Astroglial-Kir4.1 in Lateral Habenula Drives Neuronal Bursts to Mediate Depression

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Summary

Enhanced bursting activity of the lateral habenula (LHb) neurons is essential in driving depressive-like behaviors, but the cause of this increased bursts remained unknown. Here using high-throughput quantitative proteomic screen, we identified an astroglial potassium channel, Kir4.1, to be upregulated in the LHb of animal models of depression. Kir4.1 in the LHb shows a distinct expression pattern on the astrocytic membrane processes tightly wrapping around the neuronal soma. Electrophysiology and modeling data demonstrate that the level of Kir4.1 on astrocytes tightly regulates the degree of membrane hyperpolarization and the amount of burst activity of LHb neurons. Astrocyte-specific gain and loss of Kir4.1 functions in the LHb bidirectionally regulate neuronal bursting and depressive-like symptoms. Together, these results reveal a new form of glial-neural interaction in setting neuronal firing mode in a devastating psychiatric disease, and discover the therapeutic potential of targeting LHb Kir4.1 for treating major depression.

A major breakthrough in neuroscience has been the discovery that astrocytes intimately interact with neurons to support and regulate a series of essential functions to foster brain information processing¹⁻⁷. A wealth of investigations has focused on the astroglial-neural interactions at the tripartite synapses, where astrocyte processes tightly wrap around pre- and post-synaptic sites⁸. In contrast, not as much attention has been placed on astroglial-neural interaction in proximity to neuronal soma^{9,10}. Particularly, how astrocytes regulate intrinsic firing patterns of neurons, and what structural basis may underlie this regulation, are much less explored.

Despite the surging interest of lateral habenula (LHb) in negative emotion¹¹⁻²⁰, only limited attention has been given to astrocytes and their potential roles in LHb hyperfunction in depression²¹. In the accompanying paper, we demonstrate that bursting activity of LHb neurons are greatly enhanced in animal models of depression. LHb burst drives depressive-like behaviors and is a prominent target of the rapid antidepressant ketamine. However, the cause of this enhanced burst of LHb neurons remains unsolved.

Results

Kir4.1 is upregulated in LHb of animal models of depression

In an unbiased, high-throughput, quantitative proteomic screening to compare habenular protein expression of congenitally learned helpless (cLH) rats²² and wild-type SD rats, we identified Kir4.1 to be highly upregulated in the LHb of cLH rats¹¹ (1.69-fold of wild-type control, $p = 0.02$, Student's t -test, Fig. 1a). Western blot analysis confirmed that Kir4.1 had a significant increase (1.44-fold, $p = 0.009$, Paired t -test) in the membrane protein extraction of cLH habenulae (Fig. 1b). In contrast, another astrocyte-specific protein, glial fibrillary acidic protein (GFAP), did not show any change in expression (Extended Data Fig. 1a), indicating there was no astrogliosis.

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74 To test whether Kir4.1 upregulation is universal in depression, we examined an additional rat
75 model of depression, the LPS (lipopolysaccharide)-induced depression²³. One week of LPS
76 injection (0.5mg/kg, i.p.) in 3-month-old Wistar rats was sufficient to cause strong
77 depressive-like phenotype in the forced swim test (FST, Extended Data Fig. 2e)²³ and sucrose
78 preference test (SPT)²³. The Kir4.1 level was also significantly increased in the LPS-induced
79 rats (1.87-fold, $p < 0.0001$, Paired t -test, Fig. 1c). Quantitative real-time PCR revealed an
80 increase (1.2-fold, $p = 0.015$, Paired t -test) of Kir4.1 mRNA level in cLH habenulae (Fig. 1d),
81 suggesting that at least part of the protein level change is due to transcriptional upregulation.

82

83 **Age-dependent increase of LHb astrocytic Kir4.1 current and depression onset in cLH**
84 **rats**

85 Kir4.1 is a principal component of the glial Kir channel and is largely responsible for
86 mediating the K⁺ conductance and setting the resting membrane potential (RMP) of
87 astrocytes²⁴. To confirm that Kir4.1 function is indeed upregulated, we performed whole-cell
88 patch clamp onto the astrocytes in brain slices made from the LHb of cLH or SD rats.
89 Astrocytes were distinguished from neurons by their small (5–10 μ m) oval shaped somata
90 and electrophysiological features including a relatively hyperpolarized RMP (-74 ± 1 mV), a
91 low input resistance R_{in} (47 ± 6 M Ω), a linear I–V relationship and an absence of action
92 potentials in response to depolarizing current injections (Extended Data Fig. 2a-d)²⁵⁻²⁷.
93 Biocytin filling and absence of NeuN co-staining confirmed that cells fitting the above
94 criteria were indeed astrocytes (Extended Data Fig.3). We then bath applied Ba²⁺ (BaCl₂, 100
95 μ M), which selectively blocks Kir channels at sub-mM concentrations, to isolate Kir4.1
96 current²⁵ (Extended Data Fig. 2a, c). The Ba²⁺-sensitive current displayed a reversal potential
97 close to E_K (-90 mV)^{25,28} (Fig. 1e, f), indicating that it represents the K⁺ conductance. We

found that Ba^{2+} -sensitive currents in LHb astrocytes were almost doubled in cLH rats (Fig. 1e), as well as in LPS-treated rats (Extended Data Fig. 2f), at the age of P60-90.

Interestingly, the increase of Kir4.1 current and protein level was not obvious at P30 (Fig. 1f, Extended data Fig. 1b). At this age, cLH rats did not yet show depressive-like phenotypes in both the FST (Fig. 1g) and learned helpless test (LHT, Fig. 1h), suggesting that the upregulation of Kir4.1 level is concomitant with the developmental onset of the depressive-like symptoms.

Kir4.1 are expressed on astrocytic processes tightly wrapping around neuronal soma

As an inwardly rectifying K^+ channel, Kir4.1 has been strongly implicated in buffering excess extracellular K^+ in tripartite synapses^{24,29-31}. Conventional model of K^+ buffering suggests Kir4.1 to be highly expressed in astrocytic endfeet surrounding synapses³²⁻³⁴. Surprisingly, with immunohistochemistry co-labeling, Kir4.1 staining in LHb appeared to overlap with the neuronal marker NeuN at low magnification (20X, Extended Data Fig. 4a), although in the same brain slice Kir4.1 staining patterns in hippocampus were typical astrocytic-looking (Extended Data Fig. 4b). However, higher magnification imaging with single layer scanning (0.76 μ m per layer) revealed that Kir4.1 staining enveloped NeuN signals (Fig. 2a). To confirm that Kir4.1 indeed locates within astrocytes but not neurons in LHb, we separately knocked it out in either neurons or astrocytes by injecting AAV virus expressing the cre recombinase under either the neuronal promoter CaMKII or glial promoter GFAP (gfaABC1D)^{26,35} into Kir4.1^{f/f} floxed mice²⁵. The staining of Kir4.1 remained intact with neuronal knock-out, but was completely eliminated with astrocytic knock-out (Fig. 2b). Electron microscopy imaging revealed that Kir4.1-positive gold particles were distributed encircling the membrane of neuronal cell bodies (Fig. 2c, Extended Data Fig. 5a, b), as well

as in the synapses (Extended Data Fig. 5d). Consistently, whole-cell-patch recordings showed that Ba^{2+} -sensitive currents were absent in neurons but abundant in astrocytes in the LHb (Fig. 2d, Extended Data Fig. 6). Together these results suggest that Kir4.1 is mainly expressed in astrocytic processes tightly wrapping around neuronal soma and synapses in the LHb.

Overexpression of kir4.1 in LHb astrocytes increases neuronal bursts and causes strong depressive-like behaviors

To test the consequence of Kir4.1 upregulation in LHb, we used adeno-associated viruses of the 2/5 serotype (AAV2/5) that preferentially target astrocytes²⁶ together with the human GFAP (gfaABC1D) promoter^{26,35} to deliver GFP-tagged Kir4.1 channels (AAV-GFAP::Kir4.1) or AAV-GFAP::GFP as a control (Fig. 3a). 14 days after bilateral injection in the LHb at P50, AAV2/5-mediated viral transfection led to Kir4.1 and GFP expression in astrocytes throughout the LHb (Fig. 3b). The specificity of the viral infection in astrocytes was verified by co-immunostaining of NeuN and GFP: only 0.3% NeuN+ cells (n = 2668) were infected by this virus (Extended Data Fig.7). We made whole-cell recordings from either astrocytes or neurons surrounding the viral-transfected astrocytes in coronal LHb slices (Fig. 3c-e). The RMPs of both astrocytes and neurons were more hyperpolarized (Fig. 3d, e) and the percentage of bursting neurons were significantly higher (Fig.3f) in mice infected with AAV-GFAP::Kir4.1 than with AAV-GFAP::GFP.

