SI Appendix

Methods and Materials

Animals

All animals were stress- and drug-naïve at the start of testing. Mice were randomly assigned to treatment groups in all experiments. Mice acclimatized to the new environment for at least seven days prior to the start of experiments. All experimental procedures were approved by the University of Maryland, Baltimore Animal Care and Use Committee and were conducted in full accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and reported according to *ARRIVE* guidelines. Mice were used in this study, as intact circuits are required to assess the *in vivo* neurobiological mechanisms of antidepressant drug pharmacokinetics and efficacy.

Drugs

(*R,S*)-ketamine HCl (#K2753) was purchased from Sigma-Aldrich (MO, USA). (+)-MK-801 hydrogen maleate was obtained from the National Institute of Mental Health Chemical Synthesis and Drug Supply Program, USA. LY341495 disodium salt, LY379268 disodium salt, Ro 25-6981 maleate and (±)-CPP were purchased from Tocris (MN, USA). 6,6-dideuteroketamine hydrochloride ((*R,S*)-d_2-KET) and (*2R,6R*)-HNK hydrochloride were synthesized and characterized internally at the National Center for Advancing Translational Sciences (MD, USA). Absolute and relative stereochemistry for (*2R,6R*)-HNK was confirmed by small molecule x-ray crystallography (1, 2). All drugs were dissolved in 0.9% saline and administered i.p. in a volume of 7.5 ml/kg of body mass by a male experimenter.

$mGlu_{2/3}$ receptor agonist-induced hyperthermia

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Mice were transferred to an empty freshly-made home cage, containing only corn-cob bedding, and were allowed to habituate to the cage for a period of 30 min. Baseline body temperature was then measured via a rectal probe (Thermalert TH-5, Physitemp, NJ, USA). 10 min later, mice received a pre-treatment injection (i.e., vehicle; LY341495, 3 mg/kg; ketamine, 1, 3 and 10 mg/kg, (*2R,6R*)-HNK; 1, 3 and 10 mg/kg, (*R,S*)-d_2-ketamine; 3, 10 and 30 mg/kg, (+)-MK-801; 0.01, 0.03 and 0.1 mg/kg; Ro 25-6981; 10 mg/kg; (±)-CPP; 0.05 mg/kg) and returned to their individual cages. Thirty minutes later, mice received treatment (LY379268; 3 mg/kg) and were returned to the cage for another 30 min. Final rectal temperature of the mice was measured after a further 30-min period.

For CPP, the dose of 0.5 mg/kg was based on reports that this dose exerts acute (but not sustained) antidepressant-like behavioral actions in the novelty-suppressed feeding (3) and in the forced-swim test (3, 4) in mice. In addition, this dose of CPP was shown to impair learning and memory in the one-trial inhibitory avoidance test in mice (5), similar to the actions of other NMDAR antagonists including ketamine (6) and MK-801 (5).

For Ro 25-6981, the dose of 10 mg/kg was based on reports that this dose exerts antidepressant-relevant responses in rodents (7-10). At 10 mg/kg, Ro 25-6981 also induces hyperlocomotion (8), indicative of sufficient NMDAR inhibition (11).

For MK-801, the doses were based on reports of acute antidepressant-relevant actions of this NMDAR channel blocker in the forced-swim test (4). In addition, the highest dose of MK-801 we used (0.1 mg/kg) has been shown to induce NMDAR inhibition-induced hyperlocomotion, disruption of pre-pulse inhibition, as well as cognitive deficits in mice (e.g. 12, 13).

Forced-swim test (FST)

During the FST, mice were subjected to a 6-min swim session in clear Plexiglass cylinders (30 cm height x 20 cm diameter) filled with 15 cm of water (23 ± 1°C). The FST was performed in normal light conditions (800 Lux). Sessions were recorded using a digital video camera. Immobility time, defined as passive floating with no additional activity other than that necessary to keep the animal's head above water, was scored during the last 4 min of the 6-min test by a trained observer blind to the treatment groups. To assess interactions, LY379268 or vehicle was either 1) administered 10 minutes prior to ketamine, (*2R,6R*)-HNK, or vehicle with mice tested 1 hour and 24 hours later or 2) administered 4 hours after (*2R,6R*)-HNK or vehicle with mice tested 24 hours after (*2R,6R*)-HNK treatment. For the sub-effective dose treatment experiments, LY341495 and (*2R,6R*)-HNK were administered at the same time.

Inescapable shock-induced escape deficits

The inescapable shock-induced escape deficits (or learned helplessness) paradigm consisted of three different phases: inescapable shock training, escapable shock screening, and the escapable shock test. On Day 1, the animals were placed in one side of two-chambered shuttle boxes (34 cm height x 37 cm width x 18 cm depth; Coulbourn Instruments, PA, USA), with the door between the chambers closed. Following a 5-min adaptation period, 120 inescapable foot-shocks (0.45 mA, 15 sec duration, pseudo-randomized average inter-shock interval of 45 sec) were delivered through the floor. During the escapable shock screening session (Day 2), the mice were placed in one of the two chambers of the apparatus for 5 min. A shock (0.45 mA) was then delivered, and the door between the two chambers was raised simultaneously. Crossing over into the second chamber terminated the shock. If the animal did not cross over, the shock terminated after 3 sec. A total of 30 screening trials of escapable shocks were presented to each mouse with an average of 30-sec delay between each trial. Mice that developed escape deficit behavior (>5 escape failures during

the last 10 screening shocks) received the assigned drug in a randomized blinded manner 24 hours following screening (Day 3). For the experiments assessing the effect of the $mGlu_{2/3}$ receptor agonist (LY379268) on the actions of ketamine or (*2R,6R*)-HNK, pre-treatment with LY379268 preceded treatment by 10 min. During the escapable shock test phase (Day 4), the animals were placed in the shuttle boxes and, after a 5-min adaptation period, a 0.45 mA shock was delivered concomitantly with door opening for the first five trials, followed by a 2-sec delay (first shock, then door opening) for the next 40 trials. Crossing over to the second chamber terminated the shock. If the animal did not cross over to the other chamber, the shock was terminated after 24 sec. A total of 45 trials of escapable shocks were presented to each mouse with 30-sec inter-trial intervals. The number of escape failures was recorded for each mouse by computer software (Graphic State *v*3.1; Coulbourn Instruments, Whitehall, PA, USA).

Chronic social defeat stress and sucrose preference

Male C57BL/6J non-stressed control mice were maintained grouped-housed in their home cages for 10 days, concurrently with the chronic social defeat stress paradigm. Socially defeated mice underwent a 10-day chronic social defeat stress paradigm, as described elsewhere (14). Briefly, experimental mice were introduced to the home cage (43 cm length x 11 cm width x 20 cm height) of a resident aggressive retired CD-1 breeder - prescreened for aggressive behaviors - for 10 min. Following this physical attack phase, mice were transferred and housed in the opposite side of the resident's cage divided by a perforated Plexiglas divider, in order to maintain continuous, 24 h, sensory contact. This process was repeated daily for 10 days, with experimental mice being introduced to a novel aggressive CD-1 mouse each day. Following day 10, for assessing the "baseline" post-defeat sucrose preference, mice were singly housed and presented with two identical bottles containing either tap water or 1% (w/v) sucrose solution. Control mice were also

singly housed and assessed for sucrose preference. Twenty-four hours later, sucrose preference was measured and the mice that underwent social defeat stress were assigned to two groups: resilient (sucrose preference >70%) and susceptible (sucrose preference <55%). These mice, along with the non-stressed controls, were pre-treated with saline or LY379268 (3 mg/kg) and 10 min later treated with saline or (*2R,6R*)-HNK. Sucrose preference was measured for 4 days (i.e., 3 days post-treatment).

