Astroglial Kir4.1 in the lateral habenula drives neuronal bursts in depression

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Enhanced bursting activity of neurons in the lateral habenula (LHb) is essential in driving depression–like behaviours, but the cause of this increase has been unknown. Here, using a high-throughput quantitative proteomic screen, we show that an astroglial potassium channel (Kir4.1) is upregulated in the LHb in rat models of depression. Kir4.1 in the LHb shows a distinct pattern of expression on astrocytic membrane processes that wrap tightly around the neuronal soma. Electrophysiology and modelling data show that the level of Kir4.1 on astrocytes tightly regulates the degree of membrane hyperpolarization and the amount of bursting activity of LHb neurons. Astrocyte-specific gain and loss of Kir4.1 in the LHb bidirectionally regulates neuronal bursting and depression–like symptoms. Together, these results show that a glia–neuron interaction at the perisomatic space of LHb is involved in setting the neuronal firing mode in models of a major psychiatric disease. Kir4.1 in the LHb might have potential as a target for treating clinical depression.

A major breakthrough in neuroscience was the discovery that astrocytes interact intimately with neurons to support and regulate essential functions and thereby foster information processing in the brain1–8. Many investigations have focused on astroglia–neuron interactions at tripartite synapses, where astrocyte processes wrap tightly around presynaptic and postsynaptic sites9. By contrast, less attention has been placed on astroglia–neuron interactions in proximity to the neuronal soma. In particular, how astrocytes regulate the intrinsic firing patterns of neurons, and what structural basis may underlie this regulation, have been much less explored.

Despite the surging interest in the role of the LHb in depression10–18, only limited attention has been given to the influence of astrocyte dysfunction in the LHb on the aetiology of depression19. In an accompanying paper, we show that the bursting activity of LHb neurons is greatly enhanced in animal models of depression20. LHb bursting drives depressive-like behaviours and is a prominent target of the rapid antidepressant ketamine20. However, the cause of this enhanced bursting by LHb neurons remains unknown.

LHb Kir4.1 is upregulated in depression

We used an unbiased, high-throughput, quantitative proteomic screen to compare habenular protein expression between congenitally learned helpless (cLH)21 and wild-type Sprague-Dawley rats. Kir4.1 was highly upregulated in the habenulae of cLH rats22 (1.69-fold of wild-type, Extended Data Fig. 1a). Western blot analysis confirmed that Kir4.1 was significantly increased (1.44-fold, P = 0.009) in the membrane protein fraction of habenulae from cLH rats (Fig. 1a). By contrast, another astrocyte-specific protein, glial fibrillary acidic protein (GFAP), showed no change in expression (Extended Data Fig. 1b), indicating that there was no astrogliosis.

To test whether Kir4.1 upregulation is universal in depression, we examined rats in which depression was induced by treatment with lipopolysaccharide (LPS)22. One week of LPS injection (0.5 mg kg−1 per day, intraperitoneal (i.p.)) was sufficient to cause strong depressive-like phenotype in three-month-old Wistar rats in the forced swim test (FST; Extended Data Fig. 1c)22 and sucrose preference test (SPT)22. Rats with LPS-induced depression also showed a significant increase in Kir4.1 in the habenula (1.87-fold, P < 0.0001, Fig. 1b). Quantitative real-time PCR revealed an increase (1.2-fold) in Kir4.1 (also known as Kcnj10) mRNA level in habenulae from cLH rats (Extended Data Fig. 1d), suggesting that at least part of the change in protein level is due to transcriptional upregulation.

Kir4.1 is a principal component of the glial Kir channel and is largely responsible for mediating the K+ conductance and setting the membrane hyperpolarization and the amount of bursting activity of LHb neurons. Astrocyte–specific gain and loss of Kir4.1 in the LHb bidirectionally regulates neuronal bursting and depression–like symptoms. Together, these results show that a glia–neuron interaction at the perisomatic space of LHb is involved in setting the neuronal firing mode in models of a major psychiatric disease. Kir4.1 in the LHb might have potential as a target for treating clinical depression.

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Figure 1 | Kir4.1 is upregulated in the LHb in rat models of depression. a, b, Western blot analysis showing upregulation of Kir4.1 protein in membrane fraction of habenulae of cLH rats (n = 9, 9 rats for WT and cLH, respectively, a) and rats with LPS-induced depression (n = 6, 6, b). c, d, I–V plot and bar graph showing Ba2+–sensitive currents in cLH rats and wild-type controls at the age of P60–90 (c) and P30 (d). e, f, Age-dependent FST (e) and LHT (f) phenotypes of cLH rats. n = 8, 8 rats for P30, n = 10, 10; 6, 6 rats for P90 FST and LHT, respectively. Data are means ± s.e.m. *P < 0.05, ***P < 0.001, ****P < 0.0001; NS, not significant. Two-tailed paired t-test (a, b), two-tailed unpaired t-test (c, e, f) and Mann–Whitney test (d).
implicated in buffering excess extracellular K

As an inwardly rectifying K channel, Kir4.1 has been strongly implicated in buffering extracellular K in tripartite synapses. The conventional model of K’ buffering suggests that Kir4.1 is highly expressed in astrocytic endfeet surrounding synapses. Unexpectedly, immunohistochemical staining for Kir4.1 in the LHb appeared to overlap with the neuronal marker NeuN at low magnification (20×; Extended Data Fig. 4a), although in the same brain slice Kir4.1 staining in the hippocampus showed a typical astrocytic appearance (Extended Data Fig. 4b). However, higher magnification imaging with single-layer scanning (0.76 μm per layer) revealed that Kir4.1 staining enveloped NeuN signals (Fig. 2a). To confirm that Kir4.1 was located within astrocytes but not neurons in the LHb, we separately knocked it out in either neurons or astrocytes by injecting AAV viruses expressing the Cre recombinase under either the neuronal (Fig. 2f) or astrocytic (Fig. 2g) promoter. Behavioural testing and cLH rats did not yet show depression-like phenotypes in either the FST (Extended Data Fig. 1e) or SPT (Extended Data Fig. 1f), indicating that it represents the K’-sensitive currents in LHb astrocytes (Extended Data Fig. 2a–d). Biocytin filling of astrocytes from cLH or wild-type rats. Astrocytes were distinguished from neurons via the absence of NeuN co-staining confirmed that cells fitting the pattern of Kir4.1 staining, but astrocytic knockout completely blocked Kir4.1 expression in astrocytic processes that wrap tightly around neuronal somata and synapses in the LHb. Whole-cell patch-clamp recordings from either astrocytes or neurons surrounding the virally infected region showed that Kir4.1 is highly expressed in astrocytes, as indicated by the upregulation of Kir4.1 (Kir4.1) in mouse LHb (stained with antibody against GFP and Hoechst) (four independent experiments). B, Experimental paradigm for electrophysiological and behavioural testing. C–E. Astrocytic overexpression of Kir4.1 decreases RMPs of both astrocytes (n = 10, 8 astrocytes from 4, 3 mice for eGFP and Kir4.1, respectively, c) and neurons (n = 26, 29 neurons from 6, 6 mice, d) and increases the bursting population in neurons (e). F, g, Behavioural effects of expression of various viral constructs in the LHb in the FST (n = 7, 8 mice, f) and SPT (n = 6, 8 mice, g). Data are means ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Two-tailed unequal t-test (c, d, f, g) and χ² test (e).

