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Enhanced adenosine A_1 receptor and Homer1a expression in hippocampus modulates the resilience to stress-induced depression-like behavior

Tsvetan Serchov[a,*], Inna Schwarz[b], Alice Theiss[c], Lu Sun[c,d], Amrei Holz[c,e], Mate D. Döbrössy[a], Martin K. Schwarz[b], Claus Normann[c], Knut Biber[c,d,1], Dietrich van Calker[c,1]

[a] Department of Stereotactic and Functional Neurosurgery, Medical Center - University Freiburg, Faculty of Medicine, University of Freiburg, Breisacher Str. 64, 79106, Freiburg, Germany
[b] Functional Neuroconnectomics Group, Department of Experimental Epileptology and Cognition Research, Life and Brain Centre, University of Bonn, Medical School, 53105, Bonn, Germany
[c] Department for Psychiatry and Psychotherapy, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Hauptstrasse 5, 79104, Freiburg, Germany
[d] Department Biomedical Sciences of Cells & Systems, Section Molecular Neurobiology, University of Groningen, University Medical Center Groningen, 9713, AV Groningen, the Netherlands
[e] Faculty of Biology, University of Freiburg, Schaenzlestr. 1, 79104, Freiburg, Germany

HIGHLIGHTS

- Enhanced adenosine A_1 receptor (A_1R) expression in cortex has antidepressant effect.
- Hippocampal overexpression of A_1R decreases resilience to depression-like behavior.
- Upregulated A_1R expression in hippocampus increases Homer1a level and impairs LTP.
- Viral overexpression of Homer1a in hippocampus decreases stress resilience.

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ABSTRACT

Resilience to stress is critical for the development of depression. Enhanced adenosine A_1 receptor (A_1R) signaling mediates the antidepressant effects of acute sleep deprivation (SD). However, chronic SD causes long-lasting upregulation of brain A_1R and increases the risk of depression. To investigate the effects of A_1R on mood, we utilized two transgenic mouse lines with inducible A_1R overexpression in forebrain neurons. These two lines have identical levels of A_1R increase in the cortex, but differ in the transgenic A_1R expression in the hippocampus. Switching on the transgene promotes robust antidepressant and anxiolytic effects in both lines. The mice of the line without transgenic A_1R overexpression in the hippocampus (A1Hipp-) show very strong resistance towards development of stress-induced chronic depression-like behavior. In contrast, the mice of the line in which A_1R upregulation extends to the hippocampus (A1Hipp+), exhibit decreased resilience to depression as compared to A1Hipp-. Similarly, automatic analysis of reward behavior of the two lines reveals that depression resistant A1Hipp-transgenic mice exhibit high sucrose preference, while mice of the vulnerable A1Hipp + line developed stress-induced anhedonic phenotype. The A1Hipp + mice have increased Homer1a expression in hippocampus, correlating with impaired long-term potentiation in the CA1 region, mimicking the stressed mice. Furthermore, virus-mediated overexpression of Homer1a in the hippocampus decreases stress resilience. Taken together our data indicate for first time that increased expression of A_1R and Homer1a in the hippocampus modulates the resilience to stress-induced depression and thus might potentially mediate the detrimental effects of chronic sleep restriction on mood.

* Corresponding author.
E-mail address: tsvetan.serchov@uniklinik-freiburg.de (T. Serchov).
[1] The two senior authors have contributed equally to this research.

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1. Introduction

Major depression (MD) affects more than 300 million people worldwide and is associated with high individual suffering, increased risk of suicide and an enormous economic burden for the society (Eaton et al., 2008; Mrazek et al., 2014). Understanding the neurobiology of depression remains one of the foremost challenges in modern psychiatry. Stress resilience plays a critical role in regulating the development of depression and the identification of the neurobiological substrates underlying the resilience to stress is essential to the development of strategies for the prevention of stress-mediated depression.

While research on alterations in monoamines has dominated the field for years, recent studies have implicated the glutamatergic and adenosinergic systems in the neurobiology and treatment of depression (Sadek et al., 2011; Sanacora et al., 2012; van Calker and Biber, 2005; van Calker et al., 2018, 2019). Non-pharmacological treatments of depression, like sleep deprivation (SD) and electroconvulsive therapy are associated with an increased stimulation of adenosine A_1 receptor (A_1R) (Elmenhorst et al., 2007, 2009; Sadek et al., 2011; van Calker and Biber, 2005). Accordingly, it has been shown that enhanced A_1R signaling via inducible upregulation of A_1R expression in forebrain neurons of transgenic mice or specific A_1R agonist administration inhibits depressive-like behavior and that A_1R is necessary for the antidepressant effects of SD (Hines et al., 2013; Serchov et al., 2015). Moreover, we have demonstrated that the antidepressant effects of SD are mediated by A_1R-induced induction of the synaptic protein Homer1a specifically in the medial prefrontal cortex (mPFC) (Serchov et al., 2015).

While the antidepressant effect of acute SD is very fast and robust, it is only transient (Benedetti and Colombo, 2011) (Elmenhorst et al., 2017; Hines et al., 2013; Serchov et al., 2015). SD or agonist activation of A_1R lose their therapeutic effects on depression-like behavior when prolonged (Hines et al., 2013). Chronic exposure to SD is even associated with an increased risk of depression (Baum et al., 2014; Conklin et al., 2018). Chronic sleep restriction induces long-lasting increase in A_1R expression in several brain regions, including cortex and hippocampus (Kim et al., 2015), suggesting that this may underlay the negative effects on mood regulation (Novati et al., 2008). Moreover, chronic SD appears to be particularly detrimental to functions of the hippocampus and negatively affects molecular signaling important for synaptic plasticity (Areal et al., 2017; Clasadonte et al., 2014; Havekes and Abel, 2017; Kreutzmann et al., 2015; Novati et al., 2008). Thus, the long-lasting increase of A_1R expression induced by chronic sleep restriction might decrease resilience to depression-like behavior via affecting synaptic plasticity in the hippocampus.

In order to model the effects of SD on A_1R levels and their role in stress response and resilience to depression, we utilized two transgenic mouse lines with identical levels of A_1R increase in the cortex, but distinct transgenic A_1R expression in the hippocampus. We show that the mice without transgenic A_1R overexpression in the hippocampus (A1Hipp-) have strong resistance towards development of repeated swim stress-induced chronic depression-like behavior, thus providing evidence that enhanced A_1R expression in the cortex promotes stress resilience. However, mice of the strain in which A_1R is upregulated also in the hippocampus (A1Hipp+), exhibit vulnerability to develop repeated swim stress-induced chronic depression-like behavior in various tests as compared to A1Hipp-line. Moreover, the decreased resilience to stress in these mice is associated with impaired synaptic plasticity and is mediated by upregulated expression of Homer1a in the hippocampus. Thus, increased expression of A_1R and Homer1a in the hippocampus modulates stress-induced depression-like behavior and might be potential mechanism mediating the detrimental effects of chronic sleep restriction on mood.

2. Material and methods

2.1. Animals

All procedures were performed in accordance with the German animal protection law (TierSchG), FELASA (www.felasa.eu/guidelines.php), the national animal welfare body GV-SOLAS (www.gv-solas.de/index.html) guide for the care and use of laboratory animals, National Institute of Health Guide for Care and Use of Laboratory Animals, the EU Directive 2010/63/EU for animal experiments and were approved by the animal welfare committee of the University of Freiburg and University of Bonn, as well as local authorities. Animals were housed in a temperature and humidity controlled vivarium with a 12 h light-dark cycle, food and water were available *ad libitum*. Male and female mice of at least 8 weeks of age were used throughout this study. Wild type C57Bl/6J mice were obtained from a breeding colony of CEMT-Freiburg delivered from Charles River Laboratories.

2.2. Generation of adenosine A_1 receptor transgenic mice

The generation and designing of the adenosine A_1 receptor (A_1R) transgenic mouse line with tetracycline-regulated gene expression system and simultaneous expression of mouse A_1R and a red fluorescent protein mCherry, as a reporter gene was previously described (Serchov et al., 2012, 2015). The transgenic mice were genotyped by PCR using targeting primers against mCherry (For: 5′-GCCCTTCGCCTGGGACA TCC-3′, Rev: 5′-ATTACGGGGCCGTCGGAGGG-3′). For cell-specific regulation of the expression of A_1R in forebrain neurons, the responder founder animals were crossed with the CaMKII-tTA mouse line, which express the transactivator (tTA) under the control of CaMKII promoter specifically in forebrain neurons (Jackson Laboratory - B6; CBA-Tg (Camk2a-tTA)1Mmay/J) (Mayford et al., 1996). Two of the obtained F1 double-transgenic mouse lines (line 92 - exhibiting A_1 overexpression also in the hippocampus, therefore called A1Hipp + line in the following (Serchov et al., 2015) and line 52 - exhibiting no A_1R overexpression in the hippocampus, therefore called A1Hipp-line in the following), which showed highest levels and strongest doxycycline-controlled regulation of transgene expression in the cortex, were selected and used in all experiments. All mice were maintained on doxycycline (Dox) (1.5 mg/ml) containing drinking water until weaning, after which they were switched to Dox free water to allow transgene expression, unless otherwise specified. At the end of the behavior testing the transgenic expression was verified by immunohistochemistry (Suppl. Figure 1F) and qRT-PCR (Fig. 1B).

2.3. Behavioral studies

Activity and behavior of mice were observed using an automatic video tracking system for recording and analysis (VideoMot2 system V6.01) and IntelliCage system (TSE Systems, Bad Homburg, Germany), unless otherwise specified. One cohort of mice was used to perform the open field, light/dark transition and elevated plus maze tests, and another cohort was used for the classical tail suspension and forced swim tests. Both cohorts consisted of male and female mice. To avoid aggressive behaviours, a third cohort of female mice was used for the sucrose preference test performed in the IntelliCage system experiments (Alboni et al., 2017a, 2017b). The chronic behavioral despair model (CDM) (see below) was performed with all cohorts. All behavioral experiments were performed during day time with the examiners blind to treatment and genotype.