Quantitative cortical electroencephalogram (qEEG)

Surgery. Mice were anaesthetized with isoflurane (3.5%) and maintained under anesthesia (2-2.5%) throughout the surgery. Mice received analgesia (carprofen, 5 mg/kg, i.p.) prior to the start of surgery. A TA11ETA-F10 radio-telemetric transmitter (Data Sciences International, Minneapolis, MN, USA) was inserted subcutaneously and its leads were implanted over the dura above the frontal cortex (1.7 mm anterior to bregma and 1.5 mm mediolateral) and the cerebellum (6.4 mm posterior to bregma). Animals were singly housed in the behavioral qEEG room to recover from surgery for at least 7 days prior to recordings.

qEEG recordings. qEEGs were recorded using the Dataquest ART and Ponemah version 6.32 acquisition systems (Data Sciences International, Minneapolis, MN, USA) with frontal cortex qEEG recordings referenced to the cerebellum. Baseline qEEG recordings (30 min) were obtained followed by treatment injections and recordings for another 30 min. For assessing the effects of LY379268 on (*2R,6R*)-HNK-induced changes in qEEG oscillations, following baseline recordings, mice received pre-treatment (saline or LY379268) and 10 min later received saline or (*2R,6R*)-HNK (10 mg/kg) and a further 60 min of post-injection recordings were obtained. To assess the effects of (*2R,6R*)-HNK on qEEG oscillations in $mGlu_2$ and $mGlu_3$ receptor knockout mice, following

baseline (30 min) recordings, mice received a saline injection followed, 30 min later, by (*2R,6R*)-HNK; recordings continued for 60 min post-injection.

qEEG Data Analysis. qEEGs were analyzed using Neuroscore *version* 3.2.9297-1 (DSI, MN, USA). An automated analysis protocol was implemented to mark instances of invalid signal data/artifacts—defined using an amplitude detector set at greater than or equal to an absolute threshold of 0.001 mV, with a minimum duration of 1E-05 seconds, maximum duration of 10 seconds, join interval of 1 second, prepend duration of 0.01 seconds, and append duration of 0 seconds. EEG signals were exported as periodograms with the following parameters: values to 6 decimal places using 10 second epochs, Hamming windows, a Fast Fourier Transform order of 10, 50% overlap, and excluding epochs overlapping invalid data markers. These parameters yielded periodograms with frequency bins of 0.98 Hz.

Spectrograms expressed in log scales indicated as heat maps were created in MATLAB 2018a by generating average periodograms within treatment groups and normalizing over mean treatment group baseline values for each frequency bin. Data for line graphs displaying average power changes across frequencies were calculated in MATLAB by dividing power values averaged across 30 min post-treatment by power values averaged across the baseline for each animal in each frequency bin, and graphs with S.E.M. were generated using GraphPad Prism *version* 6. For line graphs displaying normalized power changes over time, oscillation power in each bandwidth (delta=1–3 Hz; theta=4–7 Hz; alpha=8–12 Hz; beta=13–29 Hz; gamma=30–80 Hz) was computed in Neuroscore, averaged into 10-min bins and divided by average baseline power values in respective bandwidths for each animal. Gamma power 1-sec representative traces during baseline and post-drug administration were acquired from Neuroscore after applying a band pass filter from 30 to 80 Hz.

Tissue distribution and clearance measurements of (*2R,6R*)-hydroxynorketamine.

For the LY379268 pre-treatment study, mice were given injections of LY379268 (3 mg/kg) and then an injection of (*2R,6R*)-HNK. For the WT vs *Grm2* $^{-/-}$ study mice were given a single injection of (*2R,6R*)-HNK. At 10, 30, or 60 minutes following (*2R,6R*)-HNK administration male mice were deeply anesthetized under 3% isoflurane and subsequently decapitated. Whole brains were immediately collected, rinsed with phosphate-buffered saline, frozen in dry ice and stored at −80°C until analysis.

The concentrations of (*2R,6R*)-HNK in brain tissue were determined by achiral liquid chromatography-tandem mass spectrometry. The analysis was accomplished using an Eclipse XDB-C18 guard column (4.6 mm × 12.5 mm) and a Varian Pursuit XRs 5 C18 analytical column (250 mm × 4.0 mm ID, 5 μm; Varian, Palo Alto, CA, USA). The mobile phase consisted of ammonium acetate (5 mM, pH 7.6) as component A and acetonitrile as component B. A linear gradient was run as follows: 0 min 20% B; 5 min 20% B; 15 min 80% B; 20 min 20% B at a flow rate of 0.4 ml/min. The total run time was 30 min per sample.

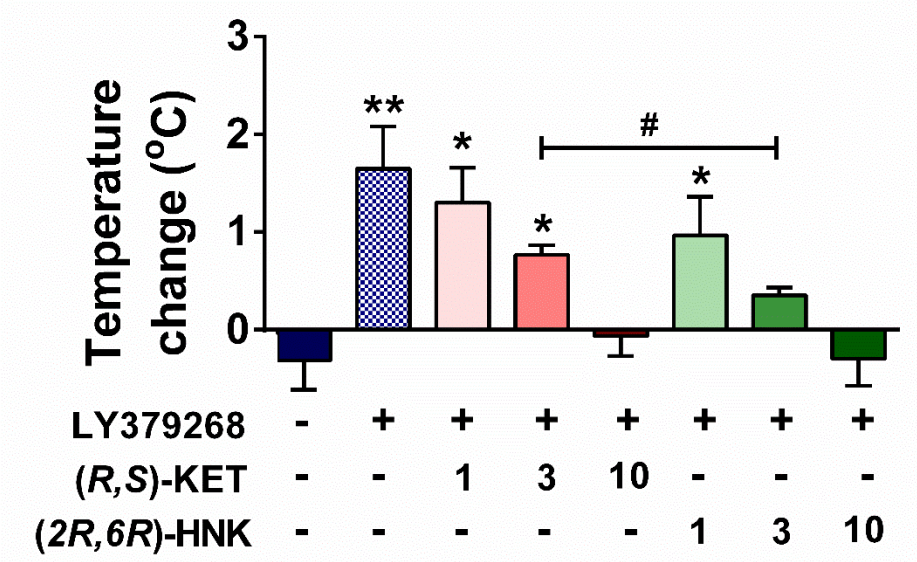
Brains were weighed and suspended in 990 μl of methanol:water (3:2, v/v). Then d4-ketamine (10 μl of 10 μg/ml) was added, and the resulting mixture was homogenized on ice with a polytron homogenizer and centrifuged at 21,000 x g for 30 min. The supernatant was collected and processed using 1-ml Oasis HLB solid phase extraction cartridges (Waters Corp., Waltham, MA). The cartridges were preconditioned with 1 ml of methanol, followed by 1 ml of water and then 1 ml ammonium acetate (10 mM, pH 9.5). The supernatants were added to the cartridges, followed by 1 ml of water, and the compounds were eluted with 1 ml of methanol. The eluent was transferred to an autosampler vial for analysis. Quality control standards were prepared at 200 ng/ml, and 500 ng/ml spiked in the corresponding matrix.

MS/MS analysis was performed using a triple quadrupole mass spectrometer model API 4000 system from Applied Biosystems/MDS Sciex equipped with Turbo Ion Spray® (TIS) (Applied Biosystems, Foster City, CA, USA). The data was acquired and analyzed using Analyst version 1.4.2 (Applied Biosystems). Positive electrospray ionization data were acquired using multiple reaction monitoring (MRM); transition 240 → 125. The TIS instrumental source settings for temperature, curtain gas, ion source gas 1 (nebulizer), ion source gas 2 (turbo ion spray) and ion spray voltage were 600°C, 25 psi, 60 psi, 60 psi and 5500 V, respectively.

