diminished, but the interaction of Homer1b/c with IP₃ receptors and with the NMDA-R signaling cascade will remain unaffected. (d) In contrast, overexpression of Homer1a (H1a) mimics a situation similar to an acute stress challenge. Here Homer1a replaces Homer1b/c at the mGluR5, thereby stimulating ligand-independent signaling. At the same time, however, the structural link of mGluR5 to the ER and to NMDA-Rs will be weakened.

Although the different experiments were performed analogously, a direct comparison of the results is hampered by differences in the mouse lines, the applied treatment (eg, gavaging), or the history of surgery. Furthermore, although we observed behavioral and neuroendocrine alterations following mGluR5/Homer1 manipulations, a direct mechanistic link of the presumably altered hippocampal function to the respective readouts is still lacking. Another still unresolved question is whether overexpression of *Homer1b/c* could have altered the response to CSDS. However, as this manipulation would have a similar effect as CSDS exposure *per se* and a further increase in *Homer1b/c* might not additionally increase mGluR5 signaling due to a ceiling effect, this experiment was not included.

In summary, our study provides compelling evidence for the involvement of the Homer1/mGluR5 complex in the emergence and regulation of stress-induced behavioral and neuroendocrine phenotypes (see Figure 5 for a schematic

overview). We demonstrated that the Homer1/mGluR5 pathway is altered by CSDS, and HPA axis function is strongly disturbed in animals that carry a total knockout of *Homer1*. We further demonstrated that stimulating ligand-independent mGluR5 activity by increased levels of *Homer1a* lead to a stress-vulnerable behavioral phenotype. Conversely, decrease of mGluR5 activity by CTEP was able to recover the stress-induced behavioral alterations. With the present data indicating an involvement of the Homer1/mGluR5 pathway in stress-related psychiatric disorder, further research to fully characterize the contributing molecular mechanisms is highly warranted.

FUNDING AND DISCLOSURE

Lothar Lindemann, Georg Jaschke, and Joseph G Wettstein are full-time employees of F. Hoffmann-La Roche AG, Basel, Switzerland. The other authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Kathrin Hafner for her excellent technical assistance. Homer1 KO mice were generously provided by Dr Paul Worley (Johns Hopkins University). Natalie Matosin thanks the Company of Biologists (UK) for their support in the form of a Traveling Fellowship. This study was supported by the Brain & Behavior Research Foundation NARSAD grant 17322. This study was also partially supported by the National Alliance for Research on Schizophrenia and Depression (grant no. 17322).