2.3.1. Open field test

The open field consisted of a square of 50 × 50 cm surrounded by a 35 cm wall, made from non-reflecting grey PVC, illuminated with 65–75 lux. The mice were placed in a central area and the behavior of the animals in the open field was recorded for 10 min. Evaluation of

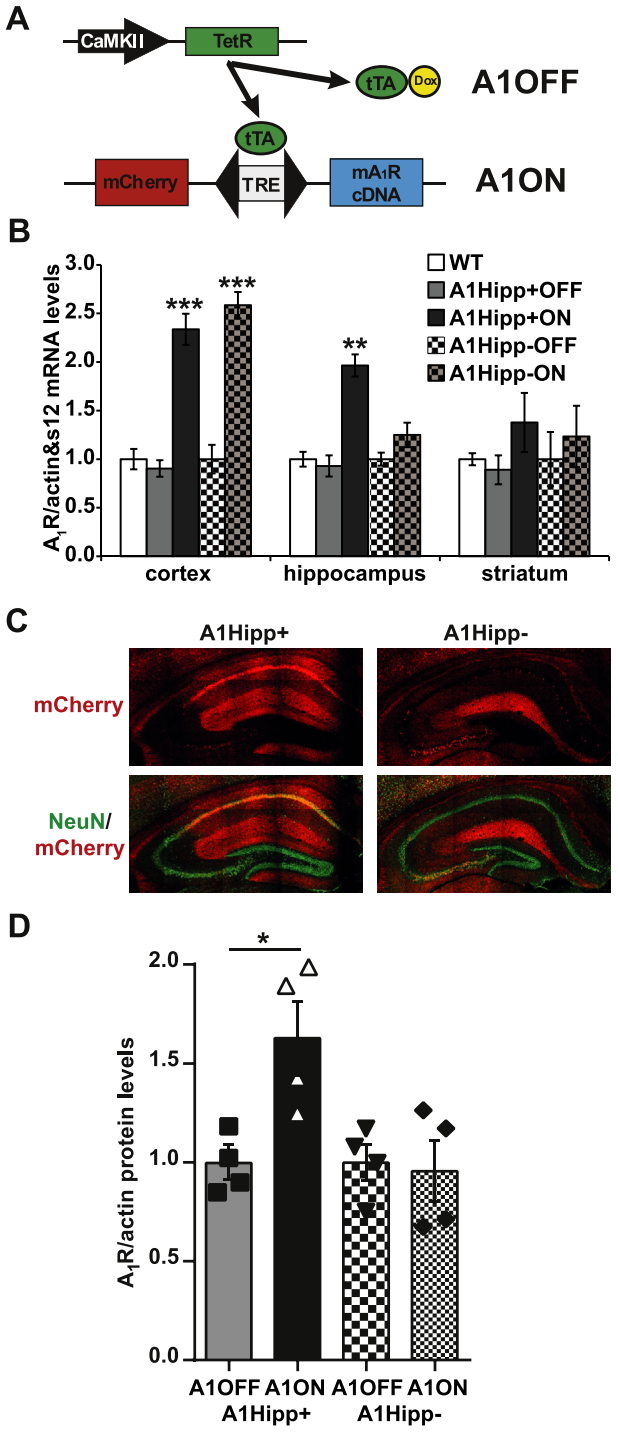

Fig. 1. Tetracycline-regulated enhanced expression of adenosine A_1 receptor in two different transgenic mouse lines. (**A**) The tetracycline-regulated (Tet-Off) expression of the mouse A_1 receptor (A_1R). Ca^{2+}/calmoduline-dependant kinase II (CaMKII) promoter controls the expression of the tetracycline transactivator (tTA) gene product, which induces the simultaneous transcription of the A_1R and mCherry specifically in forebrain neurons (A1ON), by binding to a tetracycline responsive element (TRE) containing bidirectional promoter. Thus, the gene expression could be blocked (A1OFF) by tetracycline or its stable analogue doxycycline (Dox). (**B**) Relative mRNA expression of A_1R in cortex, hippocampus and striatum of wild type (WT), A1Hipp+ and A1Hipp- mice (A1OFF – Dox treated from birth; A1ON – Dox treated from birth, followed by 4 weeks Dox withdrawal), normalized to s12, GAPDH and actin (n = 6 per group). (**C**) Spatial distribution of mCherry expression in the hippocampus of A1Hipp+ and A1Hipp- (green – neuronal nuclei marker NeuN; red – anti-mCherry/RFP staining). (**D**) A_1R protein expression in the hippocampus of A1Hipp+ and A1Hipp- normalized to actin (n = 4 per group). One-way ANOVA with Bonferroni *post hoc* test: *p < 0.05, **p < 0.01 and ***p < 0.001 in comparison to WT and A1OFF. Data are expressed as means ± SEM in all graphs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

data set included time spent in the central area of the field (10 × 10 cm squares, 9 central, 16 peripheral) and covered distance.

2.3.2. Light/dark transition test

The apparatus used for the light/dark transition test consisted of a box (21 cm × 42 cm × 25 cm) divided into a small (one third) dark compartment (5 lux) and a large (two thirds) illuminated compartment (300 lux). Mice were placed into the lit compartment and allowed to move freely between the two chambers with the door open for 5 min. The first latency to enter the dark side and the total time spent in lit compartment were recorded automatically.

2.3.3. Elevated plus maze

The elevated plus maze consisted of two open and two closed arms each of 30 × 5 cm. Closed arms were surrounded by a 15 cm high wall. All arms emerged from a central platform which was elevated 45 cm above the floor. The maze was made of non-reflecting light grey PVC. Mice were placed on the central platform, facing one of the open arms. The duration of the mice in each arm and the total travelled distance were continuously assessed during 5 min.

2.3.4. Tail suspension test (TST)

Each mouse was suspended by its tail to a horizontal bar located inside a white box (30 x 50 × 20 cm). The mouse is taped 1–1.5 cm from the tip of the tail, such that the mouse head was about 20 cm above the floor. The trial was conducted for 6 min (360 s) during which the behavior was video recorded and the amount of time spent immobile was scored by two independent persons blinded to the experimental condition of the animal. When the mice were observed to climb their tails (> 10% of total time), they were eliminated from further analyses.

2.3.5. Forced swim test (FST)

The FST was performed similarly to the previously described protocol (Serchov et al., 2015) with small modifications. Mice were subjected to two trials during which they were placed individually into transparent glass cylinder (15 cm diameter) containing 20 cm of water (22–25 °C). The first trial lasts 10 min, followed by a second 10 min test trial, which was performed 24 h later. The total duration of immobility was recorded during the last 10 min testing period. A mouse was considered to be immobile when it floated in an upright position and made only minimal movements to keep its head above water. Trials were video recorded and scored offline by an investigator blinded to the experimental outcome of each animal.

2.3.6. Sucrose preference test (SPT)

For the classical SPT, mice are presented with 2 dual bearing sipper tubes. One tube contains plain drinking water, and the second contains a 1% sucrose solution. Prior to beginning testing, mice are habituated to the presence of two drinking bottles for 3 days in their home cage. Water and sucrose solution intake is measured daily, and the positions of two bottles is switched daily to reduce any confound produced by a side bias. Sucrose preference is calculated as a percentage of the volume of sucrose intake over the total volume of fluid intake and averaged over the 3 days of testing.

2.3.7. Chronic behavioral despair model (CDM)

In order to induce chronic behavioral despair in mice we used recently described protocol (Hellwig et al., 2016; Normann et al., 2018; Serchov et al., 2015; Sun et al., 2011). The mice were subjected to

repeat swimming in a transparent cylinder (15 cm diameter) containing 20 cm of water (22–25 °C) for 10 min daily for five consecutive days (induction phase). From day 6 on, the mice were kept in the home cage without swimming for 4 weeks, after which a last swim was imposed on day32 (test phase). The immobility time of the mice was analyzed in each session. The repeated exposure to swimming significantly increased the immobility time over this 5 days period. This induced state of behavioral despair was chronically maintained for 4 weeks (till day32) and represent a model for depressive-like behavior in mice (Hellwig et al., 2016; Normann et al., 2018; Serchov et al., 2015; Sun et al., 2011).

2.3.8. Behavioral analysis in the IntelliCage

The IntelliCage system (TSE Systems, Bad Homburg, Germany) allows simultaneously analysis of spontaneous behavior, explorative behavior, activity patterns and drinking preference of up to 16 group-housed mice implanted with RFID transponders (Endo et al., 2011). The unit consists of an open common space with 4 red shelters in the center and four recording corners. Mice have free access to food in the middle of the IntelliCage, and water is available in the corners behind remotely controlled guillotine doors. Each corner houses two drinking bottles and allows the entry of only one mouse at the time. The scored parameters: the number and the durations of visits to any of the four corners, the nosepokes towards the doors and the licks on the bottles were monitored by the PC based tracking software (IntelliCage Plus, TSE Systems). Initially, the mice were allowed to adapt to the IntelliCage for at least 7 days with water available *ad libitum* in all corners. Then for 3 days the animals were habituated to the sucrose taste: in each corner one of the bottles was filled with 1% sucrose solution and the other one with water. Both doors in the corner were open allowing free choice between the bottles. Next, a nosepoke adaptation period was carried out, where all doors were closed and the mice had to perform a nosepoke to open them. The opened door closes automatically after 5 s of drinking. In all tasks involving sucrose filled bottles the positions of the bottles were exchanged every 24 h. The sucrose preference was measured with gradually increasing effort (number of nosepokes) to reach the sucrose bottles – Nosepoke SPT (Alboni et al., 2017a, 2017b). In this paradigm all doors open in response to a nosepoke and close after 5 s licking. The number of nosepokes needed to open a door to a side with a sucrose containing bottle increases (1, 2, 3, 4, 5, 6, 7) after every 24 h. For each bottle the number of licks was recorded and preference for sucrose was calculated as a percentage of the total number of licks.

2.4. Immunohistochemistry

Animals were anaesthetized with a mix of Ketamin-Rompun (Ketamine [CP Pharma] 50 mg and Rompun [Bayer Healthcare] 0.5 mg per 100 g body weight) and transcardially perfused with 50 ml of ice-cold PBS (8.1 mM Na_2HPO_4, 138 mM NaCl, 2.7 mM KCl and 1.47 mM KH_2PO_4 [pH 7.4]). Brains were removed, postfixed overnight in 4% paraformaldehyde in PBS at 4 °C and cryoprotected for 2 days in 30% sucrose in PBS at 4 °C. The brains were then frozen and 40-μm coronal sections were cut with a sliding cryostat (Leica Microsystems). Then, the free floating sections were incubated with blocking solution (0.3% Triton X-100 and 5% normal goat serum in PBS) for 1 h at 4 °C. Sections were incubated with the respective primary antibody – mouse anti-NeuN (1:1000; Millipore) and rabbit anti-RFP/mCherry (1:1000; Clontech) in blocking solution at 4 °C overnight. After 3x washing with PBS, sections were incubated with the secondary antibody – Alexa Fluor 488 goat anti-mouse IgG (1:1000) and Alexa Fluor 647 donkey anti-rabbit IgG (1:1000) (both from Invitrogen) blocking solution for 3 h at room temperature. Sections were then washed and stained with 4′, 6-diamidino-2-phenylindole (DAPI, 1:1000) for 10 min. After final washes in PBS slices were mounted on a slides using Mowiol-DABCO. All immunofluorescence images were detected and photographed using LSM-U-2 laser scanning confocal microscope and documented using ZEN 2009 software (Carl Zeiss).

2.5. Quantitative real-time PCR (qRT-PCR)

Mice from each experimental group were killed by cervical dislocation. Brains were rapidly removed, coronally cut and the following regions were dissected and quickly frozen on dry ice and stored at −80 °C until used for RNA isolation: medial prefrontal cortex (mPFC), hippocampus and striatum. The brains were always dissected by the same investigator with the assistance of a brain atlas. The RNA extraction was performed by the method of Chomczynski and Sacchi (2006). Briefly, the tissues were homogenized with guanidine thiocyanate/2-mercaptoethanol buffer and total RNA was extracted with the sodium acetate/phenol/chloroform/isoamylacohol step. Then, samples were isopropanol precipitated and washed twice with 70% ethanol. The pellets were dissolved in RNase free Tris-HCl buffer (pH 7.0), and RNA concentrations were determined by spectrophotometer (BioPhotometer; Eppendorf). Reverse transcription was performed with 1 mg of total RNA using M-MLV reverse transcriptase (Promega). The quantitative real-time PCR was done on C1000TM Thermal Cycler (CFX96 real-time PCR system, Bio-Rad) using iQ SYBR Green Supermix (BioRad). All qRT-PCR experiments were performed blinded, as the coded cDNA samples were pipetted by a technician. The target genes mRNA levels were normalized to the levels of actin, glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and s12 RNA automatically by CFX Manager software (Version 3.0.1224.1051, 2012, Bio-Rad) using the "Gene study" option via geometric averaging of multiple internal control genes (Vandesompele et al., 2002). The primer sequences used were as follows: adenosine A_1 receptor: 5′-CTGGCTACCGCTACACA TCT-3′, 5′-TCATCAGCTTTCTCCTCTGG-3'; Homer1a: 5′-CAAACACTGT TTATGGACTG-3′, 5′-TGCTGAATTGAATGTGTACC-3'; actin: 5′-CTAAG GCCAACCGTGAAAAG-3′, 5′-ACCAGAGGCATACAGGGACA-3'; GAPDH: 5′-TGTCCGTCGTGGATCTGAC-3′, 5′-CCTGCTTCACCACCTTCTTG-3'; s12: 5′-GCCCTCATCCACGATGGCCT-3′, 5′-ACAGATGGGCTTGGCGCT TGT-3'.

