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Heterozygosity for the mood disorder-associated variant Gln460Arg alters P2X7 receptor function and sleep quality

Abbreviated title: P2X7R-Q460R heterozygosity alters sleep

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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ABSTRACT

A single-nucleotide polymorphism substitution from glutamine (Gln, Q) to arginine (Arg, R) at codon 460 of the purinergic P2X7 receptor (P2X7R) has repeatedly been associated with mood disorders. The P2X7R-Gln460Arg variant per se is not compromised in its function. However, heterologous expression of P2X7R-Gln460Arg together with wild-type P2X7R has recently been demonstrated to impair receptor function. Here we show that this also applies to humanized mice co-expressing both human P2X7R variants. Primary hippocampal cells derived from heterozygous mice showed an attenuated calcium uptake upon agonist stimulation. While humanized mice were unaffected in their behavioral repertoire under basal housing conditions, mice that harbor both P2X7R variants showed alterations in their sleep quality resembling signs of a prodromal disease stage. Also healthy heterozygous human subjects showed mild changes in sleep parameters. These results indicate that heterozygosity for the wild-type P2X7R and its mood disorder associated variant P2X7R-Gln460Arg represents a genetic risk factor, which is potentially able to convey susceptibility to mood disorders.

SIGNIFICANCE STATEMENT

Depression and bipolar disorder are the most common mood disorders. The P2X7 receptor (P2X7R) regulates many cellular functions. Its polymorphic variant Gln460Arg has repeatedly been associated with mood disorders. Genetically engineered mice, with human P2X7R, revealed that heterozygous mice (i.e. they co-express the disease-associated Gln460Arg variant together with its normal version) have impaired receptor function and showed sleep disturbances. Human participants with the heterozygote genotype also had subtle alterations in their sleep profile. Our findings suggest that altered P2X7R function in heterozygote individuals disturbs sleep and might increase the risk for developing mood disorders.

INTRODUCTION

Major depression (MD) and bipolar disorder (BD) represent the most prevalent mood disorders (e.g. Collins et al., 2011). Despite their high heritability, the identification of susceptibility genes has been challenging with many discoveries failing replication. This is largely owing to the inherent phenotypic and genetic heterogeneity of these disorders as well as the difficulty in controlling for environmental factors, which interfere with disease etiology (Bosker et al., 2011).

A potential susceptibility gene for mood disorders is *P2RX7*, which is located on chromosome 12q24, a region that has been repeatedly associated with MD and BD (e.g., Degn et al., 2001;Abkevich et al., 2003). The *P2RX7* gene encodes the purinergic P2X7 receptor (P2X7R), which is a member of the P2X family of ATP-gated ion channels (Surprenant et al., 1996;Khakh and North, 2006). Unlike other family members, P2X7 subunits primarily form homotrimeric complexes (Torres et al., 1999;Nicke, 2008). The non-synonymous single nucleotide polymorphism (SNP) rs2230912 (base change 1405 A>G), which leads to a substitution of glutamine (Gln, Q) by arginine (Arg, R) at codon 460 (Gln460Arg, Q460R), has been associated with mood disorders (Barden et al., 2006;Hejjas et al., 2009;Lucae et al., 2006;McQuillin et al., 2009;Soronen et al., 2011;Nagy et al., 2008).

The P2X7R is well-known for its presence in immune, endothelia, and epithelia cells where it regulates various aspects of immune function, including expression and secretion of cytokines and other inflammatory mediators (Wiley et al., 2011). Owing to its association with mood disorders and its involvement in neuroinflammatory processes, the role of the P2X7R in the central nervous system (CNS) has been attracting increasing attention (Bartlett et al., 2014;Sperlagh and Illes, 2014). In the CNS, P2X7R expression has been detected in all of the main cell lineages including astrocytes, oligodendrocytes, microglia and neurons. Under baseline conditions, its neuronal expression is restricted to excitatory neurons of the hippocampus (Metzger et al., 2016). Pharmacological approaches and knockout (KO) mice

have demonstrated that P2X7R contributes to the regulation of various neuronal functions including neurotransmitter release and synaptic transmission (Miras-Portugal et al., 2003;Deuchars et al., 2001;Papp et al., 2004). In addition, KO mice revealed P2X7R-dependent alterations in different aspects of emotional behavior related to mood disorders (Boucher et al., 2011;Basso et al., 2009;Csolle et al., 2013).

However, several studies have not detected statistically significant associations of the Gln460Arg polymorphism with mood disorders (Lavebratt et al., 2010;Green et al., 2009;Viikki et al., 2011;Grigoroiu-Serbanescu et al., 2009;Halmai et al., 2013;Backlund et al., 2011;Yosifova et al., 2009). Therefore, we set out to functionally validate the association of the Gln460Arg polymorphism in both genetically engineered mouse models humanized for the P2X7R, and human subjects specifically genotyped for rs2230912. In contrast to the many loss- and gain-of-function polymorphisms that have been identified, the mood disorder associated P2X7R-Gln460Arg variant is not compromised in its activity (Roger et al., 2010;Aprile-Garcia et al., 2016). Similar to previous *in vitro* studies, we demonstrate here that only the co-expression of hP2X7R-Gln460Arg with hP2X7R-wild-type (WT) impairs normal receptor function in mice. Accordingly, humanized mice expressing either human WT P2X7R or hP2X7R-Gln460Arg presented no pathological findings. However, mice heterozygous for both variants exhibited significant differences in sleep parameters, which remarkably paralleled the sleep alterations seen in healthy heterozygous human subjects. Finally, the interaction of this genetic predisposition together with chronic stress as an environmental challenge revealed an increased vulnerability of heterozygous humanized P2X7R mice to develop mood disorder-like phenotypes.

MATERIALS AND METHODS

Generation of humanized P2X7R mice

Humanized P2X7R mice (*hP2RX7*) were generated by knock-in of the Gln460Arg variant of the human P2X7R cDNA to the murine *P2rx7* locus using standard gene targeting procedures in mouse embryonic stem cells as previously described (Metzger et al., 2016). Briefly, murine exon 2 was replaced by the human P2RX7 cDNA comprising exons 2-13. The variant of the human cDNA was constructed as previously described (Aprile-Garcia et al., 2016) and appeared with the following amino acid sequences at the 11 positions of previously identified haplotypes P2X7-1, P2X7-2 and P2X7-4 (Stokes et al., 2010): Val-76, Gly-150, His-155, Arg-270, Arg-276, Arg-307, Ala-348, Thr-357, Arg-460, Glu-496, Ile-568. Mice were kept on a mixed 129S2/SvPas × C57BL/6J background. General genotyping of humanized mice was performed as previously described (Metzger et al., 2016). Humanized alleles were identified by PCR using primers: 5´-GTG-GAT-GAA-TCC-CAC-ATT-AGG-ATG-GTG-3´, and 5´-TAC-TGC-CCT-TCA-CTC-TTC-GGA-AAC-3´ resulting in a 557 bp product followed by a restriction digest of the PCR product with *Pvu*II resulting in a 171 bp, 332 bp and 54 bp fragment for the humanized WT (Gln460) allele and a 503 bp and 54 bp fragment for the humanized mutant allele (Arg460).

Reverse transcriptase quantitative real-time PCR (RT-qPCR)

For quantification of mRNA expression levels, RNA was isolated using TRIzol reagent (Cat # 15596-026, Invitrogen Thermo Fisher Scientific Inc., Waltham, MA, USA) and transcribed to cDNA using the *SuperScript II Reverse Transcriptase Kit* (Cat # 18064014, Invitrogen Thermo Fisher Scientific Inc., Waltham, MA, USA) following the manufacturer's protocols. qPCR was carried out in a LightCycler96 (Roche Applied Science, Indianapolis, IN, USA) using the *Master SYBR Green kit I* (Cat # 03003230001, Roche Diagnostics, Indianapolis, IN,

USA). The following primers were used: hP2RX7-for 5´-ATG-TCA-AGG-GCC-AAG-AAG-TC-3´, hP2RX7-rev 5´-AGG-AAT-CGG-GGG-TGT-GTC-3´.

***In situ* hybridization**

For *in situ* hybridization ^{35}S-UTP labeled riboprobes were hybridized on 20 µm thick brain cryosections. The mouse-specific P2X7R probe comprises nucleotides 1215-1636 of GenBank accession no. NM_011027. The human-specific P2X7R probe comprises 1195-1616 nucleotides of Genbank accession no. NM_002562.

Interleukin-1β assay

Peritoneal macrophages were isolated as previously described (Basso et al., 2009). 3 µg/ml of lipopolysaccharide (LPS) were added, and the cells were allowed to prime for 2 h. The cells were then challenged with 1 mM of the P2X7R agonist 2´,3´-O-(benzoyl-4-benzoyl)-adenosine 5´-triphosphate (BzATP) for 30 min. Supernatants were collected and analyzed for IL-1β using an ELISA kit following the manufacturer's instructions (Cat # KMC0011C, Thermo Fisher Scientific Inc., Waltham, MA, USA).

Primary hippocampal cell culture

Primary hippocampal cultures were prepared from mice at postnatal day 2. Mice were sacrificed by decapitation and brains were dissected free of meninges and hippocampi were isolated. Subsequently tissues were dissociated and suspended in DMEM/F12 (Invitrogen) supplemented with 10% FCS and 1% penicillin/streptomycin. Primary cells were cultivated in **6-well** plates (200.000 cells per well) until they reached confluency before used for calcium imaging.

Calcium imaging

Confluent cells were trypsinized and plated at a low density of 10.000 cells/cm² on 8-well culture slides (Nunc Lab-Tek II Chamber Slide/Thermo Scientific) in order to evaluate single

cells in the measurements. After two days of recovery post trypsinization, cells were loaded for 45 min in darkness with Fluo-4 AM 6 μM (Molecular Probes) and Pluronic F-127 0.14% (Molecular Probes) in a Ca^{2+}-buffer (125 mM NaCl, 5 mM KCl, 0.4 mM CaCl2, 1 mM MgSO4, 5 mM NaHCO3, 1 mM Na2HPO4, 10 mM glucose, 20 mM Hepes, pH 7.4), and then placed on the stage of a fluorescence Olympus IX81 inverted confocal microscope. Microscope pictures were captured with the 10× UPlanSApo (0.4 numerical aperture) objective. Calcium imaging data are presented as ΔF/Fo, where Fo is the resting fluorescence (before stimulation) and ΔF is the peak change in fluorescence from resting levels.

Behavioral characterization of mice

Behavioral characterization was performed using male humanized P2X7R mice (11-13 weeks of age). All mice were single-housed for two weeks prior to the experiment under standard laboratory conditions and were maintained on a 12h light-dark cycle (lights on from 7:00 am until 7 p.m.), with food and water provided *ad libitum*. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Bavaria, Germany. Behavioral phenotyping comprised the open field (OF) test, elevated plus maze (EPM), dark-light-box (DaLi), forced swim test (FST), social approach/avoidance test and female urine sniffing test (FUST), which were conducted as previously described (Yen et al., 2012;Hartmann et al., 2012a;Malkesman et al., 2010). In the EPM. The open arm time is calculated in relation to closed arm time and is consequently depicted in percent. The open arm time was calculated as follows: open arm time /(open arm time + closed arm time) * 100. Mice were excluded from analysis in the EPM if they fell of the platforms. If mice didn't move throughout the entire course of the OF test, they were excluded from the analysis. Mice that failed to approach the cotton tip during the water and urine trial were excluded from the FUST analysis.

Chronic social defeat stress paradigm

Male mice (11-13 weeks of age) were submitted to chronic social defeat stress (CSDS) for 21 consecutive days as previously described (Hartmann et al., 2012a).

Sleep phenotyping in mice

To monitor spontaneous sleep-wake behavior, mice were chronically implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes. The polysomnographic recording setup was the same as previously reported (Kimura et al., 2010). EEG and EMG recordings (EGErA Data Acquisition System, SEA, Cologne, Germany) were performed continuously for 24 h in unrestrained adult male hP2X7R mice. Vigilance states are defined as wake, non-rapid eye movement (REM) sleep (NREMS), or REM sleep (REMS), respectively, in 4-s epochs and manually corrected if necessary.

