Negative feedback control of neuronal activity by microglia

Microglia, the brain’s resident macrophages, help to regulate brain function by removing dying neurons, pruning non-functional synapses, and producing ligands that support neuronal survival. Here we show that microglia are also critical modulators of neuronal activity and associated behavioural responses in mice. Microglia respond to neuronal activation by suppressing neuronal activity, and ablation of microglia amplifies and synchronizes the activity of neurons, leading to seizures. Suppression of neuronal activation by microglia occurs in a highly region-specific fashion and depends on the ability of microglia to sense and catabolize extracellular ATP, which is released upon neuronal activation by neurons and astrocytes. ATP triggers the recruitment of microglial protrusions and is converted by the microglial ATP/ADP hydrolysing ectoenzyme CD39 into AMP; AMP is then converted into adenosine by CD73, which is expressed on microglia as well as other brain cells. Microglial sensing of ATP, the ensuing microglia-dependent production of adenosine, and the adenosine-mediated suppression of neuronal responses via the adenosine receptor A1R are essential for the regulation of neuronal activity and animal behaviour. Our findings suggest that this microglia-driven negative feedback mechanism operates similarly to inhibitory neurons and is essential for protecting the brain from excessive activation in health and disease.
We found that the microglial response to neuronal activation is associated with reciprocal, microglia-mediated suppression of neuronal activity. Brain-wide ablation of microglia in adult mice by pharmacological inhibition of the microglia pro-survival receptor CSF1R\(^{13}\) (Fig. 1d, Extended Data Fig. 2a) had no major effect on animal behaviours at baseline\(^{24}\) (Extended Data Fig. 2b–e), but rendered animals hyper-responsive to neurostimulants at levels that normally do not cause excessive neuronal activation (Fig. 1e–g, Extended Data Fig. 2f–h). Intraperitoneal (i.p.) administration of a sub-threshold dose of kainic acid, a kainate receptor agonist that activates specific subtypes of glutamate receptors (kainate and AMPA (\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors) in the brain resulted in seizures (Racine stages above IV–V) in 90% of microglia-depleted mice compared with 11% of controls (Fig. 1e, Extended Data Fig. 2g). In line with this observation, we found that i.p. administration of picROTOXIN—an inhibitor of GABA\(_{\text{A}}\) (\(\gamma\)-aminobutyric acid A) receptors that enhances excitatory neuron activity by alleviating suppression by inhibitory neurons—resulted in significantly prolonged seizure responses in mice lacking microglia (Fig. 1f). Similar effects were seen upon stimulation of dopamine D1 receptor-expressing striatal neurons (D1 neurons). Activation of D1 receptors elicits a dose-dependent increase in motor activity and seizures in mice\(^{16}\). The pro-conversive effect of the D1 agonist depends on the downstream PKA–DARPP32–ERK signalling pathway, which increases neuronal firing frequency and is likely to involve the recurrent activation of striato-thalamo-cortical neuronal circuits\(^{13}\). Both long-term sustained ablation of microglia (for more than three weeks) and their acute depletion (for three days) by CSF1R-inhibition triggered increased motor responses (Extended Data Fig. 2h) and seizures (Fig. 1g, Extended Data Fig. 2i) in response to i.p. administration of the D1 agonist SKF81297 at subthreshold doses.

The microglia-mediated suppression of stimulus-induced neuronal activation is executed by grey matter microglia in a highly region-specific fashion. The maintenance of microglia is controlled by interleukin-34 (IL34) and colony-stimulating factor 1 (CSF1), both of which can activate CSF1R signalling and promote the survival of microglia\(^{18–20}\). In the striatum, the expression of IL34 and CSF1 is spatially separated; IL34 is expressed by D1 and D2 medium spiny neurons (MSNs) in the grey matter, whereas CSF1 is predominantly expressed by oligodendrocytes and astrocytes in white matter regions\(^{21,22}\) (Fig. 2a, b, Extended Data Fig. 3a, b). Ablation of IL34 specifically in neuronal progenitor cells (Nestin-Cre), which give rise to neurons, astrocytes, and oligodendrocytes, resulted in a selective, gene dose-dependent loss of neuron-associated microglia in striatal grey matter (Fig. 2c, d, Extended Data Fig. 3c–g). Conversely, ablation of CSF1 led to the loss of microglia predominantly in the white matter of the striatum (Fig. 2e, f, Extended Data Fig. 3h–j). Loss of IL34-maintained microglia in striatal grey matter, but not the ablation of CSF1-maintained microglia in white matter, enhanced the response to D1 agonist treatment as measured by the induction of seizures (Fig. 2g, h). Furthermore, the loss of striatal microglia had a selective impact on the responses of striatal neurons. Deletion of IL34 in either D1 or D2 neurons (Fig. 2i, Extended Data Fig. 4a–c), which causes a striatum-specific reduction in microglia of about 50%, led to exaggerated responses to D1 agonist treatment (Fig. 2j, Extended Data Fig. 4f, g). Notably, mice with striatum-specific ablation of microglia respond like wild-type mice to kainic acid and picROTOXIN (Fig. 2k, Extended Data Fig. 4h), both of which trigger seizures via the activation of cortical and hippocampal neurons (Extended Data Fig. 2f). Our data show that microglia are critical for neurostimulant-induced neuronal activity regulation in a region-specific and microglia number-dependent fashion (Extended Data Fig. 4i). The increase in neuronal responses to D1 stimulation that was induced by microglia ablation occurred in the absence of detectable changes in striatal cellular composition or D1 and D2 neuron-specific phenotypes, including morphology, intrinsic excitability, and mRNA expression patterns (Extended Data Fig. 5, Supplementary Table 3). Thus, loss of IL34 has no overall effect on neuron or glia.
phenotypes (Extended Data Fig. 5j–l) in the striatum, and the abnormal hyper-responsiveness of neurons in the absence of local microglia is not a consequence of Il34 deficiency (Extended Data Fig. 5).

Using two-photon in vivo imaging of neuronal calcium responses in the dorsal striatum (Fig. 3a, Extended Data Fig. 6a, Supplementary Video 1), we found that ablation of microglia led to increased striatal neuron synchrony and increased the probability that striatal neurons would fire simultaneously (Fig. 3b, c, Extended Data Fig. 6b). Increased neuronal synchrony has been shown to underlie seizure progression19 and is likely to contribute to seizures in microglia-deficient mice. In

sections showing IBA1 (green) nucleated (DAPI', blue) microglia.