2.6. Western blot analysis

Dissected brain tissues were homogenized in ice-cold lysis buffer (50 mM Tris-HCl [pH 7.4], 1% NP-40, 40 mM NaF, 10 mM EDTA, 0.1% SDS, 1 mM Na-orthovanadat, 1 mM PMSF and protease inhibitor cocktail). The homogenate is centrifuged at 10000g for 15 min at 4 °C. The supernatants were collected and then the protein concentration was determined with an assay kit based on the Bradford method (Bio-Rad). 40 μg protein from the lysates was mixed with 2x Laemli (50 mM Tris-HCL [pH 6.8], 2% SDS, 5% 2-Mercaptoethanol, 0.2 M DTT, 10% Glycerin, 0.04% Pyronin Y) and boiled at 95 °C. The extracts were separated on 12% SDS-PAGE and electrotransferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% nonfat dry milk in PBS-T (1% Tween20 in PBS) for 1 h at room temperature (RT) and then incubated with the respective primary antibody – rabbit anti-adenosine A_1 receptor (1:1000; #PA3-041; Thermo Scientific), rabbit anti-actin (1:5000; #A5060; Sigma), overnight at 4 °C. The membranes were washed 3 times for 5 min in PBS-T and incubated for 1 h at RT with secondary horseradish peroxidase-conjugated antibody – goat anti-rabbit IgG (1:25000; #NA9340V; GE Healthcare), diluted in PBS-T, and then washed again 3 times for 5 min in PBS-T. The signal was detected using an enhanced chemiluminiscent detection reagent kit (GE Healthcare) and a Fusion-SL imaging system (PeQlab). The immunoreactive bands along with their respective loading controls were quantified densitometrically by using the ImageJ v1.47 software (NIH, USA).

2.7. Electrophysiology recordings

Hippocampal brain slices from young adult animals (20 days–3

months postnatal) were prepared and measured as described in detail previously elsewhere (Normann et al., 2018). EPSPs were evoked by extracellular stimulation of the Schaffer collateral fibers and recorded with current-clamp whole-cell measurements in CA1 pyramidal neurons. For the LTP induction protocol, 300 EPSPs were paired with postsynaptic actions potentials induced with a short current injection into the CA1 neuron using a theta burst protocol (10 pairings with 100 Hz repeated 10 times with an interval of 200 ms; this stimulation block was repeated three times with an interval of 10 s). To calculate the mean EPSP amplitude before and 20–30 min after LTP induction, 20–30 consecutive EPSPs were averaged for each experiment. The average EPSP amplitudes in the figures represent the means from all experiments and from 4 consecutive EPSPs from all single experiments.

2.8. In vivo stereotaxic microinjections of recombinant adeno-associated viral vectors (rAAV)

For preparation of rAAV, the rat homer1a cDNA (h1a) was subcloned into an AAV backbone containing the 480 bp human synapsin core promoter, the woodchuck post-transcriptional regulatory element (WPRE), the fluorescent protein Venus (V) and the bovine growth hormone polyA sequence (Celikel et al., 2007). The same backbone carrying EGFP was used as a control. Stereotaxic injections of rAAVs, we performed as previously described (Serchov et al., 2015). Briefly, animals were anaesthetized intraperitoneally with ketamine hydrochloride (90 mg/kg)/xylazine (5 mg/kg). All pressure points and the skin incision were infused with Licain (1% lidocainhydrochlorid). 1.5 µl of either, rAAV-EGFP or rAAV-h1aV (~2 × 1011 particles/ml) were injected bilaterally into the dorsal hippocampus (1.9 mm posterior to bregma, 1.3 mm lateral from midline and 1.5 mm bellow the scull surface) (Wagner et al., 2015) using a custom-made stereotaxic frame. Viral particles were infused at a rate of 100 nl/min with a 10-µl syringe fitted with a 34G beveled needle by a microprocessor-controlled minipump (World Precision Instruments). Behavioral testing began 4 weeks after the rAAV injections.

2.9. Statistical analyses

All values are expressed as means ± SEM. Prior to statistical analyses data assumptions (for example normal distribution, similar variation between experimental groups etc.) were verified. Statistical analyses were performed with GraphPad Prism 6.07 software (GraphPad Software Inc.) using one- or two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test to compare the means of two or more groups or unpaired two-tailed Student's t-test to compare the means of two groups. A P value ≤ 0.05 was considered to be significant ($^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$). For all molecular and behavioral studies mice were randomly assigned to groups. Additionally, investigators were blinded to the treatment group until data has been collected. Sample sizes were determined on the basis of extensive laboratory experience and were verified via power analysis.

3. Results

3.1. Inducible enhanced A_1R expression in different brain areas of two transgenic mouse strains

In order to mimic the effects of SD on the A_1R levels, we generated transgenic mouse models with enhanced neuronal A_1R expression regulated by a tetracycline inducible bidirectional promoter allowing the simultaneous expression of mouse A_1R and mCherry reporter gene (Serchov et al., 2012, 2015) (Fig. 1A). Due to the random integration of the transgene cassette in the founder mouse genome, we found that the generated founder lines differed in levels and patterns of transgenic expression. For further characterization, we focused on founder line92 and line52, because these lines had the highest levels and strongest doxycycline-controlled regulation of transgene expression. In the further course of the manuscript mouse line52 and line92 will be depicted as A1Hipp-line and A1Hipp + line respectively, indicating the difference in the transgenic expression of A_1R in the hippocampus. These two lines showed identical increase of A_1R mRNA expression in cortex (A1Hipp+: 1.34 ± 0.16 vs A1Hipp-: 2.59 ± 0.14; $P = 0.2658$) (Fig. 1B). However, A1Hipp + exhibited upregulated expression of A_1R mRNA (A1Hipp+: 1.96 ± 0.11 vs A1Hipp-: 1.25 ± 0.13; $F_{6,21} = 12.23$, $P = 0.0001$) and protein (A1Hipp+: 1.64 ± 0.13 vs A1Hipp-: 1.14 ± 0.22; $F_{3,12} = 5.814$, $P = 0.0108$) in the hippocampus without doxycycline (A_1R transgene switched on, A1ON) in comparison to doxycycline treated mice (A_1R transgene switched off, A1OFF) or wildtype (WT) mice (Serchov et al., 2015). Though no significant increase of A_1R levels (A1ON) were observed in this region of A1Hipp- (Fig. 1B–D). The labelling pattern of reporter gene mCherry demonstrated that the transgenic expression in hippocampus is predominantly located in CA1 region of the A1Hipp+, while in A1Hipp-it is only in a few pyramidal cells of CA3 area and in the neuronal projections from the entorhinal cortex (Fig. 1C).

3.2. Behavioral characterization

To investigate potential behavioral differences between the two lines due to the different levels of A_1R expression in hippocampus, we subjected the mice to a battery of behavioral tests. The evaluation of the spontaneous activity and exploratory drive, assessed by the total travelled distance in the open field test showed no significant differences between the lines at both A1OFF and A1ON conditions ($F_{4,46} = 1.508$, $P = 0.2156$; Fig. 2A). As previously reported (Serchov et al., 2015), switching on the transgene (A1ON) induced anxiolytic effects in several behavioral tests for anxiety (Fig. 2A–C). However, A1Hipp- A1ON mice showed a more pronounced anxiolytic-like effect in two behaviors indicative of anxiety: time spent in the central area in open field test (A1Hipp + A1ON: 36.37 ± 1.6m vs A1Hipp- A1ON: 46.88 ± 3.35m; $F_{4,46} = 43.41$, $P < 0.0001$; Fig. 2A) and time spent in the lit compartment of dark-light box (A1Hipp + A1ON: 191.20 ± 9.13s vs A1Hipp- A1ON: 271.33 ± 11.37s; $F_{4,37} = 20.15$, $P < 0.0001$; Fig. 2C).

Enhanced A_1R expression promoted marked antidepressant effects in both lines, evident by significant decrease of the immobility time in the two basic tests for depressive-like behavior - tail suspension test (TST) ($F_{4,29} = 13.59$, $P < 0.0001$; Fig. 2D) and forced swim test (FST) ($F_{4,51} = 18.34$, $P < 0.0001$; Fig. 2E). There was no significant difference between the two strains in the depressive-like behavior in these acute tests.

We then investigated the susceptibility of the mice to develop depression-like phenotype in the chronic behavioral despair model (CDM) (Normann et al., 2018; Serchov et al., 2015; Sun et al., 2011) (Fig. 3A). In this paradigm, the mice are subjected to repetitive swimming for 5 consecutive days (induction phase), which leads to significant increase of the immobility time in days 2–5 (Fig. 3B,C) and a pronounced anhedonic phenotype, manifested by reduced sucrose preference, one of the major pathological features of depression (Fig. 4B). Moreover, several different antidepressant treatments, including chronic treatment with imipramine, 6 h SD and acute treatment with ketamine significantly reduced the immobility time during the test phase and TST, demonstrating that this model has predictive validity to antidepressant treatments (Serchov et al., 2015). WT and A1OFF mice from both strains did not show any differences in the immobility time during the induction and test phase of CDM (Fig. 3B and C). However, the enhanced A_1R expression in A1ON mice differentially affected the acquisition and the maintenance of depression-like behavioral state in the two transgenic lines (Fig. 3B and C). As published previously (Serchov et al., 2015), the A1Hipp + A1ON mice compared to WT show a delayed increase of the immobility time during the induction phase with a significant difference at days 1–4, but they finally develop the same

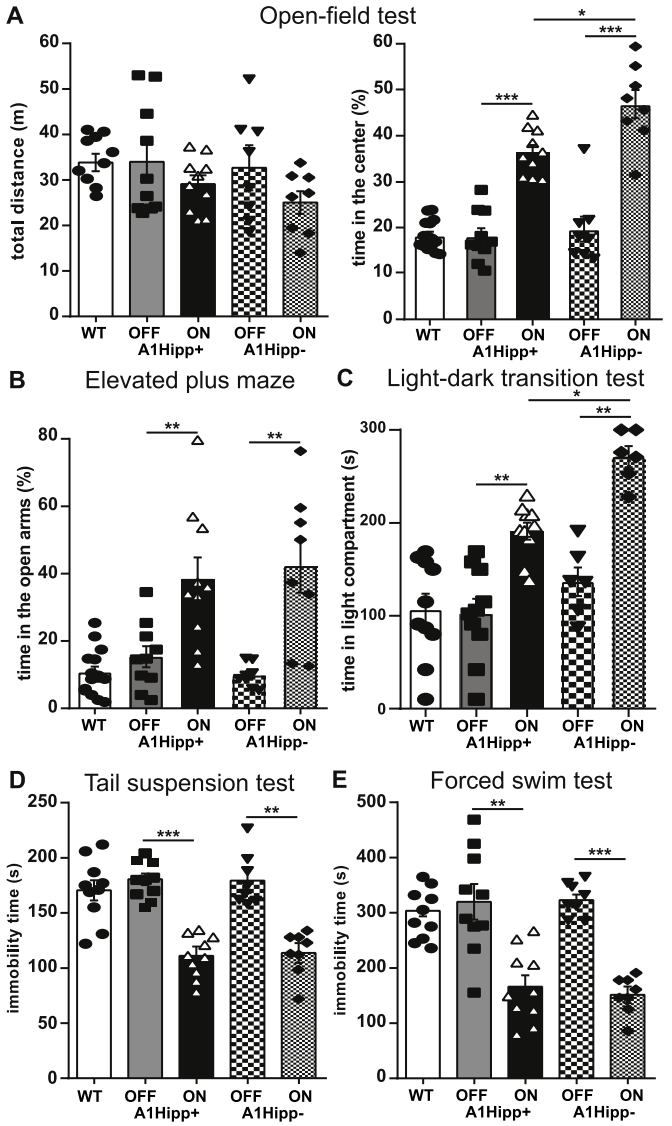

Fig. 2. Anxiolytic and antidepressant effects of enhanced A_1R expression. (**A**) Spontaneous activity and exploratory behavior expressed as total distance of movement (left) and percentage of total time spent in the inner squares of the open filed test (right). (**B**) Percentage of total time spent in the open arms of elevated plus maze. (**C**) Total time spent in the lit compartment of light-dark transition test. (**D**) Immobility time in tail suspension test (TST) and (**E**) in day 2 of forced swim test (FST). (n = 10 for WT and A1Hipp+, n = 8 for A1Hipp-) One-way ANOVA with Bonferroni *post hoc* test: **p < 0.01, ***p < 0.001 in comparison to WT and A1OFF. Data are expressed as means ± SEM in all graphs.