Sleep phenotyping in humans

The study followed the guidelines in The Declaration of Helsinki. The ethical review board approved the study and prior written informed consent was obtained from the participants. We investigated the sleep EEG recordings of n = 53 young healthy male subjects (age range 18-30 yrs) in relation to their Gln460Arg variant (rs2230912) of the *P2RX7* gene. N = 39 subjects (mean age ± s.e.m. 25.74 ± 0.44 yrs) were identified as carriers of the homozygous (A/A) gene variant, whereas n=14 subjects (mean age ± s.e.m. 25.5 ± 0.65 yrs) were heterozygous individuals (A/G genotype). No homozygous G/G carriers were identified (minor allele frequency 0.05).

Polysomnographic recordings (Comlab 32 Digital Sleep Lab) were performed according to the international 10-20 electrode system. EEG electrodes included F3, F4, C3, C4, P3, P4, O1, and O2 which were referenced against the contralateral mastoid. Sleep stages were visually scored according to the standard guidelines (Rechtschaffen and Kales, 1986). Details of the

study design used in the human polysomnographic set up are described in Adamczyk et al. (2015).

Statistical analyses

Data and statistical analysis were performed with the computer programs GraphPad Prism version 5.0 (Graphpad Prism, RRID:SCR_002798) and SPSS version 16 (SPSS, RRID:SCR_002865). All results are shown as means ± standard error of the mean (s.e.m.). Sample sizes were chosen based on previous publications reporting similar group sizes. Investigators were blinded to the experimental groups during the experiments and data analysis.

For calcium imaging, data analysis of variance (ANOVA) with repeated measures design was applied. Real-time PCR data was analyzed by one-way ANOVA with Scheffé's post hoc test. The effects of genotype on mouse sleep and of genotype and stress on behavior and neuroendocrine data were examined by two-way-multivariate ANOVA. ANOVA with repeated measures design was applied for the assessment of fur state progression and locomotion in the OF test. Tukey´s (behavioral characterization) or Bonferroni's (sleep phenotyping) post hoc test was applied whenever significant main or interaction effects were observed. Significance was accepted at $P = 0.05$. Subjects were assigned to treatment groups based on their genotype without utilizing a specific method. F test (for t tests) or Bartlett's test (for ANOVA) were performed to estimate the variation within each group of data. The statistically compared groups had similar variance.

Human sleep data were analysed as follows: The assumptions of parametric tests were checked for each investigated parameter. Normal distribution was controlled via the Kolmogorov-Smirnov test and variances were compared with the F-test. Prerequisites were considered to be violated, if the appropriate test showed a significant result at the 5% level. If

data did not fulfill these criteria they were either power transformed (when skewed to the left; sleep period time) or log transformed (when skewed to the right; all EEG spectral data), respectively prior to statistical analysis. Multivariate analysis of variance (MANOVA) was performed to compare sleep architecture and sleep continuity between genotypes (A/A, A/G). Two-way mixed model ANOVA with the between-subject factor genotype (A/A, A/G) and the within-subject factor derivation (F3A2, C3A3, P3A2 and O1A2) was performed for EEG power activity. All univariate post hoc comparisons of means between the genotypes were performed with the two-tailed unpaired t-test.

RESULTS

P2X7R function is attenuated in heterozygous humanized mice co-expressing hP2X7R-WT and hP2X7R-Gln460Arg

To investigate the impact of the mood disorder-associated Gln460Arg polymorphism *in vivo* we humanized the murine P2X7 receptor (mP2X7R) by substituting the murine exon 2 with a human cDNA expression unit, which covers exons 2-13 and in addition carries the polymorphism (hP2X7R-Gln460Arg; **Figure 1*A, B***). This strategy was chosen to maintain all regulatory elements crucial for proper temporal and spatial expression of the humanized P2X7R. To address the significant species-specific difference with respect to the receptor´s affinity towards its ligand and different modulators, we used a mouse line expressing the humanized WT P2X7R (hP2X7R-WT) instead of mP2X7R, which enabled direct comparison (**Figure 1*C*)** (Metzger et al., 2016). The temporal and spatial expression of hP2X7R variants in both humanized P2X7R mouse lines (*hP2RX7*), either homozygous for the WT hP2X7R-Gln460 ($P2rx7^{hGln460/hGln460}$, referred to as $P2rx7^{hWT}$) or for the hP2X7R-Arg460 variant ($P2rx7^{hArg460/hArg460}$, referred to as $P2rx7^{hQ460R}$) is indistinguishable from endogenous mP2X7R expression. This was demonstrated by *in situ* hybridization using species-specific riboprobes, which revealed the characteristic strong expression of P2X7R in the hippocampal CA3 region (**Figure 1*D***). We further confirmed that the expression levels of hP2X7R-WT and hP2X7R-Gln460Arg were identical thus enabling a meaningful comparison of the humanized mouse lines (**Figure 1*E***). Both lines express a fully functional hP2X7R as indicated by their capability to induce the release of IL-1β from peritoneal macrophages stimulated with LPS and BzATP (**Figure 1*F***).

Our previous *in vitro* results indicated that co-expression of hP2X7R-Q460R and hP2X7R-WT reduces receptor activity (Aprile-Garcia et al., 2016). Therefore, $P2rx7^{hWT}$ and $P2rx7^{hQ460R}$ mice were intercrossed to yield heterozygous $P2rx7^{hGln460/hArg460}$ animals (referred to as $P2rx7^{hHET}$), which were finally interbred to obtain $P2rx7^{hWT}$, $P2rx7^{hHET}$ and $P2rx7^{hQ460R}$

offspring at Mendelian rates (**Figure 2*A, B***). We used mixed primary hippocampal cell cultures to assess P2X7R function in humanized mice in more detail. BzATP stimulation revealed an attenuated calcium uptake of primary cells derived from heterozygous *hP2rx7*^{hHET} mice compared to those originating from homozygous *hP2rx7*^{hWT} or *hP2rx7*^{hQ460R} mice (RM ANOVA - time: $F_{(29, 406)} = 56.07$, $p < 0.0001$; time × genotype: $F_{(58, 406)} = 1.52$, $p = 0.012$; genotype: $F_{(2, 406)} = 8.96$, $p = 0.003$; n =6 *P2rx7*^{hWT}, 7 *P2rx7*^{hHET} and 4 *P2rx7*^{hQ460R}; **Figure 2*C***).

A comprehensive basal behavioral phenotyping of humanized mice did not reveal any significant differences in emotional behavior as assessed by the OF test (One-way ANOVA, $F_{(2,31)} = 0.84$, $p = 0.44$, n = 11-12), EPM (One-way ANOVA, $F_{(2,33)} = 0.11$, $p = 0.89$, n = 11-13), DaLi (One-way ANOVA, $F_{(2,32)} = 1.71$, $p = 0.19$, n = 11-12) or FST (One-way ANOVA, $F_{(2,33)} = 0.82$, $p = 0.45$, n = 11-12) under standard housing conditions (**Figure 2 *D-G)***

hP2X7R-WT/hP2X7R-Gln460Arg heterozygosity negatively affects sleep quality

Since impaired sleep is one of the most robust symptoms accompanying mood disorders, we additionally monitored EEG activity and assessed spontaneous sleep-wake behavior in *hP2RX7* mice. All three genotypes of *hP2RX7* mice showed unaltered nocturnal sleep-wake behavior as indicated by the normal distribution of NREMS (2-way ANOVA - genotype: $F_{(2, 360)} = 0.75$, $p = 0.75$; time: $F_{(11, 360)} = 68.13$, $p < 0.0001$; genotype x time: $F_{(22, 360)} = 1.42$, $p = 1.10$; n = 11 in all groups) and REMS (2-way ANOVA - genotype: $F_{(2, 360)} = 0.76$, $p = 0.47$; time: $F_{(11, 360)} = 73.39$, $p < 0.0001$; genotype × time: $F_{(22, 360)} = 2.04$, $p < 0.05$; n = 11 in all groups; **Figure 3 *A, B*, Table 1**). However, heterozygous mice showed a significantly higher number of episode entries to REMS during the light period (2-way ANOVA – genotype: $F_{(2, 60)} = 4.83$, $p < 0.05$; Bonferroni multiple comparisons, $p < 0.01$; **Figure 3*C*, Table 2**), suggesting a stronger drive towards REMS and more fragmented sleep cycles compared to *P2rx7*^{hWT} and *P2rx7*^{hQ460R} mice. Additionally, slow-wave activity (SWA) during NREMS,

which measures the depth of NREMS, was constantly lower in heterozygous mice (2-way ANOVA – genotype: $F_{(2, 168)} = 11.23$, $p < 0.0001$, light period; $F_{(2, 168)} = 3.85$, $p < 0.05$, dark period; n = 9-11; **Figure 3*D*, Table 1**). Accordingly, only a small amount of deep NREMS (SWS_2, containing more than 50 % of delta activity) was detected in *P2rx7*^{hHET} mice (2-way ANOVA – genotype: $F_{(2, 168)} = 15.46$, $p < 0.0001$, light period; $F_{(2, 168)} = 8.14$, $p < 0.001$, dark period; n = 9-11; **Figure 3*E*, Table 1**), suggesting a shallower NREMS stage. This finding is also reflected in the spectrograms where *P2rx7*^{hHET} mice exhibited reduced power densities in lower frequency activities of the EEG (**Figure 3*F***). These results indicate that *P2rx7*^{HET} mice have an altered sleep architecture and attenuated quality of sleep compared to *P2rx7*^{hWT} and *P2rx7*^{hQ460R} littermates.

Heterozygote human Gln460Arg carriers show more shallow sleep and lower sleep spindle frequency

Heterozygous *hP2rx7*^{hHET} mice showed signs of an increased REMS pressure together with lower sleep depth which might be indicative of a pre-symptomatic disease stage. Since sleep is readily accessible in humans, we comprehensively assessed sleep parameters in healthy subjects genotyped for the rs2230912 SNP (1405G>A). The macroscopic sleep architecture revealed marginally impaired sleep continuity in heterozygous (A/G) compared to homozygous (A/A) participants. The sleep period time was reduced whereas the sleep onset latency was increased in subjects with the heterozygous genotype (**Table 3**). In view of the impaired sleep structure in transgenic animals (more frequent entries to the REMS in heterozygous *hP2X7R* mice), as well as tendency to lower sleep continuity in humans with the A/G genotype, we investigated the influence of genotype on sleep phase stability (frequency of entries into REMS and NREMS). Since typical sleep abnormalities in depression occur at the beginning of a night's sleep (Dresler et al., 2014) we analyzed the first sleep cycle. A/G carriers showed a significant increase in the amount of entries into shallow sleep from

NREMS stage 2. Additional parameters describing the direction of NREM entries (stage 2 into shallow sleep or wakefulness; slow-wave sleep into shallow sleep or wakefulness) revealed that the sleep of A/G carriers was characterized by a significant instability of stage 2 sleep (higher numbers of entries from stage 2 sleep into shallow sleep or wakefulness) during the first sleep cycle (**Table 3**).