REFERENCES

- Anderson JJ, Bradbury MJ, Giracello DR, Chapman DF, Holtz G, Roppe J *et al* (2003). In vivo receptor occupancy of mGlu5 receptor antagonists using the novel radioligand [3H]3-methoxy-5-(pyridin-2-ylethynyl)pyridine. *Eur J Pharmacol* **473**: 35–40.
- Ango F, Prézeau L, Muller T, Tu JC, Xiao B, Worley PF *et al* (2001). Agonist-independent activation of metabotropic glutamate receptors by the intracellular protein Homer. *Nature* **411**: 962–965.
- Ary AW, Aguilar VR, Szumlanski KK, Kippin TE (2007). Prenatal stress alters limbo-corticostratial Homer protein expression. *Synapse* **61**: 938–941.
- Ary AW, Lominac KD, Wroten MG, Williams AR, Campbell RR, Ben-Shahar O *et al* (2013). Imbalances in prefrontal cortex CC-Homer1 versus CC-Homer2 expression promote cocaine preference. *J Neurosci* **33**: 8101–8113.
- Bertaso F, Roussignol G, Worley P, Bockaert J, Fagni L, Ango F (2010). Homer1a-dependent crosstalk between NMDA and metabotropic glutamate receptors in mouse neurons. *PLoS One* **5**: e9755.
- Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ *et al* (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* **311**: 864–868.
- Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL *et al* (1997). Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* **386**: 284–288.
- Busse CS, Brodtkin J, Tattersall D, Anderson JJ, Warren N, Tehrani L *et al* (2004). The behavioral profile of the potent and selective mGlu5 receptor antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) in rodent models of anxiety. *Neuropsychopharmacology* **29**: 1971–1979.
- Celikel T, Marx V, Freudenberg F, Zivkovic A, Resnik E, Hasan MT *et al* (2007). Select overexpression of homer1a in dorsal hippocampus impairs spatial working memory. *Front Neurosci* **1**: 97–110.
- Chrousos GP (2009). Stress and disorders of the stress system. *Nat Rev Endocrinol* **5**: 374–381.
- Connolly KR, Thase ME (2011). If at first you don't succeed: a review of the evidence for antidepressant augmentation, combination and switching strategies. *Drugs* **71**: 43–64.
- Cryan JF, Holmes A (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nat Rev Drug Discov* **4**: 775–790.
- de Bartolomeis A, Iasevoli F (2003). The Homer family and the signal transduction system at glutamatergic postsynaptic density: potential role in behavior and pharmacotherapy. *Psychopharmacol Bull* **37**: 51–83.
- de Kloet ER, Joëls M, Holsboer F (2005). Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* **6**: 463–475.
- Grinevich V, Jezova D, Gambaryan S, Illarionova A, Kollek A, Seeburg PH *et al* (2011). Hypertrophy and altered activity of the adrenal cortex in Homer 1 knockout mice. *Horm Metab Res* **43**: 551–556.
- Hartmann J, Wagner KV, Liebl C, Scharf SH, Wang XD, Wolf M *et al* (2012). The involvement of FK506-binding protein 51 (FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress. *Neuropharmacology* **62**: 332–339.
- Hayashi MK, Tang C, Verpelli C, Narayanan R, Stearns MH, Xu RM *et al* (2009). The postsynaptic density proteins Homer and Shank form a polymeric network structure. *Cell* **137**: 159–171.