We then assayed depressive-like phenotypes and found that mice with AAV-GFAP::Kir4.1 infection in the LHb displayed severe depressive-like behaviors, including increased immobile duration (p = 0.0002, Unpaired *t*-test) and decreased latency to immobility (p = 0.001, Unpaired *t*-test) in FST (Fig. 3g), and decreased sucrose preference in the SPT (p <

0.0001, Unpaired *t*-test, Fig. 3h), while the general locomotion was unchanged (Extended Data Fig. 8a-b).

Kir4.1-mediated K buffering regulates neuronal RMP and bursting activity

How does an astrocytic potassium channel regulate RMP and burst firing of the LHb neuron?

We hypothesize that within the highly confined extracellular space between neuronal soma and Kir4.1-positive astrocytic processes (Fig. 2), the constantly-released K⁺ from intrinsically active LHb neurons is quickly cleared by astrocytes through a Kir4.1-dependent mechanism. Accordingly, we predict that blockade of Kir4.1 should compromise K⁺ spatial buffering, resulting in increased extracellular K⁺ (K_{out}), and according to Nernst Equation, depolarized neuronal RMPs (modeled in Extended Data Fig. 9). Consistent with this prediction, blocking Kir4.1 with BaCl₂ depolarized the RMPs of LHb neurons, after about 10 min bath perfusion of BaCl₂ (Fig. 4a). The amount of changes in RMP positively correlated with the original firing rates of neurons (Fig. 4b), indicating that the more active the neuron is, the larger contribution the K⁺ buffering to its RMP. Similar amount of RMP change was induced when BaCl₂ was applied in presence of synaptic transmission blockers (picrotoxin, NBQX and AP5, Extended Data Fig. 10), suggesting that Kir4.1-dependent regulation of LHb neuronal RMP occurs mostly at neuronal cell bodies instead of at synaptic sites. Consequent to the RMP change, perfusion of BaCl₂ caused a dramatic increase of firing frequency until the neuron reached a sustained plateau of a tetanus response and stopped firing (Fig. 4c).

Vice versa, upregulation of Kir4.1 or stop neuronal firing should decrease extracellular K⁺ (K_{out}) and hyperpolarize neuronal RMPs. Indeed, overexpression of Kir4.1 in astrocytes (Fig. 3e) or blocking neuronal APs by TTX (Fig. 4d, e) both caused hyperpolarization of LHb

neurons. Overexpression of Kir4.1 in astrocytes also increased neuronal bursts (Fig. 3g). Finally, to assess a causal relationship between K_{out} and firing mode, we made current-clamp recordings of LHb neurons while lowering K_{out} from 2.75mM to 1.4mM (Fig. 4f). This led to lowered neuronal RMP by 13.7 $\pm$ 0.5mV and a direct shift of originally tonic-firing neurons (8 out of 15) into bursting mode (Fig. 4g). Consequently, percentage of bursting neurons was increased from 8% to 23% ($p < 0.0001$, Chi-square test, Fig. 4h). In summary, by increasing astrocytic Kir4.1 expression or decreasing the extracellular K^+ concentration, we were able to phenocopy in WT animals several key neuronal properties observed in the LHb of animal models of depression, namely hyperpolarized RMPs and enhanced bursts. These results indicate that enhanced capacity of extracellular K clearance due to Kir4.1 overexpression may underlie the neuronal hyperpolarization required for burst initiation.

Loss-of-function of Kir4.1 in LHb decreases neuronal bursting and rescues depressive-like phenotypes

Next, to determine whether loss-of-function of Kir4.1 in the LHb may reduce neuronal bursts and reverse depressive phenotypes, we devised two strategies, by using AAV2/5 viral vectors to express either a short hairpin RNA (shRNA) to knock down the level of Kir4.1, or a dominant negative construct to block its function in the LHb of cLH rats (Fig. 5a). We tested six shRNAs specifically targeting the Kir4.1 transcript in cell culture and chose the one with most efficient knock-down efficiency (Fig. 5b and Extended Data Fig. 11a) for viral package. To avoid an off-target effect of shRNA, we also used a dominant-negative form of Kir4.1, dnKir4.1, containing a GYG to AAA point mutation at the channel pore, which blocks K^+ channels³⁶ (Extended Data Fig. 11b, 12, Fig. 5c). We first examined the effect of Kir4.1-shRNA on glial and neural electrophysiological properties. In astrocytes infected with the

AAV-H1::Kir4.1-shRNA, we observed a dramatic change of I-V relation (Fig. 5d), a 32 mV depolarization compared with non-infected astrocytes ($p < 0.0001$, One-way ANOVA) and 41mV depolarization compared with ctrl-shRNA-infected astrocytes ($p < 0.0001$, One-way ANOVA, Fig. 5e), consistent with the literature that Kir4.1 is majorly responsible for setting the astrocytic RMPs²⁵. In neurons infected with the AAV-H1::Kir4.1-shRNA, the RMPs did not differ from neighboring non-infected neurons (because neurons do not express Kir4.1 endogenously, Fig. 5f). However, RMPs of neighboring LHb neurons from AAV-H1::Kir4.1-shRNA-infected brain slices were overall more depolarized than RMPs of those from AAV-H1::Ctrl-shRNA-infected rats ($-43 \pm 2\text{mV}$ vs. $-53 \pm 2.7\text{mV}$, $p = 0.006$, One-way ANOVA, Fig. 5f), suggesting that knock-down of Kir4.1 in astrocytes had a global impact on RMPs of neighboring neurons. Similar effects were observed in AAV-GFAP::dnKir4.1 infected LHb slices (Extended Data Fig. 11c-f). Most importantly, bursting activity in the LHb of cLH rats were significantly eliminated by AAV-H1::Kir4.1-shRNA (Fig. 5g) and AAV-GFAP::dnKir4.1 (Extended Data Fig. 11f) infection.

Behaviorally, infection of AAV-H1::Kir4.1-shRNA or AAV-GFAP::dnKir4.1 had a pronounced effect on rescuing the depressive-like phenotypes of cLH rats in three depression paradigms: reducing the immobility time ($p = 0.01$, Unpaired t -test, Fig. 5h) and increasing latency to immobility ($p = 0.0018$, Unpaired t -test, Fig. 5h) in FST, increasing bar pressing number in the LHT ($p = 0.0004$, Unpaired t -test, Fig. 5i, j), and increasing the sucrose preference score in SPT ($p = 0.04$, Unpaired t -test, Fig. 5k). The behavior scores in LHT clearly correlated with those in FST (Fig. 5l).

Here we discover an important function of Kir4.1 at the highly specialized neuron-glia interface in the LHb in regulating neuronal RMP and firing pattern. During depression, the

upregulation of Kir4.1 may cause enhanced capacity of extracellular K clearance, leading to a decrease of K_{out} and neuronal hyperpolarization (Fig. 5m). As demonstrated in our accompanying manuscript, neuronal hyperpolarization may de-inactivate T-type voltage-sensitive calcium channels (T-VSCCs) which in turn initiate NMDAR-dependent bursts to cause a stronger suppression of the downstream monoaminergic centers (Fig. 5m). These results may inspire the development of novel treatments of major depression targeting on maladaptive neuron-glia interactions in the LHb.

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Author contributions

H.H. and Y.C. designed the study. Y.C. performed the *in vitro* patch recordings; Y.Y. performed the biochemistry and immunohistochemistry experiments; Y.C., Y.Y., Y.D. and K.S. performed viral injections and behavioral experiments; Z.N., A.F. and H.B. established

248 the biophysical model; S.M. assisted cell culture experiments; G.C. and S.W. conducted the
249 EM experiments; Y.S. contributed Kir4.1^{f/f} floxed mice; S.T. and Y.L. constructed plasmids.
250 H.H. wrote the manuscript with the assistance of Y.C., Y.Y. and Z.N..