The genotype effect on NREMS EEG frequency spectrum morphology is in particular related to sleep spindles. A/G carriers exhibited an increased amount of spectral power in spindle frequency. Two-way mixed model analysis of variance (ANOVA) with the between-subject factor genotype (A/A, A/G) and the within-subject factor derivation (F3A2, C3A3, P3A2 and O1A2) performed for EEG power frequency bins in NREM sleep revealed a significant main effect of genotype in 13 Hz ($F_{(1, 51)} = 4.592$, $p = 0.037$; **Figure 4 *A, B***). Correspondingly, the mean peak frequency of sleep spindles was significantly lower in these subjects (Two-tailed unpaired *t*-test: $p = 0.001$; **Figure 4*C***). In addition, the NREMS spectrum of A/G carriers displayed a higher amount of beta frequencies at 25-26 Hz (Two-way mixed ANOVA - genotype: $F_{(1, 51)} \geq 4.614$, $p \leq .036$; **Figure 4 *A, B***). REM sleep parameters or characteristics of SWS did not differ between genotypes (**Table 3**).

hP2X7R mice respond to chronic stress

The reduced sleep quality observed in mice and to a milder degree also present in humans, led us to hypothesize that it might reflect signs of a pre-symptomatic disease stage or indicate an increased vulnerability of heterozygote individuals to develop symptoms of mood disorders. In general, the disease risk is determined by the interaction of a genetic predisposition with environmental factors such as chronic stress or severe trauma. To test this hypothesis, we subjected hP2X7R mice to three weeks of CSDS. In accordance with previous studies (Wagner et al., 2011;Wang et al., 2011;Hartmann et al., 2012a;Hartmann et al., 2012b;Gassen et al., 2014), *hP2X7R* mice showed robust physiological and neuroendocrine changes evoked

by CSDS. These included: fur quality decrease (RM ANOVA - time: $F_{(3, 116)} = 84.9$, $p < 0.001$; time × stress: $F_{(3, 116)} = 63.9$, $p < 0.001$; time × genotype: $F_{(6, 234)} = 0.74$, $p = 0.62$; time × genotype × stress: $F_{(6, 234)} = 1.1$, $p = 0.39$; stress: $F_{(1, 118)} = 190.8$, $p < 0.001$; genotype: $F_{(2, 118)} = 0.53$, $p = 0.59$; genotype × stress: $F_{(2, 118)} = 0.11$, $p = 0.68$; n = 20-23; **Figure. 5*A***), thymus atrophy (2-way ANOVA - stress: $F_{(1, 122)} = 26.14$, $p < 0.001$; genotype: $F_{(2, 122)} = 0.39$, $p = 0.68$; genotype × stress: $F_{(2, 122)} = 0.59$, $p = 0.56$; n = 20-23; **Figure 5*B***), adrenal gland enlargement (2-way ANOVA - stress: $F_{(1, 119)} = 65.88$, $p < 0.0001$; genotype: $F_{(2, 119)} = 0.12$, $p = 0.89$; genotype × stress: $F_{(2, 119)} = 1.0$, $p = 0.37$; n = 20-23; **Figure 5*C***) as well as sensitization of the hypothalamic-pituitary-adrenal (HPA) axis to a novel stressor (2-way ANOVA - stress: $F_{(1, 43)} = 20.05$, $p < 0.0001$; genotype: $F_{(2, 43)} = 0.19$, $p = 0.83$; genotype × stress: $F_{(2, 43)} = 1.16$, $p = 0.32$; n = 8-10; **Figure 5*D***). We did not detect significant genotype effects in any of the assessed parameters, indicating that the CSDS paradigm evoked similar strong effects in all three genotypes.

We then performed a wide range of behavioral tests to assess the consequences of CSDS on core endophenotypes related to mood disorders. Again, we observed no alterations in any behavioral readout in non-stressed control groups. In line with previous studies, chronically stressed *hP2RX7* mice showed decreased locomotion in the OF test compared to unstressed mice (RM ANOVA - time: $F_{(2, 50)} = 3.25$, $p = 0.05$; time × stress: $F_{(2, 50)} = 1.45$, $p = 0.24$; time × genotype: $F_{(4, 102)} = 1.36$, $p = 0.25$; time × genotype × stress: $F_{(4, 102)} = 1.45$, $p = 0.23$; stress: $F_{(1, 51)} = 10.48$, $p < 0.005$; genotype: $F_{(2, 51)} = 0.52$, $p = 0.6$; genotype × stress: $F_{(2, 51)} = 0.29$, $p = 0.75$; n = 20-23). Although, stressed $P2rx7^{hHET}$ mice appeared to show an increase in locomotion (2-way ANOVA - stress: $F_{(1, 49)} = 5.05$, $p = 0.029$; genotype: $F_{(2, 49)} = 2.37$, $p = 0.10$; genotype × stress: $F_{(2, 49)} = 0.86$, $p = 0.42$; n = 9-10) accompanied by decreased immobility (2-way ANOVA - stress: $F_{(1, 49)} = 12.34$, $p < 0.001$; genotype: $F_{(2, 49)} = 2.57$, $p = 0.09$; genotype × stress: $F_{(2, 49)} = 0.39$, $p = 0.67$;n = 9-10) during the first 5 min of the OF test, this did not reach statistical significance (**Figure 6 *A*-*C***). In the EPM, all three genotypes

displayed a similar increase in anxiety-related behavior following CSDS (2-way ANOVA - stress: $F_{(1, 117)} = 5.29$, $p = 0.023$; genotype: $F_{(2, 117)} = 1.0$, $p = 0.37$; genotype × stress: $F_{(2, 117)} = 0.44$, $p = 0.64$;n = 19-23; **Figure 6*D***). Given that impairments in social behavior, are reminiscent of various psychiatric clinical conditions including mood disorders (Nestler and Hyman, 2010), we also conducted the social avoidance test. Although 2-way ANOVA only revealed a main significant effect of stress, *P2rx7*^{hHET} and *P2rx7*^{hQ460R} mice spent less time in close proximity to the social target compared to *P2rx7*^{hWT} mice (2-way ANOVA - stress: $F_{(1, 61)} = 14.16$, $p < 0.001$; genotype: $F_{(2, 61)} = 1.9$, $p = 0.16$; genotype × stress: $F_{(2, 61)} = 0.65$, $p = 0.52$; n = 11-13; **Figure 6*E***). However, a significant stress effect was also observed during the empty wire cage-trial of the social avoidance test, implying increased anxiety-related behavior in the animals even in the absence of a novel social counterpart (2-way ANOVA - stress: $F_{(1, 61)} = 8.94$, $p = 0.004$; genotype: $F_{(2, 61)} = 2.48$, $p = 0.09$; genotype × stress: $F_{(2, 61)} = 0.25$, $p = 0.78$; n = 11-13; **Figure 6*F***). We also applied the female urine sniffing test (FUST) to assess anhedonia, but found no statistically significantly differences between genotypes following CSDS (2-way ANOVA - stress: $F_{(1, 59)} = 7.42$, $p = 0.008$; genotype: $F_{(2, 59)} = 1.46$, $p = 0.24$; genotype × stress: $F_{(2, 59)} = 0.47$, $p = 0.63$;; n = 10-12; **Figure 6*G***). CSDS had no effect on sniffing behavior when water was used as a control (**Figure 6*H***). Taken together, the analyses of *hP2RX7* mice do not provide sufficient evidence that the hP2X7R-WT/hP2X7R-Gln460Arg heterozygosity conveys an increased vulnerability to develop mood disorder-related endophenotypes in adult mice in response to chronic social defeat stress.

DISCUSSION

Modelling human genetic findings in the mouse

In recent years, considerable efforts have led to the identification of genetic variants associated with psychiatric disorders (Collins and Sullivan, 2013). However, most discoveries lack experimental validation in an appropriate genetic animal model owing to the fact that the vast majority of disease-associated SNPs are of unknown function and not conserved between humans and rodents. In contrast, non-synonymous or regulatory SNPs that directly impact amino acid sequence or gene function are exceptionally rare (Chen et al., 2006).

In the case of the P2X7R, we took advantage of the fact that the disease-associated Gln460Arg polymorphism enables us to readily model the genetic association in mice and thereby address its immediate function *in vivo*. The relevance of P2X7R *in vivo* with regards to phenotypes related to mood disorders has so far only been investigated using constitutive P2X7R KO mice (Boucher et al., 2011;Basso et al., 2009;Solle et al., 2001;Csolle et al., 2013). However, the direct translation of these findings is difficult considering that human genetic studies associated a polymorphism leading to an amino acid substitution with mood disorders but not a null allele (Hejjas et al., 2009;Lucae et al., 2006;Soronen et al., 2011;McQuillin et al., 2009;Barden et al., 2006). To interrogate the P2X7R-Gln460Arg polymorphism *in vivo* we generated humanized mice by substituting the murine P2X7R with the human P2X7R variants. We confirmed in *hP2RX7* mice previous reports demonstrating that the P2X7R-Gln460Arg variant itself is not significantly impaired in its function compared to the WT P2X7R (Roger et al., 2010). However, the co-expression of the mood disorder-associated P2X7R-Gln460Arg variant with WT P2X7R caused a significant reduction in normal receptor function, which was reflected by an attenuated calcium response of primary hippocampal cells derived from heterozygous *hP2RX7* mice. This is in line with a recent study using stably transfected HEK293 cells co-expressing both P2X7R variants, which also showed a reduced calcium uptake (Aprile-Garcia et al., 2016). Despite the

451 significantly altered P2X7R function in heterozygous *hP2RX7* mice, no significant genotype-
452 dependent behavioral alterations were detected under basal housing conditions.
453
454 **Heterozygosity affects sleep**
455 It has been shown that P2X7R expression is under circadian control and is increased
456 following sleep deprivation in humans (Backlund et al., 2012). Moreover, P2X7R directly
457 modulates the action of somnogenic cytokines including IL-1β and tumor necrosis factor α,
458 which are recognized as endogenous sleep regulatory substances (SRS; Krueger et al., 2010).
459 Here we observed that hP2X7R mice displayed normal nocturnal sleep-wake behavior.
460 However, heterozygous hP2X7R mice showed overt alterations in their sleep architecture
461 compared to homozygous littermates. Comparable impairments of sleep, including an
462 increased proportion of REMS and reduced slow-wave activity during NREMS, are often
463 seen in patients with mood disorders (Germain and Kupfer, 2008;Modell and Lauer, 2007).
464 Sleep-EEG recordings in healthy human subjects, genotyped for rs2230912, revealed NREMS
465 instability and alterations in NREMS morphology in heterozygous carriers. In particular
466 heterozygous A/G carriers showed a lower mean peak frequency of all sleep spindles in both
467 slow-wave sleep and stage 2 sleep. In view of the relevance of changes in either the number
468 or the oscillation frequency of sleep spindles in several neuropsychiatric diseases and brain
469 function in general (Christensen et al., 2015;Lopez et al., 2010;Nishida et al., 2016;Potari et
470 al., 2017;Ferrarelli et al., 2007) the observed slowing of spindle frequency may indicate a
471 subtle but sensitive sleep alteration. The prominent role of P2X7R in cytokine release
472 suggests that low EEG power seen in heterozygous hP2RX7 mice might result from
473 interference with SRSs. Moreover, the fact that changes in immune mediators such as pro-
474 inflammatory cytokines are repeatedly observed in patients with mood disorders further
475 supports a potential role of P2X7R in disease etiology (Kronfol and Remick, 2000;Stokes et
476 al., 2015). Taken together, heterozygous hP2X7R mice showed depression-like changes in

both NREMS and REMS, whereas in healthy human A/G carriers subtle sleep changes were restricted to NREMS. These findings might hint towards early disease symptoms or signs of a prodromal state which have the potential to convey increased vulnerability to develop disease (Perlis et al., 1997).

Gene × environment interactions

There is ample evidence that individual disease vulnerability is not purely genetically determined (Caspi and Moffitt, 2006) but is strongly linked to environmental factors such as stress. Sustained or chronic stress and a maladaptive stress response in combination with a genetic predisposition are able to trigger the precipitation of mood disorders, which are consequently often regarded as stress-related disorders (de Kloet et al., 2005). To test whether heterozygosity might predispose to disease development, we subjected *hP2X7R* mice to the widely used and repeatedly validated CSDS paradigm (Berton et al., 2006;Hartmann et al., 2012a). Chronic stress can have a major impact on the behavioral phenotype of animals, often resulting in diminished locomotor activity and higher anxiety-related behavior (Berton et al., 2006;Hartmann et al., 2012a). Accordingly, all genotypes of *hP2X7R* mice showed reduced locomotion in the 15-min OF test and increased anxiety in the EPM. Moreover, stressed *hP2X7R* mice showed a clearly increased social avoidance as well as signs of anhedonia. Even though heterozygous *hP2X7R* mice displayed the strongest effect to CSDS, 2-way analyses of variants did not reveal statistically significant interaction effects in any of the behavioral tests. These behavioral paradigms assessed the cardinal symptoms of mood disorders including locomotion, anxiety-related, social and anhedonic behavior (Nestler and Hyman, 2010). However, further studies evaluating different aspects of cognition, anhedonia (sucrose-preference) and anxiety (fear-memory, learned helplessness, etc.) in conjunction with other chronic stress paradigms will further refine the contribution of P2RX7 polymorphisms in mood disorders. Nevertheless, together with the observed sleep alterations,

we believe that our results provide important and relevant biological evidence that heterozygosity for wild-type P2X7R and the P2X7R-Q460R variant represents a genetic risk factor for mood disorders.