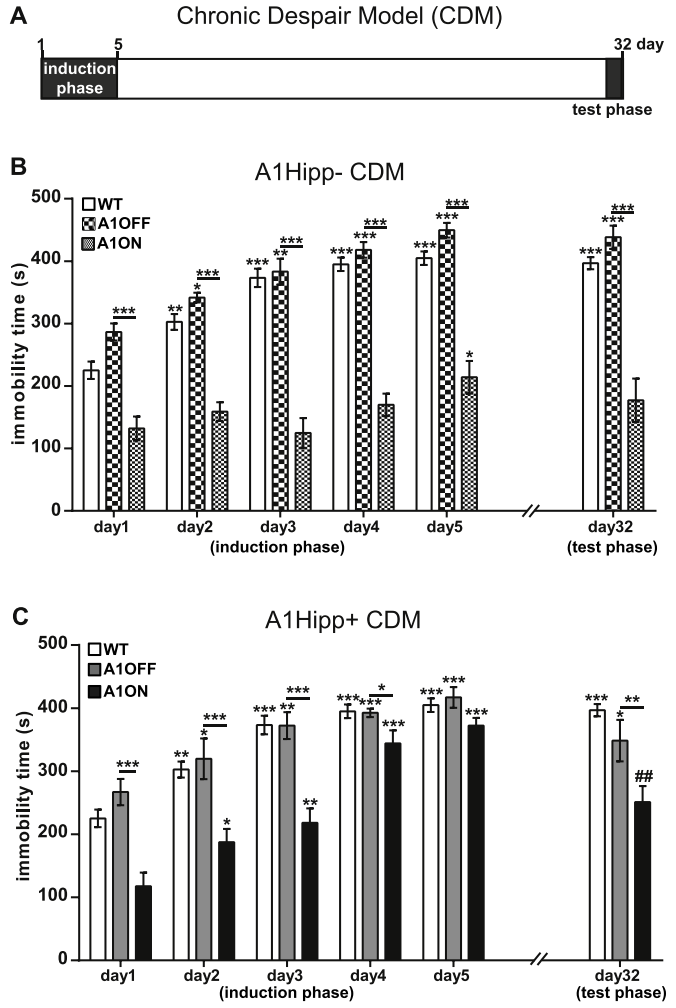

Fig. 3. Antidepressant effects and stress resilience in A1 mice in the chronically despaired model. (**A**) Schematic illustration of the experimental protocol: The mice were despaired by 10 min swim sessions for 5 consecutive days: days1–5 (induction phase). For the following 4 weeks the mice were kept undisturbed in their home cages and divided into groups: WT – wild type; A1OFF – mice with doxycycline-suppressed A_1R expression for the whole experiment; A1ON – mice with activated A_1R expression for the whole experiment. On day32 the last 10min swim session (test phase) was performed. (**B**) Immobility time spent during the induction phase (day1 to day5) and the test phase (day32) of WT (n = 20), A1Hipp- A1OFF (n = 16) and A1Hipp- A1ON (n = 18). (**C**) Immobility time spent during the induction phase (day1 to day5) and the test phase (day32) of WT (n = 20), A1Hipp + A1OFF (n = 20), A1Hipp + A1ON (n = 20). Two-way ANOVA with Bonferroni *post hoc* test: *p < 0.05, **p < 0.01, ***p < 0.001, if not indicated in comparison to day1; ##p<0.01 in comparison to day5. Data are expressed as means ± SEM in all graphs.

depressive-like behavior as WT and A1OFF mice at day 5 (Fig. 3C; genotype/A1ON effect $F_{2,269} = 41.74$, $P < 0.0001$; time effect $F_{6,269} = 38.89$, $P < 0.0001$; interaction $F_{12,269} = 4.02$, $P < 0.0001$). However, the A1Hipp- A1ON mice exhibit pronounced resistance towards development of depression-like behavior with no significant increase of the immobility time at days 2–4 of the induction phase in comparison to day 1 (Fig. 3B; genotype/A1ON $F_{2,235} = 174.7$, $P < 0.0001$; time $F_{6,235} = 15.75$, $P < 0.0001$; interaction $F_{12,235} = 5.017$, $P < 0.0001$). The maintenance of the enhanced A_1R expression in CDM A1ON mice, during the 4 weeks after the induction phase, equally reduced the immobility time during the test phase in both strains (Fig. 3B and C), demonstrating the antidepressant effect of A_1R overexpression also in CDM mice. Thus, A1Hipp- A1ON mice exhibit a pronounced resistance to triggering of depression-like behavior as compared to A1Hipp+, suggesting that the enhanced A_1R expression in the hippocampus, the only apparent difference between the two strains (Fig. 1), makes A1Hipp + mice susceptible to develop increased immobility.

3.3. Automated analyses of sucrose preference

Then, we performed sucrose preference test (SPT), as a measure of the integrity of the reward system and anhedonia. To avoid possible stress by the isolated housing, we utilized the IntelliCage system, in which spontaneous and explorative behavior and drinking preference can be monitored in whole groups of animals in an automated and unbiased manner (Endo et al., 2011). We subjected the mice to "nose-poke SPT" - an experimental paradigm for automated measurement of

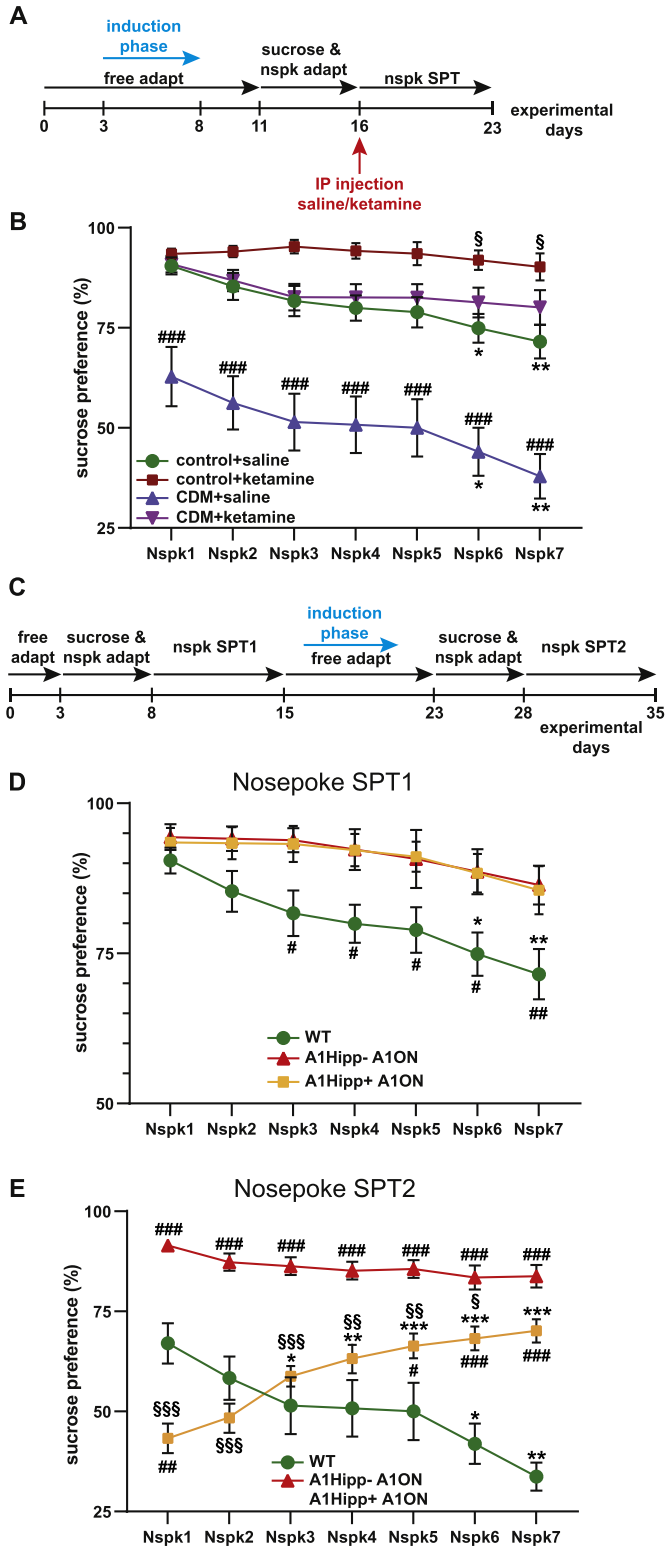

Fig. 4. Automated analyses of sucrose preference. (**A**) Schematic illustration of the experimental protocol: *free adapt* - during free adaptation, water was available in all corners *ad libitum*, doors to water were open; Then the mice were divided into 2 groups: control and chronically despaired (CDM); *induction phase* – the mice were chronically despaired by 10 min swim sessions for 5 consecutive days; *sucrose & nspk adapt* – in every corner, one bottle was filled with 1% sucrose (in two corners on left and in two corners on right side) and another bottle with water. Both doors in the corner were opened, allowing the choice between two bottles. During the nosepoke adaptation the nosepoke opened the respective door for 5s; On day 16 the mice were intraperitoneally injected with saline or 3 mg/kg ketamine; *nspk SPT* – the door opens in response to nosepoke and closes after 5s licking session. The number of nosepokes needed to open a door to a side with sucrose containing bottle increases (1, 2, 3, 4, 5, 6, 7) after every 24 h. (**B**) Sucrose preference in *nspk SPT*. (n = 8 per group, two independent experiments) Two-way ANOVA followed by Bonferroni *post hoc* test: *p < 0.05, **p<0.01 in comparison to Nspk1; ### < 0.001 in comparison to CDM + saline; § < 0.05 in comparison to control + saline; (**C**) Schematic illustration of the experimental protocol. (**D**) Sucrose preference in *nspk SPT1*. (n = 5 per group) Two-way ANOVA followed by Bonferroni *post hoc* test: *p < 0.05, **p<0.01 in comparison to Nspk1; #p<0.05, ##p < 0.01 in comparison to WT; (**E**) Sucrose preference in *nspk SPT2*. (n = 5 per group) Two-way ANOVA followed by Bonferroni *post hoc* test: *p < 0.05, **p<0.01 and ***p < 0.001 in comparison to Nspk1; #p<0.05, ##p < 0.01 and ### < 0.001 in comparison to WT; § < 0.05, §§p < 0.01, §§§p < 0.001 in comparison to A1Hipp- A1ON; Data are expressed as means ± SEM in all graphs.