**Fig. 2 | Spatial control of neuronal activity by microglia.** a, Left, cell populations in the mouse striatum (r-distributed stochastic neighbour embedding (t-SNE) plot) identified by single-nuclei RNA expression analysis (15,950 nuclei). Right, Il34 (top) or Csf1 (bottom) RNA-expressing cells. OPC, oligodendrocyte progenitor cell. b, Il34 (top left; red) and Csf1 (bottom left; red) identified by RNA in situ hybridization, DAPI nuclei (blue), grey matter (GM), and white matter (WM). Right, distribution of Il34 and Csf1 cells in GM (Il34, 93%; Csf1, 15%) and WM (Il34, 7%; Csf1, 83%) in the striatum (n = 2 and 4 mice per group, respectively). c–h, Il34fl/fl and Csf1fl/fl mice were bred to NestinCre+ mice to generate Il34fl/flNestinCre+ (purple) and Csf1fl/flNestinCre+ (blue) mice. Black, control. c, e, Striatal microglia numbers in GM and WM in control and mutant mice per frame (c, n = 4 and 3 mice; unpaired two-tailed t test; e, n = 4 and 2 mice). d, f, Representative images of control and mutant striatum sections showing IBA1 (green) nucleated (DAPI', blue) microglia. g, h, Percentages of mice with seizures in response to D1 agonist (SKF81297, 5 mg kg−1) (g, n = 15 and 10 mice; h, n = 11 and 10 mice; Fisher’s exact test). i–k, Il34fl/fl mice were bred to Drd1aCre+/− or Drd2Cre+/− mice to generate Il34fl/flDrd1aCre+ (green) and Il34fl/flDrd2Cre+ (grey) mice. i, Number of microglia per mm² in cortex, striatum, and cerebellum (n = 7, 4, and 3 mice; cortex, P = 0.38; striatum, P < 0.0001; cerebellum, P = 0.14; one-way ANOVA with Tukey’s post-hoc test). j, k, Percentage of mice with seizures within 1 h of treatment with SKF81297 (j, 5 mg kg−1) or kainic acid (k, 18 mg kg−1) (j, n = 12, 10, and 8 mice, P = 0.0014; k, n = 17, 9, and 9 mice, P = 0.40; χ² test with Bonferroni adjustment). Experiments in g were independently repeated in a second cohort with identical results. Data shown as mean ± s.e.m.
addition, we found that microglia determine the threshold for striatal neuron activation in response to D1 agonist treatment (Fig. 3d–f). Acute treatment with a low dose of D1 agonist was sufficient to induce a substantial and prolonged increase in the frequency of neuronal events, with no difference in magnitude, in animals lacking microglia as compared to control mice (Fig. 3d–f, Extended Data Fig. 6c–g).

Neuronal activation is associated with the local release of ATP by neurons and astrocytes20–23. Microglia can detect synaptic release of ATP through the surface-expressed purinergic receptor P2RY1224, which is highly expressed in forebrain microglia25 and controls ATP/ADP-dependent chemotaxis and motility of microglia3,6,23,27. Although extracellular ATP levels in the striatum at baseline (3–5 nM)13 are far below concentrations that activate P2RY12 (>100 nM)24,26, local ATP release at the synapses of activated neurons can reach concentrations up to 5–500 μM20, which is more than sufficient to trigger microglial P2RY12 signalling60,24. Using two-photon live imaging of eGFP-expressing microglia in the cortex, we tracked the positioning and velocity of microglial protrusions in vivo (Fig. 3g, h, Supplementary Video 2, Extended Data Fig. 6h–j). Depending on the level of neuronal activity, microglia can display different modes of motility: in the
absence of strong neuronal activation, microglial motility is characterized by the constant extension and retraction of microglia protrusions, which has been defined as microglial baseline or surveillance activity. The activation of neurons resulted in a decrease in microglial baseline motility indicated by reduced protrusion velocity (Fig. 3h) while enhancing microglial targeted motility (directional branch extension towards target sites), leading to increased microglia–neuron proximity (Fig. 3h). Following neuronal activation, an increased number of microglia protrusions were recruited to the synaptic boutons of active thalamocortical projection neurons in an ATP-dependent manner.
manner (Fig. 3h). The pharmacological inhibition of microglial ATP/ADP-sensing by blocking P2RY12 activity both prevented the neuronal activity-induced synaptic recruitment of microglial protrusions and restored microglial baseline motility (Fig. 3h). Our findings suggest that activity-induced synaptic ATP release can act as a local chemoattractant that leads to the targeted recruitment of microglial protrusions to activated synapses.

In addition to its role as a chemoattractant, extracellular ATP can also serve as a substrate for the ATP/ADP-hydrolysing ectoenzyme CD39 (encoded by Entpd1)28,29, the rate-limiting enzyme that catalyses ATP–AMP conversion; this is followed by conversion of AMP to adenosine (ADO)—a potent suppressor of neuronal activity30—by CD73 (encoded by Nt5e)28 and/or the tissue-nonspecific alkaline phosphatase TNAP31. ADO restrains neuronal activity by binding to pre- and postsynaptic G protein-coupled adenosine A1 receptors (A1Rs), the adenosine receptor subtype with the highest affinity for ADO in the brain (dissociation constant $K_d = 2 \text{ nM}$)32. Activation of A1Rs suppresses D1 neuron responses in the striatum both by limiting synaptic transmission via presynaptic neurotransmitter release29,33 and by suppressing the activation of postsynaptic D1 neuron signalling pathways via A1R-mediated inhibition of protein kinase A (PKA) activity33,34.

In support of the idea that ADO–A1R signalling mediates the suppressive effect of microglia on D1 neurons, we found that ex vivo isolated microglia could support the conversion of ATP to ADO (Fig. 4a). The production of ADO by microglia is suppressed by pharmacological inhibition of either CD39 (Fig. 4a), which is primarily expressed by microglia18,29 (Extended Data Fig. 7a–e), or CD73 (Fig. 4a), which is expressed by striatal microglia, albeit at levels lower than in striatal neurons as judged by CD73 mRNA and protein expression28,33,34 (Fig. 4b, Extended Data Fig. 7a, f). In line with these data, CD73 was expressed on the cell surface of CD39–microglia isolated from the forebrains of adult wild-type mice at levels higher than on microglia from CD73-deficient mice, but lower than on non-microglial CD39+ cells (Fig. 4b). These findings suggest that microglia in the striatum in vivo can contribute to the production of ADO in a cell-autonomous fashion and/or by involving neighbouring cells, including neurons. Concurrently, microglia-deficient mice show a significant decrease in extracellular ADO in the striatum (Fig. 4c). As expected to result from reduced ADO-mediated activation of A1R, a decrease in striatal microglia is associated with enhanced PKA activity in striatal D1 neurons as measured by increased phosphorylation of several PKA targets33,34 (Fig. 4d, e). These data show that microglia have a key role in the production of ADO and in ADO-mediated modulation of D1 neurons in the striatum.

We assessed the functional importance of the microglia-dependent ATP–AMP–ADO–A1R cascade in vivo by monitoring mice for seizures following interference with the individual components of the circuit (Fig. 4d). Blocking ATP/ADP-mediated microgliosis recruitment either by inactivating the P2ry12 gene (Fig. 4f) or in response to acute pharmacological inhibition of P2RY12 in the brain (Fig. 4g) triggered an increase in neuronal responses to D1 agonist treatment, supporting the idea that P2RY12 modulates neuronal activity and seizures33,34. The same effect was observed when we rendered microglia unable to convert ATP to ADO. Microglia-specific deletion of Entpd1 (which encodes CD39) in adult mice (Extended Data Fig. 5a–c) was associated with an increase in striatal neuron PKA activity (Extended Data Fig. 5d), a decrease in striatal adenosine levels (Fig. 4h) and increased susceptibility to D1 agonist-induced seizures (Fig. 4i) (left), Extended Data Fig. 5e). In addition, pharmacological inhibition of A1R activity (Fig. 4j) or D1 neuron-specific ablation of A1R expression in mice (Fig. 4k, Extended Data Fig. 5g) triggers an exaggerated D1 neuron response that recapitulates the effect of microglia ablation (Figs. 1g, 2j, 4m) or of rendering microglia unable to respond to and process ATP (Fig. 4f, g, i). Conversely, the alterations in striatal neuronal activity and seizures in mice that lack microglia (Figs. 1g, 2j, 3c, d, 4m), the microglial P2ry12-mediated ATP response (Fig. 4f, g), or microglial CD39-mediated conversion of ATP to AMP (Fig. 4i) could be reversed by the administration of an A1R agonist (Fig. 4i, l, Extended Data Figs. 6d–g, 8i, j). The increased D1 agonist-induced seizure response in mice lacking A1R in D1 neurons was not prevented by pharmacological activation of A1R receptors on non-D1 neurons (Extended Data Fig. 8h), further supporting the highly localized nature of this mechanism. Collectively, these findings suggest that the ATP–AMP–ADO–A1R cascade has a critical role in local microglia-mediated suppression of D1 neurons in the striatum.