- Hong Q, Wang YP, Zhang M, Pan XQ, Guo M, Li F *et al* (2011). Homer expression in the hippocampus of an animal model of attention-deficit/hyperactivity disorder. *Mol Med Rep* **4**: 705–712.
- Hong Q, Zhang M, Pan XQ, Guo M, Li F, Tong ML *et al* (2009). Prefrontal cortex Homer expression in an animal model of attention-deficit/hyperactivity disorder. *J Neurol Sci* **287**: 205–211.
- Jaubert PJ, Golub MS, Lo YY, Germann SL, Dehoff MH, Worley PF *et al* (2007). Complex, multimodal behavioral profile of the Homer1 knockout mouse. *Genes Brain Behav* **6**: 141–154.
- Joëls M, Baram TZ (2009). The neuro-symphony of stress. *Nat Rev Neurosci* **10**: 459–466.
- Kammermeier PJ (2008). Endogenous homer proteins regulate metabotropic glutamate receptor signaling in neurons. *J Neurosci* **28**: 8560–8567.
- Kammermeier PJ, Worley PF (2007). Homer 1a uncouples metabotropic glutamate receptor 5 from postsynaptic effectors. *Proc Natl Acad Sci USA* **104**: 6055–6060.
- Kato A, Ozawa F, Saitoh Y, Hirai K, Inokuchi K (1997). vesl, a gene encoding VASP/Ena family related protein, is upregulated during seizure, long-term potentiation and synaptogenesis. *FEBS Lett* **412**: 183–189.
- Kavalali ET, Monteggia LM (2012). Synaptic mechanisms underlying rapid antidepressant action of ketamine. *Am J Psychiatry* **169**: 1150–1156.
- Kendell SF, Krystal JH, Sanacora G (2005). GABA and glutamate systems as therapeutic targets in depression and mood disorders. *Expert Opin Ther Targets* **9**: 153–168.
- Krystal JH, Mathew SJ, D'Souza DC, Garakani A, Gunduz-Bruce H, Charney DS (2010). Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists. *CNS Drugs* **24**: 669–693.
- Krystal JH, Sanacora G, Duman RS (2013). Rapid-acting glutamatergic antidepressants: the path to ketamine and beyond. *Biol Psychiatry* **73**: 1133–1141.
- Lindemann L, Jaeschke G, Michalon A, Vieira E, Honer M, Spooren W *et al* (2011). CTEP: a novel, potent, long-acting, and orally bioavailable metabotropic glutamate receptor 5 inhibitor. *J Pharmacol Exp Ther* **339**: 474–486.
- Lominac KD, Oleson EB, Pava M, Klugmann M, Schwarz MK, Seeburg PH *et al* (2005). Distinct roles for different Homer1 isoforms in behaviors and associated prefrontal cortex function. *J Neurosci* **25**: 11586–11594.
- Mahan AL, Mou L, Shah N, Hu JH, Worley PF, Ressler KJ (2012). Epigenetic modulation of Homer1a transcription regulation in amygdala and hippocampus with pavlovian fear conditioning. *J Neurosci* **32**: 4651–4659.
- Malkesman O, Scattoni ML, Paredes D, Tragon T, Pearson B, Shaltiel G *et al* (2010). The female urine sniffing test: a novel approach for assessing reward-seeking behavior in rodents. *Biol Psychiatry* **67**: 864–871.
- Mathews DC, Henter ID, Zarate CA (2012). Targeting the glutamatergic system to treat major depressive disorder: rationale and progress to date. *Drugs* **72**: 1313–1333.
- McEwen BS (2004). Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann NY Acad Sci* **1032**: 1–7.
- Michalon A, Sidorov M, Ballard TM, Ozmen L, Spooren W, Wettstein JG *et al* (2012). Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron* **74**: 49–56.