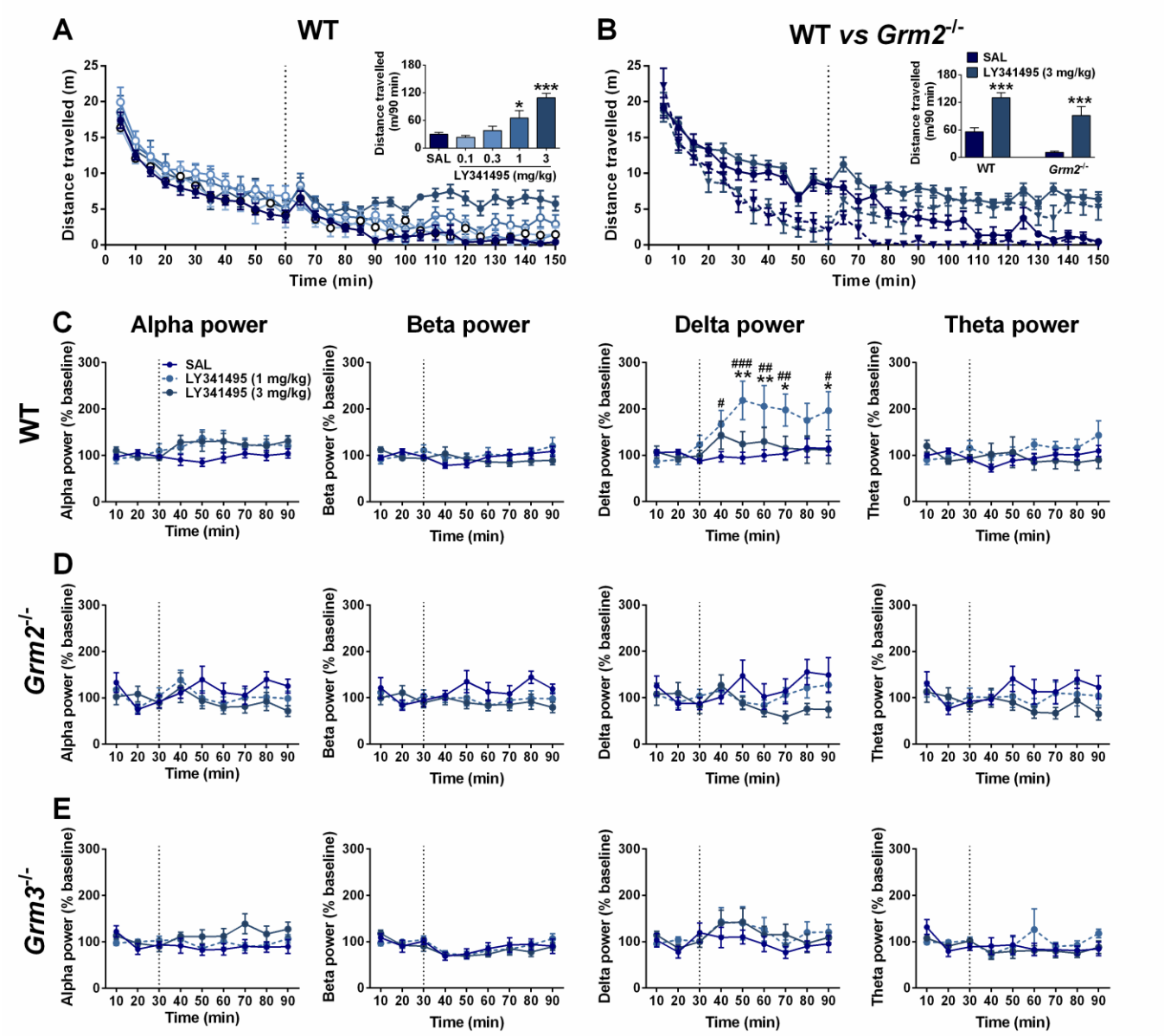
Statistical Analysis

Required sample sizes were estimated based upon our past experience performing similar experiments. For the behavioral studies a minimum of n=6/group was used for each experiment. For the qEEG measurements, each treatment group is comprised of at least 5 mice. Experimentation and analysis were performed in a manner blind to treatment assignments. Statistical outliers were determined and removed from the dataset using the ROUT method (15) (GraphPad Prism *version* 6; parameter used: Q = 1%).