A heterozygote disadvantage model

To the best of our knowledge the P2X7R is the first example suggesting a heterozygote disadvantage model for the association of a candidate SNP with psychiatric disorders (Lucae et al., 2006). It would be interesting to investigate this model in a meta-analysis similar to the recent study by Feng and colleagues (2014). Co-expression and interaction of P2X7R subunits, either with splice or polymorphic variants may represent a general mechanism for regulation of P2X7R activity and of other ion channels. However, this mechanism appears rather unique as it has not been described for any of the numerous other P2X7R variants. A first hint towards an underlying mechanism involving interaction with the P2X7R-WT emerges from a naturally occurring truncated variant of P2X7R, which is ineffective when expressed alone, but is able to hetero-oligomerize with P2X7R-WT and thereby blocks P2X7R activity (Feng et al., 2006). Similarly, C-terminally truncated variants of P2X7R, escaping inactivation in KO mice (Solle et al., 2001) and variants bearing mutations in the extracellular domain have been reported to act in a dominant negative fashion (Masin et al., 2012;Raouf et al., 2004).

Of note, P2X7 excels all other P2X family members with respect to the frequency of non-synonymous SNPs, which might to some extent reflect evolutionary adaptation related to the role of P2X7R in modulating innate immune function (Wiley et al., 2011;Sluyter and Stokes, 2011). Nevertheless, further structural insights are required to mechanistically understand the functional consequences of co-expression of hP2X7R-WT with hP2X7R-Gln460Arg and its implication in mood disorders.

Conclusions

In conclusion, the herein reported results involving studies in human participants and respective mouse models suggest that heterozygosity for P2X7R-WT and P2X7R-Gln460Arg genetically predisposes to enhanced stress vulnerability. This is causally linked to the co-expression of P2X7R-WT and P2X7R-Gln460Arg variants, which results in a significant reduction of normal P2X7R function. Our findings suggest that the alterations in P2X7R function in heterozygote mice convey disturbances in mechanisms of sleep regulation. These findings together with the subtle changes in NREMS parameters in heterozygous human subjects have the potential to open up potential novel diagnostic and therapeutic avenues. Taking into account that sleep-EEG alterations are robust predictors of an emergent depressive episode and may even precede the full-blown clinical syndrome (Steiger and Kimura, 2010), our results suggest that observed changes in sleep variables in combination with a heterozygous rs2230912 genotype could represent a predictor or biomarker for mood disorders risk. Our study provides strong evidence for a heterozygote disadvantage model, which adds a new perspective to the current knowledge of functional mechanisms underlying genetic findings in complex diseases.

546

547 REFERENCES
548

549 Abkevich V, et al. (2003) Predisposition locus for major depression at chromosome 12q22-
550 12q23.2. Am J Hum Genet 73:1271-1281.

551 Adamczyk M, Ambrosius U, Lietzenmaier S, Wichniak A, Holsboer F, Friess E (2015)
552 Genetics of rapid eye movement sleep in humans. Transl Psychiatry 5:e598.

553 Aprile-Garcia F, Metzger MW, Paez-Pereda M, Stadler H, Acuna M, Liberman AC, Senin
554 SA, Gerez J, Hoijman E, Refojo D, Mitkovski M, Panhuysen M, Stuhmer W, Holsboer F,
555 Deussing JM, Arzt E (2016) Co-Expression of Wild-Type P2X7R with Gln460Arg Variant
556 Alters Receptor Function. PLoS One 11:e0151862.

557 Backlund L, Lavebratt C, Frisen L, Nikamo P, Hukic SD, Traskman-Bendz L, Landen M,
558 Edman G, Vawter MP, Osby U, Schalling M (2012) P2RX7: expression responds to sleep
559 deprivation and associates with rapid cycling in bipolar disorder type 1. PLoS One
560 7:e43057.

561 Backlund L, Nikamo P, Hukic DS, Ek IR, Traskman-Bendz L, Landen M, Edman G,
562 Schalling M, Frisen L, Osby U (2011) Cognitive manic symptoms associated with the
563 P2RX7 gene in bipolar disorder. Bipolar Disord 13:500-508.

564 Barden N, Harvey M, Gagne B, Shink E, Tremblay M, Raymond C, Labbe M, Villeneuve A,
565 Rochette D, Bordeleau L, Stadler H, Holsboer F, Muller-Myhsok B (2006) Analysis of
566 single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to
567 P2RX7 as a susceptibility gene to bipolar affective disorder. Am J Med Genet B
568 Neuropsychiatr Genet 141B:374-382.

569 Bartlett R, Stokes L, Sluyter R (2014) The P2X7 receptor channel: recent developments and
570 the use of P2X7 antagonists in models of disease. Pharmacol Rev 66:638-675.

571 Basso AM, Bratcher NA, Harris RR, Jarvis MF, Decker MW, Rueter LE (2009) Behavioral
572 profile of P2X7 receptor knockout mice in animal models of depression and anxiety:
573 relevance for neuropsychiatric disorders. Behav Brain Res 198:83-90.

574 Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova
575 NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ (2006) Essential role of
576 BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311:864-868.

577 Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma D, van VT, Willemsen G,
578 DeRijk RH, de Geus EJ, Hoogendijk WJ, Sullivan PF, Penninx BW, Boomsma DI, Snieder
579 H, Nolen WA (2011) Poor replication of candidate genes for major depressive disorder
580 using genome-wide association data. Mol Psychiatry 16:516-532.

581 Boucher AA, Arnold JC, Hunt GE, Spiro A, Spencer J, Brown C, McGregor IS, Bennett MR,
582 Kassiou M (2011) Resilience and reduced c-Fos expression in P2X7 receptor knockout
583 mice exposed to repeated forced swim test. Neuroscience 189:170-177.

584 Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: joining forces with
585 neuroscience. Nat Rev Neurosci 7:583-590.

586 Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C,
587 McEwen BS, Hempstead BL, Lee FS (2006) Genetic variant BDNF (Val66Met)
588 polymorphism alters anxiety-related behavior. Science 314:140-143.

589 Christensen JA, Nikolic M, Warby SC, Koch H, Zoetmulder M, Frandsen R, Moghadam KK,
590 Sorensen HB, Mignot E, Jennum PJ (2015) Sleep spindle alterations in patients with
591 Parkinson's disease. Front Hum Neurosci 9:233.

592 Collins AL, Sullivan PF (2013) Genome-wide association studies in psychiatry: what have we
593 learned? Br J Psychiatry 202:1-4.

594 Collins PY, et al. (2011) Grand challenges in global mental health. Nature 475:27-30.

595 Csolle C, Ando RD, Kittel A, Goloncser F, Baranyi M, Soproni K, Zelena D, Haller J,
596 Nemeth T, Mocsai A, Sperlagh B (2013) The absence of P2X7 receptors (P2rx7) on non-
597 haematopoietic cells leads to selective alteration in mood-related behaviour with
598 dysregulated gene expression and stress reactivity in mice. Int J Neuropsychopharmacol
599 16:213-233.

600 de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat
601 Rev Neurosci 6:463-475.

602 Degn B, Lundorf MD, Wang A, Vang M, Mors O, Kruse TA, Ewald H (2001) Further
603 evidence for a bipolar risk gene on chromosome 12q24 suggested by investigation of
604 haplotype sharing and allelic association in patients from the Faroe Islands. Mol Psychiatry
605 6:450-455.

606 Deuchars SA, Atkinson L, Brooke RE, Musa H, Milligan CJ, Batten TF, Buckley NJ, Parson
607 SH, Deuchars J (2001) Neuronal P2X7 receptors are targeted to presynaptic terminals in
608 the central and peripheral nervous systems. J Neurosci 21:7143-7152.

609 Dresler M, Spoormaker VI, Beitinger P, Czisch M, Kimura M, Steiger A, Holsboer F (2014)
610 Neuroscience-driven discovery and development of sleep therapeutics. Pharmacol Ther
611 141:300-334.

612 Feng WP, Zhang B, Li W, Liu J (2014) Lack of association of P2RX7 gene rs2230912
613 polymorphism with mood disorders: a meta-analysis. PLoS One 9:e88575.

614 Feng YH, Li X, Wang L, Zhou L, Gorodeski GI (2006) A truncated P2X7 receptor variant
615 (P2X7-j) endogenously expressed in cervical cancer cells antagonizes the full-length P2X7
616 receptor through hetero-oligomerization. J Biol Chem 281:17228-17237.

617 Ferrarelli F, Huber R, Peterson MJ, Massimini M, Murphy M, Riedner BA, Watson A, Bria P,
618 Tononi G (2007) Reduced sleep spindle activity in schizophrenia patients. Am J Psychiatry
619 164:483-492.

620 Gassen NC, Hartmann J, Zschocke J, Stepan J, Hafner K, Zellner A, Kirmeier T,
621 Kollmannsberger L, Wagner KV, Dedic N, Balsevich G, Deussing JM, Kloiber S, Lucae S,
622 Holsboer F, Eder M, Uhr M, Ising M, Schmidt MV, Rein T (2014) Association of FKBP51
623 with priming of autophagy pathways and mediation of antidepressant treatment response:
624 evidence in cells, mice, and humans. PLoS Med 11:e1001755.

625 Germain A, Kupfer DJ (2008) Circadian rhythm disturbances in depression. Hum
626 Psychopharmacol 23:571-585.

627 Green EK, Grozeva D, Raybould R, Elvidge G, Macgregor S, Craig I, Farmer A, McGuffin P,
628 Forty L, Jones L, Jones I, O'Donovan MC, Owen MJ, Kirov G, Craddock N (2009)
629 P2RX7: A bipolar and unipolar disorder candidate susceptibility gene? Am J Med Genet B
630 Neuropsychiatr Genet 150B:1063-1069.

631 Grigoroiu-Serbanescu M, et al. (2009) Variation in P2RX7 candidate gene (rs2230912) is not
632 associated with bipolar I disorder and unipolar major depression in four European samples.
633 Am J Med Genet B Neuropsychiatr Genet 150B:1017-1021.

634 Halmai Z, Dome P, Vereczkei A, bdul-Rahman O, Szekely A, Gonda X, Faludi G, Sasvari-
635 Szekely M, Nemoda Z (2013) Associations between depression severity and purinergic
636 receptor P2RX7 gene polymorphisms. J Affect Disord 150:104-109.

637 Hartmann J, Wagner KV, Dedic N, Marinescu D, Scharf SH, Wang XD, Deussing JM,
638 Hausch F, Rein T, Schmidt U, Holsboer F, Muller MB, Schmidt MV (2012a) Fkbp52
639 heterozygosity alters behavioral, endocrine and neurogenetic parameters under basal and
640 chronic stress conditions in mice. Psychoneuroendocrinology 37:2009-2021.

641 Hartmann J, Wagner KV, Liebl C, Scharf SH, Wang XD, Wolf M, Hausch F, Rein T,
642 Schmidt U, Touma C, Cheung-Flynn J, Cox MB, Smith DF, Holsboer F, Muller MB,
643 Schmidt MV (2012b) The involvement of FK506-binding protein 51 (FKBP5) in the
644 behavioral and neuroendocrine effects of chronic social defeat stress. Neuropharmacology
645 62:332-339.

646 Hejjas K, Szekely A, Domotor E, Halmai Z, Balogh G, Schilling B, Sarosi A, Faludi G,
647 Sasvari-Szekely M, Nemoda Z (2009) Association between depression and the Gln460Arg
648 polymorphism of P2RX7 gene: a dimensional approach. Am J Med Genet B
649 Neuropsychiatr Genet 150B:295-299.

650 Khakh BS, North RA (2006) P2X receptors as cell-surface ATP sensors in health and disease.
651 Nature 442:527-532.

652 Kimura M, Muller-Preuss P, Lu A, Wiesner E, Flachskamm C, Wurst W, Holsboer F,
653 Deussing JM (2010) Conditional corticotropin-releasing hormone overexpression in the
654 mouse forebrain enhances rapid eye movement sleep. Mol Psychiatry 15:154-165.

655 Kronfol Z, Remick DG (2000) Cytokines and the brain: implications for clinical psychiatry.
656 Am J Psychiatry 157:683-694.