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Methods

Animals. Male cLH rats (3-4 weeks or 8-12 weeks of age) and age-matched male Sprague Dawley rats (SLAC Laboratory Animal Co., Shanghai) were used. The cLH rats were screened by learned helpless test^{1,2} for breeding as previously described¹. Male Wistar rats (SLAC Laboratory Animal Co., Shanghai, 12 weeks) were used for establishing the LPS-induced depressive-like rat model. Male adult (7-8 weeks of age) C57BL/6 mice (SLAC) or Kir4.1^{f/f} floxed mice (originally obtained from Dr. Ken McCarthy at University of North Carolina) were used for virus injection the immunohistochemistry experiments. Animals were group-housed two/cage for rats and four/cage for mice under a 12-h light-dark cycle (light on from 7 a.m. to 7 p.m.) with free access to food and water *ad libitum*. All animal studies and experimental procedures were approved by the Animal Care and Use Committee of the animal facility at Zhejiang University.

Western Blot. The habenular membrane fraction and whole protein was extracted as previously described¹¹. Animals were anesthetized using 10% chloral hydrate, and habenular tissue was quickly dissected from the brain and homogenized in lysis buffer (320 mM sucrose, 4 mM HEPES pH7.4, 1 mM MgCl₂ and 0.5 mM CaCl₂, 5 mM NaF, 1 mM Na₃VO₄, EDTA-free, Protease Inhibitor cocktail tablets (Roche) on ice. The lysis buffer used for extracting the total protein of HEK293TN cell contained 50mM Tris (pH 7.4), 150mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS and Protease Inhibitor cocktail tablets (Roche). After protein concentration measurement by BCA assay, 10~20μg proteins for each lane was separated on a 10% SDS-PAGE gel and transferred for western blot analysis. Anti-Kir4.1 (1:1000, Alomone labs), anti-GFAP (1:1000, Sigma) and anti-tubulin (1:5000, Bio-Rad) antibodies, and high sensitive ECL reagent (GE Healthcare) were used. All the bands were analyzed with Quantity one or Image J.

Immunohistochemistry. Animals were anesthetized using 10% chloral hydrate, and then perfused transcardially with ice-cold PBS (pH 7.4) followed by 4% paraformaldehyde. After overnight post fix in 4% paraformaldehyde solution, brains were cryoprotected in 30% sucrose for 1 day (for mice) or 3 days (for rats). Coronal sections (40 μ m) were cut on a microtome (Leica) and collected in PBS and stored at 4°C for further using. The antibodies used were rabbit anti-Kir4.1 extracellular peptide (1:200, Alomone labs), mouse anti-GFAP (1:500, Sigma), mouse anti-NeuN (1:500, Millipore), Rabbit anti NeuN (1:500, Millipore), mouse anti-S100b (1:500, Sigma), chicken anti-GFP (1:1000, Abcam), Mouse anti-Flag (1:1000, Beyotime), Alexa Fluor 488 goat anti-rabbit IgG, Alexa Fluor 488 goat anti-chicken IgG, Alexa Fluor 546 goat anti-mouse IgG (all 1:1000, Invitrogen). Specifically, for Kir4.1 staining, the rabbit anti-Kir4.1 extracellular peptide antibody was incubated for 48-72 h and the other primary antibodies were incubated for 36-48 h. For the antibody absorption experiments, the rabbit anti-Kir4.1 extracellular peptide antibody was pre-adsorbed with the Kir4.1 antigen by mixing at the weight ratio of 1:2 for 24 h. Slices were counterstained with Hoechst in the final incubation step to check the injection site. Fluorescent image acquisition was performed with an Olympus Fluoview FV1000 confocal microscope and a Nikon A1 confocal microscope.

Cell transfection and cell culture. HEK293 cells (gift from Jianhong Luo) were used for the electrophysiology recording and HEK293TN cells (Taitool Bioscience, China) were used for western blot analysis. Cells used in this study were authenticated and checked for mycoplasma contamination. The plasmids used were pAAV-Ubi-Kir4.1-2A-eGFP, pAAV-Ubi-dnKir4.1 (GYG to AAA)-2A-eGFP, pAAV-CAG-eGFP, pAAV-H1-Kir4.1-shRNA-CAG-eGFP and pAAV-H1-Luciferase-shRNA-CAG-eGFP. HEK293 cells were trypsinized for 5 min using 0.25% trypsin (wt/vol), and replated onto glass coverslips in fresh

DMEM:F12 medium (Life Technologies) containing 1 mM nonessential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), and 10% FBS (Hyclone). Cells were transfected using Lipofectamine 2000 (Invitrogen) according to standard protocols, then cultured for 5-6 h in well plates at 37 °C in a humid atmosphere of 5% CO₂ and 95% air. Cells were cultured for another 48 h before western blot or electrophysiology experiments.

Plasmid constructs. The pAAV-Ubi-Kir4.1-2A-eGFP plasmid was assembled by homologous recombination of AAV backbone linearized from the AAV-Ubi-CaMKII-2A-eGFP plasmid¹¹ by PCR and Kir4.1 amplified from pZac2.1- gfaABC1D-EGFP-Kir4.1 plasmid (AddGene). The pAAV-Ubi-dnKir4.1 (GYG to AAA)-2A-eGFP plasmid was made by PCR-based mutagenesis using pAAV-Ubi-Kir4.1-2A-eGFP as a backbone (Fw: 5'-AAAGATGGCCGGAGCAACGTGAGAATGGAGCATATTGCT-3'; Rev: 5'-GCTGATGTAGCGGAAGGCGGCGGCAATGGTGGTCTGGGATTTCGAGGGA-3'). The pAAV-gfaABC1D- dnKir4.1 (GYG to AAA)-2A-EGFP was assembled by homologous recombination of pZac2.1 gfaABC1D backbone linearized from the pZac2.1- gfaABC1D-EGFP-Kir4.1 plasmid (AddGene) by PCR and Kir4.1dn-2A-EGFP sequence amplified from pAAV-Ubi-dnKir4.1 (GYG to AAA)-2A-eGFP plasmid. The pAAV-H1-Kir4.1-shRNA-CAG-eGFP was constructed using a vector (provide by Taitool Bioscience, China), which contains a CAG promoter driving eGFP and a H1 promoter driving shRNA expression. We designed 6 shRNA sequences by RNAi designer online software (Invitrogen) as indicated below:

- 1) 5'-GGACGACCTTCATTGACAT-3';
- 2) 5'-GCTACAAGCTTCTGCTCTTCT-3';
- 3) 5'-GCTCTTCTCGCCAACCTTTAC-3';
- 4) 5'-CCGGAACCTTCCTTGCAAA-3';

5) 5'-GCGTAAGAGTCTCCTCATTGG-3'

6) 5'-GCCCTTAGTGTGCGCATTA-3'.

We then tested the knock-down efficiency by Western blot of Kir4.1 from HEK293TN cells which were co-transfected with Flag-tagged-Kir4.1 plasmid (pAAV-CMV-betaGlobin-Kir4.1-eGFP-3Flag) and each of the six shRNA plasmids. Based on our western blot result (Extended Figure 11a), we chose the fifth sequence, 5'-GCGTAAGAGTCTCCTCATTGG-3', for Kir4.1-shRNA virus package.

Electron microscopic immunohistochemistry. Four mice were deeply anesthetized with 1% sodium pentobarbital intraperitoneally (50 mg/kg body weight) and perfused transcardially with 20ml saline, followed by 40ml ice-cold mixture of 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1M PB for 1 h. Brainstems were removed and postfixed by immersion in the same fixative for 4 h at 4°C. Serial coronal sections of 50 µm thickness were prepared with a vibratome (VT 1000S, Leica), and approximately 18-20 sections, including the LHb region, were collected from each brain.