resting membrane potential (RMP) in astrocytes. To confirm that Kir4.1 function is indeed upregulated in rat models of depression, we performed whole-cell patch-clamp recordings onto LHb astrocytes from cLH or wild-type rats. Astrocytes were distinguished from neurons by their small (5–10 μm) soma and electrophysiological features (Extended Data Fig. 2a–d; see Methods). Biocytin filling and the absence of NeuN co-staining confirmed that cells fitting the above criteria were indeed astrocytes (Extended Data Fig. 3). We then bath-applied Ba²⁺ (BaCl₂, 100 μM), which selectively blocks Kir channels at sub-millimolar concentrations, to isolate Kir4.1 currents (Extended Data Fig. 2a, c). The Ba²⁺-sensitive current displayed a reversal potential close to the equilibrium potential of K⁺ (−90 mV) (Fig. 1c, d), indicating that it represents the K’ conductance. We found that Ba²⁺-sensitive currents in LHb astrocytes were almost doubled in astrocytes from cLH (Fig. 1c) and LPS-treated rats (Extended Data Fig. 2f) at postnatal days (P60–90).

Notably, the increase in Kir4.1 current and protein level was not obvious in LHb rats at P30 (Fig. 1d, Extended Data Fig. 1e). At this age, LHb rats did not yet show depression-like phenotypes in either the FST (Fig. 1e) or the learned helplessness test (LHT; Fig. 1f), suggesting that the upregulation of Kir4.1 is concomitant with the developmental onset of the depression-like symptoms.

**Kir4.1 on astrocytes encircles neuronal soma**

As an inwardly rectifying K’ channel, Kir4.1 has been strongly implicated in buffering extracellular K’ in tripartite synapses. The conventional model of K’ buffering suggests that Kir4.1 is highly expressed in astrocytic endfeet surrounding synapses. Unexpectedly, immunohistochemical staining for Kir4.1 in the LHb appeared to overlap with the neuronal marker NeuN at low magnification (20×; Extended Data Fig. 4a), although in the same brain slice Kir4.1 staining in the hippocampus showed a typical astrocytic appearance (Extended Data Fig. 4b). However, higher magnification imaging with single-layer scanning (0.76 μm per layer) revealed that Kir4.1 staining enveloped NeuN signals (Fig. 2a). To confirm that Kir4.1 was located within astrocytes but not neurons in the LHb, we separately knocked it out in either neurons or astrocytes by injecting AAV viruses expressing the Cre recombinase under either the neuronal (Fig. 2f) or astrocytic (Fig. 2g) promoter. Behavioural testing and cLH rats did not yet show depression-like phenotypes in either the FST (Extended Data Fig. 1e) or SPT (Extended Data Fig. 1f), indicating that it represents the K’-sensitive currents in LHb astrocytes (Extended Data Fig. 2a–d). Biocytin filling of astrocytes from cLH or wild-type rats. Astrocytes were distinguished from neurons via the absence of NeuN co-staining confirmed that cells fitting the pattern of Kir4.1 staining, but astrocytic knockout completely blocked Kir4.1 expression in astrocytic processes that wrap tightly around neuronal somata and synapses in the LHb. Whole-cell patch-clamp recordings from either astrocytes or neurons surrounding the virally infected region showed that Kir4.1 is highly expressed in astrocytes, as indicated by the upregulation of Kir4.1 (Kir4.1) in mouse LHb (stained with antibody against GFP and Hoechst) (four independent experiments). B, Experimental paradigm for electrophysiological and behavioural testing. C–E. Astrocytic overexpression of Kir4.1 decreases RMPs of both astrocytes (n = 10, 8 astrocytes from 4, 3 mice for eGFP and Kir4.1, respectively, c) and neurons (n = 26, 29 neurons from 6, 6 mice, d) and increases the bursting population in neurons (e). F, g, Behavioural effects of expression of various viral constructs in the LHb in the FST (n = 7, 8 mice, f) and SPT (n = 6, 8 mice, g). Data are means ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Two-tailed unequal t-test (c, d, f, g) and χ² test (e).

**Figure 2 | Kir4.1 is expressed on astrocytic processes that wrap tightly around neuronal somata in LHb.** 

A, Immunohistochemistry signals of Kir4.1 envelope neuronal somata as indicated by white arrows (three independent experiments). B, The pan-soma Kir4.1 signals remain intact in the LHb of Kir4.1 floxed mice injected with AAV-CaMKIIa:eGFP-Cre, but are eliminated in mice injected with AAV-GFP:eGFP-Cre (three independent experiments). C, Immunogold electron microscopy of Kir4.1. Red arrows indicate gold signals surrounding a neuronal soma (three independent experiments). D, I–V plots of the Ba²⁺-sensitive Kir4.1 current recorded in LHb astrocytes and neurons. Top left, representative resting membrane potential (RMP) in astrocytes

Kir4.1 gain-of-function causes depression

To test the consequences of Kir4.1 upregulation in the LHb, we used adeno-associated viruses of the 2/5 serotype (AAV2/5), which preferentially target astrocytes, together with the human GFA (gfaABC1D) promoter to deliver eGFP-tagged Kir4.1 channel (AAV-GFA::Kir4.1) or eGFP alone (AAV-GFA::eGFP) as a control (Fig. 3a). Twenty-one days after bilateral injection into the LHb at P50, AAV2/5-mediated viral transfection led to expression of Kir4.1 and eGFP in astrocytes throughout the LHb (Fig. 3a). The specificity of the viral infection in astrocytes was verified by co-immunostaining of NeuN and eGFP: only 0.3% of NeuN⁺ cells (n = 2,668) were infected by this virus (Extended Data Fig. 7a). We made whole-cell patch-clamp recordings from either astrocytes or neurons surrounding the virally transfected astrocytes in coronal LHb slices (Fig. 3b–e). The RMPs of
BaCl$_2$ after perfusion with BaCl$_2$ rats, and tonic, respectively, firing of LHb neurons? We hypothesize that within the highly con of bursting firing (4 of 9 neurons; or a prolonged plateau potential within a burst and eventually cessation of neuronal activity (5 of 9 neurons; firing to burst-firing mode after [K]$_{out}$ is reduced from normal (2.75 mM) by 50% (to 1.4 mM). g, h. Reducing [K]$_{out}$ by 50% decreases neuronal RMPs (n = 15 neurons from 4 rats, sampled 1 min after change in [K]$_{out}$ g) and increases bursting neuronal population (n = 33, 40 neurons from 4, 6 rats for normal and reduced [K]$_{out}$, respectively). h. Data are means ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Two-tailed paired t-test (a, c, d, g), χ$^2$ test (b) and linear regression test (b, e).