This novel microglia-controlled negative feedback mechanism is also likely to operate in other brain regions. Indeed, we found that microglia could reduce cortical neuron firing rates and seizures in response to glutamate receptor stimulation in a CD39– and A1R-dependent fashion in vitro (Extended Data Fig. 9) and in vivo (Extended Data Fig. 8f). Microglia-driven neurosuppression is likely to have a key role in constraining excessive neuronal activation that cannot be sufficiently suppressed by inhibitory neurons alone. This potent mechanism may also allow microglia to relay changes in the state of the local or peripheral
environment to neurons and thereby to direct specific behavioural responses. Microglia can respond directly to pro-inflammatory signals that arise from the periphery37,38 or are generated locally in the brain during neuro-inflammation or neurodegeneration39,40. It is conceivable that the downregulation of P2ry12 and Entpd1 expression in reactive microglia that is seen during various inflammatory24,40 and neurodegenerative diseases, including Alzheimer’s and Huntington’s diseases7 (summarized in Extended Data Fig. 10a–g) contributes to the pathological increases in neuron excitability and behavioural alterations that are associated with these disorders7,41,42 (Extended Data Fig. 10h–j). It is further tempting to speculate that abnormal neuronal functions during sickness behaviour or depression, which have been linked to aberrant activation of microglia13, might reflect changes in the ATP–AMP–ADO metabolic pathway or in neuronal A1R-mediated signalling responses.

Online content
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Mice were housed two to five animals per cage with a 12-h light–dark cycle (lights on from 0700 to 1900 h) at constant temperature (23 °C) and humidity (±50%) with ad libitum access to food and water. All animal protocols were approved by the IACUC at Icahn School of Medicine at Mount Sinai and were performed in accordance with NIH guidelines.

For brain-wide microglia ablation, adult C57Bl/6 wild-type mice (Jackson Laboratory, stock number 000664) between 8 and 16 weeks of age were treated with the CSF1R inhibitor PLX562244 (1,200 ppm chow, Mount Sinai and were performed in accordance with NIH guidelines.

Mice were anaesthetized with CO2 followed by decapitation. Brain regions of interest were rapidly dissected, frozen in liquid nitrogen and stored at −80 °C until further processing. RNA extraction from frozen samples was performed using the TRIzol/chloroform technique according to the manufacturer’s instructions (Invitrogen Corporation, Carlsbad, CA). After extraction, RNA was precipitated overnight at −80 °C in isopropanol with 0.15 M sodium acetate and Glycolblue (Life Technologies). The pellet was washed twice with 70% ethanol, air-dried, and resuspended in nuclese-free water. cDNA was prepared from total RNA using the High Capacity RNA-to-cDNA kit (Applied Biosystems).

Relative gene expression of the cDNA was assayed by qPCR (StepOne Software, ThermoFisher) using pre-designed recommended TaqMan gene expression assays from Applied Biosystems and following the manufacturer’s recommendations (IL34, Csf1, Gapdh, Entpd1, Adora1, Ccl3, Cd4, Kdm6b, Adhr1, Ccl24, Kcnk13, Lkbkb). Cycle counts for mRNA quantification were normalized to Gapdh. Relative expression (ΔCt) and quantification (RQ = 2 − ΔΔCt) for each mRNA were calculated using the ΔΔCt method as suggested.

Translating ribosome affinity purification

This approach relies on the genetic labelling of the ribosomal protein L10a with enhanced green fluorescent protein (eGFP) in a cell type-specific fashion followed by eGFP-based immunoaffinity purification of the ribosome-associated mRNAs25,59,60. The microglia-specific TRAP approach allows us to assess rapid changes in microglial ribosomal RNA association in the absence of aberrant microglial activation following tissue dissection and cell isolation25. To assess changes in microglia in response to CaMKII∗ neuron activation and inhibition using the DREADD approach, microglia-specific eGFP–L10a expression in response to CNO was induced with 0.25 mg/kg CNO 2 h before use of an Alzheimer’s disease mouse model, 5xfAD 57 mice were purchased from Jackson Laboratory/MMRRC (stock number 034840-JAX).

All mice were used for experiments backcrossed to the C57Bl/6j background for at least five generations. If not otherwise specified, Cre-negative littermate controls were used as controls. Unless otherwise specified, male and female mice were used for all experiments (only male mice were used for social interaction behaviour, live two-photon imaging of calcium transients in neurons, and live two-photon imaging of microglial process velocity and contact with boutons).

Routine genotyping was performed by tail biopsy and PCR as previously described25,52,58.
cyclohexamide (Sigma)). RNA was purified from beads directly using Biosystems). Homogenates were centrifuged for 10 min at 2,000 g at 4 °C to pellet large cell debris. NP-40 (EMD Biosciences, CA) and 1,2-diheptanoyl-sn-glycero-3-phosphocholine (Avanti Polari Lipids, AL) were added to the supernatant at final concentrations of 1% and 30 mM, respectively. After incubation on ice for 5 min, the lysate was centrifuged for 10 min at 13,000g to pellet insoluble material. Mouse monoclonal anti-GFP antibodies (clones 19C5 and 19F7, Antibody and Bioresource Core Facility, Memorial Sloan Kettering, NY) and biotinylated protein L (GenScript, Piscataway, NJ)–streptavidin MyOne T1 Dynabeads (Invitrogen) were added to the supernatant, and the mixture was incubated at 4 °C with end-over-end rotation overnight. Beads were collected on a magnetic rack and washed four times with high-salt polysome wash buffer (10 mM HEPES (pH 7.3), 350 mM KCl, 5 mM MgCl2, 1% NP-40, 0.5 mM dithiothreitol (Sigma), and 100 μg/mL cyclohexamide (Sigma)). RNA was purified from beads directly using RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions.

**RNA sequencing**

RNA purification from TRAP samples and from 5% of their corresponding unbound fractions was performed using RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions and used for subsequent sequencing. RNA integrity was assayed using an RNA Pico chip on Bioanalyzer 2100 using 2100 Expert Software (Agilent, Santa Clara, CA) and only samples with RIN >9 were considered for subsequent analysis. Double-stranded cDNA was generated from 1–5 ng RNA using Nugen Ovation V2 kit (NuGEN, San Carlos, CA) according to the manufacturer’s instructions. cDNA (500 ng per sample) was sonicated to obtain fragments of 200 base pairs using the Covaris S2 system (duty cycle, 10%; intensity, 5.0; bursts per second, 200; duration: 120 s; mode, frequency sweeping; power, 23 W; temperature, 5.5–6 °C; Covaris Inc., Woburn, MA). These fragments were used to produce libraries for sequencing by a TrueSeq DNA Sample kit (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. The quality of the libraries was assessed using the 2200 TapeStation (Agilent). Multiplexed libraries were directly loaded on a NextSeq 500 (Illumina) with high-output single-read sequencing using 75 cycles. Raw sequencing data were processed using Illumina bcl2fastq2 Conversion Software v2.17.