Kir4.1 was detected by the immunogold-silver staining. Briefly, sections were blocked with blocking buffer (5% BSA, 5% NGS and 0.05% Triton X-100 in PBS), and then incubated overnight in the primary antibodies of rabbit anti-Kir4.1 (1:200) diluted with solution containing 1% BSA, 1% NGS and 0.05% Triton X-100. The secondary antibody was anti-rabbit IgG conjugated to 1.4 nm gold particles (1:100, Nanoprobes, Stony Brook, NY) for 4 h. After rinsing, sections were post fixed in 2% glutaraldehyde in PBS for 45 min. Silver enhancement was performed in the dark with HQ Silver Kit (Nanoprobes) for visualization of Kir4.1 immunoreactivity. Before and after the silver enhancement step, sections were rinsed several times with de-ionized water.

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354 Immuno-labeled sections were fixed with 0.5% osmium tetroxide in 0.1 M PB for 1 h,
355 dehydrated in graded ethanol series, then in propylene oxide, and finally flat-embedded in
356 Epon 812 between sheets of plastic. After polymerization, acrylic sheets were then peeled
357 from the polymerized resin, and flat-embedded sections were examined under the light
358 microscope. Three to four sections containing Kir4.1 immunoreactivity in the LHb were
359 selected from each section, trimmed under a stereomicroscope, and glued onto blank resin
360 stubs. Serial ultrathin sections were cut with an Ultramicrotome (Leica EM UC6, Germany)
361 using a diamond knife (Diatome, PA) and mounted on formvar-coated mesh grids (6-8
362 sections/grid). They were then counterstained with uranyl acetate and lead citrate, and
363 observed under a JEM-1230 electron microscope (JEOL LTD, Japan) equipped with CCD
364 camera and its application software (832 SC1000, Gatan, PA).

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366 **Stereotaxic surgery and virus injection.** cLH rats (postnatal 50-60 days) were deeply
367 anesthetized by using 4% pentobarbital. Mice (postnatal 50-60 days) were deeply
368 anesthetized by ketamine (100 mg/kg of body weight) and xylazine (8 mg/kg). Animals were
369 placed on a stereotactic frame (RWD Instruments, China). A small volume of virus was
370 injected into bilateral LHb (for rats LHb: AP, -3.7 mm from bregma; ML, ± 0.7 mm; DV, -
371 4.55 mm from the brain surface; for mice: AP, -1.72 mm from bregma; ML, ± 0.46 mm; DV,
372 -2.62 mm from the brain surface) using a pulled glass capillary with a pressure microinjector
373 (Picospritzer III, Parker, USA) at a slow rate of 0.1 μ l/min. After the injection was completed,
374 the capillary was left for an additional 10 minutes before it was then slowly withdrawn
375 completely. After surgery, animals were recovered from anesthesia under a heat pad.

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AAV-CaMKII::EGFP-Cre (AAV2/1-CaMKII-HI-EGFP-Cre, 0.2 μ l, bilateral into LHb, University of Pennsylvania vector core, Pennsylvania, USA), AAV2/5-GFAP::EGFP-Cre (AAV2/5-gfaABC1D-EGFP-Cre, titer: 4.74×10^{12} v.g./ml, dilution: 1:2, 0.2 μ l, bilateral into LHb, Taitool Bioscience, China), AAV-GFAP::Kir4.1 (AAV2/5-gfaABC1D-EGFP-Kir4.1, titer: 9.19×10^{12} v.g./ml, dilution: 1:5, 0.2 μ l, bilateral into LHb, Taitool Bioscience, China), AAV-GFAP::GFP (AAV2/5-gfaABC1D-EGFP, titer: 1.61×10^{13} v.g./ml, dilution: 1:5, 0.2 μ l, bilateral into LHb, University of Massachusetts, Guangping Gao Lab, USA), AAV-H1::Kir4.1-shRNA (AAV2/5-H1-Kir4.1-shRNA-CAG-EGFP, titer: 3.04×10^{13} v.g./ml, dilution: 1:10, 0.2 μ l, bilateral into LHb, Taitool Bioscience, China), AAV-H1::Ctrl-shRNA (AAV2/5-H1-Luciferase-shRNA-CAG-EGFP, titer: 1.46×10^{13} v.g./ml, dilution: 1:5, 0.2 μ l, bilateral into LHb, Taitool Bioscience, China), AAV2/5-gfaABC1D-dnKir4.1-2A-EGFP (GYG to AAA) (titer: 4.15×10^{13} v.g./ml, 0.2 μ l, bilateral into LHb, Taitool Bioscience, China). All viral vectors were aliquot and stored at -80 °C until use.

Depression model and Behavior assay

LPS-induced depression. The LPS-induced depression model was conducted as previously described²². Wistar male rats (3 months) were used for the experiments. LPS (Sigma, L-2880) dissolved in sterile 0.9% saline was intraperitoneally injected into Wistar rats, at a dosage of 0.5 mg/kg. This dosage was used to stimulate a subclinical infection without inducing obvious inflammation and other apparent impairments in animals. Saline or LPS was injected between 09:30 and 10:30 a.m. daily for 7 days. The forced swim test was performed 24 hours after the last injection. The habenular tissue was dissected 24 hours after the behavioral test and eletrophysiology experiments.

Learned helpless test (LHT). Male juvenile (P30) or adult (P90) cLH rats were tested in a lever-pressing task to evaluate the learned helpless (LH) phenotype¹¹. A cue-light-illuminated lever in the shock chamber was presented, which can terminate the shock when rats pressed the lever. 15 escapable shocks (0.8 mA) were delivered with a 24 s inter-shock interval. Each shock lasted up to 60 s unless the rat pressed the lever to terminate the shock. Out of the 15 trials, rats which failed to press the lever for more than 10 trials were defined as “learned helplessness” (LH), and rats with less than 5 failures were defined as “non-learned helplessness” (NLH).

Forced swim test (FST). Animals were individually placed in a cylinder (12 cm diameter, 25 cm height for mice; 20 cm diameter, 50 cm height for rats) of water (23-25 °C) and swam for 6 min under normal light³. Water depth was set to prevent animals from touching the bottom by tails and hind limbs. Animal behaviors were videotaped from the side. The immobility time during the last 4 min test was counted offline by an observer blind of the animal treatments. Immobility was defined as time when animals remained floating or motionless with only movements necessary for keeping balance in the water. For rats, an additional pre-test was conducted 24 h before the test, during which rats were individually placed in a cylinder of water with conditions described above for 15 min.

Sucrose preference test (SPT). Animals were single housed and habituated with two bottles of water for 2 days, followed by two bottles of 2% sucrose for 2 days³. Animals were then water deprived for 24 h and then exposed to one bottle of 2% sucrose and one bottle of water for 2 h in the dark phase. Bottle positions were switched after 1 h. Total consumption of each fluid was measured and sucrose preference was defined as the ratio of sucrose consumption divided by total consumptions of water and sucrose.

Open field test (OFT). Animals were placed in the center of an arena (40 cm x 40 cm x 40.5 cm for mice; and 100 cm x 100 cm x 40 cm for rats) in a room with dim light for 10 min. A video camera positioned directly above the arena was used to track the movement of each animal (Any-maze, Stoelting, US).

LHb brain slice preparation. Rats (P25-30 or P60-90) and mice (P90) were anesthetized with isoflurane and 10% chloral hydrate, and then perfused with 20 ml ice-cold ACSF (oxygenated with 95 % O₂ + 5% CO₂) containing (mM): 125 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 1 MgCl₂ and 25 Glucose, with 1 mM pyruvate added. The brain was removed as quickly as possible after decapitation and put into chilled and oxygenated ACSF. Coronal slices containing habenular (350µm- and 300µm- thickness for rats and mice, respectively) were sectioned in cold ACSF by a Leica 2000 vibratome and then transferred to ACSF at 32°C for incubation and recovery for 1h and then transferred to room temperature. ACSF was continuously gassed with 95% O₂ and 5% CO₂. Slices were allowed to recover for at least 1 hour before recording.