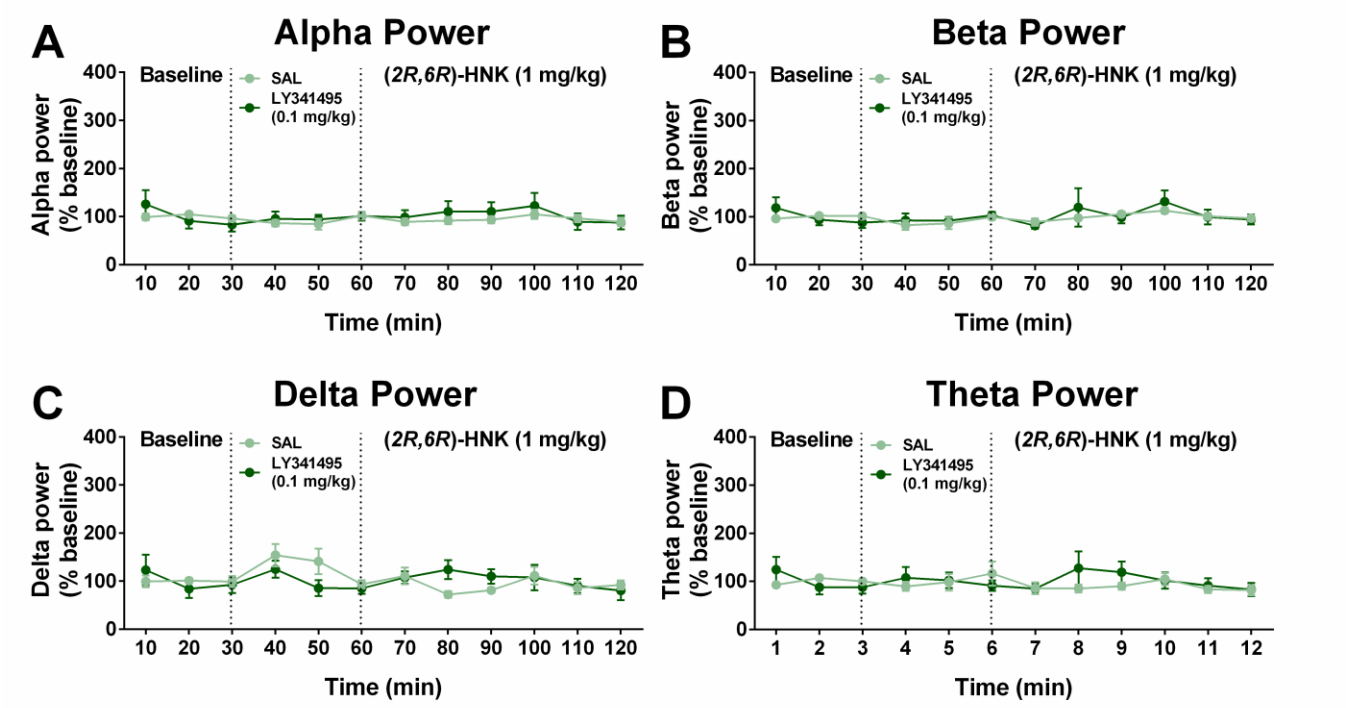
Figures



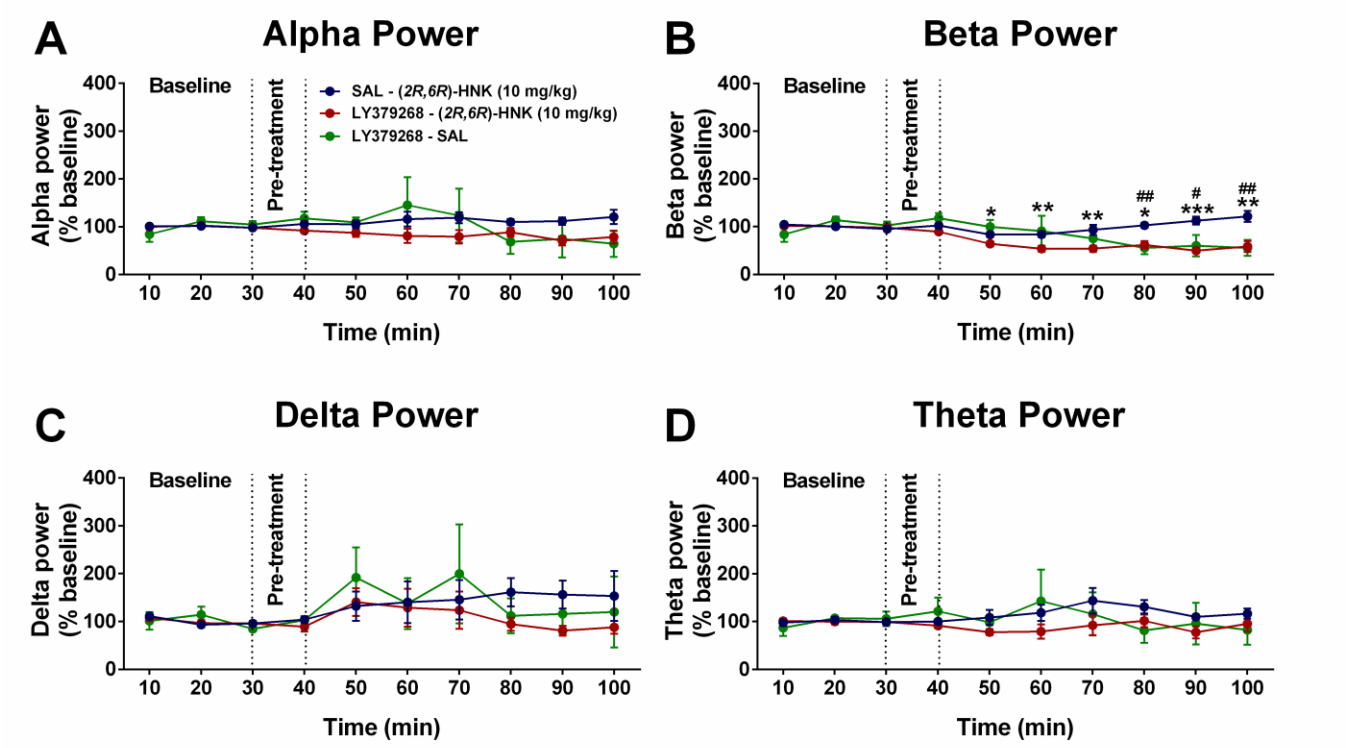
SI Appendix Fig. S1 (Related to Fig. 1): (*2R,6R*)-hydroxynorketamine is more potent than (*R,S*)-ketamine in preventing mGlu$_{2/3}$ receptor agonist-induced hyperthermia in mice. While both (*2R,6R*)-hydroxynorketamine (HNK) and (*R,S*)-ketamine (KET) prevented mGlu$_{2/3}$ receptor agonist (LY379268; 3 mg/kg)-induced hyperthermia in mice, (*2R,6R*)-HNK was more potent to induce this effect at the dose of 3 mg/kg. Data are the mean ± S.E.M. * $p<0.05$; ** $p<0.01$; vs control; ## $p<0.01$ *vs* (*2R,6R*)-HNK (3 mg/kg). See *SI Appendix, Table S1* for statistical analyses and *n* numbers.



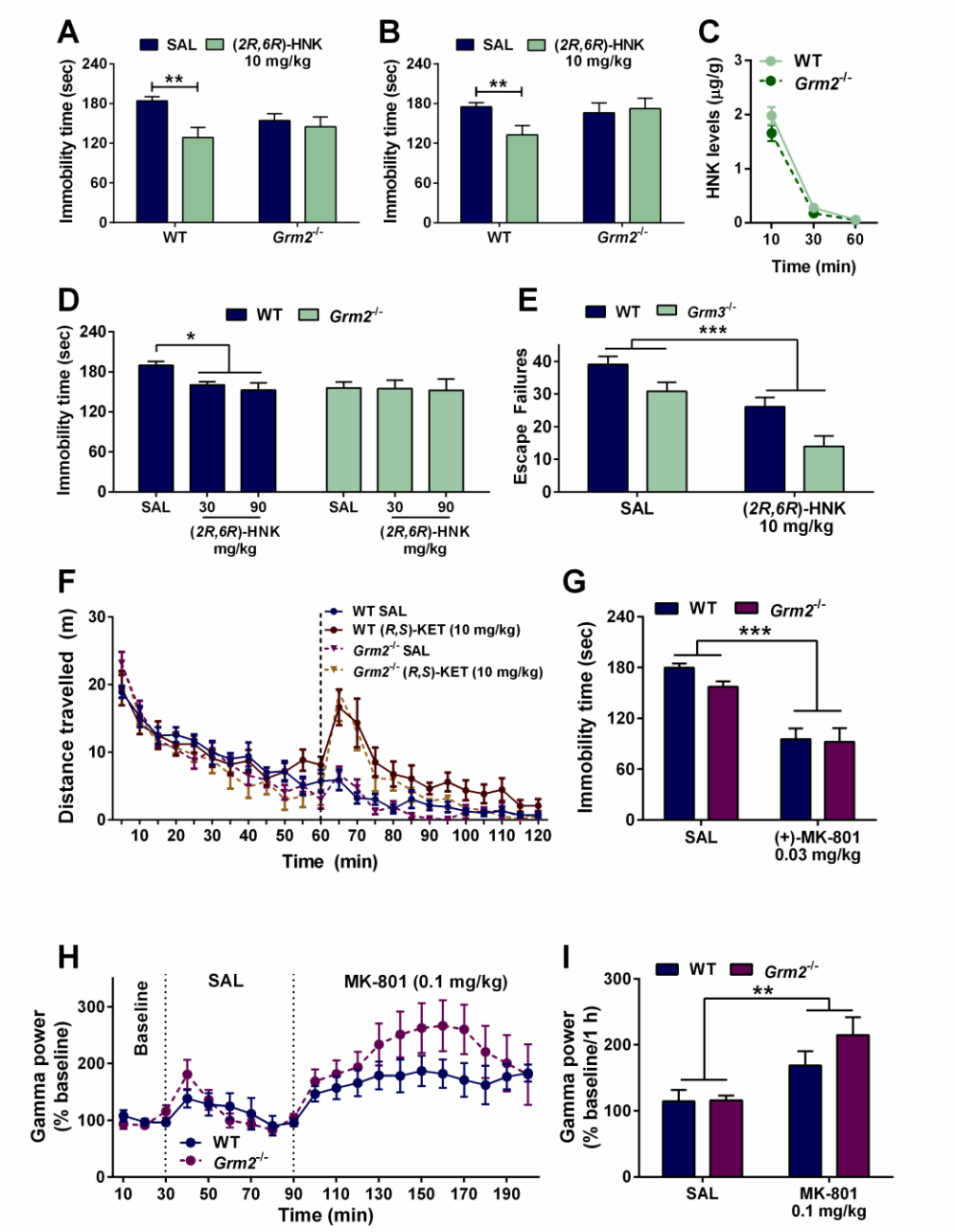
SI Appendix, Fig. S2 (Related to Fig. 2): LY341495 administration induces similar changes in locomotion and low frequency cortical qEEG oscillations in WT, *Grm2*$^{-/-}$ and *Grm3*$^{-/-}$ mice. (A) Following a 60 min baseline period, an injection of LY341495 induced hyperlocomotion at the doses of 1 and 3 mg/kg in wild-type (WT) mice. **(B)** Similar to the WT mice, administration of LY341495 (3 mg/kg) induced a significant hyperlocomotor effect in *Grm2* $^{-/-}$ mice. **(C)** In WT mice, administration of LY341495 at 1 mg/kg induced an increase in delta qEEG power, whereas 3 mg/kg did not induce any changes in alpha, beta or theta oscillations. **(D-E)** Administration of LY341495 (1 or 3 mg/kg) to *Grm2* $^{-/-}$ and *Grm3* $^{-/-}$ mice did not result in changes in alpha, beta, delta, or theta qEEG power. Data are the mean ± S.E.M. * $p<0.05$; ***$p<0.001$ *vs* controls. Dashed lines denote injection times. For panel C (delta power), * $p<0.05$, ** $p<0.01$, *vs* 30 min baseline time point for LY341495 (1 mg/kg); # $p<0.05$, ## $p<0.01$, ### $p<0.001$ *vs* SAL. See *SI Appendix, Table S1* for statistical analyses and *n* numbers.