Figure 4 | Kir4.1-dependent potassium buffering regulates neuronal RMP and bursting in LHb. a, d. Changes in neuronal RMPs caused by BaCl$_2$ (100 µM, n = 7, 11, 12 neurons from 3, 3, 3 rats for silent, bursting and tonic, respectively, a) or TTX (1 µM, n = 13, 9, 9 neurons from 2, 2, 2 rats, d) in different neuronal types. RMPs were measured 15 min or 5 min after perfusion with BaCl$_2$ or TTX, respectively, when the RMPs had stabilized. b, e. Changes in neuronal RMPs after BaCl$_2$ (n = 14, 9 neurons from 3, 3 rats for tonic and burst group, respectively) or TTX (n = 9, 9 neurons from 2, 2 rats, e) treatment 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), 9 neurons from both astrocytes and neurons were more hyperpolarized (Fig. 3c, d) and the percentage of bursting neurons was significantly higher (Fig. 3e, P < 0.0001) in mice infected with AAV-GFAP::Kir4.1 than in mice infected with AAV-GFAP::eGFP.

We then assayed depression-like phenotypes and found that mice with AAV-GFAP::Kir4.1 infection in the LHb displayed severe depression-like behaviors, including increased immobile duration and decreased latency to immobility in the FST (Fig. 3f), and decreased sucrose preference in the SPT (Fig. 3g), while general locomotion was unchanged (Extended Data Fig. 7c, d). Kir4.1 regulates neuronal RMP and bursting How does an astrocytic K$^+$ channel regulate the RMP and burst firing of LHb neurons? We hypothesize that within the highly confined extracellular space between neuronal somata 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 spatial buffering of K$^+$, resulting in increased extracellular K$^+$ ([K]$_{out}$), and according to the Nernst equation, depolarized neuronal RMPs (modelled in Extended Data Fig. 8). Consistent with this prediction, blockade of Kir4.1 by bath perfusion with BaCl$_2$ for about 10 min depolarized the RMPs of LHb neurons (Fig. 4a). The degree of change 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 makes to its RMP. A similar amount of RMP change was induced when BaCl$_2$ was applied in the presence of synaptic transmission blockers (picrotoxin, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoline-7-sulfonamide (NBQX) and 2-amino-5-phosphonopentanoic acid (AP5); Extended Data Fig. 9a–c), suggesting that Kir4.1-dependent regulation of RMP in LHb neurons occurs mostly at neuronal cell bodies instead of at synapses. Consequent to the RMP change and accumulation of [K]$_{out}$, perfusion with BaCl$_2$ caused either a shift in the spiking pattern from bursting to tonic firing and eventually to cessation of neuronal activity (5 of 9 neurons; Fig. 4c), or a prolonged plateau potential within a burst and eventually cessation of bursting firing (4 of 9 neurons; Extended Data Fig. 9d).

By contrast, upregulation of Kir4.1 or prevention of neuronal firing should decrease [K]$_{out}$ and hyperpolarize neuronal RMPs. Indeed, overexpression of Kir4.1 in astrocytes (Fig. 3d) or blockage of neuronal action potentials by tetrodotoxin (TTX; Fig. 4d, e) caused hyperpolarization of LHb neurons. Overexpression of Kir4.1 in astrocytes also increased neuronal bursting (Fig. 3e). Finally, to assess a causal relationship between [K]$_{out}$ and firing mode, we made current-clamp recordings from LHb neurons while lowering [K]$_{out}$ by 50% (from 2.75 mM to 1.4 mM, Fig. 4f). This led to a reduction in neuronal RMP of 13.7 ± 0.5 mV (Fig. 4g) and a direct shift of originally tonic-firing neurons (8 out of 15) into bursting mode. Consequently, the percentage of bursting neurons was increased from 8% to 23% (Fig. 4h). Thus, by increasing astrocytic Kir4.1 expression or decreasing extracellular K$^+$ concentration, we were able to phenocopy in wild-type 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 extracellular K$^+$ clearance resulting from overexpression of Kir4.1 might underlie the neuronal hyperpolarization required for burst initiation.

Kir4.1 loss-of-function rescues depression To determine whether loss-of-function of Kir4.1 in the LHb could reduce neuronal bursts and reverse depressive phenotypes, we devised two strategies. We used AAV2/5 viral vectors to express either a short hairpin RNA (shRNA) to knock down the level of Kir4.1, or a dominant-negative form of Kir4.1 (dnKir4.1) (Extended Data Fig. 10a) for viral packaging. To avoid an off-target effect of the shRNA, we also used a dominant-negative construct to block its function, in the LHb of cLH rats (Fig. 5a). We tested six shRNAs that specifically targeted the Kir4.1 transcript in cell culture and chose the one with highest knockdown efficiency (Fig. 5b and Extended Data Fig. 10a) for viral packaging. To avoid an off-target effect of the shRNA, we also used a dominant-negative form of Kir4.1 (dnKir4.1), which contains a GYG-to-AAA point mutation at the channel pore, which blocks K$^+$ channels$^{12}$ (Fig. 5a, c, Extended Data Figs 7b, 10b). We first examined the effect of Kir4.1-shRNA on glial and neural electrophysiological properties. In astrocytes infected with AAV-H1::Kir4.1-shRNA, we observed a marked change in the current–voltage (I–V) relationship (Fig. 5d) and a depolarization of 41 mV compared with astrocytes infected with control shRNA (Fig. 5e), consistent with previous findings that Kir4.1 is mainly responsible for...
setting astrocytic RMPs\textsuperscript{84}. The RMPs of neurons infected with AAV-H1::Kir4.1-shRNA did not differ from those of neighbouring non-infected neurons (because neurons do not express Kir4.1 endogenously, Fig. 5f). However, the RMPs of neighbouring LHb neurons in AAV-H1::Kir4.1-shRNA-infected brain slices were overall more depolarized than the RMPs of neurons from rats infected with the control shRNA (Fig. 5f), suggesting that knock-down of Kir4.1 in astrocytes had a widespread impact on the RMPs of neighbouring neurons. Similar effects were observed in LHb slices infected with AAV-GFAP::dnKir4.1 (Extended Data Fig. 10c–f). Notably, bursting activity in the LHb of cLH rats was eliminated by infection with AAV-H1::Kir4.1-shRNA (Fig. 5g) or AAV-GFAP::dnKir4.1 (Extended Data Fig. 10f).