***In vitro* electrophysiological recording.** For LHb neuron recordings, currents were measured under whole-cell patch clamp using pipettes with a typical resistance of 5–6 MΩ filled with internal solution containing (mM) 105 K-Gluconate, 30 KCl, 4 Mg-ATP, 0.3 Na-GTP, 0.3 EGTA, 10 HEPES and 10 Na-phosphocreatine, with pH set to 7.35. For the biocytin filling, 5mg/ml biocytin was added in the internal solution. The external ACSF solution contained (in mM) 125 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 1 MgCl₂ and 25 Glucose. Cells were visualized with infrared optics on an upright microscope (BX51WI, Olympus). A MultiClamp 700B amplifier and pCLAMP10 software were used for electrophysiology (Axon Instruments). The series resistance and capacitance was compensated

451 automatically after stable Giga seal were formed. The spontaneous neuronal activity was
452 recorded under current-clamp ($I = 0$ pA) for consecutive 60 s. RMP was determined during
453 the silent period of the neuronal spontaneous activity.

454
455 To test TTX ($1\ \mu\text{M}$, Sigma) and BaCl_2 ($100\ \mu\text{M}$, Sigma) effect onto neuronal RMP and
456 spontaneous activity, baselines were recorded for at least for 3 min. Drug were then perfused,
457 the arrival of the drug was precisely indicated with a bubble that was pre-added before the
458 transition from normal ACSF to drug added ACSF. TTX effect on LHb neuronal RMP and
459 spontaneous activity was stabilized after several minutes while BaCl_2 effect on neuronal RMP
460 and spontaneous activity took more than 10 min to stabilize. We thus analyzed the drug effect
461 of TTX and BaCl_2 at the time point of 5min and 15 min respectively.

462
463 **Astrocytic whole-cell patch clamp and Kir4.1 current isolation.** Astrocytes were
464 distinguished from neurons by their small ($5\text{-}10\ \mu\text{m}$) oval-shaped somata and by distinct
465 electrophysiological features: a hyperpolarized RMP and a low input resistance, a linear I-V
466 relationship and an absence of action potentials in response to increased injection currents.
467 BaCl_2 ($100\ \mu\text{M}$, Sigma) were applied to isolate Kir4.1 current which is subtracted from the IV
468 curve recorded from -120mV to 0mV . Biocytin (Sigma) 5mg/ml was dissolved into the
469 patch-clamp pipette solution. After electrophysiological characterization, cells are held for at
470 least 30 minutes in voltage clamp and constantly injected with a hyperpolarization current
471 ($500\ \text{ms}$, $50\ \text{pA}$, $0.5\ \text{Hz}$, $30\ \text{min}$) to allow biocytin filling (performed at $34\ ^\circ\text{C}$). Subsequently,
472 slices were fixed overnight in 4% paraformaldehyde at $4\ ^\circ\text{C}$. The antibodies used were rabbit
473 anti-NeuN ($1:500$, Millipore), Alexa Fluor 546 donkey anti-rabbit IgG ($1:1000$, Invitrogen),
474 Cy2-conjugated Streptavidin ($1:1000$, Jackson ImmunoResearch). Fluorescent image
475 acquisition was performed with a Nikon A1 confocal microscope.

476 **Tri-compartment model.**

477 Tri-compartment model was constructed including the neuron, the astrocyte and the
 478 extracellular space. The model is based on ionic fluxes between three compartments. Na^+ and
 479 K^+ voltage-gated channel, and leak channel were recruited into the neuron as:

480
$$dV_N/dt = (I_{app} - I_K - I_{Na} - I_{Leak,N})/c_N$$

481 where V_N is the neuronal membrane potential, c_N is the neuronal capacitance. I_{app} is the
 482 external current applied to the neuron. I_{Na} and I_K are the fast Na^+ and K^+ currents responsible
 483 for the generation of action potentials; $I_{Leak,N}$ are responsible for the neuronal resting
 484 membrane potential. Kir4.1 channel on the extracellular and vessel side and leak channel
 485 were recruited into the astrocyte as:

486
$$dV_A/dt = (-I_{Kir} - I_{Kir,vess} - I_{Leak,A})/c_A$$

487 where V_A is the astrocytic membrane potential, c_A is the astrocytic capacitance. I_{Kir} are
 488 outward during the resting state, while become inward when K^+ equilibrium potential
 489 exceeds the astrocytic membrane potential. $I_{Kir,vess}$ accounts for the K^+ outflow from the
 490 astrocyte from the vessel side. $I_{Leak,A}$ are responsible for the astrocytic resting membrane
 491 potential.

492 The equations of the K^+ in the three compartments are described by:

493
$$d[K^+]_o/dt = (I_{kir} + I_K)/(F \cdot vol_o) - 2 \cdot (i_{pump,N} + i_{pump,A}) - d_{K_o} \cdot ([K^+]_o - K_{o_o})$$

494
$$d[K^+]_N/dt = -I_K/(F \cdot vol_N) + 2 \cdot i_{pump,N} \cdot vol_o/vol_N - d_{K_N} \cdot ([K^+]_N - K_{N_o})$$

495
$$d[K^+]_A/dt = (-I_{kir} - I_{kir,vess})/(F \cdot vol_A) + 2 \cdot i_{pump,A} \cdot vol_o/vol_A - d_{K_A} \cdot ([K^+]_A - K_{A_o})$$

496 The extracellular K^+ is mainly contributed by I_K . Released extracellular K^+ from the neuron
 497 is taken up by through Kir4.1 channels and Na^+/K^+ pump.

498 The equations of the Na^+ in the three compartments are described by:

499
$$d[Na^+]_o/dt = I_{Na}/(F \cdot vol_o) + 3 \cdot (i_{pump,N} + i_{pump,A}) - d_{Na_o} \cdot ([Na^+]_o - Na_{o_o})$$

500
$$d[Na^+]_N/dt = -I_{Na}/(F \cdot vol_N) - 3 \cdot i_{pump,N} \cdot vol_o/vol_N - d_{Na_N} \cdot ([Na^+]_N - Na_{N_o})$$

501
$$d[Na^+]_A/dt = -3 \cdot i_{pump,A} \cdot vol_o/vol_A - d_{Na_A} \cdot ([Na^+]_A - Na_{A_o})$$

See supplementary information for detailed formulas.

Statistical analyses. Required sample sizes were estimated based on our past experience performing similar experiments. Animals were randomly assigned to treatment groups. Analyses were performed in a manner blinded to treatment assignments in all behavioral experiments. Statistical analyses were performed using GraphPad Prism software v6. By pre-established criteria, values were excluded from the analyses if the viral injection or drug delivering sites were out of LHb. All statistical tests were two-tailed, and significance was assigned at $P < 0.05$. Normality and equal variances between group samples were assessed using the D'Agostino & Pearson omnibus normality test and Brown–Forsythe tests respectively. When normality and equal variance between sample groups was achieved, one-way ANOVAs (followed by Bonferroni's multiple comparisons test), or t test were used. Where normality or equal variance of samples failed, Mann-Whitney U test were performed. Linear regression test, Chi-square test was used inappropriate situations. The sample sizes, specific statistical tests used, and the main effects of our statistical analyses for each experiment are reported in Supplementary Table 1.

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Figure 1 | Kir4.1 is upregulated in LHb of animal models of depression.

a, Volcano plot of high-throughput proteomic screen identifies differentially expressed proteins in habenula of cLH rats. Ln (fold change) was Ln transformed value of the normalized protein ratio of cLH and control. Significance Z score was calculated as the average normalized ratio minus two folds of standard deviation. Proteins in the shaded areas have more than 50% significant change¹¹. Kir4.1 is one of the eight upregulated proteins identified. Dash lines indicate fold change of 50%.

b, c, Western blot analysis showing upregulation of Kir4.1 protein in membrane fraction of habenula of cLH (**b**) and LPS-induced depression rat models (**c**). Tubulin is used as loading control. Numbers in the bars indicate the number of animals used.

d, QPCR analysis of Kir4.1 mRNA in habenula.

e, f, I-V plot and bar graph showing Ba²⁺-sensitive current in cLH rats and their wild type controls at the age of P60-90 (**e**) and P30 (**f**).

g, h, Age-dependent forced swim (FST, **g**) and learned helpless (LHT, **h**) phenotypes of cLH rats. Low number of lever press and high immobility time indicate depressive-like phenotype in P90 cLH rats.

Data are means $\pm$ SEM. *P < 0.05, **P < 0.01, ****P < 0.0001; n.s., not significant (See Supplementary Table 1 for statistical analysis and n numbers).