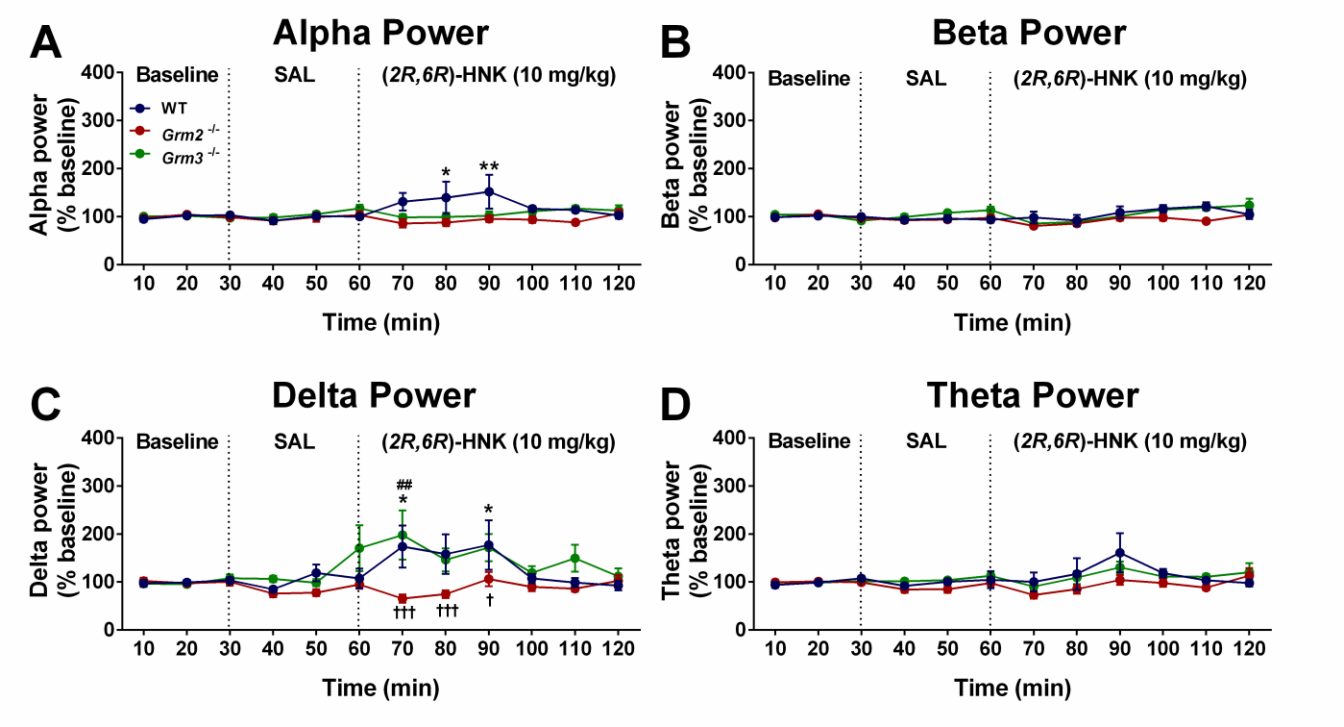
SI Appendix, Fig. S3 (Related to Fig. 3): Effects of sub-effective doses of the $mGlu_{2/3}$ receptor antagonist LY341495 and (*2R,6R*)-hydroxynorketamine on < 30Hz cortical qEEG oscillations. Administration of sub-effective doses of LY341495 (0.1 mg/kg) and (*2R,6R*)-hydroxynorketamine (HNK) (1 mg/kg), did not induce any changes on **(A)** alpha, **(B)** beta, **(C)** delta or **(D)** theta qEEG oscillations in mice. Data are the mean ± S.E.M. See *SI Appendix, Table S1* for statistical analyses and *n* numbers.



SI Appendix, Fig. S4 (Related to Fig. 4): Effects of mGlu$_{2/3}$ receptor activation prior to (*2R,6R*)-hydroxynorketamine treatment on < 30Hz cortical qEEG oscillations. Pre-treatment with saline (SAL) or LY379268 (3 mg/kg), 10 min before administration of (*2R,6R*)-hydroxynorketamine (HNK) (10 mg/kg) did not induce any significant changes on **(A)** alpha, **(C)** delta or **(D)** theta qEEG oscillations in mice. **(B)** Administration of LY379268 10 min prior to (*2R,6R*)-HNK induced a decrease in the power of qEEG oscillations in the beta range compared with baseline. Data are the mean ± S.E.M. * p<0.05; ** p<0.01; *** p<0.001 *vs* 30-min baseline time point for the LY379268 – (*2R,6R*)-HNK group. # p<0.05; ## p<0.01 *vs* 30-min baseline time point for the LY379268 – SAL group. See *SI Appendix, Table S1* for statistical analyses and *n* numbers.



SI Appendix, Fig. S5 (Related to Fig. 5): mGlu$_2$, but not mGlu$_3$ receptor or NMDAR-inhibition- dependent actions of (*2R,6R*)-hydroxynorketamine. Administration of (*2R,6R*)-HNK (10 mg/kg) decreased immobility time in female wild-type (WT), but not *Grm2* (*Grm2*$^{-/-}$) knockout animals **(A)** 1- and **(B)** 24-hours post-injection. **(C)** Administration of (*2R,6R*)-hydroxynorketamine (HNK; 10 mg/kg) to wild-type and *Grm2*$^{-/-}$ mice resulted in equivalent brain HNK levels. **(D)** Administration of higher doses of (*2R,6R*)-HNK (i.e., 30 and 90 mg/kg) induced anti-immobility actions in WT, but not *Grm2*$^{-/-}$ mice in the forced-swim test 1 h after administration. **(E)** Administration of (*2R,6R*)-HNK (10 mg/kg) decreased shuttle escape failures 24 h following inescapable electrical shock in both WT and *Grm3*$^{-/-}$ mice. **(F)** (*R,S*)-ketamine (KET; 10 mg/kg) after a 60 min baseline period induced equipotent hyperlocomotor effects in WT and *Grm2*$^{-/-}$ mice. Administration of the NMDAR antagonist (+)-MK-801 (0.1 mg/kg) **(G)** reduced immobility time in the 1 h forced-swim test and **(H, I)** enhanced cortical qEEG gamma oscillations in mice. Data are the mean ± S.E.M. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Dashed lines denote injection times. See *SI Appendix, Table S1* for statistical analyses and *n* numbers.