657 Krueger JM, Taishi P, De A, Davis CJ, Winters BD, Clinton J, Szentirmai E, Zielinski MR
658 (2010) ATP and the purine type 2 X7 receptor affect sleep. J Appl Physiol (1985)
659 109:1318-1327.

660 Lavebratt C, Aberg E, Sjoholm LK, Forsell Y (2010) Variations in FKBP5 and BDNF genes
661 are suggestively associated with depression in a Swedish population-based cohort. J Affect
662 Disord 125:249-255.

663 Lopez J, Hoffmann R, Armitage R (2010) Reduced sleep spindle activity in early-onset and
664 elevated risk for depression. J Am Acad Child Adolesc Psychiatry 49:934-943.

665 Lucae S, Salyakina D, Barden N, Harvey M, Gagne B, Labbe M, Binder EB, Uhr M, Paez-
666 Pereda M, Sillaber I, Ising M, Bruckl T, Lieb R, Holsboer F, Muller-Myhsok B (2006)
667 P2RX7, a gene coding for a purinergic ligand-gated ion channel, is associated with major
668 depressive disorder. Hum Mol Genet 15:2438-2445.

669 Malkesman O, Scattoni ML, Paredes D, Tragon T, Pearson B, Shaltiel G, Chen G, Crawley
670 JN, Manji HK (2010) The female urine sniffing test: a novel approach for assessing
671 reward-seeking behavior in rodents. Biol Psychiatry 67:864-871.

672 Masin M, Young C, Lim K, Barnes SJ, Xu XJ, Marschall V, Brutkowski W, Mooney ER,
673 Gorecki DC, Murrell-Lagnado R (2012) Expression, assembly and function of novel C-
674 terminal truncated variants of the mouse P2X7 receptor: re-evaluation of P2X7 knockouts.
675 Br J Pharmacol 165:978-993.

676 McQuillin A, Bass NJ, Choudhury K, Puri V, Kosmin M, Lawrence J, Curtis D, Gurling HM
677 (2009) Case-control studies show that a non-conservative amino-acid change from a
678 glutamine to arginine in the P2RX7 purinergic receptor protein is associated with both
679 bipolar- and unipolar-affective disorders. Mol Psychiatry 14:614-620.

680 Metzger MW, Walser SM, Aprile-Garcia F, Dedic N, Chen A, Holsboer F, Arzt E, Wurst W,
681 Deussing JM (2016) Genetically dissecting P2rx7 expression within the central nervous
682 system using conditional humanized mice. Purinergic Signal [Epub ahead of print].

683 Miras-Portugal MT, az-Hernandez M, Giraldez L, Hervas C, Gomez-Villafuertes R, Sen RP,
684 Gualix J, Pintor J (2003) P2X7 receptors in rat brain: presence in synaptic terminals and
685 granule cells. Neurochem Res 28:1597-1605.

686 Modell S, Lauer CJ (2007) Rapid eye movement (REM) sleep: an endophenotype for
687 depression. Curr Psychiatry Rep 9:480-485.

688 Nagy G, Ronai Z, Somogyi A, Sasvari-Szekely M, Rahman OA, Mate A, Varga T, Nemoda Z
689 (2008) P2RX7 Gln460Arg polymorphism is associated with depression among diabetic
690 patients. Prog Neuropsychopharmacol Biol Psychiatry 32:1884-1888.

691 Nestler EJ, Hyman SE (2010) Animal models of neuropsychiatric disorders. Nat Neurosci
692 13:1161-1169.

693 Nicke A (2008) Homotrimeric complexes are the dominant assembly state of native P2X7
694 subunits. Biochem Biophys Res Commun 377:803-808.

695 Nishida M, Nakashima Y, Nishikawa T (2016) Slow sleep spindle and procedural memory
696 consolidation in patients with major depressive disorder. Nat Sci Sleep 8:63-72.

697 Papp L, Vizi ES, Sperlagh B (2004) Lack of ATP-evoked GABA and glutamate release in the
698 hippocampus of P2X7 receptor-/- mice. Neuroreport 15:2387-2391.

699 Perlis ML, Giles DE, Buysse DJ, Tu X, Kupfer DJ (1997) Self-reported sleep disturbance as a
700 prodromal symptom in recurrent depression. J Affect Disord 42:209-212.

701 Potari A, Ujma PP, Konrad BN, Genzel L, Simor P, Kormendi J, Gombos F, Steiger A,
702 Dresler M, Bodizs R (2017) Age-related changes in sleep EEG are attenuated in highly
703 intelligent individuals. Neuroimage 146:554-560.

704 Raouf R, Chakfe Y, Blais D, Speelman A, Boue-Grabot E, Henderson D, Seguela P (2004)
705 Selective knock-down of P2X7 ATP receptor function by dominant-negative subunits. Mol
706 Pharmacol 65:646-654.

707 Rechtschaffen A, Kales AA (1986) A Manual of Standardized Terminology, Techniques and
708 Scoring System For Sleep Stages of Human Subjects. Los Angeles.

709 Roger S, Mei ZZ, Baldwin JM, Dong L, Bradley H, Baldwin SA, Surprenant A, Jiang LH
710 (2010) Single nucleotide polymorphisms that were identified in affective mood disorders
711 affect ATP-activated P2X7 receptor functions. J Psychiatr Res 44:347-355.

712 Sluyter R, Stokes L (2011) Significance of P2X7 receptor variants to human health and
713 disease. Recent Pat DNA Gene Seq 5:41-54.

714 Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, Koller BH, Griffiths RJ, Gabel CA
715 (2001) Altered cytokine production in mice lacking P2X(7) receptors. J Biol Chem
716 276:125-132.

717 Soronen P, Mantere O, Melartin T, Suominen K, Vuorilehto M, Rytsala H, Arvilommi P,
718 Holma I, Holma M, Jylha P, Valtonen HM, Haukka J, Isometsa E, Paunio T (2011) P2RX7
719 gene is associated consistently with mood disorders and predicts clinical outcome in three
720 clinical cohorts. Am J Med Genet B Neuropsychiatr Genet 156B:435-447.

721 Sperlagh B, Illes P (2014) P2X7 receptor: an emerging target in central nervous system
722 diseases. Trends Pharmacol Sci.

723 Steiger A, Kimura M (2010) Wake and sleep EEG provide biomarkers in depression. J
724 Psychiatr Res 44:242-252.

725 Stokes L, Fuller SJ, Sluyter R, Skarratt KK, Gu BJ, Wiley JS (2010) Two haplotypes of the
726 P2X(7) receptor containing the Ala-348 to Thr polymorphism exhibit a gain-of-function
727 effect and enhanced interleukin-1beta secretion. FASEB J 24:2916-2927.

728 Stokes L, Spencer SJ, Jenkins TA (2015) Understanding the role of P2X7 in affective
729 disorders-are glial cells the major players? Front Cell Neurosci 9:258.

730 Surprenant A, Rassendren F, Kawashima E, North RA, Buell G (1996) The cytolytic P2Z
731 receptor for extracellular ATP identified as a P2X receptor (P2X7). Science 272:735-738.

732 Torres GE, Egan TM, Voigt MM (1999) Hetero-oligomeric assembly of P2X receptor
733 subunits. Specificities exist with regard to possible partners. J Biol Chem 274:6653-6659.

734 Viikki M, Kampman O, Anttila S, Illi A, Setala-Soikkeli E, Huuhka M, Mononen N,
735 Lehtimaki T, Leinonen E (2011) P2RX7 polymorphisms Gln460Arg and His155Tyr are
736 not associated with major depressive disorder or remission after SSRI or ECT. Neurosci
737 Lett 493:127-130.

738 Wagner KV, Wang XD, Liebl C, Scharf SH, Muller MB, Schmidt MV (2011) Pituitary
739 glucocorticoid receptor deletion reduces vulnerability to chronic stress.
740 Psychoneuroendocrinology 36:579-587.

741 Wang XD, Chen Y, Wolf M, Wagner KV, Liebl C, Scharf SH, Harbich D, Mayer B, Wurst
742 W, Holsboer F, Deussing JM, Baram TZ, Muller MB, Schmidt MV (2011) Forebrain
743 CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic
744 remodeling. Neurobiol Dis 42:300-310.

745 Wiley JS, Sluyter R, Gu BJ, Stokes L, Fuller SJ (2011) The human P2X7 receptor and its role
746 in innate immunity. Tissue Antigens 78:321-332.

747 Yen YC, Mauch CP, Dahlhoff M, Micale V, Bunck M, Sartori SB, Singewald N, Landgraf R,
748 Wotjak CT (2012) Increased levels of conditioned fear and avoidance behavior coincide
749 with changes in phosphorylation of the protein kinase B (AKT) within the amygdala in a
750 mouse model of extremes in trait anxiety. Neurobiol Learn Mem.

751 Yosifova A, Mushiroda T, Stoianov D, Vazharova R, Dimova I, Karachanak S, Zaharieva I,
752 Milanova V, Madjirova N, Gerdjikov I, Tolev T, Velkova S, Kirov G, Owen MJ,
753 O'Donovan MC, Toncheva D, Nakamura Y (2009) Case-control association study of 65
754 candidate genes revealed a possible association of a SNP of HTR5A to be a factor
755 susceptible to bipolar disease in Bulgarian population. J Affect Disord 117:87-97.
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759 **FIGURE LEGENDS**

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761 **Figure 1.** Establishment of humanized P2X7R mice (*hP2RX7*). ***A***, Targetings strategy for
762 knock-in of the human *P2RX7* cDNA into the mouse *P2rx7* locus. Partial restriction maps
763 (only relevant *Bam*HI (B) sites are depicted) of the wild-type *P2rx7* locus, targeting vector,
764 mutant locus following homologous recombination and *loxP* flanked humanized locus after
765 Flp recombinase-mediated deletion of the neomycin selection marker (pA, 4 ×
766 polyadenylation signal). ***B***, Southern blot analysis of genomic DNA from an embryonic stem
767 cell clone targeted with the human P2X7R-Q460R construct, which was used to generate
768 $P2rx7^{Q460R}$ mice. The targeted allele is indicated by the presence of an additional 7.4-kb
769 fragment. ***C***, General strategy to substitute the murine P2X7R by the human wild-type (WT)
770 receptor or its P2X7R-Q460R variant. ***D***, The mRNA expression of human P2X7R in knock-
771 in mice fully recapitulates endogenous expression of murine P2X7R as demonstrated by *in*
772 *situ* hybridization on coronal brain sections. Depicted are photomicrographs of the
773 hippocampus (scale bar = 100 μm) with magnifications of the hippocampal CA3 region
774 shown below. ***E***, Human P2X7R mRNA is expressed at identical levels in the cortex (Ctx),
775 hippocampus (Hip) and cerebellum (Cb) of heterozygous $P2rx7^{+/hWT}$ and $P2rx7^{+/Q460R}$ mice (n
776 = 6). ***E***, Human P2X7R mRNA is expressed at identical levels in the cortex (Ctx),
777 hippocampus (Hip) and cerebellum (Cb) of heterozygous $P2rx7^{+/hWT}$ and $P2rx7^{+/Q460R}$ mice (n
778 = 6). ***F***, Validation of hP2X7R functionality in $P2rx7^{hWT}$ and $P2rx7^{hQ460R}$ mice as determined
779 by interleukin-1β (IL-1β) release. Peritoneal macrophages of $P2rx7^{hWT}$ and of $P2rx7^{hQ460R}$
780 mice are able to secrete IL-1β in response to LPS stimulation and subsequent treatment with
781 the P2X7R agonist BzATP (n = 4). Data are expressed as mean ± s.e.m.

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Figure 2. Generation and basal characterization of heterozygous humanized P2X7R mice. ***A***, Scheme illustrating the generation of *P2rx7*^{hWT}, *P2rx7*^{hHET} and *P2rx7*^{hQ460R} mice. ***B***, Genotyping was performed by PCR and subsequent restriction digest of the 557 bp PCR product with *Pvu*II. ***C***, Calcium uptake following BzATP treatment of primary hippocampal cells is attenuated in heterozygous *hP2rx7*^{hHET} mice. For each primary culture preparation, three independent measurements were performed; (RM ANOVA, p* < 0.05, n = 4-7). Basal behavioral characterization of hP2X7R mice revealed (***D***) no differences in the total distance travelled of the open field (OF) test, in the time spent in the (***E***) open arm of the elevated plus maze (EPM) or (***F***) lit zone time of the dark-light box test (DaLi), and (***G***) in total immobility during the forced swim test (FST) (One-way ANOVA, n = 11-12). Data are expressed as mean ± s.e.m.