Figure 2 | Kir4.1 is expressed on astrocytic processes tightly wrapping around neuronal soma in LHB.

a, Immunohistochemistry signals of Kir4.1 envelope neuronal soma as indicated by white arrows.

b, The pan-soma Kir4.1 signals remain intact in LHB of Kir4.1^{ff} floxed mice injected with AAV2/1-CaMKII::EGFP-Cre, but are eliminated when injected with AAV2/5-GFAP::EGFP-Cre (GFAP: human astrocyte-specific GFAP promoter, gfaABC1D).

c, Immunogold electron microscopy of Kir4.1. Red arrows indicate gold signals surrounding a neuronal soma.

d, I-V plots of the Ba²⁺ sensitive Kir4.1 current recorded in LHB astrocytes and neurons, with representative traces shown in up-left and statistic bar graph of current recorded when cells are held at -120mV shown in up-right.

Data are means $\pm$ SEM. ****P < 0.0001 (See Supplementary Table 1 for statistical analysis and n numbers).

Figure 3 | Astrocytic kir4.1 overexpression increases neuronal bursts in LHb and causes depressive-like phenotypes.

a, Schematics of AAV vectors engineered to overexpress Kir4.1 or a GFP control and under a GFAP promoter.

b, Illustration of bilateral viral injection of AAV-GFAP::Kir4.1 in mouse LHb (stained with antibody against GFP and Hoechst).

c, Experimental paradigm for electrophysiology and behavioral testing.

d-f, Astrocytic overexpression of Kir4.1 decreases RMPs of both astrocytes (**d**) and neurons (**e**) and increases the bursting population in neurons (**f**).

g, h, Behavioral effects of expressing various viral constructs in LHb in forced swim test (FST) (**g**) and sucrose preference test (SPT) (**h**).

Data are means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (See Supplementary Table 1 for statistical analysis and n numbers).

Figure 4 | Kir4.1-dependent potassium buffering regulates neuronal RMP and bursting in LHB.
a, d, Changes of neuronal RMPs caused by BaCl₂ (100 μM, **a**) or TTX (1 μM, **d**) in different neuronal types. RMPs are measured at 15min or 5min after perfusion of BaCl₂ or TTX, respectively, when the RMPs stabilize.
b, e, Changes of neuronal RMPs after BaCl₂ (**b**) or TTX (**e**) treatments correlate with original firing rates of tonic-firing neurons (blue) or intra-burst frequencies of bursting neurons (red).
c, Representative trace (left) and bar graph (right) showing BaCl₂ effect (sampled at 15mins after drug perfusion) onto bursting neurons. Spikes in bursting mode are marked in blue. Spikes in tonic-firing mode are marked in black.
f, Example (left) and percentage of LHB neurons transforming from tonic- to burst- firing mode after K_{out} is switched from normal (2.75 mM) to half (1.4mM) (sampled at 1min after drug perfusion).
g, h, Lowering K_{out} to half decreases neuronal RMPs (**g**) and increases bursting neural population (**h**).

Data are means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n.s., not significant (See Supplementary Table 1 for statistical analysis and n numbers).

Figure 5 | Loss of function of Kir4.1 in LHb decreases neuronal bursting and rescues depressive-like phenotypes of cLH rats.

a, Schematics of the AAV vector engineered to overexpress shRNA or dominant negative form of Kir4.1. H1: human H1 promoter. CAG: The CMV early enhancer/chicken beta actin promoter. Three point mutations in the dnKir4.1 and their locations are indicated.

b, Western blot and quantification showing efficient knock-down of Kir4.1 by shRNA in HEK293T cells.

c, Experimental paradigm for behavioral testing of cLH rats infected by virus (top) and illustration of bilateral expression of AAV-GFAP::dnKir4.1 in LHb (stained with antibody against GFP and Hoechst).

d-g, AAV-Kir4.1-shRNA caused a shift of reverse potential from -72mV to -40mV in astrocytes (**d**), depolarization of astrocytes (**e**) and neurons in viral infected region (**f**), and abolished neuronal bursting (**g**). Floating bars for membrane slope conductance in **d** is calculated from the I/V plots (between -120 and +40 mV).

h-k, Behavioral effects of expressing various viral constructs in the LHb of cLH rats in FST (**h**), LHT (**i**, **j**) and SPT (**k**). **j**: Percentage of rats in each category. LH: learned helpless rats with <5 lever presses; NLH, non-learned helpless rats with >10 lever presses.
l, FST immobility is highly correlated with learned helpless phenotype.
m, A model for mechanisms of depression and ketamine treatment at LHb. Upregulation of Kir4.1 on astrocytic processes surrounding neuronal soma leads to enhanced K⁺ buffering at the tight neuron-glia junction, decreased K_{out} and hyperpolarized neuronal RMP. Consequently, de-inactivation of T-VSCCs initiates NMDAR-dependent bursts, causing a stronger output of LHb to trigger depression. Ketamine blockade of NMDARs stops bursts and relieves depression.
Data are means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n.s., not significant (See Supplementary Table 1 for statistical analysis and n numbers).

Extended data

Extended Data Figure 1 | Kir4.1 is unchanged in p30 cLH rats.

a, Western blot analysis showing no change of GFAP protein in habenula of cLH rats at the age of P60-90.

b, Western blot analysis showing no change of Kir4.1 protein in membrane fraction of habenula in cLH rats at the age of P30.

Data are means $\pm$ SEM. n.s., not significant (See Supplementary Table 1 for statistical analysis and n numbers).

Extended Data Figure 2 | Ba-sensitive Kir4.1 current is upregulated in LHb of adult cLH and LPS-induced depressive-like animals.

a, c, Representative traces showing linear I-V curve in a typical astrocyte before (upper) and after (middle) Ba²⁺ perfusion under voltage steps (-130mV to -30 mV, step by 10mV, 2s duration, holding at -70mV). Subtraction of the two led to Ba-sensitive Kir current (bottom) in P60-90 (**a**) and P30 (**c**) cLH rats.

b, d, I-V plot of astrocytes in cLH rats and SD controls at the age of P60-90 (**b**) and P30 (**d**).

e, LPS injection (500 µg/kg, i.p., for 7 days) induces increased immobility time and decreased latency to immobility in FST.

f, I-V plot and bar graph showing Ba²⁺-sensitive current in LPS injected Wistar rats and their saline controls at the age of P60-90.

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Extended Data Figure 3 | Biocytin intercellular filling and double staining with NeuN confirm the identity of electrophysiologically identified neurons and astrocytes.

a, b, A neuron (**a**) and an astrocyte (**b**) in LHb slices are first identified based on their specific morphology (astrocytes: 5-10μm diameter; neuron: ~15μm diameter) and physiological properties. The neuron fires at a depolarizing voltage step (**a**), whereas the astrocyte shows a steady-state I-V relationship and a lack of spiking activity (**b**). After electrophysiological characterization, cells are held for at least 30 minutes in voltage clamp and constantly injected with a hyperpolarization current (500ms, 50pA, 0.5Hz, 30min) to allow biocytin filling.

c-h, Biocytin labeled neurons and astrocytes subsequently confirmed by co-labeling with NeuN.

c, d, Biocytin signals in a single neuron (**c**) or a group of astrocytes due to diffusion through gap junctions (**d**).

e, f, NeuN signals.

g, h, Colabeling of NeuN with the neuron (indicated by white arrow, **g**) but not astrocytes (**h**).

Note that all biocytin filled neurons (n = 18) show colabel with NeuN and all biocytin filled astrocytes (n = 11) do not colabel with NeuN.

Extended Data Figure 4 | Expression pattern of Kir4.1 in LHb and hippocampus.

a, b, Kir4.1 co-immunostaining with neuronal marker (NeuN) or astrocytic marker (S100b and GFAP) in LHb (**a**) or hippocampus (**b**). Bottom two panels are staining with the same kir4.1 antibody pre-incubated with the antigen peptide, demonstrating the specificity of Kir4.1 antibody.

Extended Data Figure 5 | Electron microscopy immunohistochemistry of Kir4.1 staining.

a-c, Many Kir4.1 immunograins (arrows) surround the neuronal soma (**a**, **b**) and the vascular endothelial cell (**c**).

d, The Kir4.1 grains (arrows) also surround the axon-dendritic synapses, but are rare near the synaptic zones as indicated by the postsynaptic densities (arrowheads).

e, Insert shows Kir4.1 immunograins near a gap junction.

s: neuronal soma; t: axon terminal. Scale bars= 0.5 μ m.