Behaviourally, infection with AAV-H1::Kir4.1-shRNA or AAV-GFAP::dnKir4.1 caused a pronounced reduction in the depression-like phenotypes of cLH rats in three depression paradigms: it reduced immobility in the FST (Fig. 5h), increased bar pressing number in the LHT (Fig. 5i, j), and increased sucrose preference in the SPT (Fig. 5k). The behaviour scores in the LHT correlated with those in the FST (Fig. 5i).

**Concluding remarks**

Here we describe an important function of Kir4.1 in regulating neuronal RMP and firing pattern at the highly specialized neuron–glia interface in the LHb. During depression, upregulation of Kir4.1 may cause enhanced extracellular K\textsuperscript{+} clearance, leading to a decrease in [K\textsubscript{out}] and neuronal hyperpolarization (Fig. 5m). As demonstrated in the accompanying publication\textsuperscript{86}, neuronal hyperpolarization may de-inactivate T-type voltage-sensitive calcium channels (T-VSCCs), which in turn initiate NMDAR-dependent bursts and thereby increase suppression of downstream monoaminergic centres (Fig. 5m). These results may inspire the development of new treatments for major depression targeting maladaptive neuron–glia interactions in the LHb.
We also expect that the perisomatic K⁺ buffering mechanism described here may have a more widespread function.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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**Author Contributions** 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 behavioural experiments; Z.N., A.F. and H.B. established the biophysical model; S.M. assisted with cell culture technical support on electromicroscopy; C. Liu and C.-J. Shen for help with immunohistochemistry; S.-M. Duan, Y.-D. Zhou, J.-W. Zhao, X.-H. Zhang and B. MacVicar for advice on experimental design; and C. Giamm and P. Magistrati for comments on the manuscript.

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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.) were used. The cLH rats were screened using the learned helpless test for breeding as previously described. Male Wistar rats (SLAC Laboratory Co, 12 weeks) were used for establishing the LPS-induced depressive-like rat model. Male adult (7–8 weeks of age) C57Bl/6 mice (SLAC) were used for virus injection in the behaviour experiments. Kir4.1 floxed (Kirs1(+/−)) mice (originally obtained from K. McCarthy at University of Oxford) were used for virus injection and the immunohistochemistry experiments. Animals were group-housed two per cage for rats and four per cage for mice under a 12 h light–dark cycle (light on from 7 a.m. to 7 p.m.) with 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 blotting. The hubenar membrane fraction and whole proteins were extracted as previously described. Animals were anesthetized using isofothane, and hubenar tissue was quickly dissected from the brain and homogenized in lysis buffer (320 mM sucrose, 4 mM HEPES pH 7.4, 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 cells contained 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS and protease inhibitor cocktail tablets (Roche). After protein concentration measurement by BCA assay, 10–20 µg protein for each lane was separated on a 10% SDS-PAGE gel and transferred for western blot analysis. Rabbit anti-Kir4.1 intracellular peptide (1:1,000, Alomone labs), mouse anti-GFAP (1:1,000, Sigma), mouse anti-α-tubulin (1:5,000, Sigma) and mouse anti-GAPDH-HRP (1:5,000, KangChen Bio-tech Inc.) antibodies, and high-sensitivity ECL reagent (GE Healthcare) were used. All the bands were analysed with Quantity One on ImageJ.

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 µm) were cut on a microtome (Leica) and collected in PBS and stored at 4°C for further use. 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-GFAP (1:1,000, Abcam), mouse anti-Flag (1:1,000, Beyotime), Alexa Fluor 488 goat anti-rabbit IgG, Alexa Fluor 488 goat anti-chicken IgG, Alexa Fluor 560 goat anti-mouse IgG (all 1:1,000, 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 extracellular antigen by mixing with a weight ratio of 1:2 for 24 h. Slices for checking the injection site were counterstained with Hoechst in the final incubation step. Fluorescent image acquisition was performed with an Olympus Fluoview FV1000 confocal microscope and a Nikon A1R confocal microscope.

Cell transfection and cell culture. Human embryonic kidney (HEK293) cells (gift from J. Luo) were used for the electrophysiology recording and HEK293TN cells (Taitoo Bioscience) 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 and pAAV-H1-Luciferase-shRNA-CAG-eGFP, HEK293 or HEK293 TN cells were grown in 2A-eGFP, pAAV-CAM-eGFP, pAAV-H1-Kir4.1-shRNA-CAG-eGFP and pAAV-GFAP::eGFP-Cre (AAV2/5-gfaABC1D-eGFP-Cre, titre: 4.74 × 10^12 v.g./ml, dilution: 1:2; 0.2 µl, bilateral into LHb, Taiboo Bioscience), AAV-GFAP::eGFP-Cre (AAV2/5-gfaABC1D-eGFP-Cre, titre: 4.15 × 10^12 v.g./ml, 0.2 µl, bilateral into LHb, Taiboo Bioscience). All virus vectors were aliquoted and stored at −80°C until use.

Depression model and behaviour assay. LPS-induced depression. The LPS-induced depression model was used as previously described. Wistar male rats contained a CAG promoter driving eGFP and an H1 promoter driving shRNA expression. We designed six shRNA sequences using RNAi designer online software (http://rnaidesigner.thermofisher.com/rnaexpress; Invitrogen) as indicated below:

1. 5′-GAGGACGCTTTCATGCACT-3′
2. 5′-GCTACAAGGTTCTGGTCTTCT-3′
3. 5′-GCTCTTCTGCGCAACCATTTAC-3′
4. 5′-GCGAGAATCCTTGGCAAA-3′
5. 5′-GCTTAAGGCTCCTGCTATT-3′
6. 5′-GCCCTTAACTGTCGCCATTA-3′

We then tested the knockdown 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 Data Fig. 10a), we chose the fifth sequence, 5′-GCGTAAGGCTCCTGCTATT-3′, for the 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 20 ml saline, followed by 40 ml ice-cold mixture of 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1 M 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 immunogold-silver staining. In brief, sections were blocked with blocking buffer (5% BSA, 5% NGS and 0.05% Triton X-100 in PBS), and then incubated overnight with primary antibodies (rabbit anti-Kir4.1 extracellular peptide; 1:200, Alomone labs) 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) 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 deionized water.