Figure 3. Heterozygosity for hP2X7R-WT with hP2X7R-Gln460Arg alters sleep parameters in *hP2RX7* mice. No genotype differences were observed in the amount of (***A***) NREMS or (***B***) REMS across the 24-h recording period (2-h mean ± s.e.m.). ***C***, Architecture of REMS was significantly altered in *P2rx7*^{hHET} mice that showed more frequent entries to the REMS epoch during the light period (12-h mean ± s.e.m.; *$P < 0.05$). ***D***, Slow-wave activity (SWA) during NREMS was significantly attenuated in heterozygous mice (2-h mean ± s.e.m.; *$P < 0.05$, **$P < 0.001$). ***E***, *P2rx7*^{hHET} mice rarely entered SWS_2 across the entire 24 h (2-h mean ± s.e.m.; **$P < 0.001$). ***F***, Hypnograms and spectrograms of representative animals for each genotype. *P2rx7*^{hHET} mice show suppression of EEG power in lower frequency bands indicating a lower sleep quality.

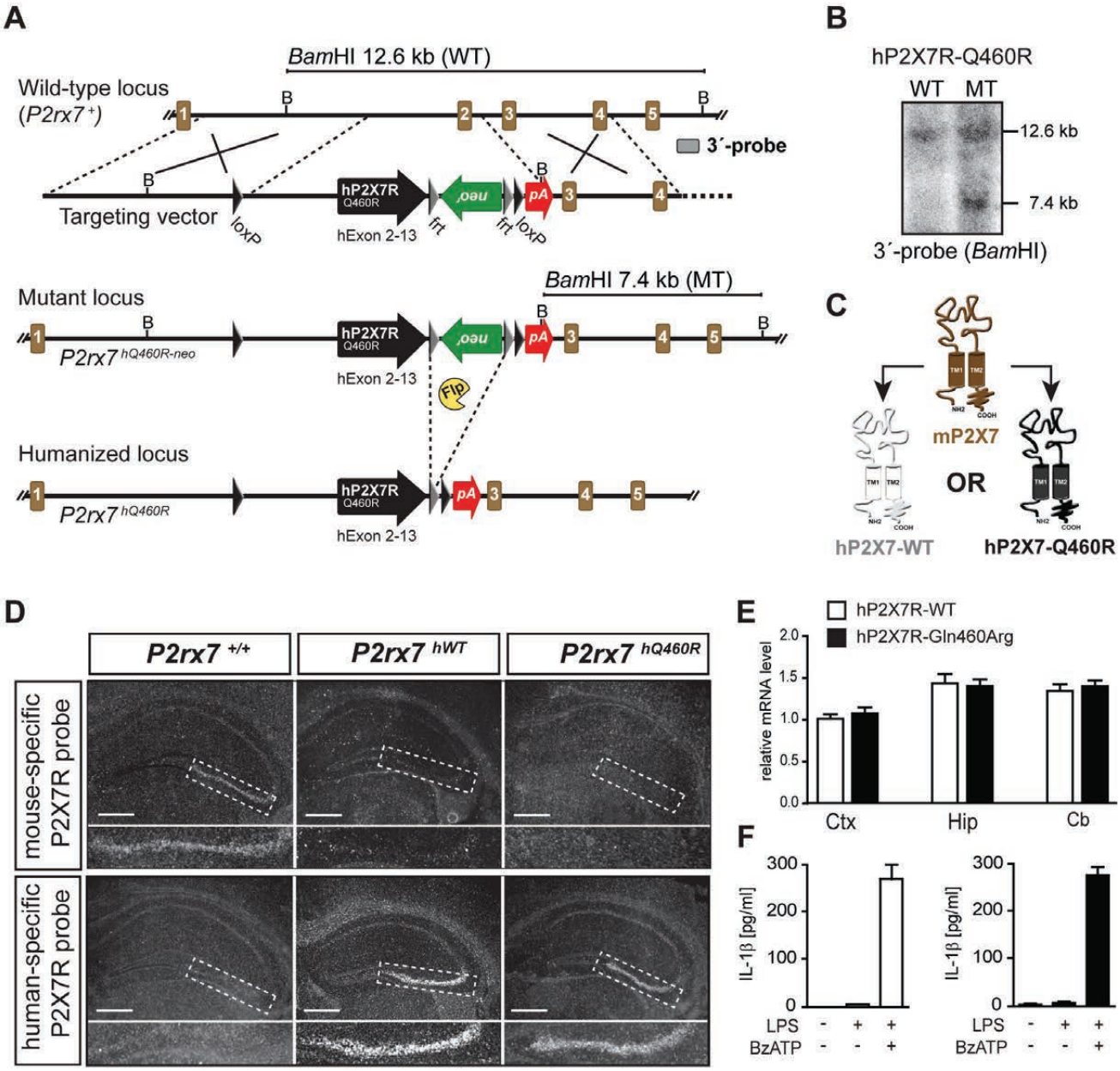
Figure 4. Heterozygosity alters sleep parameters in humans. ***A***, Electroencephalogram (EEG) power spectra (log-transformed) during non-rapid eye movement sleep (NREMS) between homozygous (A/A) and heterozygous (A/G) healthy participants. EEG power density in the

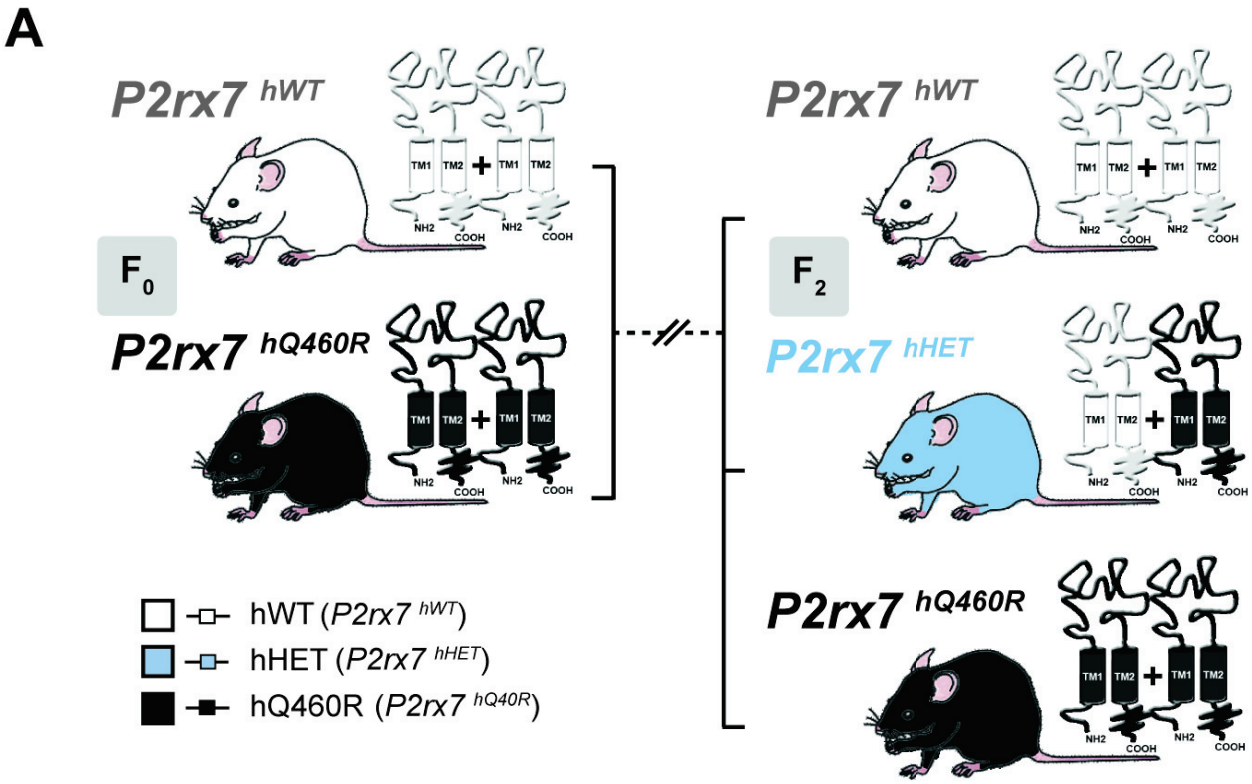
heterozygous genotype was significantly higher in the 13, 25 and 26 Hz bin (2-way mixed ANOVA with the between-subject factor genotype and the within-subject factor derivation for F3A2, C3A2, P3A2 and O1A2; *$P < 0.05$). ***B***, The genotype effect on power density was most prominent in parietal EEG derivation (P3A2) in 13 Hz bin. ***C***, 13 Hz frequency in human EEG overlaps with sleep spindles activity, whose frequency usually varies between 12 and 15 Hz. Heterozygous A/G carriers showed a significantly lower mean peak frequency of all sleep spindles detected in parietal (P3A2) EEG derivation within NREMS (2-tailed unpaired t-test, **$P = 0.001$).

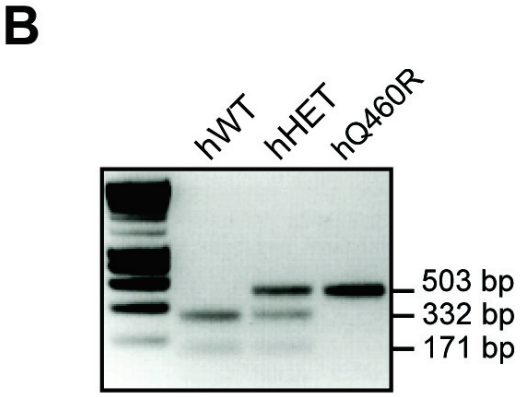
Figure 5. Chronic social defeat stress (CSDS) induces robust physiological and neuroendocrine changes independent of genotype. ***A***, CSDS led to a decrease in fur quality from day four onwards, depicted by an increase in fur state index ***B***, Thymus weights were significantly reduced in stressed animals compared to their littermate controls. ***C,*** An increase in relative adrenal weights was observed in all mice subjected to CSDS ***D***, CSDS led to an enhanced corticosterone response following a forced swim test. Data are expressed as mean ± s.e.m; *$p < 0.05$.