**Bioinformatic analysis of RNA-seq data**

Raw sequencing reads were mapped to the mouse genome (mm9) using the TopHat2 package (v2.1.0)44. Reads were counted using HTSeq-count (v0.6.0)44 against the Ensembl v67 annotation. The read alignment, read counting as well as quality assessment using metrics such as total mapping rate, mitochondrial and ribosomal mapping rates were done in parallel using an in-house workflow pipeline called SpeCtRA44. The raw counts were processed through a variance-stabilizing transformation (VST) procedure using the DESeq2 package (v2.1.0)45 to obtain transformed counts. The top 500 most variable genes across all samples on the basis of the VST procedure using the DESeq2 package (v2.1.0)45 were normalized to the mean across all samples (log2RPKM) was normalized to the mean across all samples. These fragments were used to produce libraries for sequencing by a TrueSeq DNA Sample kit (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. The quality of the libraries was assessed using the 2200 TapeStation (Agilent). Multiplexed libraries were directly loaded on a NextSeq 500 (Illumina) with high-output single-read sequencing using 75 cycles. Raw sequencing data were processed using Illumina bcl2fastq2 Conversion Software v2.17.

**Striatal nuclei isolation**

Striatal nuclei were isolated as previously described25. Mice were killed with CO2, and brain regions were quickly dissected and homogenized in 0.25 M sucrose, 150 mM KCl, 5 mM MgCl2, 20 mM tricine pH 7.8 with a glass Dounce homogenizer (1984-1002, Kimble Chase, Vineland, NJ). All buffers were supplemented with 0.15 mM spermine, 0.5 mM spermidine, and EDTA-free protease inhibitor cocktail (11836170001, Roche). The homogenate was then spun through a 29% iodixanol cushion. The resulting nuclear pellet was resuspended in 0.25 M sucrose, 150 mM KCl, 5 mM MgCl2, 20 mM tricine pH 7.8, supplemented with 200 μM DyeCycle Ruby (V10304, Invitrogen) and 10% donkey serum (017-000-121, Jackson Immunoresearch, West Grove, PA). Striatal nuclei were sorted in a BD FACS Aria II cell sorter by gating for the lowest DyeCycle Ruby population, which indicates singlet nuclei.

**Single-nucleus RNA library preparation and sequencing**

Single-nucleus RNA-seq (snRNA-seq) was performed on these samples using the Chromium platform (10x Genomics, Pleasanton, CA) with the 3’ gene expression (3’ GEX) V2 kit, using a targeted input of ~5,000 nuclei per sample. In brief, gel-bead in emulsions (GEMs) were generated on the sample chip in the Chromium controller. Barcoded cDNA was extracted from the GEMs using Post-GEM RT-cleanup and amplified for 12 cycles. Amplified cDNA was fragmented and subjected to end-repair, poly-A-tailing, adaptor ligation, and 10x-specific sample indexing following the manufacturer’s protocol. Libraries were quantified using Bioanalyzer (Agilent) and QuBit (Thermofisher) analysis and then sequenced in paired-end mode on a HiSeq 2500 instrument (Illumina, San Diego, CA) targeting a depth of 50,000–100,000 reads per nucleus.

The raw read data were demultiplexed, aligned and analysed using 10X Cell Ranger (v2.1.0). To capture unspliced pre-mRNA in the single-nucleus RNA expression assay, intronic regions in the 10X Cell Ranger mm10 v1.2.0 reference were marked as exonic as suggested by 10X for pre-mRNA reference generation. Data from two libraries were aggregated together with read depth normalized according to the number of mapped reads per nucleus of each library. Unique molecular identifier (UMI) threshold of 250 was manually selected to include the microglial population, resulting in a total of 15,950 sequenced nuclei. The median number of UMIs detected per nucleus was 1,707, with a median of 1,075 genes per nuclei. Doublet populations were manually excluded from the analysis. Unique populations were manually annotated using known markers for striatal cell types31 using the 10X Loupe Cell Browser (v2.0.0) (10X Genomics). Expression of individual genes was visualized by t-SNE on the Loupe browser, with
In situ hybridization was performed as previously described. Mice were calculated for each sequenced nucleus using R and graphed by normalized expression values (UMIs per 10^3 UMIs) for selected genes. For analysis of CD39 and CD73, single-cell suspensions were prepared from neonatal pups (4–6 forebrains, age P5–P7). For analysis of CD39 and CD73, single-cell suspensions were prepared from neonatal pups and stained with YFP and CD73. Forward and side scatter were used to gate on a defined population of cells to exclude debris and also to select single cells. Live cells were determined as YFP negative in Cx3cr1fl/fl;YFPmice, microglia were identified by YFP expression. FACS plots show gating on live cells that correspond to microglia, which made up 6–7% of all cells in samples prepared from adult forebrain.

One-hundred per cent of YFP+ cells were CD39+. No cells were YFP−/CD39+. Therefore, CD39−/YFP− were used to identify microglia in subsequent FACS experiments. Wild-type and Nt5e−/− mice, single, live cells were identified by forward and side scatter gating as well as lack of DAPI expression. Wild-type and Nt5e−/− mice were stained with CD39−/A700 and gated on YFP+ cells. Nt5e−/− mice were used in parallel to determine the specificity of the CD73−/PE antibody. Seven per cent of live cells were CD39+ microglia in both wild-type and Nt5e−/− mice. No cells were CD39+ in Nt5e−/− mice. CD39− cells were identified using gates created from Nt5e−/− mice, IgG isotype control samples, and unstained samples.

Immunofluorescence staining
Immunostaining was performed as previously described. Male wild-type mice aged 3 months fed with a control or PLX5622 diet for 3 weeks, 4–8-month-old male and female CaMKII-IActo-CHRM3 Cx3cr1fl/flCd11b+/− mice and CaMKII-IActo-CHRM4 Cx3cr1fl/flCd11b+/− mice and CaMKII-IActo-CHRM4 Cx3cr1fl/flCd11b+/− mice were stained with Nestin, I344/Drd1a−/−, I344/Drd2−/−, Csf1−/−, Nt5e−/−, and Cdf190/Cx3cr1fl/flCd11b+/− mice and littermate controls were anesthetized with ketamine (120 mg/kg) and xylazine (24 mg/kg) and perfused transcardially with 10 ml PBS and 40 ml 4% parafomaldehyde (Electron Microscopy Sciences). Fixed brains were removed and dehydrated in 5%, 15%, and 30% sucrose in PBS. Following dehydration, brains were frozen in Neg-50 (Thermo Scientific) on dry ice and stored at −80 °C until further processing. Brains were cut using a cryostat and 25-μm sections were mounted on SuperFrost Plus microscope slides (Fisher Scientific).Slides were stored at −80 °C until staining. Slides were washed with PBS, permeabilized with PBS + 0.2% Triton X-100 (PBST) and blocked with 2% normal goat serum in PBST for one hour at room temperature. Slides were incubated with primary antibodies (IBAI (1:500) 019-19741, Wako; CD11b (1:500) MCA711GT, Biocad; GFP (1:2,000) ab6556, Abcam; GFP (1:500) ab193970, Abcam; NeuN (1:500) MAB377, Millipore; NeuN (1:500) ABIN91, Millipore, cFOS (1:1,000) ab190289, Abcam; GFAP (1:500) G3893, Sigma; Olig2 (1:500) sc-293163, Santa Cruz; P2RY12 (1:1,000) AS-5043A, Anaspec) in 2% normal goat serum in PBST overnight at 4 °C. Slides were washed in PBST and incubated with Alexa Fluor conjugated secondary antibodies (Alexa Fluor 488-, 546-, and 647-labelled goat anti-mouse, goat anti-rat, goat anti-chicken, or goat anti-rabbit IgGs (H+L): 1:500, Thermo Scientific) in 2% normal goat serum in PBST for 1 h at room temperature. Slides were washed and coveredslips using Prolong Gold anti-fade with DAPI (Invitrogen) and dried overnight. Imaging was performed using a Zeiss LSM 780 Confocal Microscope (Zeiss, Oberkochen, DE). For z-stack images, 20-μm z-stack confocal images were acquired at 20% Percoll (GE Healthcare), and cultured cells were also used for immunoblotting.