reward behavior with gradually increasing effort to reach the sucrose solution (for IntelliCage experimental design see material and methods and Fig. 4A and C) (Alboni et al., 2017a, 2017b). In this paradigm the mice needed to nosepoke in order to get restricted access (5 s per drinking session) to the bottles. The number of nosepokes necessary to open the door to the sucrose increase gradually after every 24 h. Initially, we evaluated this paradigm by comparing the sucrose preference of WT control animals and CDM mice after acute injection of 3 mg/kg ketamine or saline as control (Fig. 4A and B). As expected, the naive saline injected mice showed high sucrose preference with gradual decrease of the sucrose preference correlating with the increased effort to get access to the sucrose (treatment $F_{3,16} = 129.6$, $P < 0.0001$; Nspk $F_{6,96} = 5.02$, $P < 0.0001$; interaction $F_{18,96} = 0.5669$, $P = 0.9168$). The ketamine injected naïve mice displayed significantly higher sucrose preference in comparison to control saline treated mice. In contrast, the CDM saline injected mice exhibited a pronounced anhedonic phenotype with marked decrease of the sucrose preference associated with the increased number of nosepokes to get access to the sucrose (Fig. 4B). The ketamine treatment, which has rapid and sustained antidepressant effect up to one week (Autry et al., 2011), inhibited the anhedonic phenotype of CDM mice (Fig. 4B).

Next, we tested the sucrose preference of the A1ON mice (Fig. 4C). Since, we did not observe any significant differences between A1OFF and WT mice in any of the performed behavioral tests, we excluded the A1OFF mice from the SPT measurements, because they have to be continuously treated with doxycycline in the drinking water, which in turn has bitter taste and creates artifacts in the final interpretation of the data. During the initial nosepoke SPT1, performed with naïve mice, A1ON transgenic mice from both strains demonstrated significantly higher sucrose preference than WT controls (Fig. 4D; genotype $F_{2,70} = 26.40$, $P < 0.0001$; Nspk $F_{6,70} = 4.668$, $P = 0.0004$; interaction $F_{12,70} = 0.4606$, $P = 0.932$). Likewise, the A1Hipp- A1ON animals subjected to the CDM paradigm show high sucrose preference compared to WT CDM mice (Fig. 4E; genotype $F_{2,70} = 128.5$, $P < 0.0001$; Nspk $F_{6,70} = 0.4825$, $P = 0.8194$; interaction $F_{12,70} = 6,454$, $P < 0.0001$). While CDM A1Hipp + A1ON mice initially showed diminished sucrose preference similar to WT CDM mice, they later developed high sucrose preference equal to A1Hipp-mice (Fig. 4E). These data corroborate that chronically despaired A1Hipp + A1ON strain is anhedonic, while A1ON mice from A1Hipp-line, resistant to CDM, do not exhibit an anhedonic phenotype. However, these data also show that A1Hipp + A1ON mice, made anhedonic in CDM, regain sucrose preference already after 2 weeks (Nspk7). This corresponds with the regain of reduced immobility time in the test phase observed in A1Hipp + A1ON (Fig. 3C).

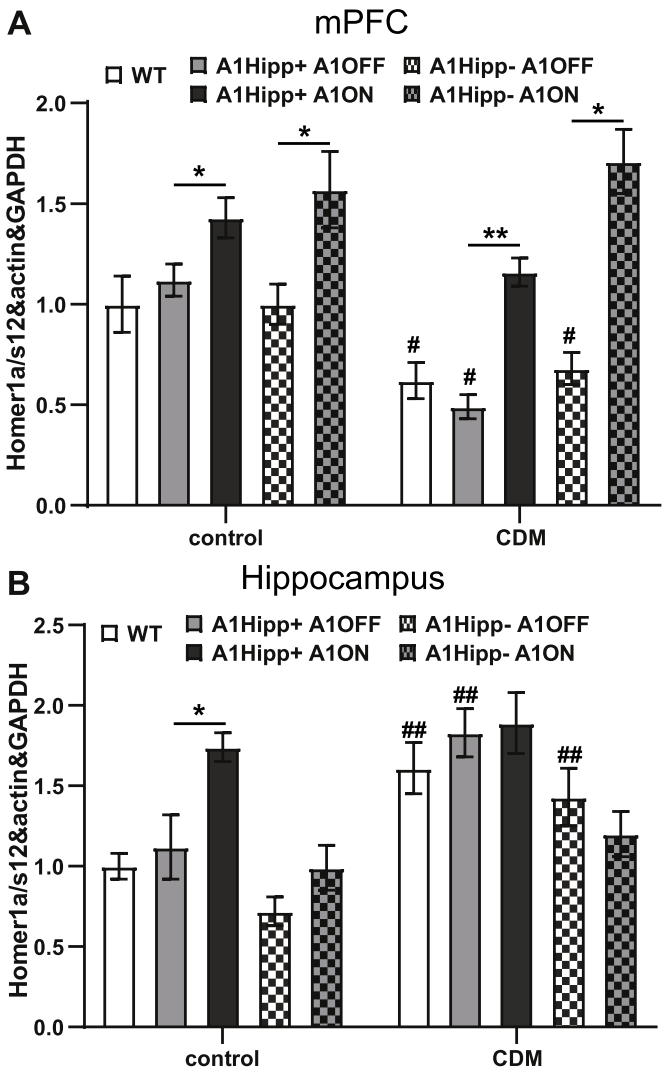

Fig. 5. Enhanced A_1 expression increases Homer1a levels. qRT-PCR analysis of relative Homer1a mRNA expression levels in medial prefrontal cortex (mPFC) (**A**) and hippocampus (**B**) of control and mice subjected to the chronic despair (CDM) paradigm (n = 6 per group). Two-way ANOVA with Bonferroni *post hoc* test: *p < 0.05, **p < 0.01; #p < 0.05, ##p < 0.01 in comparison to the respective CDM group.

3.4. Homer1a expression in cortex and hippocampus of A1Hipp + mice as compared to A1Hipp-mice

Considering the potential mechanism mediating the behavioral differences between the two A_1R transgenic lines, we analyzed the expression of Homer1a (Serchov et al., 2015, 2016). We found an increased Homer1a mRNA expression in the mPFC of A1ON mice from both lines, as compared to WT and A1OFF mice under control conditions (A1Hipp + A1ON: 1.43 ± 0.10, A1Hipp- A1ON: 1.57 ± 0.19 vs WT: 1.00 ± 0.14, A1Hipp + A1OFF: 1.12 ± 0.08, A1Hipp- A1OFF: 1.00 ± 0.10) or when mice were subjected to the CDM (A1Hipp + A1ON: 1.16 ± 0.07, A1Hipp- A1ON: 1.71 ± 0.16 vs WT: 0.62 ± 0.09, A1Hipp + A1OFF: 0.49 ± 0.06, A1Hipp- A1OFF: 0.68 ± 0.08) (genotype $F_{4,30} = 70.83$, $P < 0.0001$; treatment $F_{1,30} = 13.34$, $P < 0.0001$; interaction $F_{4,30} = 9.70$, $P < 0.0001$) (Fig. 5A). As expected, WT CDM and A1OFF CDM mice showed decreased Homer1a levels in mPFC compared to the controls. Different results were obtained in the hippocampus. A1Hipp + A1ON mice, expressing transgenic A_1R in hippocampus, showed enhanced Homer1a mRNA levels in this region as compared to A1 OFF and WT mice (Fig. 5B; genotype $F_{4,30} = 6.201$, $P = 0.0006$; treatment $F_{1,30} = 26.15$, $P < 0.0001$; interaction $F_{4,30} = 4.114$, $P = 0.0069$). However both A1Hipp- A1ON and A1Hipp- A1OFF mice did not differ from WT. Moreover, in contrast to the findings in the mPFC, CDM mice demonstrated not reduced but rather significantly increased hippocampal Homer1a mRNA levels, corresponding with the enhanced depression-like behavior (Fig. 5B). This effect was much more pronounced in WT and A1Hipp+ (A1ON and A1OFF) than in A1Hipp- A1 OFF and not significant in A1Hipp- A1 ON mice (Fig. 5B).

Taken together these data demonstrate that enhanced A_1R expression relates with increased Homer1a levels in both lines. Moreover, induction of chronic depression-like behavioral state in CDM mice decreases Homer1a expression in the mPFC, but increases it in the hippocampus, suggesting for differential brain region-specific regulation of Homer1a by stress.

3.5. Disrupted synaptic plasticity in the hippocampus of A1Hipp + mice

Since long-term potentiation (LTP) in the hippocampus can be modulated by Homer1a and in animal models of depression (Liu et al., 2017; Normann et al., 2007, 2018; Rozov et al., 2012), we measured LTP induced in hippocampal brain slices from adult WT and A1Hipp + A1ON animals by an associative pairing paradigm (Fig. 6A). Since, we did not observed any significant differences between A1OFF and WT mice in any of the performed behavioral tests, as well as no significant upregulation of A_1R and Homer1a expression in the hippocampus of A1Hipp- A1ON, these mice were not included in the patch clamp experiments. In WT control mice, this resulted in a persistent increase of EPSP amplitudes to 171.6 ± 15.1% of baseline amplitudes ($p < 0.05$, Fig. 6B). One week after the CDM protocol, LTP induction was blocked (CDM: 97.8 ± 8.2% of baseline, $p > 0.5$; $p < 0.05$ vs. WT controls; Fig. 6C and D). In A1Hipp + A1ON mice, this protocol caused only a short-term potentiation, but no sustained LTP (99.5 ± 15.8% of baseline, $p > 0.5$, Fig. 6C). Nearly identical results were found in A1Hipp + A1ON mice after CDM (108.2 ± 21.8% of baseline, $p > 0.5$, $p < 0.5$ vs. A1Hipp + A1ON control, Fig. 6C and D). These results show that the induction of a sustained LTP in control A1Hipp + A1ON mice, which exhibit transgenic A_1R expression in hippocampus, is compromised to a level similar to that of swim stressed WT animals after CDM.

3.6. Viral overexpression of Homer1a in the hippocampus of A1Hipp-mice increases depression-like behavior

To address directly the potential role of increased Homer1a expression in the hippocampus on depressive-like behavior in these transgenic lines, we used recombinant adeno-associated viral vectors (rAAVs) carrying either Homer1a, or EGFP, as a control (Serchov et al., 2015). A1Hipp- A1ON mice were bilaterally stereotaxically injected with either AAV-Homer1a, or AAV-EGFP into the dorsal hippocampus (Fig. 7A). The mice were behaviorally tested 4 weeks after the injections (see for experimental design Fig. 7A). rAAV-mediated overexpression of Homer1a did not affect spontaneous locomotor activity ($t_{4,4} = 0.1728$, $p = 0.8671$, Fig. 7B) and anxiety-like behavior ($t_{4,4} = 1.092$, $p = 0.3068$, Fig. 7C) of the mice in open field test. However, Homer1a injected A1Hipp- A1ON mice showed faster development of increased depressive-like behavior in CDM (treatment $F_{1,40} = 15.21$, $P < 0.0001$; time $F_{4,40} = 67.43$, $P < 0.0001$; interaction $F_{4,40} = 7.36$, $P = 0.0002$, Fig. 7D) and TST ($t_{4,4} = 2.741$, $P = 0.0254$, Fig. 7E) in comparison to the control A1Hipp- A1ON mice expressing EGFP. In addition, AAV-Homer1a expression causes significant decrease in sucrose preference ($t_{4,4} = 2.911$, $p = 0.0196$, Fig. 7F). These data indicate that inducing overexpression of Homer1a in the hippocampus of A1Hipp-mice results in a phenotype, which is more vulnerable to develop depression-like behavior in the CDM paradigm and mimicking the phenotype of A1Hipp + mice.