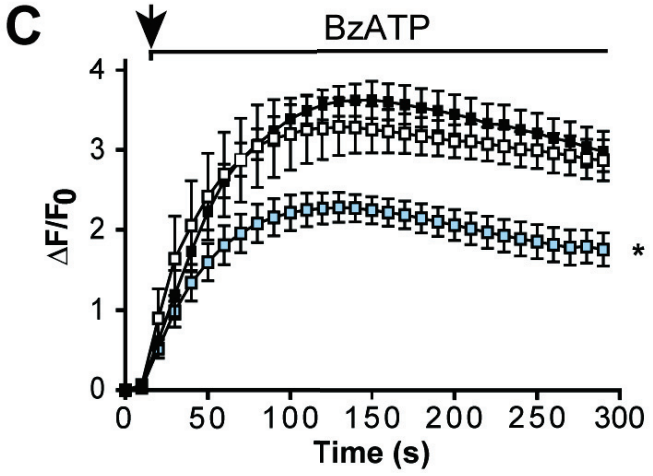
Figure 6. Chronic social defeat stress (CSDS) induces robust behavioral deficits in *hP2RX7* mice. CSDS induced an overall decrease in locomotion in hP2X7R mice in the open field (OF) when assessed for the entire test duration (15 min, ***A***), as well as in the initial 5 min (***B***). Accordingly, immobility in the OF was significantly increased during the first 5 min of the OF test (***C***). ***D***, *hP2RX7* mice showed an enhanced anxiety response to CSDS in the elevated plus maze. ***E***, CSDS evoked a decreased interaction time with the social target in *hP2RX7* mice during the social avoidance paradigm. ***F***, However, *hP2RX7* mice also spent significantly less time interacting with the empty wire cage during the first trial of the social avoidance task. ***G***, During the female urine sniffing test, a decrease in time spent sniffing

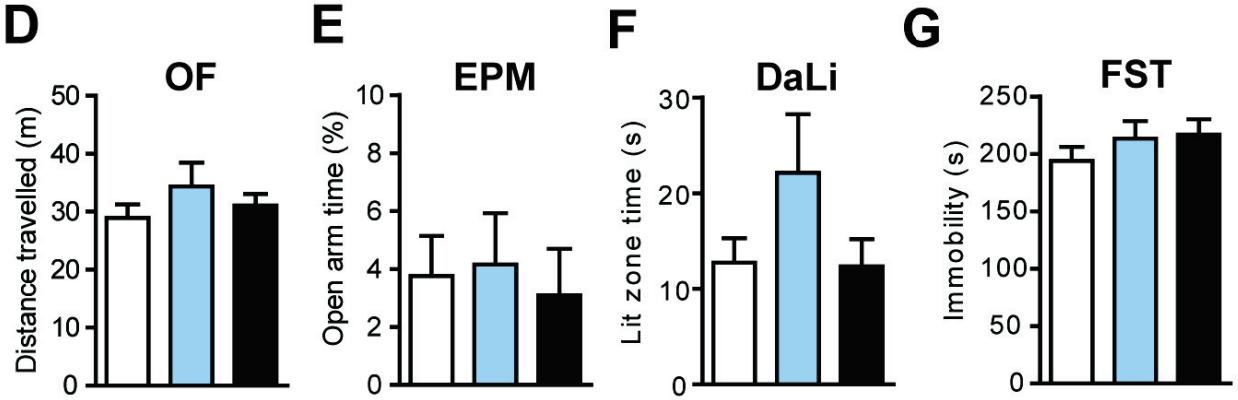
estrus female urine was observed in all stressed *hP2RX7* animals compared to non-stressed controls. ***H***, No significant genotype and/or condition differences were detectable in the water trial of the female urine sniffing test. Data are expressed as mean ± s.e.m. *$p < 0.05$.