Microglia flow cytometry
FACS analysis was used to determine microglial CD39 and CD73 surface expression as well as the purity of CD11b−/bead-isolated microglia. For confirmation of the purity of the CD11b−/bead-isolated microglia, single-cell suspensions were prepared from neonatal pups (4–6 forebrains, age P5–P7). For analysis of CD39 and CD73 expression on adult microglia, single-cell suspensions were prepared from the forebrains of wild-type, Cx3cr1fl/flCd11b+/− mice, which express cytosolic YFP under the microglial Cx3cr1 promoter, and Nt5e−/− (CD39-deficient) mice.

Tissue was rapidly dissected and mechanically dissociated in HBSS and centrifuged over 20% Percoll (GE Healthcare), and nonmonoclear
2-μm intervals, with a 40×/1.3 oil objective at 0.6 or 1× zoom. For single plane images, 4.6-μm images were acquired with 20×/0.8 or 40×/1.3 objectives at 0.6 or 1× zoom. Image processing was performed using Zen 2012 software (Zeiss). Cell counting was done using the ImageJ cell counter tool by counting cFOS+ cells or IBA1+ cells that overlapped with DAPI nuclei. Graphs show the average number of cells in white matter and grey matter per mouse (n = 2–4 mice, 4–11 images per mouse). Striatal images were taken at 20× as described above. ImageJ was used to calculate the percentage of the image that was white matter or grey matter.

**Immunoblotting**

Western blot analysis was performed as previously described. Male and female age-matched mice were used. Mice were anesthetized with CO2, followed by decapitation, and the region of interest was rapidly dissected and frozen in liquid nitrogen and stored at −80 °C until further processing. For analysis of DARPP32 phosphorylation, mice were killed, brains were immediately frozen in liquid nitrogen, and frozen striatum punches were extracted. Samples were sonicated at 4 °C in 1% SDS solution supplemented with protease inhibitor (Roche, Switzerland) and PhosStop phosphatase inhibitor (Roche, Switzerland), and boiled for 10 min. The protein concentration was determined using a BCA protein assay kit (ThermoFisher Scientific, USA) according to the manufacturer’s instructions. Protein samples were diluted in an equal volume of 2× LDS sample buffer (Invivogen) and supplemented with DTT to a final concentration of 200 mM (Sigma).

**Tissue protein samples (20–40 μg)** were separated on 4–12% Bolt Bis-tris precast denaturing gels or 10% NuPAGE Bis-tris precast denaturing gels (Invitrogen, USA) and transferred onto PVDF membranes. Membranes were blocked for 1 h and probed with primary antibodies diluted in 5% milk-TBST solution or 2% BSA overnight at 4 °C (DARPP32 (1:1,000), Novus, Cat#NB300-304; DARPP32-TH3R34 (1:1,000), Cell Signaling, Cat#124383; DARPP32-TH3R75 (1:1,000), clone cc911; DARPP32 (1:5,000), clone 6a kindly provided by A. Nairn and P. Greengard; IL34 (1:1,000), Abcam, Cat#AF5195; GLUR1-SER845 (1:1,000), Cell Signaling, Cat#1385; GLUR1 (1:1,000), Millipore, Cat#MAB2263; DRD1 (1:1,000), Abcam, Cat#ab20066; CD39 (1:1,000), R&D Systems, Cat#AF4398; CD73 (1:500), Cell Signaling, Cat#13660; H3 (1:5,000), Abcam, Cat#ab1791; IBA1 (1:1,000), Wako, Cat#016-20001; P2RY12 (1:1,000), Anaspec, Cat#AS-S5043A). Membranes were then washed and probed with horseradish-peroxidase conjugated anti-mouse (GE), anti-rabbit IgG (GE), anti-rat IgG (Invitrogen) or anti-sheep IgG (Jackson ImmunoResearch, USA) secondary antibodies (all:1:10,000) for one hour at room temperature. Membranes were developed using enhanced chemiluminescence substrate (PerkinElmer, USA) and exposed on film. Exposed films were scanned, and protein bands were quantified using ImageJ Software (NIH, USA). All values were plotted relative to littersmate control samples.

For immunoblotting of CD11b+ isolated microglia, cells were counted on a haemocytometer and centrifuged at 300g for 15 min to pellet cells. Cells were resuspended in 100 μl lysis buffer (see above), sonicated, and boiled for ten minutes. The lysed cells were concentrated by centrifugation (Protein Concentrator Tubes, 3K, Pierce) and diluted in an equal volume of 2× LDS sample buffer (Invitrogen) supplemented with DTT (final concentration 200 mM) to a final concentration of 50,000 cells per μl. Increasing numbers of cells were loaded along with whole tissue samples of control and CD73− striatal lysate (5 ng). Samples were run, transferred, and incubated in primary and secondary antibodies as described above. Membranes were developed using chemiluminescence substrate (PerkinElmer, IBA, H3) or with SuperSignal West Femto Maximum Sensitivity Substrate (ThermoScientific) for less abundant proteins (CD73).

**Slice biotinylation assay of membrane-bound proteins**

Slice biotinylation was adapted from previously described protocols. Ten-week-old male and female IL34−/− and IL34−/−Drd1α−/− mice were anesthetized with isoflurane and the brain was rapidly removed and sliced (300 μm) in prechilled, pre-saturated (95%/5% O2/CO2) 1× sucrose artificial cerebrospinal fluid (SACSF) on a vibratome. Slices were recovered for 45 min at 31 °C in oxygenated artificial cerebrospinal fluid (ACSF). Following recovery, slices were incubated with 1.0 mg/ml sulfo-NHS-SS-biotin on ice for 45 min. Slices were washed 3 times with ice-cold ACSF and incubated for 10 min in ice-cold ACSF. Slices were then washed 3 times with ice-cold quencher buffer (ACSF, 100 mM glycine and incubated twice for 25 min on ice to quench excess free biotin. Slices were then washed in ice-cold ACSF three times and the striatum was micro-dissected. Tissue was pelleted by centrifuging at 200g for 1 min. Supernatant was removed and tissue was resuspended in 300 μl ice-cold RIPA buffer (Thermo Scientific) with protease inhibitors (Roche) and pipetted to break up tissues. To complete tissue lysis, samples were incubated for 30 min at 4 °C with end-over-end rotation. Debris was pelleted by spinning at 1,800g for 15 min. Supernatant was collected and heated at 98 °C for 10 min and sample concentration was assessed using a BCA protein assay kit (Thermo Fisher Scientific, USA) according to the manufacturer’s instructions. A portion of protein was aliquoted and diluted in an equal volume of 2× LDS sample buffer (Invitrogen) and supplemented with DTT to a final concentration of 200 mM (Sigma) to serve as input. Streptavidin MyOne T1 Dynabeads (Invitrogen) were washed in RIPA/PI buffer and added to a known concentration of protein lysate and were rotated at 4 °C overnight. The following day, samples were spun down and biotinylated and unbound fractions were magnetically separated and collected. Biotinylated proteins were eluted off magnetic beads in 2× LDS sample buffer supplemented with DTT by boiling for 5 min at 95 °C and separated on a magnet. Equal amounts of protein (5–20 μg) were loaded for immunoblotting.