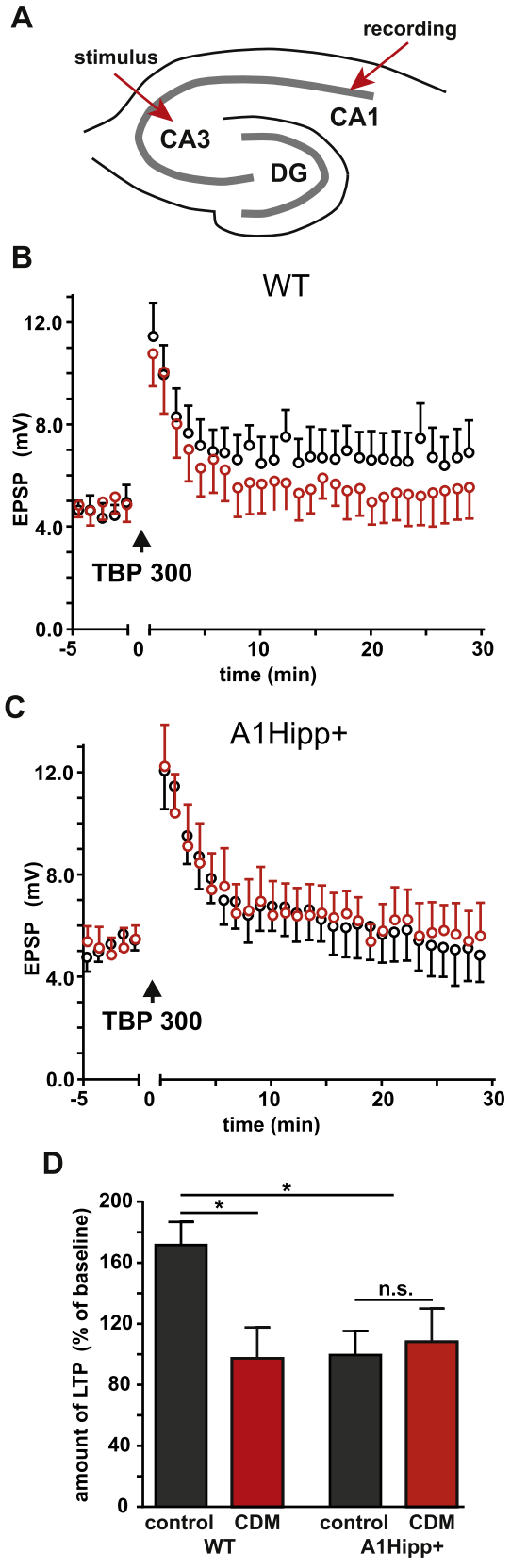

Fig. 6. Enhanced A_1R expression disrupts synaptic plasticity in the hippocampus of A1Hipp + line. (**A**) Schematic representation of the experimental setup for cellular LTP reccordings. LTP induction in adult WT (**B**) and A1Hipp + A1ON (**C**) mice under control conditions (black) and one week after CDM (red) (n = 6 per group) averaged EPSPs ± SEM, TBP 300: theta burst pairing of 300 EPSP-AP pairs. (**D**) Averaged amount of LTP in all groups 20–30 min after LTP induction. Two-way ANOVA with Bonferroni *post hoc* test: *p < 0.05. Data are expressed as means ± SEM for all graphs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

In order to investigate the role of A_1R in the brain and mimic the effects of SD on mouse behavior, we have generated multiple transgenic mouse lines with conditional up-regulation of A_1R selectively in forebrain neurons via CaMKII promoter and Tet-OFF system, to avoid possible artifacts of the transgene throughout development. Each line had a unique expression pattern, due to the random integration site of the transgene into the founder mouse genome. In this study, we have selected two founder mouse lines with identical levels of A_1R increase in the cortex, comparable to the upregulation induced by seizures or sleep deprivation in mice and humans (Biber et al., 2008; Elmenhorst et al., 2007, 2009; Vanore et al., 2001). However, both lines have distinct pattern of transgenic expression in the hippocampus altering their depression- and anxiety-like behavior.

Our detailed behavioral analysis demonstrates that both A_1R lines have no significant behavioral differences (also in comparison to WT), when the transgene-expression is switched off (A1OFF). Moreover, doxycycline treatment does not cause any significant changes in the behavior, including locomotor and explorative behavior in open field test, anxiety-like behavior in elevated plus maze and light-dark transition test, depression-like behavior in FST and TST (Suppl. Fig. 1A–E), as well as the development of chronic depression-like state in the CDM paradigm (Serchov et al., 2015). This clearly indicates that the described behavioral phenotypes are specifically triggered by enhanced A_1R expression and not influenced by any genetic artifacts due the random integration of the transgene into the mouse genome or by long treatment with doxycycline.

Corroborating our previous findings (Serchov et al., 2015), we show that up-regulation of A_1R elicited a robust anxiolytic effect in both transgenic lines without significantly affecting the spontaneous locomotor activity and exploratory behavior. Moreover, our results are in line with the role of A_1R in controlling anxiety behavior in both rodents and humans, since individuals with increased anxiety also show increases in A_1R binding *in vivo* (Hohoff et al., 2014; Prediger et al., 2006). The A1Hipp-mice, which had no transgenic expression of A1R in the hippocampus, showed stronger anxiolytic effect, in comparison to the A1Hipp + mice with transgenic upregulated A_1R in this region. In human clinical populations, as well as in animal models, depression often correlates with increased anxiety (Ho et al., 2002; Lamers et al., 2011). Thus the observed less anxious phenotype of A1Hipp-mice might be a result of the comorbidity between less depression-prone and less anxious behaviors.

The two basic tests for depressive-like behavior TST and the classical FST demonstrated strong antidepressant effects of the enhanced A_1R expression (Serchov et al., 2015), with no significant difference between the two strains (Fig. 2D and E). However, there are marked differences between both strains in their vulnerability to development of depressive-like behavior after repeated stress. Whereas A1ON A1Hipp + mice develop depression-like behavior in the CDM (although later that WT mice) A1ON A1Hipp-mice are completely resistant to triggering depressive-like phenotype (Fig. 3). These differences between the two strains become even more evident by the sucrose preference analysis utilizing the IntelliCage system. WT mice submitted to CDM show in this test the expected antidepressant-like effect of ketamine (Fig. 4B; Serchov et al., 2015). Control (naïve) mice with upregulated A_1R expression from both strains show no significant differences among each other in the sucrose preference test, but have as expected higher sucrose intake in comparison to WT (Fig. 4D).

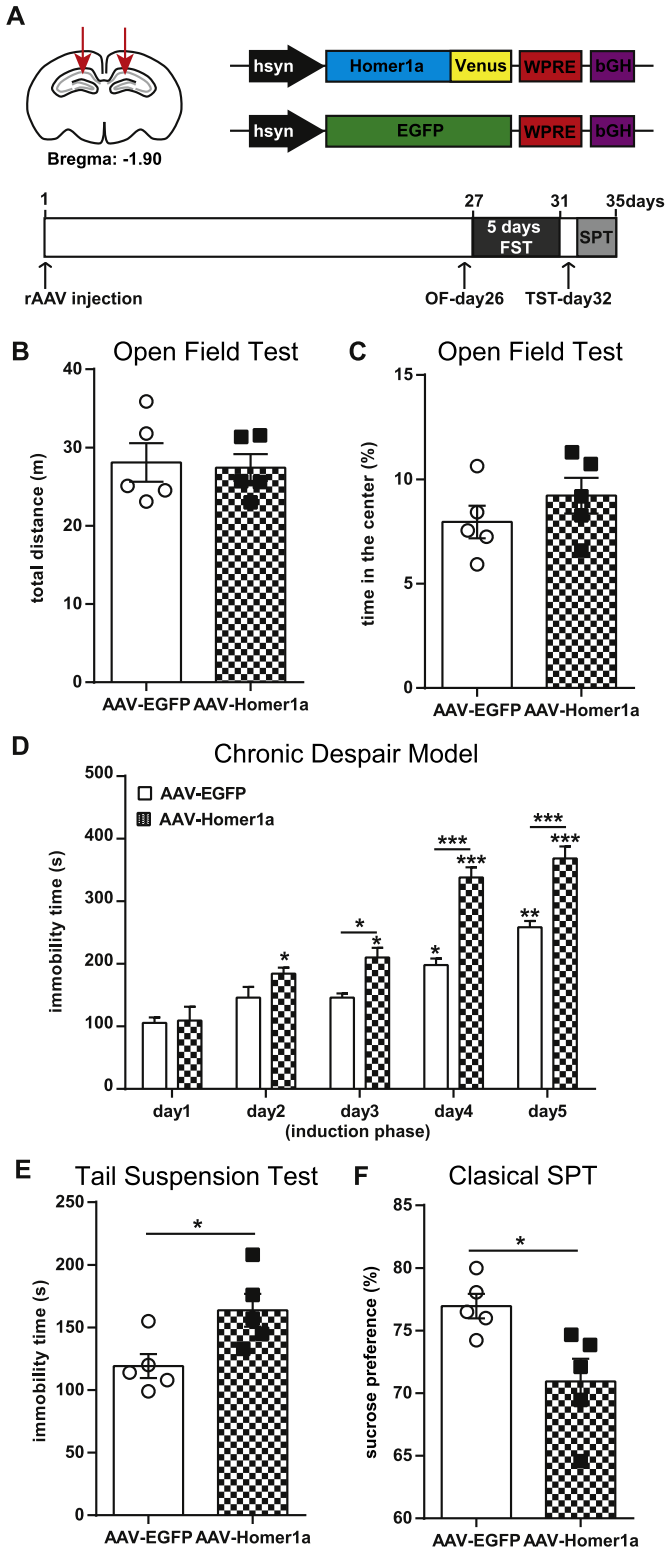

Fig. 7. Intra-hippocampal injections of rAAV-Homer1a decrease the resistance to development of depression-like behavior in A1Hipp- A1ON mice. (**A**) Schematic illustration of the experimental protocol: A1Hipp- A1ON were stereotaxically bilaterally injected with rAAV-Homer1a or rAAV-EGFP as a control (top right) into dorsal hippocampus (top left). The mice were kept undisturbed in their home cages for 4 weeks and then subjected to open field test (OF) (day26), then despaired by 10 min swim sessions for 5 consecutive days (day27 to day31), followed by TST (day32) and SPT (day32 to day35). Spontaneous activity in OF: total distance of movement (**B**) and the percentage of total time spent in the inner squares of the arena (**C**) of A1Hipp- A1ON mice injected with of AAV-EGFP (n = 5) or AAV-Homer1a (n = 5) into dorsal hippocampus. (**D**) Immobility time spent during the induction phase of CDM (n = 5 per group). Two-way ANOVA with Bonferroni *post hoc* test: *p < 0.05, **p < 0.01, ***p < 0.001. (**E**) Immobility time in TST. (**F**) Sucrose preference in SPT. (n = 5 per group) Student t-test: *p < 0.05. Data are expressed as means ± SEM in all graphs.