Extended Data Figure 6 | Kir4.1 is expressed in astrocytes but not neurons in LHb.

a, Schematics showing sequence of drug application and recording after a neuron or astrocyte is patched.

b, Representative traces showing a linear I-V curve in a typical astrocyte under voltage steps (-130mV to -30 mV, step by 10mV, 2s duration, holding at -70mV, protocol demonstrated on left) (upper panel). I-V curves of the same cell after addition of TTX (1uM), ZD7288 (50uM) and 4AP (1mM, middle), and further addition of Ba²⁺ (100uM, bottom) are shown below.

c, Representative traces showing a non-linear I-V curve in a typical neuron under voltage steps (-130mV to -30 mV, step by 10mV, 2s duration, holding at -70mV, protocol demonstrated on left) (upper panel). I-V curves of the same cell after addition of TTX, ZD7288 and 4AP (middle), and further addition of Ba²⁺ (bottom) are shown below.

Extended Data Figure 7 | Characterization of cell-type specificity of GFAP promoter driven expression of Kir4.1 in mouse LHb

Double immunofluorescence for mouse NeuN (red) and GFP (green) in the coronal section of LHb brain slices infected with AAV-GFAP::Kir4.1 (AAV2/5-gfaABC1D-EGFP-Kir4.1) virus. Left are examples of anterior, middle and posterior coronal section of LHb. Numbers in the bottom right corner are the number of merged cells/ number of NeuN+ cells in the viral-infected area. Right are zoom-in images of the white square area in left. Note that there is only one infected neuron, as indicated by the white arrow, in all three fields of view.

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Extended Data Figure 9 | Simulation of the dynamic potassium buffering effect of Kir4.1 in the tri-compartment model.

a, Schematic representation of a tri-compartment model involving neuron, astrocyte and extracellular space in between.

b-d, Effects of increasing Kir4.1 expression level on extracellular K^+ concentration (**b**), neuronal membrane potential (**c**) and astrocytic membrane potential (**d**). Ctrl: control condition with 1x Kir4.1 conductance; Depr: depression condition with 2x Kir4.1 conductance. Grey shaded areas indicate application of 10 Hz tonic stimulation to neurons. Note that under this neuronal firing condition, Kout is lower, and neuron and astrocyte are more hyperpolarized in Depr than in Ctrl.

e-g, Effects of *in silico* TTX (blocking APs, $g_{Na} = 0$) or Ba^{2+} (blocking Kir4.1, $g_{Kir4.1} = 0$) treatments on extracellular K^+ concentration (**e**), neuronal membrane potential (**f**) and astrocytic membrane potential (**g**) when neurons are under 10 Hz tonic stimulation. Grey shaded areas indicate *in silico* application of drugs. Note that TTX and Ba^{2+} cause opposite changes to Kout, neuronal membrane potential and astrocytic membrane potential.

Neuronal spikes are not shown for clarity of presentation.

Extended Data Figure 10 | BaCl₂ caused depolarization of neuronal RMP in presence of synaptic transmitter blockers.

a, b, Representative trace (a) and bar graph (b) showing effect of BaCl₂ (100uM) perfusion onto tonic-firing neurons which have been bathed with transmitter blockers (100 uM picrotoxin, 10 uM NBQX and 100 uM AP5).

c, Bar graph showing the level of RMP depolarization caused by BaCl₂ in presence or absence of transmitter blockers.

Data are means $\pm$ SEM. ***P < 0.001, n.s., not significant (See Supplementary Table 1 for statistical analysis and n numbers)

Extended Data Figure 11 | Characterization of Kir4.1 loss-of-function constructs.

a, Flag-tagged-Kir4.1 plasmid (pAAV-CMV-betaGlobin-Kir4.1-eGFP-3Flag) was co-transfected with pAAV-vector expressing 6 different shRNAs (see Methods) of Kir4.1 or the negative control (shRNA of luciferase) into HEK293TN cell. Based on the knock-down efficiency as shown in the western blot, Kir4.1-shRNA-5 was chosen for viral package.

b, I-V plot showing Kir4.1 currents recorded in HEK293 cells transfected with pAAV-Kir4.1 together with negative control pAAV-GFP or pAAV-dnKir4.1 plasmid. Bars represent the current values recorded at -160mV.

c, I-V plot and bar graph showing Ba2+-sensitive currents blocked by AAV-dnKir4.1 in both cLH and SD rats.

d-f, AAV-dnKir4.1 caused a depolarization of RMP in astrocytes (d) and neurons in viral infected area (e), and abolished neuronal bursting (f) in both cLH and SD rats.

Data are means $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (See Supplementary Table 1 for statistical analysis and n numbers).

Extended Data Figure 12 | Characterization of cell-type specificity of GFAP promoter driven expression of dnKir4.1 in rat LHb.

Double immunofluorescence for rat NeuN (red) and GFP (green) in the coronal section of LHb brain slices infected with AAV-GFAP::dnKir4.1 (AAV2/5-gfaABC1D-dnKir4.1-2A-eGFP) virus. Left are examples of anterior, middle and posterior coronal section of LHb. Numbers in the bottom right corner are the number of merged cells/ number of NeuN+ cells in the viral-infected area. Right are zoom-in images of the white square area in left. Note that there is only one infected neuron, as indicated by the white arrow, in all three fields of view.

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	Value	Unit	Parameter
R	8.31	$\text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$	Gas constant
T	308	K	Absolute temperature
F	96485	$\text{C} \cdot \text{mol}^{-1}$	Faraday constant
dt	0.1	ms	Step time
c_N	30	pF	Neuronal capacitance
c_A	1	pF	Astrocytic capacitance
g_K	20	nS	Neuronal K^+ channel conductance
g_{Na}	120	nS	Neuronal Na^+ channel conductance
$g_{\text{Leak,N}}$	0.5	nS	Neuronal leak conductance
$V_{\text{Leak,N}}$	$\square 50$	mV	Neuronal leak potential
$i_{\text{max,N}}$	0.12	$\text{mM} \cdot \text{ms}^{-1}$	Neuronal Na^+/K^+ pump rate
g_{Kir}	12	nS	Astrocytic Kir4.1 channel conductance on extracellular side
$g_{\text{Kir_vess}}$	12.5	nS	Astrocytic Kir4.1 channel conductance on vessel side
$g_{\text{Leak,A}}$	0.1	nS	Astrocytic leak conductance
$V_{\text{Leak,A}}$	$\square 40$	mV	Astrocytic leak potential
$i_{\text{max,A}}$	0.12	$\text{mM} \cdot \text{ms}^{-1}$	Astrocytic Na^+/K^+ pump rate
V_h	178.46	mV	Kir4.1 channel parameter
V_s	119.47	mV	Kir4.1 channel parameter
$V_{\text{rest,A}}$	-10	mV	Kir4.1 channel parameter
km_K	3.0	mM	Na^+/K^+ pump parameter
km_{Na}	10.	mM	Na^+/K^+ pump parameter
V_N	$\square 63.76$	mV	Initial neuronal membrane potential
V_A	$\square 87.08$	mV	Initial astrocyte membrane potential
$[\text{K}^+]_N$	135.04	mM	Initial neuronal K^+ concentration
$[\text{K}^+]_o$	3.34	mM	Initial extracellular K^+ concentration
$[\text{K}^+]_A$	135.00	mM	Initial astrocytic K^+ concentration
$[\text{Na}^+]_N$	11.16	mM	Initial neuronal Na^+ concentration