**Neuronal culture**

Primary neuronal culture was performed as previously described. Embryonic day 18 (E18) timed-pregnant female mice were anesthetized with CO2 and killed by cervical dislocation. In a dissection hood, 10–12 embryos per experiment were collected through an incision in the mother’s abdomen, taken out of the amniotic sacs, and decapitated in ice-cold Hank’s balanced salt solution (HBSS). Using fine scissors and forceps, brains were rapidly dissected and the cortex cleared from meninges and isolated under a dissection microscope. Cortices were collected in ice-cold HBSS and kept on ice until all embryos had been dissected. In a tissue culture hood, HBSS was removed and the cortex tissue was digested using 0.25% Trypsin-EDTA for 15 min at 37 °C, then treated with DNaseI for 10 min at 37 °C. The tissue was dissociated by serial triturations with a 25-ml serological pipette, followed by triturations with 10- and 5-ml serological pipettes. The cell suspension was washed once with DMEM, supplemented with 10% FBS and 1% penicillin–streptomycin, and passed through a 40-μm cell strainer before being counted on a haemocytometer. Single cells were seeded on poly-β-lysine (0.1 mg/ml)-coated wells at a density of 104 cells per well on a 12-well plate. Cells were grown in neurobasal medium, supplemented with B27 supplement, N2 supplement, and 0.5 mM glutamine and maintained at 37 °C in 5% CO2.

**Axion recording**

For multiple electron array (MEA) recordings, AccuSpot Classic MEA 48 plates with 16 microelectrodes per well were used (M768-KAP-48A, Axion). Plates were pre-incubated at 37 °C for one hour with 5 μg of 0.1%
PEI(P3143, Sigma) in borate buffer (boric acid (A73-500, Fisher), sodium tetraborate (221732, Sigma)). After incubation, plates were washed four times with 200 μl of H2O and dried overnight in the hood with the lid cracked. On the next day, neurons were cultured as described above, and the dried Axion plate was coated with 20 μg/ml laminin in neurobasal medium (L2020, Sigma) mid-way through the neuronal culture. Five microliters of laminin was dropped into each well of the plate and incubated at 37 °C for 1–2 h without letting the plate dry. Laminin was removed directly before seeding the well with neurons. Neurons were diluted to 12 million cells/ml and 5 μl was dropped onto each well to seed 60,000 neurons per well. The plate was incubated for 1 h and then 250 μl DMEM medium, supplemented with 10% FBS and 1% penicillin-streptomycin, was added to each well. On the next day, 250 μl neurobasal medium supplemented with B27 supplement, N2 supplement, and 0.5 mM-glutamine was added to the wells. A half-medium change with neurobasal medium with supplements was performed every 3 days. Microglia were isolated using the MACS Miltenyi system as described29 and 100,000 isolated microglia were added to each well of the MEA plate after 12 days in vitro (DIV12). Baseline Axion recordings were performed at DIV14 on the Axion Maestro MEA reader and the electrical activity of the culture was recorded for 10 min and analysed using AxIS software. On DIV14, two hours after baseline recording, the MEA plate was treated with combinations of the following compounds: 10 μM glutamate (Sigma), 100 nM A1R agonist (CPA, Tocris), 100 nM A1R antagonist (DCPCX, Sigma), 10 μM adenosine (Sigma), 200 μM CD39 inhibitor (2h pretreatment, ARL 67156, Tocris). Recordings were performed 1 h after treatment. For analysis, wells were excluded if the mean firing rate was <1 Hz or if the well contained inactive electrodes (<6 active electrodes).

Adenosine assay
Mice were anaesthetized with isoflurane and striata were rapidly dissected, snap frozen, and stored at −80 °C until processing. Weighed tissue was sonicated in 1× PBS and adenosine was measured by fluorometric assay (MET-5090, Cell BioLabs) following the manufacturer’s instructions.

Two-photon imaging of neuronal activity
Surgery. Male mice were anaesthetized with an isoflurane gas/carbon mixture (5% for induction and 1.5% for maintenance during surgery) and carefully placed in a stereotaxic frame (David Kopf Instruments, CA, USA). Body temperature was maintained at 36–37 °C and ophthalmic ointment was applied to prevent the eyes from drying. After shaving and sterilizing the skin with chlorhexidine, a midline incision was made with a sterile scalpel. Then a surgical scissor was used to cut off extra skin and further expose the skull surface. The skull surface was wiped and cleaned with autoclaved cotton swabs. Bregma and lambda were identified and levelled to be on the same Z-axis. Then a craniotomy hole was drilled over the striatum (antero-posterior: −0.1 mm, medio-lateral: −1.5 mm relative to bregma) with drill bits (#73 size, Kyocera). AA9 Syn-GCaMP6s (600 μl, Addgene, #100843) was injected into the striatum (antero-posterior: −0.1 mm, medio-lateral: −1.5 mm, dorso-ventral: −2.85 mm relative to bregma) using a blunt 35-gauge microinjection needle within a 10-μl microsyringe (NanoFil, World Precision Instruments, FL, USA), which was controlled by a microsyringe pump (UMP3, World Precision Instruments) and a controller (Micro4, World Precision Instruments). The AAV was injected at a rate of 50 nl/min.

Following viral injection, a 23-gauge needle was mounted to a stereotaxic cannula holder (Doric lenses) and lowered to 0.5 mm above the injection site (antero-posterior: −0.1 mm, medio-lateral: −1.5 mm, dorso-ventral: −2.25 mm relative to bregma). After the needle was held at that location for 5 min, it was retracted and lowered to the coordinates mentioned above several times to create the path for GRIN lens implantation. After complete retraction of the 23-gauge needle, a GRIN lens of 0.6 mm diameter, 7.3 mm length (INSCOPIX, INC, CA, USA) was assembled with a ferruleusing super glue (Loctite) and mounted to a stereotaxic cannula holder (Doric lenses), then lowered to 0.15 mm above the injection site (antero-posterior: −0.1 mm, medio-lateral: −1.5 mm, dorso-ventral: −2.7 mm relative to bregma). A layer of adhesive cement (C&B Metabond, Parkell Inc.) was applied to the skull surface to strongly hold the implanted ferrule. After the adhesive cement had completely dried, a thick layer of dental cement (Lang Dental) was applied to build a head cap. Before the head cap fully solidified, we applied some super glue to the bottom surface of the head ring and placed the head ring around the exposed ferrule. Then we wrapped the exposed upper surface of the lens with a small piece of parafilm, then added KWIK-SIL on top to protect the lens. Mice were given 1 mg/kg buprenorphine SR and 5 mg/kg ketoprofen subcutaneously (s.c.) intraoperatively and received 30 mg/kg ibuprofen in their home cage water for seven days post-surgery.

Habituation to head-fixation and i.p. injection. After a 1–2-week recovery from the surgery, the mice were randomly divided into two groups: one group received PLXS622 and one group received control chow (lacking inhibitor).

The mice were later put under water restriction (1.5 ml/day) and were handled and habituated daily to head-fixation and immobilization for 2 weeks. They were immobilized in a polyethylene tube and head-fixed in the future recording environment under the two-photon microscope. During this habituation period, we increased the head-fixation period from 3 min to 40 min gradually. After they showed no signs of stress and drank water provided randomly during the 40-min session, we switched to no-water-provided head-fixation habituation for future recording.

After habituation to head-fixation, we also performed daily habituation to i.p. injection before the head-fixation session. Mice were injected i.p. with a microlitre volume equivalent to 10× the body weight in grams (10 × BW μl) of saline, matching the volume to be injected in future imaging sessions, and then performed head-fixation. This habituation was performed for -1 week until the mice showed a reduction in clear signs of stress upon handling and i.p. injection.

In vivo two-photon imaging. We use a customized two-photon microscope with a galvo-galvo scanner for imaging. The setting we used through the session below is 4 Hz scan rate at 194 × 194 pixels. The imaging protocol lasted for 7 days and included 3 sessions. Session 1: 3 days of baseline recording. We injected 10 × BW μl of saline i.p., started recording 10 min after the injection and imaged for 10 min. For analysis of baseline neuronal activity, data were pooled from three days of 10-min baseline (saline injection) recordings.