Correspondingly, the induction of chronic state of increased immobility time in CDM triggered temporary anhedonia in A1Hipp + A1ON mice, as evident from decreased sucrose preference (Fig. 4E) while A1Hipp-A1ON mice showed no anhedonic phenotype. The reduction of sucrose preference in the A1Hipp+ was, however, only transient (Fig. 4E). This further indicates that enhanced A_1R expression elicits "therapeutic" effects in mice with already induced chronic depression-like behavioral symptoms. Thus, we provide the first evidence that enhanced A_1R expression in the cortex promotes stress resilience, while upregulated A_1R expression in hippocampus of the A1Hipp + does not have a general pro-depressive effect, but rather influences the response to stress and vulnerability to develop depression-like symptoms.

As a potential mechanism mediating the behavioral effects of enhanced A_1R expression, we considered a role of Homer1a. We have shown previously that the antidepressant effects of SD and of activation of A1R are mediated by induction of Homer1a specifically in the mPFC and that Homer1a is upregulated by stimulation of A_1R (Serchov et al., 2015, 2016). The level of Homer1a expression in the mPFC inversely correlates with depression-like behavior (Serchov et al., 2015, 2016). The data presented here fully corroborate these findings. They show that, after the CDM procedure, the CDM resistant A1Hipp-mice have higher Homer1a mRNA levels in the mPFC as compared to the CDM sensitive mice of the A1Hipp + line (Fig. 4A). As well in the hippocampus of the A1Hipp + enhanced A_1R expression (A1ON) is associated with upregulation of Homer1a (Fig. 5B, control) and A1Hipp-mice, which have no transgenic expression of A_1R in the hippocampus do not show an increase of Homer1a in the hippocampus. We therefore hypothesized that the increased resilience to depression-like behavior of the A1Hipp-mice as compared to the A1Hipp + mice might be due to the lack of Homer1a upregulation in the hippocampus of A1Hipp-A1ON line (Fig. 5B). Consistently, it has been reported previously that overexpression of Homer1a in the hippocampus of WT mice leads to an increased vulnerability to chronic social defeat stress (Wagner et al., 2015). To test this hypothesis we investigated mice with rAAV-mediated Homer1a overexpression (Fig. 7). Indeed, rAAV-mediated Homer1a overexpression in the hippocampus of the A1Hipp-mice impedes the resistance towards the development of depression-like phenotype shown in this line in several tests. Thus, the finding that Homer1a expression in the hippocampus alters the stress response might explain the behavioral differences between the two transgenic lines.

Several lines of evidence show that hippocampal long-term synaptic plasticity is compromised by behavioral stress in animal models of depression and in humans with major depressive disorder and is rescued by antidepressants (Castren, 2013; Holderbach et al., 2007; Normann et al., 2007, 2018). Consistently, we found impaired induction of LTP in the acute hippocampal slices from chronically despaired WT mice. Our data are in line with results from other groups showing that different forms of stress blocks LTP (Artola et al., 2006; Pavlides et al., 2002). Importantly, both A_1R activation and Homer1a overexpression in hippocampus exert an inhibitory effect on LTP (Florian et al., 2011; Rozov et al., 2012; zur Nedden et al., 2011). Consistently, non-stressed mice from the A1Hipp+, also showed impaired LTP similar to CDM WT and CDM A1Hipp + mice. The impaired LTP induction of the A1Hipp + mice did not affect spatial learning or recognition and working memory (Serchov et al., 2015); however, it

might nevertheless contribute to the behavioral effects after CDM and increase the vulnerability of these mice to induction of depression-like behavior.

Homer1a was demonstrated to be a key regulator of metabotropic glutamate receptor 5 (mGluR5), which has been implicated in the pathophysiology of mood disorders (Holz et al., 2019; Hughes et al., 2013; Kammermeier, 2008; Tronson et al., 2010). Enhanced Homer1a levels reduce mGluR5 interactions with the longer Homer scaffolds and cause a prolonged ligand-independent activation of mGluR5 and its downstream signaling (Holz et al., 2019; Kammermeier, 2008). Indeed, disrupted Homer1/mGluR5 interactions are implicated in the regulation of the stress response, including the vulnerability to chronic social stress (Wagner et al., 2015), enhancement of the stress effects on fear (Tronson et al., 2010) and the acute stress-induced cognitive deficits (Wagner et al., 2013). Furthermore, several antagonists of mGluR5 have been proposed as novel agents for treatment of depression (Hughes et al., 2013).

In summary, we demonstrate for first time that the upregulation of A_1R/Homer1a in cortex and hippocampus can elicit different effects on the stress-induced depression. Accordingly, the modulation of mGluR5, the most prominent interacting partner of Homer1, exhibits both antidepressant and pro-depressant effects correlating with the relative extent of change in glutamatergic and GABAergic neurons respectively (Holz et al., 2019; Lee et al., 2015). Likewise, one night of sleep deprivation elicits robust but transient antidepressant effects (Hines et al., 2013; Serchov et al., 2015), while chronic exposure to sleep restriction is associated with increased risk of depression (Baum et al., 2014; Conklin et al., 2018). Indeed, chronic sleep restriction induces long-lasting increase in A_1R expression in several brain regions, including hippocampus (Kim et al., 2015), similarly to our A_1R transgenic mouse models. Moreover, several reports have shown hippocampal upregulation of Homer1a after different periods of sleep deprivation (Maret et al., 2007; Serchov et al., 2015). Similarly, the increased mGluR5 availability and enhanced downstream signaling after sleep loss correlates with behavioral and encephalographic biomarkers of elevated sleep need (Hefti et al., 2013; Holst et al., 2017). In addition, chronic sleep deprivation appears to be particularly detrimental to the hippocampus function, where it negatively affects the structural and synaptic plasticity in this brain region (Areal et al., 2017; Havekes and Abel, 2017; Kreutzmann et al., 2015). Thus these data suggest that the increased expression of A_1R and Homer1a in hippocampus may mediate the detrimental effects of chronic sleep restriction on the resilience to stress-induced depression. Future studies examining the interaction between mPFC and hippocampus will further extend our understanding of how different neuronal circuits with their respective neurotransmitters promote stress resilience.

Author contributions

Conceptualization, T.S., C.N., K.B., D.v.C.; Methodology, T.S., M.K.S, C.N.; Investigation, T.S., A.T., I.S., L.S. A.H.; Writing-Original draft, T.S., C.N., D.v.C.; Writing-Review and Editing, T.S., C.N., A.H., M.D.D., K.B., D.v.C.; Funding Acquisition, T.S., K.B., D.v.C.; Supervision, C.N., K.B., D.v.C.

Declaration of competing interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2019.107834.

References

Alboni, S., van Dijk, M., Poggini, S., Milior, G., Perrotta, M.L., Drenth, T., Brunello, N., Wolfer, D., Limatola, C., Amrein, I., Cirulli, F., Maggi, L., Branchi, I., 2017a. Hippocampus-related effects of fluoxetine treatment under stressful vs enriched conditions. Mol. Psychiatry 22, 483.

Alboni, S., van Dijk, R.M., Poggini, S., Milior, G., Perrotta, M., Drenth, T., Brunello, N., Wolfer, D.P., Limatola, C., Amrein, I., Cirulli, F., Maggi, L., Branchi, I., 2017b. Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. Mol. Psychiatry 22, 635.

Areal, C.C., Warby, S.C., Mongrain, V., 2017. Sleep loss and structural plasticity. Curr. Opin. Neurobiol. 44, 1–7.

Artola, A., von Frijtag, J.C., Fermont, P.C., Gispen, W.H., Schrama, L.H., Kamal, A., Spruijt, B.M., 2006. Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. Eur. J. Neurosci. 23, 261–272.

Autry, A.E., Adachi, M., Nosyreva, E., Na, E.S., Los, M.F., Cheng, P.F., Kavalali, E.T., Monteggia, L.M., 2011. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 475, 91–95.

Baum, K.T., Desai, A., Field, J., Miller, L.E., Rausch, J., Beebe, D.W., 2014. Sleep restriction worsens mood and emotion regulation in adolescents. JCPP (J. Child Psychol. Psychiatry) 55, 180–190.

Benedetti, F., Colombo, C., 2011. Sleep deprivation in mood disorders. Neuropsychobiology 64, 141–151.

Biber, K., Pinto-Duarte, A., Wittendorp, M.C., Dolga, A.M., Fernandes, C.C., Von Frijtag Drabbe Kunzel, J., Keijser, J.N., de Vries, R., Ijzerman, A.P., Ribeiro, J.A., Eisel, U., Sebastiao, A.M., Boddeke, H.W., 2008. Interleukin-6 upregulates neuronal adenosine A1 receptors: implications for neuromodulation and neuroprotection. Neuropsychopharmacology 33, 2237–2250.

Castren, E., 2013. Neuronal network plasticity and recovery from depression. JAMA Psychiatr. 70, 983–989.

Celikel, T., Marx, V., Freudenberg, F., Zivkovic, A., Resnik, E., Hasan, M.T., Licznerski, P., Osten, P., Rozov, A., Seeburg, P.H., Schwarz, M.K., 2007. Select overexpression of homer1a in dorsal hippocampus impairs spatial working memory. Front. Neurosci. 1, 97–110.

Chomczynski, P., Sacchi, N., 2006. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nat. Protoc. 1, 581–585.

Clasadonte, J., McIver, S.R., Schmitt, L.I., Halassa, M.M., Haydon, P.G., 2014. Chronic sleep restriction disrupts sleep homeostasis and behavioral sensitivity to alcohol by reducing the extracellular accumulation of adenosine. J. Neurosci. 34, 1879–1891.

Conklin, A.I., Yao, C.A., Richardson, C.G., 2018. Chronic sleep deprivation and gender-specific risk of depression in adolescents: a prospective population-based study. BMC Public Health 18, 724.

Eaton, W.W., Martins, S.S., Nestadt, G., Bienvenu, O.J., Clarke, D., Alexandre, P., 2008. The burden of mental disorders. Epidemiol. Rev. 30, 1–14.

Elmenhorst, D., Basheer, R., McCarley, R.W., Bauer, A., 2009. Sleep deprivation increases A(1) adenosine receptor density in the rat brain. Brain Res. 1258, 53–58.

Elmenhorst, D., Elmenhorst, E.M., Hennecke, E., Kroll, T., Matusch, A., Aeschbach, D., Bauer, A., 2017. Recovery sleep after extended wakefulness restores elevated A1 adenosine receptor availability in the human brain. Proc. Natl. Acad. Sci. U. S. A. 114, 4243–4248.

Elmenhorst, D., Meyer, P.T., Winz, O.H., Matusch, A., Ermert, J., Coenen, H.H., Basheer, R., Haas, H.L., Zilles, K., Bauer, A., 2007. Sleep deprivation increases A1 adenosine receptor binding in the human brain: a positron emission tomography study. J. Neurosci. 27, 2410–2415.

Endo, T., Maekawa, F., Voikar, V., Haijima, A., Uemura, Y., Zhang, Y., Miyazaki, W., Suyama, S., Shimazaki, K., Wolfer, D.P., Yada, T., Tohyama, C., Lipp, H.P., Kakeyama, M., 2011. Automated test of behavioral flexibility in mice using a behavioral sequencing task in IntelliCage. Behav. Brain Res. 221, 172–181.