SI Appendix, Fig. S6 (Related to Fig. 5): Effect of (*2R,6R*)-hydroxynorketamine administration on < 30Hz qEEG oscillations in wild-type, *Grm2*$^{-/-}$, and *Grm3*$^{-/-}$ mice. Following a 30 min baseline recording and a further 30-min recording after a saline (SAL) injection, (*2R,6R*)-hydroxynorketamine (HNK; 10 mg/kg) administration induced an increase in **(A)** alpha power in wild-type mice and in **(C)** delta power in wild-type (WT) and *Grm3*$^{-/-}$ mice compared with their respective baseline. No other effects were observed in **(B)** beta, or **(D)** theta oscillations. Data are the mean ± S.E.M. * $p<0.05$; ** $p<0.01$ *vs* 30-min baseline time point for WT mice; ## $p<0.01$ *vs* 30-min baseline time point for *Grm3*$^{-/-}$ mice; †$p<0.05$; †††$p<0.001$ between WT and *Grm3*$^{-/-}$ *vs* *Grm2*$^{-/-}$ mice. Dashed lines denote injection times. See *SI Appendix, Table S1* for statistical analyses and *n* numbers.

Table

SI Appendix, Table S1: Relevant statistical analyses

Fig. 1		Sample size (figure order)							
B	LY341495 effect on LY379268-induced hyperthermia	*n = 5 / group*	two-way ANOVA	**Pre-Treatment effect**		**Treatment effect**		**Interaction**	
				$F_{[1, 16]} = 12.81$	$p < 0.01$	$F_{[1, 16]} = 4.32$	$p = 0.05$	$F_{[1, 16]} = 9.01$	$p < 0.01$
C	(*R,S*)-KET effect on LY379268-induced hyperthermia	*n = 6 / group*	two-way ANOVA	$F_{[1, 20]} = 8.00$	$p < 0.05$	$F_{[1, 20]} = 15.46$	$p < 0.001$	$F_{[1, 20]} = 7.24$	$p < 0.05$
D	(+)-MK-801 effect on LY379268-induced hyperthermia	*n = 6,6,6,6,6,5,6,6*	two-way ANOVA	$F_{[3, 39]} = 0.21$	$p = 0.89$	$F_{[1, 39]} = 33.42$	$p < 0.001$	$F_{[3, 39]} = 0.38$	$p = 0.77$
E	Ro 25 and (±)-CPP effect on LY379268-induced hyperthermia	*n = 6 / group*	two-way ANOVA	$F_{[2, 30]} = 3.91$	$p < 0.05$	$F_{[1, 30]} = 42.92$	$p < 0.001$	$F_{[2, 30]} = 8.31$	$p < 0.01$
G	(*R,S*)-d_2-KET effect on LY379268-induced hyperthermia	*n = 6,6,6,6,7,6,6,7*	two-way ANOVA	$F_{[3, 42]} = 0.11$	$p = 0.96$	$F_{[1, 42]} = 34.23$	$p < 0.001$	$F_{[3, 42]} = 1.10$	$p < 0.37$
H	(*2R,6R*)-HNK effect on LY379268-induced hyperthermia	*n = 6,6,5,7*	two-way ANOVA	$F_{[1, 20]} = 8.57$	$p < 0.01$	$F_{[1, 20]} = 14.27$	$p < 0.01$	$F_{[1, 20]} = 7.22$	$p < 0.05$
I	(*2R,6R*)-HNK effect on LY379268-induced hyperthermia								
	• WT mice	*n = 7,6,7,8*	two-way ANOVA	$F_{[1, 23]} = 5.38$	$p < 0.05$	$F_{[1, 23]} = 17.85$	$p < 0.001$	$F_{[1, 23]} = 22.40$	$p < 0.001$
	• *Grm2*$^{-/-}$ mice	*n = 7 / group*	two-way ANOVA	$F_{[1, 24]} = 0.07$	$p = 0.80$	$F_{[1, 24]} = 0.03$	$p = 0.86$	$F_{[1, 24]} = 0.60$	$p = 0.45$
	• *Grm3*$^{-/-}$ mice	*n = 6,7,6,7*	two-way ANOVA	$F_{[1, 22]} = 18.50$	$p < 0.001$	$F_{[1, 22]} = 19.25$	$p < 0.001$	$F_{[1, 22]} = 19.55$	$p < 0.001$
Fig. 2				**Treatment effect**					
A	LY341495 – 1 hour FST	*n = 8/group*	one-way ANOVA	$F_{[4, 35]} = 8.75$	$p < 0.001$				
B	LY341495 – 24 hours FST	*n = 9,9,8,8,8,8*	one-way ANOVA	$F_{[5, 44]} = 4.92$	$p < 0.01$				
F	LY341495 effect on cortical qEEG gamma power			**Treatment effect**		**Time effect**		**Interaction**	
	• WT mice	*n = 10,8,8*	two-way RM ANOVA	$F_{[2, 25]} = 19.48$	$p < 0.001$	$F_{[8, 200]} = 31.56$	$p < 0.001$	$F_{[16, 200]} = 8.57$	$p < 0.001$
	• *Grm2*$^{-/-}$ mice	*n = 9 / group*	two-way RM ANOVA	$F_{[2, 24]} = 0.45$	$p = 0.64$	$F_{[8, 192]} = 10.95$	$p < 0.001$	$F_{[16, 192]} = 0.99$	$p = 0.47$
	• *Grm3*$^{-/-}$ mice	*n = 7,7,6*	two-way RM ANOVA	$F_{[2, 17]} = 6.17$	$p < 0.01$	$F_{[8, 136]} = 9.76$	$p < 0.001$	$F_{[16, 136]} = 3.10$	$p < 0.001$
Fig. 3				**LY341495 effect**		**Treatment effect**		**Interaction**	
A	Sub-effective (*R,S*)-KET_LY341495; 1 hour FST	*n = 9,8,7,9,8,8*	two-way ANOVA	$F_{[1, 43]} = 6.64$	$p < 0.05$	$F_{[2, 43]} = 2.94$	$p = 0.06$	$F_{[2, 43]} = 2.78$	$p = 0.07$
B	Sub-effective (*R,S*)-KET_LY341495; 24 hours FST	*n = 9,8,8,9,8,8*	two-way ANOVA	$F_{[1, 44]} = 6.18$	$p < 0.05$	$F_{[2, 44]} = 0.67$	$p = 0.51$	$F_{[2, 44]} = 2.58$	$p = 0.08$
C	Sub-effective (*2R,6R*)-HNK_LY341495; 1 hour FST	*n = 9,8,9,9*	two-way ANOVA	$F_{[1, 31]} = 8.72$	$p < 0.01$	$F_{[1, 31]} = 6.17$	$p < 0.05$	$F_{[1, 31]} = 0.99$	$p = 0.33$
D	Sub-effective (*2R,6R*)-HNK_LY341495; 24 hours FST	*n = 9,8,9,9*	two-way ANOVA	$F_{[1, 31]} = 6.57$	$p < 0.05$	$F_{[1, 31]} = 5.29$	$p < 0.05$	$F_{[1, 31]} = 5.01$	$p < 0.05$
				Treatment effect		**Time effect**		**Interaction**	
H	Sub-effective (*2R,6R*)-HNK_LY341495; qEEG gamma	*n = 7,6*	two-way RM ANOVA	$F_{[1, 11]} = 9.08$	$p < 0.05$	$F_{[11, 121]} = 12.69$	$p < 0.001$	$F_{[11, 121]} = 7.54$	$p < 0.001$
Fig. 4				**Pre-treatment effect**		**Treatment effect**		**Interaction**	
A	Effect of LY379268 on (*R,S*)-KET 1 hour FST	*n = 9,10,9,9*	two-way ANOVA	$F_{[1, 33]} = 7.65$	$p < 0.01$	$F_{[1, 33]} = 9.55$	$p < 0.01$	$F_{[1, 33]} = 4.04$	$p = 0.05$
B	Effect of LY379268 on (*R,S*)-KET 24 hours FST	*n = 9,10,9,9*	two-way ANOVA	$F_{[1, 33]} = 3.74$	$p = 0.06$	$F_{[1, 33]} = 6.00$	$p < 0.05$	$F_{[1, 33]} = 5.67$	$p < 0.05$
C	Effect of LY379268 on (*2R,6R*)-HNK 1 hour FST	*n = 7,9,8,10*	two-way ANOVA	$F_{[1, 30]} = 0.70$	$p = 0.41$	$F_{[1, 30]} = 5.59$	$p < 0.05$	$F_{[1, 30]} = 4.81$	$p < 0.05$
D	Effect of LY379268 on (*2R,6R*)-HNK 24 hours FST	*n = 10,10,10,9*	two-way ANOVA	$F_{[1, 35]} = 0.79$	$p = 0.38$	$F_{[1, 35]} = 7.72$	$p < 0.01$	$F_{[1, 35]} = 7.03$	$p < 0.05$
E	Effect of LY379268 on (*2R,6R*)-HNK LH	*n = 11,10,11,10*	two-way ANOVA	$F_{[1, 38]} = 1.77$	$p = 0.19$	$F_{[1, 38]} = 3.66$	$p = 0.06$	$F_{[1, 38]} = 6.80$	$p < 0.05$
F	Effect of LY379268 on (*2R,6R*)-HNK Sucrose preference			**Experimental group effect**		**Day effect**		**Interaction**	
	• Non-stressed controls	*n = 6,5,6,5*	two-way RM ANOVA	$F_{[3, 18]} = 0.14$	$p = 0.94$	$F_{[4, 72]} = 3.45$	$p < 0.05$	$F_{[12, 72]} = 0.79$	$p = 0.66$
	• CSDS resilient	*n = 6,5,6,5*	two-way RM ANOVA	$F_{[3, 18]} = 0.42$	$p = 0.74$	$F_{[4, 72]} = 6.99$	$p < 0.001$	$F_{[12, 72]} = 0.65$	$p = 0.79$
	• CSDS susceptible	*n = 6,6,7,7*	two-way RM ANOVA	$F_{[3, 22]} = 9.00$	$p < 0.05$	$F_{[4, 88]} = 107.3$	$p < 0.001$	$F_{[12, 88]} = 1.90$	$p < 0.05$
G	(*2R,6R*)-HNK brain levels: SAL *vs* LY379268 pre-treatment	*n = 4 / time point*	No stats (n<5)						
				Experimental group effect		**Time effect**		**Interaction**	
K	Effect of LY379268 on (*2R,6R*)-HNK qEEG gamma power	*n = 6,7,5*	two-way RM ANOVA	$F_{[2, 15]} = 7.12$	$p < 0.01$	$F_{[9, 135]} = 9.54$	$p < 0.001$	$F_{[18, 135]} = 3.17$	$p < 0.001$
Fig. 5				**Treatment effect**		**Genotype effect**		**Interaction**	
A	(*2R,6R*)-HNK 1 hour FST; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 8,9,8,8,8,9*	two-way ANOVA	$F_{[1, 44]} = 13.10$	$p < 0.001$	$F_{[2, 44]} = 7.71$	$p < 0.01$	$F_{[2, 44]} = 6.75$	$p < 0.01$
B	(*2R,6R*)-HNK 24 hours FST; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 8,9,8,8,8,9*	two-way ANOVA	$F_{[1, 44]} = 10.41$	$p < 0.001$	$F_{[2, 44]} = 3.60$	$p < 0.05$	$F_{[2, 44]} = 5.85$	$p < 0.01$