Immunolabelled sections were fixed with 0.5% osmium tetroxide in 0.1 M PB for 1 h, dehydrated in graded ethanol series and then in propylene oxide, and finally flat-embedded in Epon 812 between sheets of plastic. After polymerization, acrylic sheets were then peeled from the polymerized resin, and flat-embedded sections were examined under the light microscope. Three to four sections containing Kir4.1 immunoreactivity in the LHb were selected from each brain, trimmed under a stereomicroscope, and glued onto blank resin stubs. Serial ultrathin sections were cut with an Ultracutomicrotome (Leica EM UC6, Germany) using a diamond knife (Diatome) and mounted on formvar-coated mesh grids (6–8 sections per grid). They were then counterstained with uranyl acetate and lead citrate, and observed under a JEM-1230 electron microscope (JEOL) equipped with a CCD camera and its application software (832 SCI1000).

Stereoatomic surgery and virus injection. cLH rats (P50–60) were deeply anesthetized using 4% pentobarbital. Microns (P50–60 days) were deeply anesthetized using ketamine (100 mg/kg of body weight) and xylazine (8 mg/kg). Animals were injected using a stereotaxic frame (RWD model) and glued onto blank resin stubs. Serial ultrathin sections were cut with an Ultracutomicrotome (Leica EM UC6, Germany) using a diamond knife (Diatome) and mounted on formvar-coated mesh grids (6–8 sections per grid). They were then counterstained with uranyl acetate and lead citrate, and observed under a JEM-1230 electron microscope (JEOL) equipped with a CCD camera and its application software (832 SCI1000).

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Astrocytic whole-cell patch-clamp recordings and Kir4.1 current isolation after perfusion started, respectively. Neurons stabilized after a few minutes, whereas the effect of BaCl$_2$ on TTX action potentials in response to increased injection currents of 34-35. BaCl$_2$ (100 μM, Sigma) was applied to isolate Kir4.1 current, which was subtracted from the I-V curve recorded from -120 mV to 0 mV. Biocytin (Sigma, 5 mg/ml) was dissolved into the patch-clamp pipette solution. After electrophysiological characterization, cells were held for at least 30 min in voltage clamp and constantly injected with a hyperpolarization current (500 ms, 50 pA, 0.5 Hz, 30 min) to allow biocytin filling (performed at 34 °C). Subsequently, slices were fixed overnight in 4% paraformaldehyde at 4 °C. The antibodies used were rabbit anti-NeuN (1:500, Millipore), Alexa Fluor 564 donkey anti-rabbit IgG (1:1,000, Invitrogen) and Cy2-conjugated streptavidin (1:1,000, Jackson ImmunoResearch). Fluorescent image acquisition was performed with a Nikon A1 confocal microscope.

Tri-compartment model. A tri-compartment model was constructed including the neuron, the astrocyte and the extracellular space. The model was based on ionic fluxes between these three compartments. Na$^+$ and K$^+$ voltage-gated channels, and leak channels were recruited into the neuron as $dV_{s}/dt=(I_{app}-I_{Na}-I_{K}-I_{Kir})/C_{m}$, where $V_{m}$ is the neuronal membrane potential, $I_{Na}$ is the neuronal capacitance, $I_{Na}$ is an external current applied to the neuron, $I_{K}$ and $I_{K}$ are the fast Na$^+$ and K$^+$ currents of the action potentials, respectively, and $I_{Kir}$ is responsible for the astrocyte resting membrane potential. To trigger an action potential, we applied an external step current of amplitude 5 nA and duration 0.1 s. Kir4.1 channels on the extracellular and vessel side and leak channels were recruited into the astrocyte as $dV_{a}/dt=(-I_{Kir,vess}-I_{Kir,vess}-I_{Kir,vess})/C_{a}$, where $V_{a}$ is the astrocytic membrane potential, $I_{Kir}$ is the astrocytic capacitance, $I_{Kir,vess}$ and $I_{Kir,vess}$ account for the K$^+$ flow on the side of the neuron and vessel, respectively ($I_{Kir}$ is outward during the resting state, and becomes inward when K$^+$ equilibrium potential exceeds the astrocytic membrane potential), and $I_{Kir,vess}$ is responsible for the astrocyte resting membrane potential.

The dynamics for K$^+$ in the three compartments are described by:

$$d[K+]_{Na}/dt=(I_{K}+I_{Na})/(F \times V_{Ol})-2(I_{pump,N}+I_{pump,A})$$

$$-d_{Ka}(I_{K}+I_{Na})$$

$$d[K+]_{Na}/dt=-I_{K}/(F \times V_{Ol})+2I_{pump,N} \times V_{Ol}/V_{0b}$$

$$-d_{Ka}(I_{K}+I_{Na})$$

$$d[K+]_{Na}/dt=(I_{K}+I_{Na})/(F \times V_{Ol})+2I_{pump,N} \times V_{Ol}/V_{0a}$$

$$-d_{Ka}(I_{K}+I_{Na})$$

where $[K+]_{Na}$, $[K+]_{Na}$ and $[K+]_{Na}$ are the extracellular, neuronal and astrocytic K$^+$ concentrations, respectively; $F$ is the Faraday constant; $V_{Ol}$, $V_{0b}$ and $V_{0a}$ are the volumes of the extracellular, astrocytic and neuronal compartments, respectively; and $I_{pump,N}$ and $I_{pump,A}$ are the fluxes through the Na$^+$-K$^+$ pump of the neuron and astrocyte membranes, respectively. The term $d(I_{K}+I_{Na})$ describes the diffusion of K$^+$ in the considered compartment, where $d_{Ka}$, $d_{Ka}$, $d_{Ka}$ and $d_{Ka}$ are the rates of extracellular, neuronal and astrocytic K$^+$ effective flux; $K_{0b}, K_{0b}$ and $K_{0b}$ are the extracellular, neuronal and astrocytic K$^+$ concentrations expected at equilibrium, respectively. The extracellular K$^+$ is mainly contributed by $I_{K}$. Released extracellular K$^+$ from the neuron is taken up by Kir4.1 channels and Na$^+/K^+$ pumps. The dynamics for Na$^+$ in the three compartments are described by:

$$d[Na+]_{Na}/dt=I_{Na}/(F \times V_{Ol})+3I_{pump,N}+I_{pump,A}$$

$$-d_{Na}(Na+]_{Na})$$

$$d[Na+]_{Na}/dt=-I_{Na}/(F \times V_{Ol})-3I_{pump,N} \times V_{Ol}/V_{0b}$$

$$-d_{Na}(Na+]_{Na})$$

$$d[Na+]_{Na}/dt=-3I_{pump,N} \times V_{Ol}/V_{0a}-d_{Na}(Na+]_{Na})$$

See Supplementary Information (Model Description and Supplementary Table 2) for detailed formulation.