Florian, C., Vecsey, C.G., Halassa, M.M., Haydon, P.G., Abel, T., 2011. Astrocyte-derived adenosine and A1 receptor activity contribute to sleep loss-induced deficits in hippocampal synaptic plasticity and memory in mice. J. Neurosci. 31, 6956–6962.

Havekes, R., Abel, T., 2017. The tired hippocampus: the molecular impact of sleep deprivation on hippocampal function. Curr. Opin. Neurobiol. 44, 13–19.

Hefti, K., Holst, S.C., Sovago, J., Bachmann, V., Buck, A., Ametamey, S.M., Scheidegger, M., Berthold, T., Gomez-Mancilla, B., Seifritz, E., Landolt, H.P., 2013. Increased metabotropic glutamate receptor subtype 5 availability in human brain after one night without sleep. Biol. Psychiatry 73, 161–168.

Hellwig, S., Brioschi, S., Dieni, S., Frings, L., Masuch, A., Blank, T., Biber, K., 2016. Altered microglia morphology and higher resilience to stress-induced depression-like behavior in CX3CR1-deficient mice. Brain Behav. Immun. 55, 126–137.

Hines, D.J., Schmitt, L.I., Hines, R.M., Moss, S.J., Haydon, P.G., 2013. Antidepressant effects of sleep deprivation require astrocyte-dependent adenosine mediated signaling. Transl. Psychiatry 3, e212.

Ho, Y.J., Eichendorff, J., Schwarting, R.K., 2002. Individual response profiles of male Wistar rats in animal models for anxiety and depression. Behav. Brain Res. 136, 1–12.

Hohoff, C., Garibotto, V., Elmenhorst, D., Baffa, A., Kroll, T., Hoffmann, A., Schwarte, K., Zhang, W., Arolt, V., Deckert, J., Bauer, A., 2014. Association of adenosine receptor

gene polymorphisms and in vivo adenosine A1 receptor binding in the human brain. Neuropsychopharmacology 39, 2989–2999.
Holderbach, R., Clark, K., Moreau, J.L., Bischofberger, J., Normann, C., 2007. Enhanced long-term synaptic depression in an animal model of depression. Biol. Psychiatry 62, 92–100.
Holst, S.C., Sousek, A., Hefti, K., Saberi-Moghadam, S., Buck, A., Ametamey, S.M., Scheidegger, M., Franken, P., Henning, A., Seifritz, E., Tafti, M., Landolt, H.P., 2017. Cerebral mGluR5 availability contributes to elevated sleep need and behavioral adjustment after sleep deprivation. Elife 6.
Holz, A., Mulsch, F., Schwarz, M.K., Hollmann, M., Dobrossy, M.D., Coenen, V.A., Bartos, M., Normann, C., Biber, K., van Calker, D., Serchov, T., 2019. Enhanced mGlu5 signaling in excitatory neurons promotes rapid antidepressant effects via AMPA receptor activation. Neuron.
Hughes, Z.A., Neal, S.J., Smith, D.L., Sukoff Rizzo, S.J., Pulicicchio, C.M., Lotarski, S., Lu, S., Dwyer, J.M., Brennan, J., Olsen, M., Bender, C.N., Kouranova, E., Andree, T.H., Harrison, J.E., Whiteside, G.T., Springer, D., O'Neil, S.V., Leonard, S.K., Schechter, L.E., Dunlop, J., Rosenzweig-Lipson, S., Ring, R.H., 2013. Negative allosteric modulation of metabotropic glutamate receptor 5 results in broad spectrum activity relevant to treatment resistant depression. Neuropharmacology 66, 202–214.
Kammermeier, P.J., 2008. Endogenous homer proteins regulate metabotropic glutamate receptor signaling in neurons. J. Neurosci. 28, 8560–8567.
Kim, Y., Elmenhorst, D., Weisshaupt, A., Wedekind, F., Kroll, T., McCarley, R.W., Strecker, R.E., Bauer, A., 2015. Chronic sleep restriction induces long-lasting changes in adenosine and noradrenaline receptor density in the rat brain. J. Sleep Res. 24, 549–558.
Kreutzmann, J.C., Havekes, R., Abel, T., Meerlo, P., 2015. Sleep deprivation and hippocampal vulnerability: changes in neuronal plasticity, neurogenesis and cognitive function. Neuroscience 309, 173–190.
Lamers, F., van Oppen, P., Comijs, H.C., Smit, J.H., Spinhoven, P., van Balkom, A.J., Nolen, W.A., Zitman, F.G., Beekman, A.T., Penninx, B.W., 2011. Comorbidity patterns of anxiety and depressive disorders in a large cohort study: The Netherlands Study of Depression and Anxiety (NESDA). J. Clin. Psychiatry 72, 341–348.
Lee, K.W., Westin, L., Kim, J., Chang, J.C., Oh, Y.S., Amreen, B., Gresack, J., Flajolet, M., Kim, D., Aperia, A., Kim, Y., Greengard, P., 2015. Alteration by p11 of mGluR5 localization regulates depression-like behaviors. Mol. Psychiatry 20, 1546–1556.
Liu, W., Ge, T., Leng, Y., Pan, Z., Fan, J., Yang, W., Cui, R., 2017. The role of neural plasticity in depression: from Hippocampus to prefrontal cortex. Neural Plast. 2017, 6871089.
Maret, S., Dorsaz, S., Gurcel, L., Pradervand, S., Petit, B., Pfister, C., Hagenbuchle, O., O'Hara, B.F., Franken, P., Tafti, M., 2007. Homer1a is a core brain molecular correlate of sleep loss. Proc. Natl. Acad. Sci. U. S. A. 104, 20090–20095.
Mayford, M., Bach, M.E., Huang, Y.Y., Wang, L., Hawkins, R.D., Kandel, E.R., 1996. Control of memory formation through regulated expression of a CaMKII transgene. Science 274, 1678–1683.
Mrazek, D.A., Hornberger, J.C., Altar, C.A., Degtiar, I., 2014. A review of the clinical, economic, and societal burden of treatment-resistant depression: 1996-2013. Psychiatr. Serv. 65, 977–987.
Normann, C., Frase, S., Haug, V., von Wolff, G., Clark, K., Munzer, P., Dorner, A., Scholliers, J., Horn, M., Vo Van, T., Seifert, G., Serchov, T., Biber, K., Nissen, C., Klugbauer, N., Bischofberger, J., 2018. Antidepressants rescue stress-induced disruption of synaptic plasticity via serotonin transporter-independent inhibition of L-type calcium channels. Biol. Psychiatry 84, 55–64.
Normann, C., Schmitz, D., Furmaier, A., Doing, C., Bach, M., 2007. Long-term plasticity of visually evoked potentials in humans is altered in major depression. Biol. Psychiatry 62, 373–380.
Novati, A., Roman, V., Cetin, T., Hagewoud, R., den Boer, J.A., Luiten, P.G., Meerlo, P., 2008. Chronically restricted sleep leads to depression-like changes in neurotransmitter receptor sensitivity and neuroendocrine stress reactivity in rats. Sleep 31, 1579–1585.
Pavlides, C., Nivon, L.G., McEwen, B.S., 2002. Effects of chronic stress on hippocampal long-term potentiation. Hippocampus 12, 245–257.
Prediger, R.D., da Silva, G.E., Batista, L.C., Bittencourt, A.L., Takahashi, R.N., 2006. Activation of adenosine A1 receptors reduces anxiety-like behavior during acute ethanol withdrawal (hangover) in mice. Neuropsychopharmacology 31, 2210–2220.
Rozov, A., Zivkovic, A.R., Schwarz, M.K., 2012. Homer1 gene products orchestrate Ca (2+)-permeable AMPA receptor distribution and LTP expression. Front. Synaptic Neurosci. 4, 4.
Sadek, A.R., Knight, G.E., Burnstock, G., 2011. Electroconvulsive therapy: a novel hypothesis for the involvement of purinergic signalling. Purinergic Signal. 7, 447–452.
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.
Serchov, T., Atas, H.C., Normann, C., van Calker, D., Biber, K., 2012. Genetically controlled upregulation of adenosine A(1) receptor expression enhances the survival of primary cortical neurons. Mol. Neurobiol. 46, 535–544.
Serchov, T., Clement, H.W., Schwarz, M.K., Iasevoli, F., Tosh, D.K., Idzko, M., Jacobson, K.A., de Bartolomeis, A., Normann, C., Biber, K., van Calker, D., 2015. Increased signaling via adenosine A1 receptors, sleep deprivation, imipramine, and ketamine inhibit depressive-like behavior via induction of Homer1a. Neuron 87, 549–562.
Serchov, T., Heumann, R., van Calker, D., Biber, K., 2016. Signaling pathways regulating Homer1a expression: implications for antidepressant therapy. Biol. Chem. 397, 207–214.
Sun, P., Wang, F., Wang, L., Zhang, Y., Yamamoto, R., Sugai, T., Zhang, Q., Wang, Z., Kato, N., 2011. Increase in cortical pyramidal cell excitability accompanies depression-like behavior in mice: a transcranial magnetic stimulation study. J. Neurosci. 31, 16464–16472.
Tronson, N.C., Guzman, Y.F., Guedea, A.L., Huh, K.H., Gao, C., Schwarz, M.K., Radulovic, J., 2010. Metabotropic glutamate receptor 5/Homer interactions underlie stress effects on fear. Biol. Psychiatry 68, 1007–1015.
van Calker, D., Biber, K., 2005. The role of glial adenosine receptors in neural resilience and the neurobiology of mood disorders. Neurochem. Res. 30, 1205–1217.
van Calker, D., Serchov, T., Normann, C., Biber, K., 2018. Recent insights into antidepressant therapy: distinct pathways and potential common mechanisms in the treatment of depressive syndromes. Neurosci. Biobehav. Rev. 88, 63–72.
van Calker, D., Biber, K., Domschke, K., Serchov, T., 2019. The role of adenosine receptors in mood and anxiety disorders. J. Neurochem. 151 (1), 11–27.
Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3 RESEARCH0034.
Vanore, G., Giraldez, L., Rodriguez de Lores Arnaiz, G., Girardi, E., 2001. Seizure activity produces differential changes in adenosine A1 receptors within rat hippocampus. Neurochem. Res. 26, 225–230.
Wagner, K.V., Hartmann, J., Labermaier, C., Hausl, A.S., Zhao, G., Harbich, D., Schmid, B., Wang, X.D., Santarelli, S., Kohl, C., Gassen, N.C., Matosin, N., Schieven, M., Webhofer, C., Turck, C.W., Lindemann, L., Jaschke, G., Wettstein, J.G., Rein, T., Muller, M.B., Schmidt, M.V., 2015. Homer1/mGluR5 activity moderates vulnerability to chronic social stress. Neuropsychopharmacology 40, 1222–1233.
Wagner, K.V., Hartmann, J., Mangold, K., Wang, X.D., Labermaier, C., Liebl, C., Wolf, M., Gassen, N.C., Holsboer, F., Rein, T., Muller, M.B., Schmidt, M.V., 2013. Homer1 mediates acute stress-induced cognitive deficits in the dorsal hippocampus. J. Neurosci. 33, 3857–3864.
zur Nedden, S., Hawley, S., Pentland, N., Hardie, D.G., Doney, A.S., Frenguelli, B.G., 2011. Intracellular ATP influences synaptic plasticity in area CA1 of rat hippocampus via metabolism to adenosine and activity-dependent activation of adenosine A1 receptors. J. Neurosci. 31, 6221–6234.