$[\text{Na}^+]_o$	145.95	mM	Initial extracellular Na^+ concentration
$[\text{Na}^+]_A$	7.00	mM	Initial astrocytic Na^+ concentration
Vol_o	0.01	pL	Extracellular volume
Vol_N	1.08	pL	Neuronal volume
Vol_A	1.08	pL	Astrocytic volume
d_{K_N}	0.0001	ms^{-1}	K^+ effective flux rate in the neuron
d_{K_O}	0.001	ms^{-1}	K^+ effective flux rate in the extracellular space
d_{K_A}	0.1	ms^{-1}	K^+ effective flux rate in the astrocyte
d_{Na_N}	0.0001	ms^{-1}	Na^+ effective flux rate in the neuron
d_{Na_O}	0.01	ms^{-1}	Na^+ effective flux rate in the extracellular space
d_{Na_A}	0.01	ms^{-1}	Na^+ effective flux rate in the astrocyte
$[\text{K}^+]_{N_0}$	135	mM	Neuronal potassium concentration of the effective flux
$[\text{K}^+]_{O_0}$	3.5	mM	Extracellular potassium concentration of the effective flux
$[\text{K}^+]_{A_0}$	135	mM	Astrocytic potassium concentration of the effective flux
$[\text{Na}^+]_{N_0}$	12.0	mM	Neuronal sodium concentration of the effective flux
$[\text{Na}^+]_{O_0}$	144	mM	Extracellular sodium concentration of the effective flux
$[\text{Na}^+]_{A_0}$	7.0	mM	Astrocytic sodium concentration of the effective flux

8 Model description

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10 The mathematical description of I_K and I_{Na} were achieved through a Hodgkin-Huxley style
 11 derivation of forward and backward rate equations^{1,2}:

$$I_K = g_K \cdot n^4 \cdot (V_n - E_{Kn})$$

$$I_{Na} = g_{Na} \cdot m^3 \cdot h \cdot (V_n - E_{Nan})$$

12 where g_K and g_{Na} are the conductance of fast Na^+ and K^+ channel; E_{Kn} and E_{Nan} are the
 13 neuronal equilibrium potential for K^+ and Na^+ . The gate variables m , h , and n are dimensionless
 14 activation and inactivation variables, which describe the activation and inactivation processes of
 15 the sodium and potassium channels, each of which is governed by the following differential
 16 equations:

$$dn/dt = \alpha_n(V_n) \cdot (1 - n) - \beta_n(V_n) \cdot n$$

$$dm/dt = \alpha_m(V_n) \cdot (1 - m) - \beta_m(V_n) \cdot m$$

$$dh/dt = \alpha_h(V_n) \cdot (1 - h) - \beta_h(V_n) \cdot h$$

17 where the forward and the backward rate α and β describe the transition between the closed and
 18 open state of gate. The function of α and β are given by:

$$\alpha_n(V_n) = 0.01 \cdot (V_n + 55) / (1 - \exp[(-V_n - 55)/10])$$

$$\beta_n(V_n) = 0.125 \cdot \exp[(-V_n - 65)/80]$$

$$\alpha_m(V_n) = 0.1 \cdot (V_n + 40) / (1 - \exp[(-V_n - 40)/10])$$

$$\beta_m(V_n) = 4 \cdot \exp[(-V_n - 65)/18]$$

$$\alpha_h(V_n) = 0.07 \cdot \exp[(-V_n - 65)/20]$$

$$\beta_h(V_n) = 1 / (\exp[(-V_n - 35)/10] + 1)$$

19 The neuronal membrane potential is highly dependent on the neuronal equilibrium potential for K^+
 20 and Na^+ , which are given by the Nernst equations:

$$E_{Kn} = R \cdot T / F \cdot \ln([K^+]_O / [K^+]_N)$$

$$E_{Nan} = R \cdot T / F \cdot \ln([Na^+]_O / [Na^+]_N)$$

21 where $[K^+]_N$ and $[K^+]_O$ are the neuronal and extracellular K^+ concentration; $[Na^+]_N$ and
 22 $[Na^+]_O$ are the neuronal and extracellular Na^+ concentration; R , T and F are the gas constant,
 23 absolute temperature and faraday constant.

24 Neuronal leak current is described by:

$$I_{\text{Leak,N}} = g_{\text{Leak,N}} \cdot (V_n - E_{\text{Leak,N}})$$

25 where $g_{\text{Leak,N}}$ is the neuronal leak conductance and $E_{\text{Leak,N}}$ is the neuronal leak potential.

26 The formula of Kir4.1 channel was modified from a previous model³:

$$I_{\text{Kir}} = g_{\text{Kir}} \cdot \sqrt{[K^+]_o} / (1 + \exp((V_A + V_{\text{restA}} - E_{\text{Ka}} + V_h)/V_s)) (V_A + V_{\text{restA}} - E_{\text{Ka}})$$

$$I_{\text{Kir,vess}} = g_{\text{Kir,vess}} \cdot \sqrt{[K^+]_{O,0}} / (1 + \exp((V_A + V_{\text{restA}} - E_{\text{Ka,vess}} + V_h)/V_s)) (V_A + V_{\text{restA}} - E_{\text{Ka,vess}})$$

27 where g_{Kir} and $g_{\text{Kir,vess}}$ are the conductance of Kir4.1 channel on the extracellular and vessel

28 side respectively.

29 Astrocytic equilibrium potential for K^+ and Na^+ are given by:

$$E_{\text{Ka}} = R \cdot T/F \cdot \ln([K^+]_o/[K^+]_A)$$

$$E_{\text{Naa}} = R \cdot T/F \cdot \ln([Na^+]_o/[Na^+]_A)$$

30 where $[K^+]_A$ and $[Na^+]_A$ are the astrocytic K^+ and Na^+ concentration.

31 The astrocyte membrane potential equation also comprises a leak term:

$$I_{\text{Leak,A}} = g_{\text{Leak,A}} \cdot (V_A - E_{\text{Leak,A}})$$

32 The equation of the Na^+/K^+ pump depends on the extracellular K^+ and intracellular Na^+

33 concentrations as:

$$i_{\text{pump,N}} = i_{\text{max,N}} \cdot (1 + km_k/[K^+]_o)^{-2} \cdot (1 + km_{Na}/[Na^+]_N)^{-3}$$

$$i_{\text{pump,A}} = i_{\text{max,A}} \cdot (1 + km_k/[K^+]_o)^{-2} \cdot (1 + km_{Na}/[Na^+]_A)^{-3}$$

34 where $i_{\text{max,N}}$ and $i_{\text{max,A}}$ are the Na^+/K^+ pump rate for neuron and astrocyte respectively.

35 The kinetics of ion concentrations are calculated as:

$$d[K^+]_o/dt = (I_{\text{Kir}} + I_K)/(F \cdot \text{Vol}_o) - 2i_{\text{pump,A}} - 2i_{\text{pump,N}} - d_{K,O} \cdot ([K^+]_o - [K^+]_{O,0})$$

$$d[K^+]_N/dt = -I_K/(F \cdot \text{Vol}_N) + 2i_{\text{pump,N}} \cdot \text{Vol}_O/\text{Vol}_N - d_{K,N} \cdot ([K^+]_N - [K^+]_{N,0})$$

$$d[K^+]_A/dt = (-I_{\text{Kir}} - I_{\text{Kir,vess}})/(F \cdot \text{Vol}_A) + 2i_{\text{pump,A}} \cdot \text{Vol}_O/\text{Vol}_A - d_{K,A} \cdot ([K^+]_A - [K^+]_{A,0})$$

$$d[Na^+]_o/dt = I_{Na}/(F \cdot \text{Vol}_o) + 3i_{\text{pump,A}} + 3i_{\text{pump,N}} - d_{Na,O} \cdot ([Na^+]_o - [Na^+]_{O,0})$$

$$d[Na^+]_N/dt = -I_{Na}/(F \cdot \text{Vol}_N) - 3i_{\text{pump,N}} \cdot \text{Vol}_O/\text{Vol}_N - d_{Na,N} \cdot ([Na^+]_N - [Na^+]_{N,0})$$

$$d[Na^+]_A/dt = -3i_{\text{pump,A}} \cdot \text{Vol}_O/\text{Vol}_A - d_{Na,A} \cdot ([Na^+]_A - [Na^+]_{A,0})$$

36 where Vol_O , Vol_N and Vol_A are the volumes of the extracellular, neuronal and astrocytic

37 compartments. The term $d \cdot ([X] - [X_0])$ accounts for the diffusion effect, where d is the rate of this

38 effective flux, $[X]$ is the ionic concentration and $[X_0]$ is the equilibrium concentration.

39 Simulations were solved numerically with an explicit Runge-Kutta Prince-Dormand 8th-9th order
40 method with the GSL library in cython and python. The parameters used in the model are
41 presented in Supplementary Table 1.
42

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