Statistical analyses. The required sample sizes were estimated on the basis of our past experience performing similar experiments. Animals were randomly assigned to treatment groups. Analyses were performed blinded to treatment assignments in all behavioural experiments. Statistical analyses were performed using GraphPad Prism software v6. Pre-established criteria, values were excluded from the
analyses if the viral injection or drug delivering sites were out of the 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 and 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 $t$-tests were used. Where normality or equal variance of samples failed, Mann–Whitney $U$ tests were performed. Linear regression tests and $\chi^2$ tests were used in appropriate situations. The sample sizes, specific statistical tests used, and main effects of our statistical analyses for each experiment are reported in Supplementary Table 1.

**Data availability.** The data that support the findings of this study are available from the corresponding author upon reasonable request.

Extended Data Figure 1 | Habenular protein expression in rat models of depression. 

**a**, Volcano plot of high-throughput proteomic screen identifies proteins that are differentially expressed in the habenulae of cLH rats versus wild-type rats. Ln (fold change) is ln-transformed value of the normalized protein ratio of cLH and control. Significance value was calculated as the average normalized ratio minus two folds of s.d. Proteins in the shaded areas have more than 50% significant change. Kir4.1 is one of the eight upregulated proteins identified. Dashed lines indicate fold change of 50%. 

**b**, Western blot analysis showing no change in GFAP protein in habenulae of cLH rats at P60–90. n = 4, 4 rats for control and cLH, respectively. 

**c**, LPS injection (500 µg kg⁻¹ i.p. for 7 days) induces increased immobile time and decreased latency to immobility in the FST. n = 8, 9 rats for saline and LPS, respectively. 

**d**, QPCR analysis of Kir4.1 mRNA in habenulae. Two-tailed paired t-test. n = 5, 5 rats for control and cLH, respectively. 

**e**, Western blot analysis showing no change in Kir4.1 protein in membrane fraction of habenulae in cLH rats at P30. n = 6, 6. 

Data are means ± s.e.m., *P < 0.05, n.s., not significant. Two-tailed paired t-test (b, d, e); two-tailed unpaired t-test (c).
Extended Data Figure 2 | Ba\(^{2+}\)-sensitive Kir4.1 current is upregulated in LHb of adult cLH rats and adult LPS-injected Wistar rats.

a, c, Representative traces showing linear I–V curve in a typical astrocyte before (upper) and after (middle) Ba\(^{2+}\) perfusion under voltage steps (−130 mV to −30 mV, step by 10 mV, 2 s duration, holding at −70 mV). Subtraction of the two led to Ba\(^{2+}\)-sensitive Kir current (bottom) at P60–90 (a) and P30 (c) in cLH rats. b, d, I–V plots of astrocytes in cLH rats and controls at P60–90 (b) and P30 (d). e, I–V plots of astrocytes in LPS-injected Wistar rats and saline controls at P60–90. f, I–V plot and bar graph showing Ba\(^{2+}\)-sensitive currents in LPS-injected Wistar rats and saline-injected controls at P60–90. Two-tailed unpaired t-test. \(n=7\), 6 astrocytes from 2, 2 rats for saline and LPS, respectively. Data are means ± s.e.m., \(* P < 0.05\).
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 were first identified on the basis of their specific morphology (astrocytes: 5–10 μm diameter; neurons: ~15 μm 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 were held for at least 30 min in voltage clamp and constantly injected with a hyperpolarization current (500 ms, 50 pA, 0.5 Hz, 30 min) to allow biocytin filling. 

c–h, Biocytin-labelled neurons and astrocytes subsequently confirmed by co-labelling with NeuN. c, d, Biocytin signals in a single neuron (c) or a group of astrocytes owing to diffusion through gap junctions (d) (four independent experiments). e, f, NeuN signals (four independent experiments). g, h, Colabelling of NeuN with the neuron (indicated by white arrow, g) but not astrocytes (h) (four independent experiments). Note that all biocytin-filled neurons (n = 18) 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 the LHb and hippocampus. a, b, Kir4.1 co-immunostaining with neuronal marker (NeuN) or astrocytic marker (S100b and GFAP) in the LHb (a) or hippocampus (b). Bottom two panels show staining with the same Kir4.1 antibody pre-incubated with the antigen peptide, demonstrating the specificity of the Kir4.1 antibody (two independent experiments).
Extended Data Figure 5 | Electron microscopy immunohistochemistry of Kir4.1 staining. a, b, Many Kir4.1 immunograins (arrows) surround the neuronal soma. c, Kir4.1 grains (arrows) also surround axon–dendrite synapses, but are rare near the synaptic zones as indicated by the postsynaptic densities (arrowheads). d, Kir4.1 immunograins are also detected surrounding a vascular endothelial cell. e, Inset shows Kir4.1 immunograins near a gap junction. s, neuronal soma; t, axon terminal. Scale bars, 0.5 μm. Three independent experiments.
Kir4.1 is expressed in astrocytes but not neurons in the LHb.

**a**. Schematics showing sequence of drug application and recording after a neuron or astrocyte is patched. Representative traces showing a linear $I-V$ curve in a typical astrocyte under voltage steps ($-130 \text{ mV}$ to $-30 \text{ mV}$, step by $10 \text{ mV}$, $2 \text{ s}$ duration, holding at $-70 \text{ mV}$, protocol demonstrated on left, upper panel). $I-V$ curves of the same cell after addition of TTX (1 $\mu$M), 4-aminopyridine (4AP, 1 mM) and 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride (ZD7288, 50 $\mu$M) (middle) and further addition of Ba$^{2+}$ (100 $\mu$M, bottom) are shown below.

**b**. Representative traces showing a nonlinear $I-V$ curve in a typical neuron under voltage steps ($-120 \text{ mV}$ to $-40 \text{ mV}$, step by $10 \text{ mV}$, $2 \text{ s}$ duration, holding at $-60 \text{ mV}$, 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 Ba$^{2+}$ (bottom) are shown below.