C	(*R,S*)-KET 1 hour FST; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 10/group*	two-way ANOVA	$F_{[1, 44]} = 7.10$	*p < 0.05*	$F_{[2, 44]} = 0.001$	*p = 0.97*	$F_{[2, 44]} = 2.98$	*p = 0.09*
				Genotype effect		**Time effect**		**Interaction**	
G	(*2R,6R*)-HNK qEEG; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 13,14,13*	two-way RM ANOVA	$F_{[2, 37]} = 1.97$	*p = 0.15*	$F_{[11, 407]} = 9.02$	*p < 0.001*	$F_{[22, 407]} = 1.71$	*p < 0.05*
SI Appendix, Fig. S1				**Treatment effect**					
A	(*2R,6R*)-HNK 1 hour FST; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 6 / group*	two-way ANOVA	$F_{[7, 40]} = 6.46$	*p < 0.001*				
SI Appendix, Fig. S2				**Treatment effect**					
A	OFT; LY341495 dose response – WT mice	*n = 8,7,8,8,8*	one-way ANOVA	$F_{[4, 34]} = 12.39$	*p < 0.001*				
				Treatment effect		**Genotype effect**		**Interaction**	
B	OFT; LY341495 – WT vs *Grm2*$^{-/-}$ mice	*n = 9,10,9,7*	two-way ANOVA	$F_{[1, 31]} = 50.05$	*p < 0.001*	$F_{[1, 31]} = 14.71$	*p < 0.001*	$F_{[1, 31]} = 0.01$	*p = 0.77*
				Treatment effect		**Time effect**		**Interaction**	
C	Alpha power – WT mice	*n = 12,8,7*	two-way RM ANOVA	$F_{[2, 24]} = 2.18$	*p = 0.13*	$F_{[8, 192]} = 2.05$	*p < 0.05*	$F_{[16, 192]} = 1.44$	*p = 0.12*
D	Beta power – WT mice	*n = 12,8,7*	two-way RM ANOVA	$F_{[2, 24]} = 0.41$	*p = 0.67*	$F_{[8, 192]} = 1.17$	*p = 0.32*	$F_{[16, 192]} = 1.47$	*p = 0.11*
E	Delta power – WT mice	*n = 12,8,7*	two-way RM ANOVA	$F_{[2, 25]} = 4.11$	*p < 0.05*	$F_{[8, 200]} = 4.34$	*p < 0.001*	$F_{[16, 200]} = 2.75$	*p < 0.001*
F	Theta power – WT mice	*n = 12,8,7*	two-way RM ANOVA	$F_{[2, 24]} = 0.86$	*p = 0.44*	$F_{[8, 192]} = 0.93$	*p = 0.49*	$F_{[16, 192]} = 1.70$	*p = 0.05*
G	Alpha power – *Grm2*$^{-/-}$ mice	*n = 9 / group*	two-way RM ANOVA	$F_{[2, 24]} = 2.32$	*p = 0.12*	$F_{[8, 192]} = 1.55$	*p = 0.14*	$F_{[16, 192]} = 0.93$	*p = 0.54*
H	Beta power – *Grm2*$^{-/-}$mice	*n = 9 / group*	two-way RM ANOVA	$F_{[2, 24]} = 3.76$	*p < 0.05*	$F_{[8, 192]} = 0.81$	*p = 0.60*	$F_{[16, 192]} = 0.90$	*p = 0.57*
I	Delta power – *Grm2*$^{-/-}$mice	*n = 9 / group*	two-way RM ANOVA	$F_{[2, 24]} = 2.79$	*p = 0.08*	$F_{[8, 192]} = 1.40$	*p = 0.20*	$F_{[16, 192]} = 1.39$	*p = 0.15*
J	Theta power – *Grm2*$^{-/-}$mice	*n = 9 / group*	two-way RM ANOVA	$F_{[2, 24]} = 2.50$	*p = 0.08*	$F_{[8, 192]} = 1.02$	*p = 0.42*	$F_{[16, 192]} = 0.67$	*p = 0.82*
K	Alpha power – *Grm3*$^{-/-}$ mice	*n = 7,7,6*	two-way RM ANOVA	$F_{[2, 17]} = 2.25$	*p = 0.14*	$F_{[8, 136]} = 1.25$	*p = 0.28*	$F_{[16, 136]} = 1.39$	*p = 0.16*
L	Beta power – *Grm3*$^{-/-}$mice	*n = 7,7,6*	two-way RM ANOVA	$F_{[2, 17]} = 0.22$	*p = 0.81*	$F_{[8, 136]} = 6.48$	*p < 0.001*	$F_{[16, 136]} = 0.66$	*p = 0.83*
M	Delta power – *Grm3*$^{-/-}$mice	*n = 7,7,6*	two-way RM ANOVA	$F_{[2, 17]} = 0.86$	*p = 0.44*	$F_{[8, 136]} = 2.32$	*p < 0.05*	$F_{[16, 136]} = 0.59$	*p = 0.89*
N	Theta power – *Grm3*$^{-/-}$mice	*n = 7,7,6*	two-way RM ANOVA	$F_{[2, 17]} = 0.55$	*p = 0.59*	$F_{[8, 136]} = 1.84$	*p = 0.07*	$F_{[16, 136]} = 1.01$	*p = 0.45*
SI Appendix, Fig. S3				**Treatment group effect**		**Time effect**		**Interaction**	
A	Alpha power – Sub-effective LY341495 and (*2R,6R*)-HNK	*n = 7,6*	two-way RM ANOVA	$F_{[1, 11]} = 0.80$	*p = 0.39*	$F_{[11, 121]} = 0.89$	*p = 0.55*	$F_{[11, 121]} = 0.51$	*p = 0.90*
B	Beta power – Sub-effective LY341495 and (*2R,6R*)-HNK	*n = 7,6*	two-way RM ANOVA	$F_{[1, 11]} = 0.28$	*p = 0.61*	$F_{[11, 121]} = 1.41$	*p = 0.18*	$F_{[11, 121]} = 0.51$	*p = 0.89*
C	Delta power – Sub-effective LY341495 and (*2R,6R*)-HNK	*n = 7,6*	two-way RM ANOVA	$F_{[1, 11]} = 0.07$	*p = 0.80*	$F_{[11, 121]} = 1.70$	*p = 0.08*	$F_{[11, 121]} = 1.44$	*p = 0.16*
D	Theta power – Sub-effective LY341495 and (*2R,6R*)-HNK	*n = 7,6*	two-way RM ANOVA	$F_{[1, 11]} = 0.36$	*p = 0.56*	$F_{[11, 121]} = 0.71$	*p = 0.73*	$F_{[11, 121]} = 1.04$	*p = 0.42*
SI Appendix, Fig. S4				**Treatment group effect**		**Time effect**		**Interaction**	
A	Alpha power – Effect of LY379268 on (*2R,6R*)-HNK	*n = 6,7,5*	two-way RM ANOVA	$F_{[2, 15]} = 1.30$	*p = 0.30*	$F_{[9, 135]} = 1.17$	*p = 0.32*	$F_{[18, 135]} = 1.28$	*p = 0.21*
B	Beta power – Effect of LY379268 on (*2R,6R*)-HNK	*n = 6,7,5*	two-way RM ANOVA	$F_{[2, 15]} = 5.66$	*p < 0.05*	$F_{[9, 135]} = 6.13$	*p < 0.001*	$F_{[18, 135]} = 3.76$	*p < 0.001*
C	Delta power – Effect of LY379268 on (*2R,6R*)-HNK	*n = 6,7,5*	two-way RM ANOVA	$F_{[2, 15]} = 0.49$	*p = 0.62*	$F_{[9, 135]} = 1.95$	*p < 0.05*	$F_{[18, 135]} = 0.66$	*p = 0.85*
D	Theta power – Effect of LY379268 on (*2R,6R*)-HNK	*n = 6,7,5*	two-way RM ANOVA	$F_{[2, 15]} = 1.31$	*p = 0.30*	$F_{[9, 135]} = 0.57$	*p = 0.82*	$F_{[18, 135]} = 0.79$	*p = 0.71*
SI Appendix, Fig. S5				**Treatment effect**		**Genotype effect**		**Interaction**	
A	(*2R,6R*)-HNK effect in females; 1 hour FST	*n = 9,8,9,8*	two-way ANOVA	$F_{[1, 30]} = 7.43$	*p < 0.05*	$F_{[1, 30]} = 0.31$	*p = 0.58*	$F_{[1, 30]} = 3.74$	*p = 0.06*
B	(*2R,6R*)-HNK effect in females; 24 hours FST	*n = 9,8,9,7*	two-way ANOVA	$F_{[1, 29]} = 1.90$	*p = 0.18*	$F_{[1, 29]} = 1.36$	*p = 0.25*	$F_{[1, 29]} = 3.46$	*p = 0.07*
C	(*2R,6R*)-HNK brain levels_ WT *vs Grm2*$^{-/-}$ mice	*n = 3-4 / time point*	No stats (n<5)						
D	Higher doses (*2R,6R*)-HNK FST	*n = 9,8,8,8,7,8*	two-way ANOVA	$F_{[2, 42]} = 2.12$	*p = 0.13*	$F_{[1, 42]} = 2.39$	*p = 0.13*	$F_{[2, 42]} = 1.57$	*p = 0.22*
E	(*2R,6R*)-HNK effect in the LH; WT *vs Grm3*$^{-/-}$ mice	*n = 8,10,7,9*	two-way ANOVA	$F_{[1, 30]} = 27.35$	*p < 0.001*	$F_{[1, 30]} = 12.71$	*p < 0.01*	$F_{[1, 30]} = 0.47$	*p = 0.50*
G	MK-801, 1 hour FST; WT *vs Grm2*$^{-/-}$ mice	*n = 9,8,7,7*	two-way ANOVA	$F_{[1, 27]} = 54.58$	*p < 0.001*	$F_{[1, 27]} = 1.65$	*p = 0.21*	$F_{[1, 27]} = 0.92$	*p = 0.35*
I	MK-801, qEEG gamma power; WT *vs Grm2*$^{-/-}$ mice	*n = 5,7,5,7*	two-way ANOVA	$F_{[1, 10]} = 21.98$	*p < 0.001*	$F_{[1, 10]} = 1.03$	*p = 0.33*	$F_{[1, 10]} = 1.87$	*p = 0.20*
SI Appendix, Fig. S6				**Genotype effect**		**Time effect**		**Interaction**	
A	(*2R,6R*)-HNK qEEG alpha power; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 13,14,13*	two-way RM ANOVA	$F_{[2, 37]} = 2.40$	*p = 0.10*	$F_{[11, 407]} = 1.16$	*p = 0.31*	$F_{[22, 407]} = 1.97$	*p < 0.01*
B	(*2R,6R*)-HNK qEEG beta power; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 13,14,13*	two-way RM ANOVA	$F_{[2, 37]} = 1.74$	*p = 0.19*	$F_{[11, 407]} = 4.99$	*p < 0.001*	$F_{[22, 407]} = 1.49$	*p = 0.07*
C	(*2R,6R*)-HNK qEEG delta power; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 13,14,13*	two-way RM ANOVA	$F_{[2, 37]} = 4.03$	*p < 0.05*	$F_{[11, 407]} = 3.48$	*p < 0.001*	$F_{[22, 407]} = 1.86$	*p < 0.05*
D	(*2R,6R*)-HNK qEEG theta power; WT *vs Grm2*$^{-/-}$ *vs Grm3*$^{-/-}$	*n = 13,14,13*	two-way RM ANOVA	$F_{[2, 37]} = 1.63$	*p = 0.21*	$F_{[11, 407]} = 3.03$	*p < 0.01*	$F_{[22, 407]} = 0.87$	*p = 0.64*

Raw data for all the analyses are provided in *Dataset S1*. *Abbreviations*: CSDS, chronic social defeat stress; FST, forced-swim test; HNK, hydroxynorketamine; KET, ketamine; OFT, open-field test; qEEG, quantitative electroencephalogram; RM, repeated measures; SAL, saline; WT, wild-type.

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