- Mutlu O, Gumuslu E, Ulak G, Celikyurt IK, Kocurk S, Kir HM *et al* (2012). Effects of fluoxetine, tianeptine and olanzapine on unpredictable chronic mild stress-induced depression-like behavior in mice. *Life Sci* **91**: 1252–1262.
- Nestler EJ, Hyman SE (2010). Animal models of neuropsychiatric disorders. *Nat Neurosci* **13**: 1161–1169.
- Palaniyappan L, Insole L, Ferrier N (2009). Combining antidepressants: a review of evidence. *APT* **15**: 90–99.
- Palucha A, Branski P, Szewczyk B, Wieronska J, Klak K, Pilc A (2005). Potential antidepressant-like effect of MTEP, a potent and highly selective mGluR5 antagonist. *Pharmacol Biochem Behav* **81**: 901–906.
- Pilc A, Chaki S, Nowak G, Witkin JM (2008). Mood disorders: regulation by metabotropic glutamate receptors. *Biochem Pharmacol* **75**: 997–1006.
- Popoli M, Yan Z, McEwen BS, Sanacora G (2012). The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat Rev Neurosci* **13**: 22–37.
- Prins J, Olivier B, Korte SM (2011). Triple reuptake inhibitors for treating subtypes of major depressive disorder: the monoamine hypothesis revisited. *Expert Opin Investig Drugs* **20**: 1107–1130.
- Rietschel M, Mattheisen M, Frank J, Treutlein J, Degenhardt F, Breuer R *et al* (2010). Genome-wide association-, replication-, and neuroimaging study implicates HOMER1 in the etiology of major depression. *Biol Psychiatry* **68**: 578–585.
- Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D *et al* (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry* **163**: 1905–1917.
- Sagvolden T, Russell VA, Aase H, Johansen EB, Farshbaf M (2005). Rodent models of attention-deficit/hyperactivity disorder. *Biol Psychiatry* **57**: 1239–1247.
- Sanacora G, Treccani G, Popoli M (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology* **62**: 63–77.
- Savignac HM, Finger BC, Pizzo RC, O'Leary OF, Dinan TG, Cryan JF (2011). Increased sensitivity to the effects of chronic social defeat stress in an innately anxious mouse strain. *Neuroscience* **192**: 524–536.
- Scharf SH, Sterlemann V, Liebl C, Müller MB, Schmidt MV (2013). Chronic social stress during adolescence: interplay of paroxetine treatment and ageing. *Neuropharmacology* **72C**: 38–46.
- Schmidt MV, Sterlemann V, Ganea K, Liebl C, Alam S, Harbich D *et al* (2007). Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. *Psychoneuroendocrinology* **32**: 417–429.
- Schmidt MV, Trümbach D, Weber P, Wagner K, Scharf SH, Liebl C *et al* (2010). Individual stress vulnerability is predicted by short-term memory and AMPA receptor subunit ratio in the hippocampus. *J Neurosci* **30**: 16949–16958.
- Shiraishi-Yamaguchi Y, Furuichi T (2007). The Homer family proteins. *Genome Biol* **8**: 206.
- Szumliński KK, Lominac KD, Kleschen MJ, Oleson EB, Dehoff MH, Schwarz MK *et al* (2005). Behavioral and neurochemical phenotyping of Homer1 mutant mice: possible relevance to schizophrenia. *Genes Brain Behav* **4**: 273–288.
- Szumliński KK, Dehoff MH, Kang SH, Frys KA, Lominac KD, Klugmann M *et al* (2004). Homer proteins regulate sensitivity to cocaine. *Neuron* **43**: 401–413.
- Thase ME (2006). Preventing relapse and recurrence of depression: a brief review of therapeutic options. *CNS Spectr* **11**: 12–21.
- Tronson NC, Guzman YF, Guedea AL, Huh KH, Gao C, Schwarz MK *et al* (2010). Metabotropic glutamate receptor 5/Homer interactions underlie stress effects on fear. *Biol Psychiatry* **68**: 1007–1015.
- Tu JC, Xiao B, Naisbitt S, Yuan JP, Petralia RS, Brakeman P *et al* (1999). Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* **23**: 583–592.
- Tu JC, Xiao B, Yuan JP, Lanahan AA, Leoffert K, Li M *et al* (1998). Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. *Neuron* **21**: 717–726.
- Wagner KV, Hartmann J, Mangold K, Wang XD, Labermaier C, Liebl C *et al* (2013). Homer1 mediates acute stress-induced cognitive deficits in the dorsal hippocampus. *J Neurosci* **33**: 3857–3864.
- Wagner KV, Marinescu D, Hartmann J, Wang XD, Labermaier C, Scharf SH *et al* (2012). Differences in FKBP51 regulation following chronic social defeat stress correlate with individual stress sensitivity: influence of paroxetine treatment. *Neuropsychopharmacology* **37**: 2797–2808.
- Wagner KV, Wang XD, Liebl C, Scharf SH, Müller MB, Schmidt MV (2011). Pituitary glucocorticoid receptor deletion reduces vulnerability to chronic stress. *Psychoneuroendocrinology* **36**: 579–587.
- Wang XD, Chen Y, Wolf M, Wagner KV, Liebl C, Scharf SH *et al* (2011). Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic remodeling. *Neurobiol Dis* **42**: 300–310.
- Webhofer C, Gormanns P, Reckow S, Lebar M, Maccarrone G, Ludwig T *et al* (2013). Proteomic and metabolomic profiling reveals time-dependent changes in hippocampal metabolism upon paroxetine treatment and biomarker candidates. *J Psychiatr Res* **47**: 289–298.
- Yim YS, Lee J, Kim GT, Song T, Kim CH, Kim DG (2012). Hippocampal mGluR5 predicts an occurrence of helplessness behavior after repetitive exposure to uncontrollable stress. *Neurosci Lett* **519**: 62–66.
- Yuan JP, Kiselyov K, Shin DM, Chen J, Shcheynikov N, Kang SH *et al* (2003). Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors. *Cell* **114**: 777–789.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)