Session 2: 3 days of recording after SKF81297 injection. We injected 10 × BW μl of SKF81297 solution (3 mg/kg, diluted in saline) i.p. and started the recording 10 min after injection. A low dose of SKF81297 was used to avoid confounds induced by seizure activity. The imaging lasted for 30 min, analysed in 10-min bins, to capture the whole post-injection dynamics. For the analysis of neuronal activity in response to SKF81297, data were pooled across three days of imaging for each 10-min bin starting 10 min after injection (first bin: 10–20 min after injection, second bin: 20–30 min after injection, and third bin: 30–40 min after injection).

Session 3: 1 day of recording after i.p. injection of mixed solution of SKF81297 (3 mg/kg, diluted in saline) and CPA (0.1 mg/kg, diluted in saline). The recording was started 10 min after injection and lasted for 30 min. For the analysis of neuronal activity in response to co-administration of SKF81297 and CPA, data were pooled from the 30-min imaging session.

Calcium imaging data analysis. The calcium imaging data were originally acquired as a DAT file in our customized system and later processed into TIF files. The data were first rigid motion corrected using a custom script adapted from NoRMCorre (https://github.com/flatironinstitute/NoRMCorre); see ref. 77 for technical details.
The motion-corrected data were later run through a pipeline adapted from Suite2P (https://github.com/cortex-lab/Suite2P) for automatic ROI detection and spike deconvolution. To account for out-of-focus contamination from background signals \( (F_{bg}) \), a fraction \( d = 0.7 \) of the background was subtracted from the raw fluorescence \( (F_{raw}) \). The relative change in fluorescence was calculated as \( \Delta F/F = (F - F_{bg})/F_{bg} \), where \( F \) is the background-corrected fluorescence \( F = F_{raw} - dF_{bg} \) and \( F_{bg} \) is the median of the \( F \) distribution.

To identify significant calcium events, we used a peak detection algorithm that identifies maxima in the derivative of the \( \Delta F/F \) signal implemented in Matlab (peakfinder\(^*\)). The identified maxima must be above a threshold, defined as the mean plus 3 s.d. of the \( \Delta F/F \) distribution.

To understand how the similarity between calcium traces of neurons varies as a function of their distance from each other, we used a method described before\(^8\) which involves calculating the Pearson's correlation coefficient (PCC) between two time series traces \( x \) and \( y \), where \( PCC = \frac{\sum_{i=1}^{N}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{N}(x_i - \bar{x})^2 \sum_{i=1}^{N}(y_i - \bar{y})^2}} \). \( \bar{x} \) and \( \bar{y} \) denote the mean and s.d., respectively. The PCC was calculated for pairs of neurons where the distance between them was calculated as the Euclidean distance between the centres of their somas.

Synchrony was calculated as previously defined\(^8\). In brief, onsets of calcium transients were identified with a threshold crossing of 2 ± s.d. of the calcium trace baseline. In order to account for uncertainty associated with threshold detection, each event is represented as a 750-ms (3-frame) pulse centred at the onset of the calcium trace. We defined two events to be synchronous if they overlapped at least at one time instant. The binary matrix was then used to calculate synchrony between two cells as the average of the ratio of the number of times both cells were simultaneously active to the total activations of each cell.

### Two-photon imaging of microglial protrusion and synaptic terminals

For imaging of microglia and synaptic terminals, male Cx3cr1\(^{eGFP}\)- mice (8–10 weeks) derived from the C57BL/6J strain, in which enhanced green fluorescent protein (eGFP) expresses under the microglial Cx3cr1 promoter, were used. For Ca\(^{2+}\) imaging of synaptic terminals, male C57BL/6J mice (6–10 weeks) were used.

#### Surgery and virus injection

Mice were anaesthetized with mixture of ketamine (74 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). The skull was exposed and disinfected, and a custom-made head plate was firmly exposed and disinfected, and a custom-made head plate was firmly fastened onto the skull. Mice were allowed to recover for 1 day before the following craniotomy and viral injection.

For virus injection, a circular craniotomy (2 mm diameter) was performed over the left primary motor cortex (centred at 0.2 mm anterior and 1 mm lateral from bregma) under isoflurane (1%) anaesthesia. For imaging of microglia and synaptic terminals, mixed AAV solution (Addgene; AAV8.hSyn.mH3D(Gq).mCherry; 5 × 10\(^7\) vector genomes/ml, UPenn Vector Core; AAV2/1-CAG-FLEX-TdTomato: 7.6 × 10\(^7\) vector genomes/ml, and AAV2/1-CaMKII-Cre: 2.94 × 10\(^7\) vector genomes/ml, diluted 1:10,000 in saline) was injected into the ventral lateral nucleus of the thalamus (1 mm anterior and 1 mm lateral from bregma, 3,600 μm deep). For Ca\(^{2+}\) imaging of synaptic terminals, AAV2/1-Syn-GCaMP6s: 2.7 × 10\(^7\) vector genomes/ml (1:2 diluted in saline) and AAV8.hSyn.hM3D(Gq).mCherry were injected into the ventral lateral nucleus of the thalamus (1 mm anterior and 1 mm lateral from bregma, 3,600 μm deep). To exclude the effects of CNO, control mice with the same AAV2/1-CAG-FLEX-TdTomato and AAV2/1-CaMKII-Cre cocktail but omitting AAV8.hSyn.hM3D(Gq).mCherry were used. Virus was filled into a glass capillary with filament (GDC-1; Narishige, Tokyo, Japan) and injected at the stereotaxic coordinates over 5 min. After the injection, a double glass window comprising 2- and 4.5-mm glass coverslips (Matsunami Glass, Osaka, Japan) joined together with an ultraviolet curable adhesives (NOR-61, Norland) was implanted over the craniotomy.

#### Two-photon image analysis

Analysis was performed using ImageJ (1.52v; NIH). All images were corrected for focal plane and depth direction displacements using HyperStackReg (Ved Sharma, 2015–2016). For quantification of interactions between microglia and pre-synapses, 2-μm-diameter ROIs were manually defined around axonal boutons, and mean intensity in the green channel (microglia) within each ROI was measured for all frames. To normalize this value, mean intensity was divided by the average intensity of five control frames within the same ROI, which were recorded before drug stimulation. Microglial contacting onto boutons was further demonstrated by measuring the PCC value of red and green channels with the ImageJ Coloc 2 plugin. To assess microglial motility in time-lapse images, the ImageJ built-in tool Manual Tracking was used to track the tips of primary processes in each frame. Microglial tips were identified by eGFP expression (Cx3cr1\(^{eGFP}\)-mice). We tracked 7–9 microglia tips per microglia for 2–4 microglia per mouse. We measured the trajectories of primary processes that were defined as branches emanating directly from the cell body. To track the process tips, we first identified the tips by projecting 20 × 1-μm \( z \)-slices and the farthest point of each primary processes was plotted with ImageJ software. The ImageJ built-in tool Manual Tracking was used to track the tips of processes for each frame.

#### Confirmation of viral targeting and CNO-mediated activation of neurons

##### Two-photon Ca\(^{2+}\) imaging

All imaging was conducted 5 weeks after the virus injection to acquire sufficient virus expression and brain tissue recovery to prevent microglial activation.