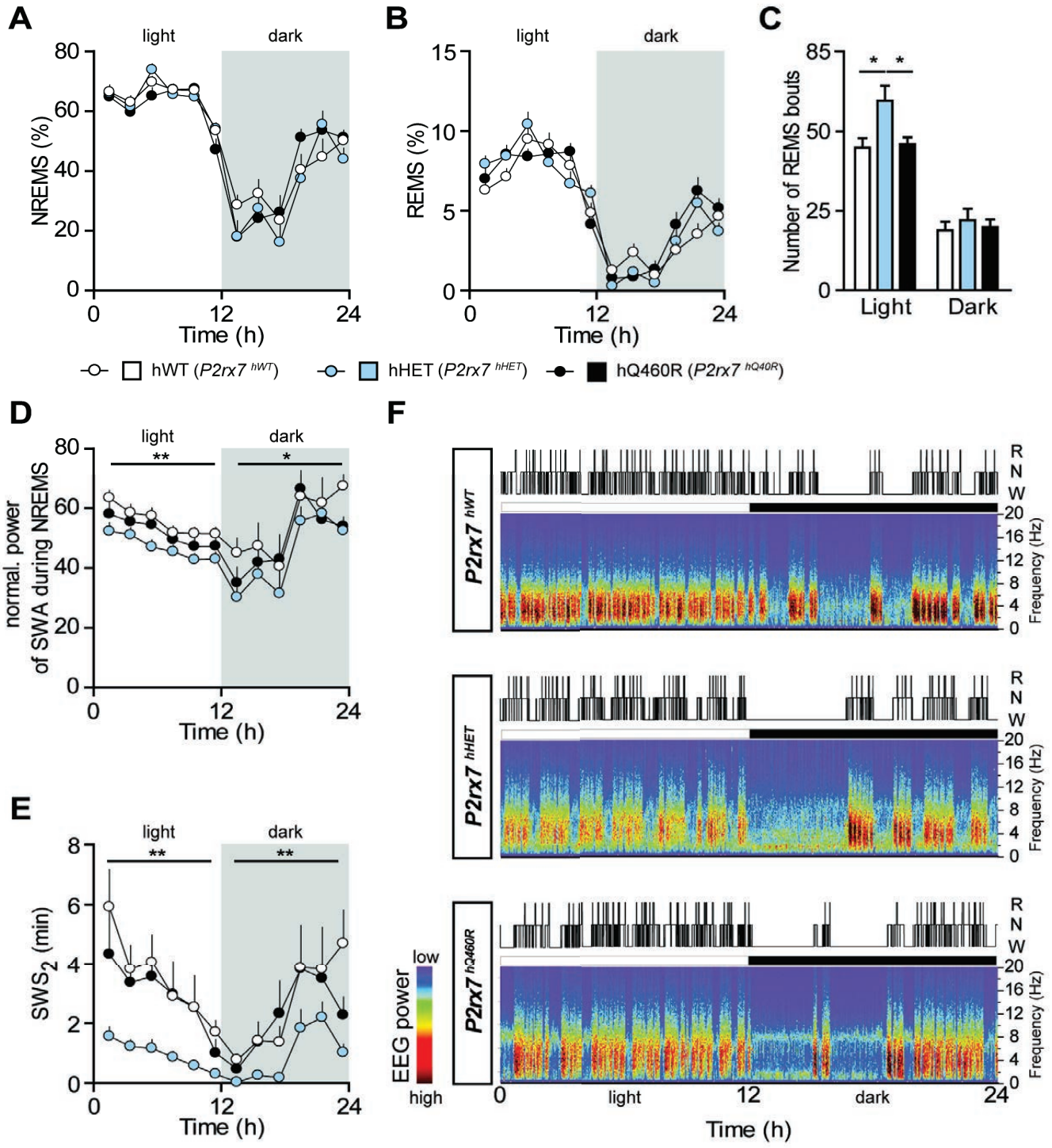


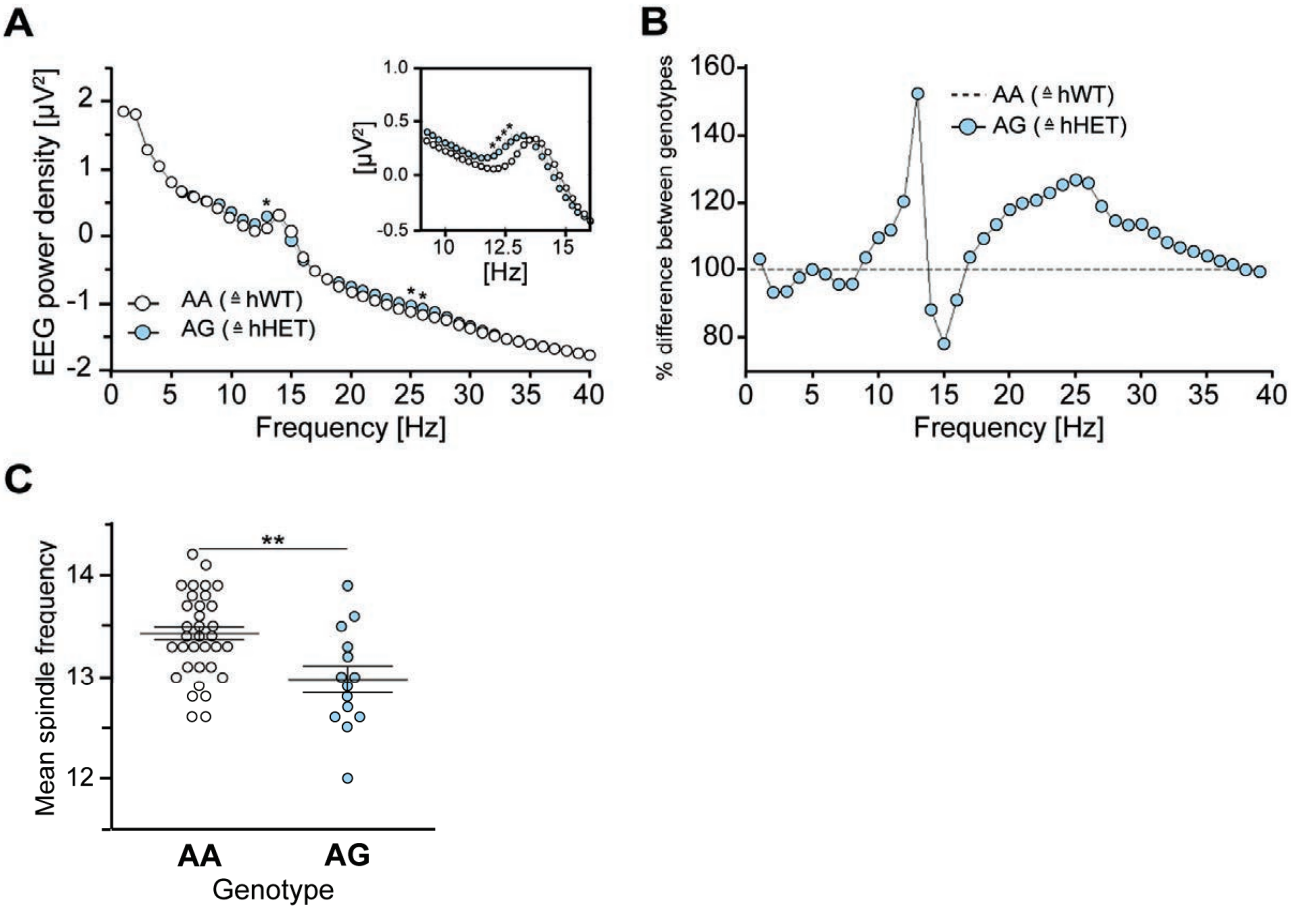


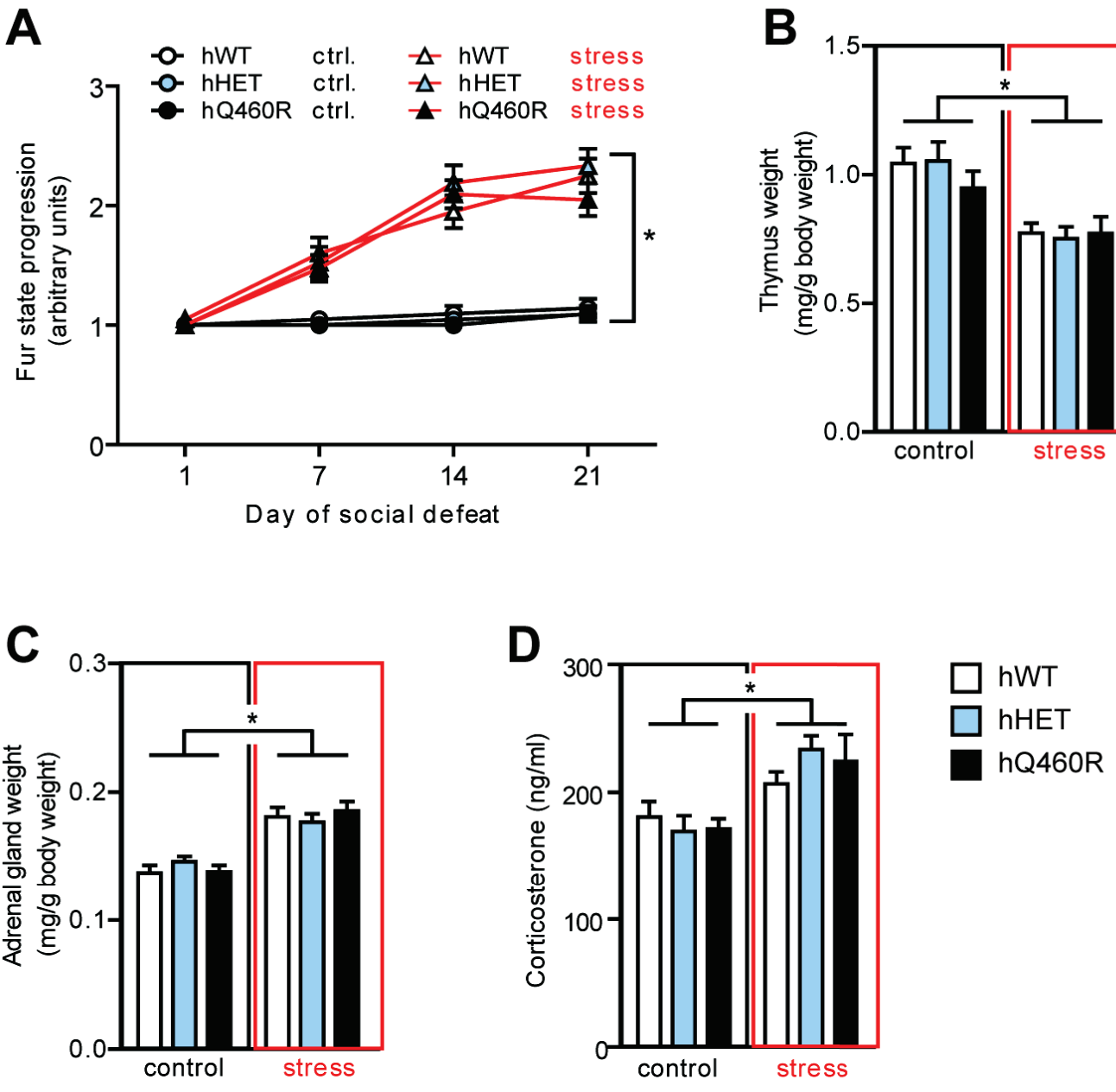












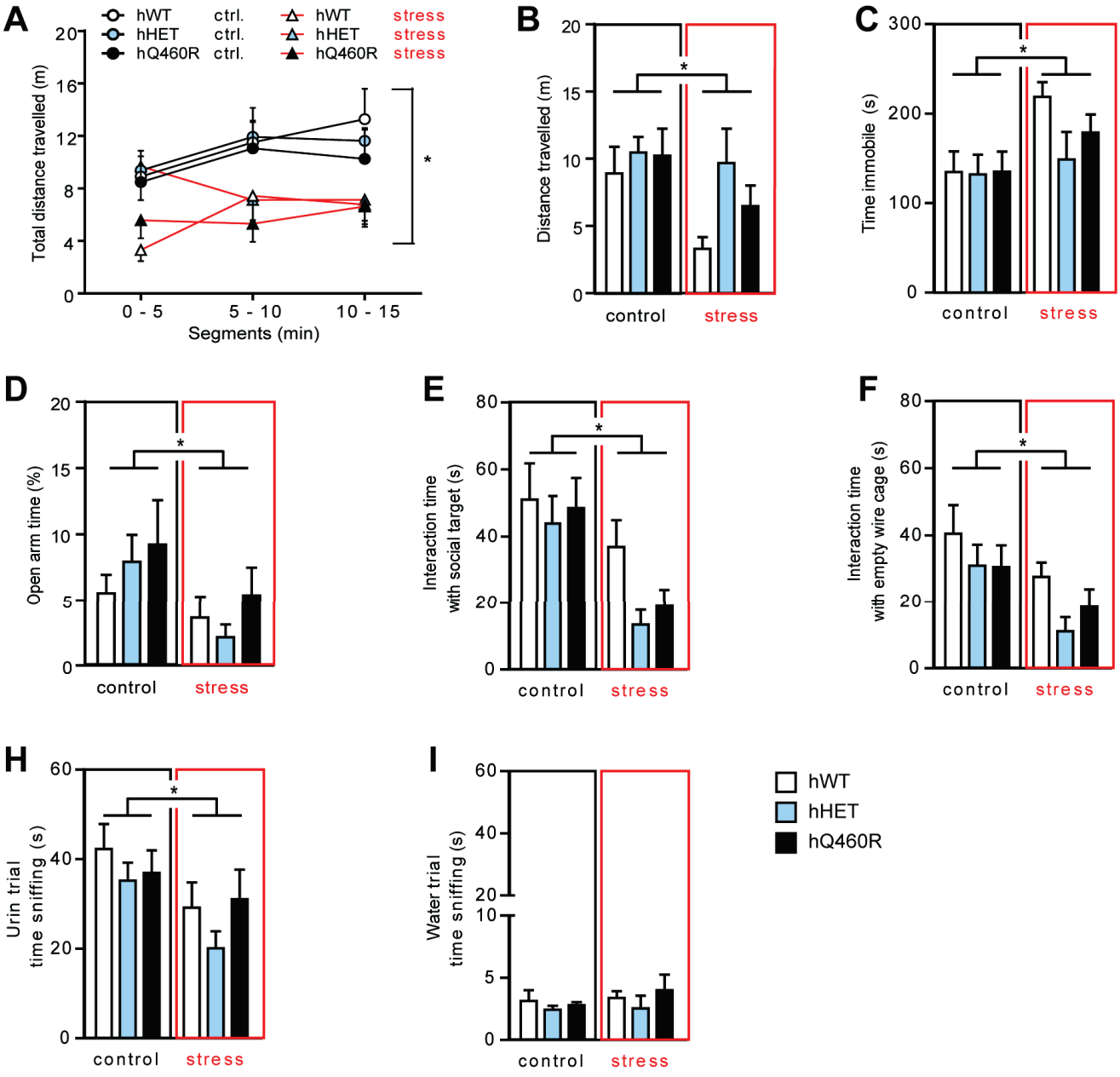


Table 1

Amounts of wakefulness, NREMS and REMS, normalized SWA and SWS_2 across the light-dark cycle in *P2rx7*^{hWT}, *P2rx7*^{hHET} and *P2rx7*^{hQ460R} mice.

	Light					Dark				
	Wake (%)	NREMS (%)	REMS (%)	SWA	SWS_2 (min)	Wake (%)	NREMS (%)	REMS (%)	SWA	SWS_2 (min)
hWT	27.83 ± 2.89	64.24 ± 2.36	7.93 ± 0.62	60.19 ± 1.59	3.49 ± 0.43	63.96 ± 5.04	33.09 ± 4.34	2.38 ± 0.59	60.88 ± 3.29	2.66 ± 0.48
hHET	30.61 ± 2.93	61.83 ± 2.44	7.56 ± 0.55	46.92 ± 1.14	0.95 ± 0.13	59.02 ± 5.64	37.35 ± 4.93	3.10 ± 0.70	44.30 ± 3.74	0.92 ± 0.25
hQ460R	28.13 ± 2.66	64.41 ± 2.21	7.46 ± 0.53	51.98 ± 1.40	2.96 ± 0.34	60.38 ± 3.69	36.62 ± 3.18	2.57 ± 0.45	49.37 ± 3.34	2.29 ± 0.39
P-value	0.7506	0.6871	0.8299	**< 0.0001**	**< 0.0001**	0.7609	0.7479	0.6780	**0.0059**	**0.0076**

Data are mean values ± s.e.m.; n = 9, *P2rx7*^{hWT} mice (hWT), n = 11, *P2rx7*^{hHET} (hHET) and *P2rx7*^{hQ460R} (hQ460R) mice. Comparisons of the 12-h intervals of the light and the dark period between genotypes were performed by one-way ANOVA factor 'genotype', followed by post hoc Bonferroni's test. *P*-values in bold type indicate statistical significance.

Table 2

Average numbers and durations of wakefulness, non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS) bouts under the baseline conditions in *P2rx7*^{hWT}, *P2rx7*^{hHET} and *P2rx7*^{hQ460R} mice.

	Mean duration (min)			*P*-value	Number of bouts			*P*-value
	hWT	hHET	hQ460R		hWT	hHET	hQ460R	
Light								
Wake	1.03 ± 0.11	0.95 ± 0.11	1.17 ± 0.07	0.2556	195.2 ± 14.94	210.7 ± 14.87	188.6 ± 6.50	0.4137
NREMS	2.31 ± 0.19	2.08 ± 0.12	2.28 ± 0.08	0.3839	201.3 ± 14.86	221.7 ± 15.24	195.5 ± 6.4	0.2907
REMS	1.19 ± 0.05	0.98 ± 0.06	1.18 ± 0.05	**0.0157**	44.89 ± 2.94	59.64 ± 4.66	46.00 ± 2.14	**0.0086**
Dark								
Wake	3.16 ± 1.08	3.40 ± 0.71	3.18 ± 0.50	0.9662	140.0 ± 16.81	135.3 ± 17.31	137.7 ± 13.11	0.9622
NREMS	1.81 ± 0.20	1.71 ± 0.19	1.99 ± 0.14	0.4909	141.2 ± 17.03	138.5 ± 17.69	134.8 ± 12.96	0.9622
REMS	1.03 ± 0.08	0.84 ± 0.07	1.12 ± 0.08	**0.0347**	18.88 ± 2.71	22.0 ± 3.69	19.91 ± 2.13	0.7673

Data are mean values ± s.e.m.; n = 9, *P2rx7*^{hWT} (hWT) mice, n = 11, *P2rx7*^{hHET} (hHET) and *P2rx7*^{hQ460R} (hQ460R) mice. Comparisons of the 12-h intervals of the light and the dark period between genotypes were performed by one-way ANOVA factor 'genotype', followed by post hoc Bonferroni's test. *P*-values in bold type indicate statistical significance

Table 3

Sleep continuity, sleep architecture and sleep phase stability

	A/A (n = 39)	A/G (n = 14)	*P**
Sleep continuity			
Total sleep time	402.73 ± 5.19	382.29 ± 11.92	0.07
Sleep period time	464.96 ± 2.59	449.79 ± 8.56	**0.04**
Sleep efficiency (%)**	86 ± 1	82 ± 2	0.11
Sleep onset latency	15.15 ± 2.18	29.75 ± 8.55	**0.05**
All night sleep architecture			
NREM sleep	311.00 ± 4.58	291.89 ± 10.20	NS
Slow wave sleep	88.22 ± 4.67	87.29 ± 8.84	
Slow wave sleep latency	12.72 ± 1.21	11.89 ± 1.31	
Stage 1 sleep	33.91 ± 3.11	35.14 ± 3.73	
Stage 2 sleep	222.78 ± 5.09	204.61 ± 8.77	
Wakefulness	25.40 ± 3.43	28.04 ± 6.43	
REM sleep	90.50 ± 3.87	89.25 ± 6.46	
REM latency	82.00 ± 6.51	94.50 ± 14.91	
1st sleep cycle architecture			
NREM sleep	73.42 ± 5.50	78.32 ± 7.68	NS
Slow wave sleep	40.51 ± 3.35	39.61 ± 4.90	
Stage 1 sleep	3.14 ± 0.46	7.82 ± 2.86	
Stage 2 sleep	32.91 ± 3.11	38.71 ± 4.68	
Wakefulness	2.21 ± 0.60	7.18 ± 4.41	
REM sleep	11.19 ± 1.12	14.68 ± 3.90	
All night sleep phase stability			
# of entries into REM sleep	13.87 ± 0.66	13.36 ± 1.90	NS
# of entries into NREM sleep	25.62 ± 1.37	28.64 ± 2.75	
Switches from stage 2 into stage 1/wake	16.41 ± 1.28	21.07 ± 2.69	
Switches from SWS into stage 1/wake	2.74 ± 0.35	2.50 ± 0.45	
1st sleep cycle sleep phase stability			
# of entries into REM sleep	1.95 ± 0.16	2.50 ± 0.42	0.21
# of entries into NREM sleep	3.41 ± 0.41	6.71 ± 1.37	**0.01**
Switches from stage 2 into stage 1/wake	2.05 ± 0.34	5.14 ± 1.26	**0.01**
Switches from SWS into stage 1/wake	1.05 ± 0.18	1.29 ± 0.27	0.29

Group mean ± s.e.m. of sleep characteristics in minutes. Multivariate analysis of variance (MANOVA) revealed marginal effect of genotype on sleep continuity, which tended to be worse in subjects with A/G genotype ($F_{4,48}$ = 2.388, P = 0.06). MANOVA did not detect genotype differences in sleep architecture in the whole night as well as during the first sleep cycle. Also, MANOVA revealed significant lower sleep phase stability in subjects with A/G genotype in the first sleep cycle ($F_{2,50}$ = 2.361, P = 0.047). *In case of significant MANOVA outcome, 2-tailed unpaired post hoc t-tests; **Sleep efficiency is the ratio of sleep to time spent in bed showed as percentage. REM: rapid eye movement, NREM: non-rapid eye movement, SWS: slow-wave sleep.