Extended Data Figure 6 | Kir4.1 is expressed in astrocytes but not neurons in the 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 ($-130 \text{ mV}$ to $-30 \text{ mV}$, step by $10 \text{ mV}$, $2 \text{ s}$ duration, holding at $-70 \text{ mV}$, protocol demonstrated on left, upper panel). $I-V$ curves of the same cell after addition of TTX (1 $\mu$M), 4-aminopyridine (4AP, 1 mM) and 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride (ZD7288, 50 $\mu$M) (middle) and further addition of Ba$^{2+}$ (100 $\mu$M, bottom) are shown below. c, Representative traces showing a nonlinear $I-V$ curve in a typical neuron under voltage steps ($-120 \text{ mV}$ to $-40 \text{ mV}$, step by $10 \text{ mV}$, $2 \text{ s}$ duration, holding at $-60 \text{ mV}$, 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 Ba$^{2+}$ (bottom) are shown below.
Extended Data Figure 7 | Characterization of cell-type specificity of GFAP promoter, and locomotion. a, b, Double immunofluorescence for NeuN (red) and eGFP (green) in the coronal section of LHb brain slices infected with AAV-GFAP::Kir4.1 (AAV2/5-gfaABC1D-eGFP-Kir4.1) virus in mice (three independent experiments, a) or AAV-GFAP::dnKir4.1 (AAV2/5-gfaABC1D-dnKir4.1-2A-eGFP) virus in cLH rats (two independent experiments, b). Left, examples of anterior, middle and posterior coronal sections of LHb. Numbers in the bottom right corner are the number of merged cells/number of NeuN+ cells in the virus-infected area. Right, zoomed-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. c, d, Overexpression of Kir4.1 in the LHb of C57 mice does not affect locomotion. n = 7, 8 mice for eGFP and Kir4.1, respectively. e, f, Overexpression of Kir4.1-shRNA in the LHb of cLH rats does not affect locomotion activities. n = 7, 7 rats for control and Kir4.1 shRNA, respectively. Data are means ± s.e.m; n.s., not significant. Two-tailed unpaired t-test (c-f).
Extended Data Figure 8 | 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 (see Methods for details). 

**b**–**d**, Effects of increasing Kir4.1 expression on $[\text{K}]_{\text{out}}$ (**b**), neuronal membrane potential (**c**) and astrocytic membrane potential (**d**). Ctrl, control condition with $1 \times$ Kir4.1 conductance; Depr, depression condition with $2 \times$ Kir4.1 conductance. Grey shaded areas indicate application of 10 Hz tonic stimulation to neurons. Note that under this neuronal firing condition, $[\text{K}]_{\text{out}}$ is lower, and neuron and astrocyte are more hyperpolarized in the depression condition than the control. 

**e**–**g**, Effects of *in silico* TTX (blocking action potentials, $g_{\text{Na}} = 0$) or $\text{Ba}^{2+}$ (blocking Kir4.1, $g_{\text{Kir4.1}} = 0$) treatments on $[\text{K}]_{\text{out}}$ (**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 $\text{Ba}^{2+}$ cause opposite changes to $[\text{K}]_{\text{out}}$, neuronal membrane potential and astrocytic membrane potential. Neuronal spikes are not shown for clarity of presentation.

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Extended Data Figure 9 | BaCl₂ caused depolarization of neuronal RMP in the presence of synaptic transmitter blockers. 

**a, b,** Representative trace (a) and bar graph (n = 9 neurons from 3 rats; b) showing effect of BaCl₂ (100μM) perfusion onto tonic-firing neurons that have been bathed with transmitter blockers (100μM picrotoxin, 10μM NBQX and 100μM AP5). 

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

**d,** Representative trace showing effect of BaCl₂ (sampled 15 min after drug perfusion) on bursting neurons (n = 4 out of 9 neurons from 3 rats). Spikes in bursting and tonic-firing mode are shown in blue and black, respectively. Data are means ± s.e.m., ***P < 0.001, n.s., not significant. Two-tailed paired t-test (b) and two-tailed unpaired t-test (c).
Extended Data Figure 10 | 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 six different shRNAs (see Methods) of Kir4.1 or the negative control (shRNA of luciferase) into HEK293 TN cells. On the basis of knockdown efficiency as shown in the western blot, Kir4.1-shRNA-5 was chosen for viral package (two independent experiments).

b, I–V plot showing Kir4.1 currents recorded in HEK293 cells transfected with pAAV-Kir4.1 together with negative control pAAV-eGFP or pAAV-dnKir4.1 plasmid. Bars represent the current values recorded at −160 mV. n = 18, 15 HEK293 cells for eGFP and dnKir4.1, respectively. c, I–V plot and bar graph showing Ba²⁺-sensitive currents blocked by AAV-dnKir4.1 in both cLH and wild-type rats. d–f, AAV-dnKir4.1 caused depolarization of RMP in astrocytes (n = 9, 8, 9, 6 astrocytes from 2, 4, 2, 4 rats for wild-type eGFP, wild-type dnKir4.1, cLH eGFP and cLH dnKir4.1, respectively; d) and neurons in viral infected area (n = 54, 48, 45, 58 neurons from 2, 4, 2, 4 rats, e), and abolished neuronal bursting (f) in both cLH and wild-type rats. Data are means ± s.e.m.; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Two-tailed unpaired t-test (b–e) and χ² test (f).
Life Sciences Reporting Summary

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1. Sample size
   Describe how sample size was determined. 
   Required sample sizes were estimated based on our past experience performing similar experiments.

2. Data exclusions
   Describe any data exclusions.
   Values were excluded from the analyses if the viral injection sites were out of LHb.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   The experiment for each experiment was successfully repeated for at least two times.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   Animals were randomly assigned to treatment groups.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   Analysis were performed in a manner blinded to treatment assignments in all behavioral experiments.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   n/a  Confirmed

   The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   A statement indicating how many times each experiment was replicated
   The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
   A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
Software

Describe the software used to analyze the data in this study.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

cLH rats need permission from Fritz Henn. Other unique materials used are readily available from the authors or commercially available.

Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Rabbit anti-Kir4.1 (intracellular) (Alomone labs, APC-035), rabbit anti-Kir4.1 (extracellular) (Alomone labs, APC-165), mouse anti-α tubulin (Sigma, T6074), mouse anti-GAPDH-HRP (KangChen Bio-tech Inc, KC-5G5), mouse anti-GFAP (Sigma, G3893), mouse anti-NeuN (Millipore, MABN140), mouse anti-S100B (Sigma, AMAB91038), chicken anti-GFP (Abcam, ab13970), mouse anti-Flag (Beyotime, AF519).

Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293 cells (gift from Jianhong Luo) were used for the electrophysiology recording and HEK293TN cells (Taitool Bioscience, China) were used for western blot analysis.

b. Describe the method of cell line authentication used.

Identity of the cell lines were frequently checked by their morphological features.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cells used in this study were checked for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

Animals and human research participants

Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

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, for breeding as previously described. 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 Kir4.1f/f floxed mice (originally obtained from Dr. Ken McCarthy at University of North Carolina) were used for virus injection the immunohistochemistry experiments.

Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No human research participants.
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Model description

The mathematical description of $I_K$ and $I_{Na}$ are achieved through a Hodgkin-Huxley style derivation of forward and backward rate equations:\cite{Hodgkin1952, Huxley1952}:

$$I_K = g_K n^4 (V_N - E_{KN})$$
$$I_{Na} = g_{Na} m^3 h (V_N - E_{NaN})$$

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

$$\frac{dn}{dt} = \alpha_n(V_N) (1 - n) - \beta_n(V_N) n$$
$$\frac{dm}{dt} = \alpha_m(V_N) (1 - m) - \beta_m(V_N) m$$
$$\frac{dh}{dt} = \alpha_h(V_N) (1 - h) - \beta_h(V_N) h$$

where the forward and the backward rate $\alpha$ and $\beta$ describe the transition between the closed and open state of gate. The function of $\alpha$ and $\beta$ are given by:

$$\alpha_n(V_N) = 0.01 (V_N + 55) / (1 - \exp([-V_N - 55] / 10))$$
$$\beta_n(V_N) = 0.125 \exp([-V_N - 65] / 80)$$
$$\alpha_m(V_N) = 0.1 (V_N + 40) / (1 - \exp([-V_N - 40] / 10))$$
$$\beta_m(V_N) = 4 \exp([-V_N - 65] / 18)$$
$$\alpha_h(V_N) = 0.07 \exp([-V_N - 65] / 20)$$
$$\beta_h(V_N) = 1 / (\exp([-V_N - 35] / 10) + 1)$$

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

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

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

Neuronal leak current is described by:

\[ I_{\text{Leak}, N} = g_{\text{Leak}, N} (V_N - E_{\text{Leak}, N}) \]

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

The formula of Kir4.1 channel are modified from a previous model:\(^1\)

\[
I_{\text{Kir}} = g_{\text{Kir}} \sqrt{([K^+]_O) / (1 + \exp((V_A + V_{\text{rest}, A} - E_{KA} + V_h) / V_s))} (V_A + V_{\text{rest}, A} - E_{KA})
\]

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

where \( g_{\text{Kir}} \) and \( g_{\text{Kir, vess}} \) are the conductance of Kir4.1 channel at the side of neuron and vessel respectively.

Astrocytic equilibrium potential for K\(^+\) and Na\(^+\) are given by:

\[ E_{KA} = R \frac{T \ln([K^+]_O / [K^+]_A)}{F} \]

\[ E_{K_{A, \text{vess}}} = R \frac{T \ln([K^+]_{O, 0} / [K^+]_A)}{F} \]

\[ E_{NaA} = R \frac{T \ln([Na^+]_O / [Na^+]_A)}{F} \]

where \([K^+]_A\) and \([Na^+]_A\) are the astrocytic K\(^+\) and Na\(^+\) concentration.

The astrocyte membrane potential equation also comprises a leak term:

\[ I_{\text{Leak}, A} = g_{\text{Leak}, A} (V_A - E_{\text{Leak}, A}) \]

The equation of the Na\(^+\)/K\(^+\) pump depends on the extracellular K\(^+\) and intracellular Na\(^+\) concentrations as:

\[
i_{pump, N} = i_{\text{max}, N} (1 + k_{mk} / [K^+]_O)^2 (1 + k_{mNa} / [Na^+]_A)^3
\]

\[
i_{pump, A} = i_{\text{max}, A} (1 + k_{mk} / [K^+]_O)^2 (1 + k_{mNa} / [Na^+]_A)^3
\]

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

Simulations were solved numerically with an explicit Runge-Kutta Prince-Dormand 8th-9th order method with the GSL library in cython and python. The parameters used in the model are presented in Supplementary Table 2.

References


### Supplementary Table 2: Model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>8.31</td>
<td>J·mol⁻¹·K⁻¹</td>
<td>Gas constant</td>
</tr>
<tr>
<td>T</td>
<td>308</td>
<td>K</td>
<td>Absolute temperature</td>
</tr>
<tr>
<td>F</td>
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<td>C·mol⁻¹</td>
<td>Faraday constant</td>
</tr>
<tr>
<td>dt</td>
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<td>ms</td>
<td>Step time</td>
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<td>c_N</td>
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<td>pF</td>
<td>Neuronal capacitance</td>
</tr>
<tr>
<td>c_A</td>
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<td>pF</td>
<td>Astrocytic capacitance</td>
</tr>
<tr>
<td>g_K</td>
<td>20</td>
<td>nS</td>
<td>Neuronal K⁺ channel conductance</td>
</tr>
<tr>
<td>g_Na</td>
<td>120</td>
<td>nS</td>
<td>Neuronal Na⁺ channel conductance</td>
</tr>
<tr>
<td>g_Leak_N</td>
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<td>nS</td>
<td>Neuronal leak conductance</td>
</tr>
<tr>
<td>V_Leak_N</td>
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<td>mV</td>
<td>Neuronal leak potential</td>
</tr>
<tr>
<td>i_max,N</td>
<td>0.12</td>
<td>mM·ms⁻¹</td>
<td>Neuronal Na⁺/K⁺ pump rate</td>
</tr>
<tr>
<td>g_Kir</td>
<td>12.5</td>
<td>nS</td>
<td>Astrocytic Kir4.1 channel conductance on extracellular side</td>
</tr>
<tr>
<td>g_Kir_vess</td>
<td>12.5</td>
<td>nS</td>
<td>Astrocytic Kir4.1 channel conductance on vessel side</td>
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<tr>
<td>V_A</td>
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<td>[Κ⁺]_N</td>
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<td>Unit</td>
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<tr>
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<td>Extracellular volume</td>
</tr>
<tr>
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<td>pL</td>
<td>Neuronal volume</td>
</tr>
<tr>
<td>$\text{Vol}_A$</td>
<td>1.08</td>
<td>pL</td>
<td>Astrocytic volume</td>
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<tr>
<td>$d_{K,N}$</td>
<td>0.0001</td>
<td>ms$^{-1}$</td>
<td>$\text{K}^+$ effective flux rate in the neuron</td>
</tr>
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<tr>
<td>$d_{Na,O}$</td>
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<td>mM</td>
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</tbody>
</table>