The two-photon microscopy setup was composed of a laser scanning system (NIS-Elements; Nikon Instech Co., Ltd, Tokyo, Japan), a mode-locked Ti:Sapphire Chameleon Ultra II laser (Coherent, Santa Clara, CA) set at 950 nm and a water-immersion objective lens (25×, N.A. 1.10; Nikon Instech Co., Ltd). XYT imaging was conducted over the primary motor cortex, and the imaging plane was within 100–150 μm of the surface. The 1,024 × 1,024 pixel imaging field was 129.96 μm × 129.96 μm with a pixel size of 0.1269 μm. Z-step size was 1 μm, each XY frame duration was 1 min and the whole imaging session was 3 h long. For DREADDs excitation or microglial P2Y12R inhibition, CNO (Tocris Bioscience, Bristol, UK; 5 mg/kg) or clodipogrel (Sanofi-Aventis; 100 mg/kg) was dissolved in saline and freshly prepared before every injection. CNO, clodipogrel and CNO + clodipogrel imaging sessions were started immediately after the intravenous administration of CNO to mice. Note that the mice used for each experiment were totally naive for all drugs. In control imaging, mice were treated with saline injection. Mice were imaged for 3 h immediately after the injection.

##### Two-photon Ca\(^{2+}\) image analysis

Analysis was performed using ImageJ (1.52v; NIH). All images were corrected for focal plane and depth direction displacements using HyperStackReg (Ved Sharma, 2015–2016). For quantification of interactions between microglia and pre-synapses, 2-μm-diameter ROIs were manually defined around axonal boutons, and mean intensity in the green channel (microglia) within each ROI was measured for all frames. To normalize this value, mean intensity was divided by the average intensity of five control frames within the same ROI, which were recorded before drug stimulation. Microglial contacting onto boutons was further demonstrated by measuring the PCC value of red and green channels with the ImageJ Coloc 2 plugin. To assess microglial motility in time-lapse images, the ImageJ built-in tool Manual Tracking was used to track the tips of primary processes in each frame. Microglial tips were identified by eGFP expression (Cx3cr1\(^{eGFP}\)-mice). We tracked 7–9 microglia tips per microglia for 2–4 microglia per mouse. We measured the trajectories of primary processes that were defined as branches emanating directly from the cell body. To track the process tips, we first identified the tips by projecting 20 × 1-μm \( z \)-slices and the farthest point of each primary processes was plotted with ImageJ software. The ImageJ built-in tool Manual Tracking was used to track the tips of processes for each frame.

#### Confirmation of viral targeting and CNO-mediated activation of neurons

##### Two-photon Ca\(^{2+}\) imaging

All imaging was conducted 5 weeks after the virus injection to acquire sufficient virus expression and brain tissue recovery to prevent microglial activation.

The two-photon microscopy setup was composed of a laser scanning system (NIS-Elements; Nikon Instech Co., Ltd, Tokyo, Japan), a mode-locked Ti:Sapphire Chameleon Ultra II laser (Coherent, Santa Clara, CA) set at 950 nm and a water-immersion objective lens (25×, N.A. 1.10; Nikon Instech Co., Ltd). XYT imaging was conducted over the primary motor cortex, and the imaging plane was within 100–150 μm of the surface. The 1,024 × 1,024 pixel imaging field was 129.96 μm × 129.96 μm with a pixel size of 0.1269 μm. Z-step size was 1 μm, each XY frame duration was 1 min and the whole imaging session was 3 h long. For DREADDs excitation or microglial P2Y12R inhibition, CNO (Tocris Bioscience, Bristol, UK; 5 mg/kg)
was dissolved in saline and freshly prepared before every experiment. Saline and CNO imaging sessions were started 30 min after the intravenous administration of drug or the same amount of saline to mice.

**Ca²⁺ imaging analysis.** Analysis was performed using ImageJ (1.52v; NIH) and MATLAB (2019b: The Mathworks, Natick, MA). All images were corrected for focal plane using TurboReg. Axonal terminal identification, Ca²⁺ activity extraction and quantification of area under the curve (AUC) were conducted using customized MATLAB scripts.

**Electrophysiological recordings of spontaneous excitatory postsynaptic currents (sEPSCs).**

**Brain slice preparation for physiology recordings.** Eight- to twelve-week-old male and female Il13<sup>fl/flDrd1aCre<sup>/Drd1atdTomato<sup> mice were used for all electrophysiology experiments. Mice were anesthetized with isoflurane followed by transcardial perfusion with oxygenated (95% O₂/5% CO₂) N-methyl-d-glucamine (NMDG) HEPES solution (in mM): 92 NaNGD, 2.5 KCl, 1.2 NaH₂PO₄, 30 NaHCO₃, 20 HEPES, 25 glucose, 5 Na⁺ ascorbate, 2 thiourea, 3 Na⁺ pyruvate, 10 MgSO₄ 7H₂O, 0.5 CaCl₂, 2 H₂O, with pH adjusted to 7.3–7.4, 300–310 mOsm). The brain was quickly removed into ice-cold NMDG HEPES solution for 1 min. Coronal slices (200 μm thick) containing the striatum were cut with a vibratome (Leica VT1200S, Germany). We then moved slices into a pre-warmed (32 °C) recovery chamber and carried out the stepwise Na⁺ spike-in procedure, then kept the slices at room temperature for at least 1 h, in the following solution: 95% O₂/5% CO₂-equilibrated HEPES holding solution containing the following (in mM): 92 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 30 NaHCO₃, 20 HEPES, 25 glucose, 5 Na⁺ ascorbate, 2 thiourea, 3 Na⁺ pyruvate, 2 MgSO₄ 7H₂O, 2 CaCl₂, and 2 H₂O.

**Voltage-clamp electrophysiology.** Recordings were made under an upright microscope (Scientifica SliceScope Pro 2000, Scientifica, UK) equipped with infrared differential interference contrast optics for visualization. Slices were transferred to a recording chamber superfused with standard recording ACSF containing (in mM) 124 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 24 NaHCO₃, 5 HEPES, 12.5 glucose, 2 MgSO₄ 7H₂O and 2 CaCl₂, 2H₂O, adjusted to pH 7.3–7.4, 295–305 mOsm. Recordings were performed at 32 °C. Patch pipettes were made from borosilicate glass capillary tubing (1B150F-4; World Precision Instruments) using a micropipette puller (PC-10; Narishige, Japan).

For measurements of sEPSCs in the striatum, the internal recording pipette solution was potassium-based and contained the following (in mM): K⁺ gluconate 130, KCl 4, EGTA 0.3, HEPES 10, MgATP 4, Na₂GTP 0.3, phosphocreatine 10; pH adjusted to 7.3–7.4, 295–305 mOsm. Solution was ACSF + 100 μM picrotoxin. sEPSCs were recorded from neurons (dSPNs) visualized by ribosomal eGFP expressed in dSPNs and dendritic arborization analysed using Neurolucida software (MBF Bioscience).

**Microdialysis**

C57/B16 male mice were put on PLX5622 or control chow (n = 5 PLX5622 diet, n = 5 control diet) for one week. Dialysis probes were implanted and mice recovered for one week before commencing microdialysis collection. Microdialysis guide canulae (Harvard Apparatus, Holliston, MA) were stereotaxically implanted into the striatum (A/P: +1.4 mm; M/L: −1.0 mm; D/V: −3.8 mm from skull). Microdialysis experiments were conducted after a one-week recovery period following guide cannula implantation. Dialysis tubing was flushed before initial use with 70% EtOH for 5 min, followed by dH₂O via syringe pump (Model R99-E, Razel Scientific Instruments, Saint Albans, VT) at a flow rate of 1 μl/min. The tubing was then attached to the microdialysis probe (Cuprophan 6kD, membrane length 1 mm; Harvard Apparatus, Holliston, MA), which was primed by placing the probe in ACSF (pH 7.4: 148 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl₂, 0.85 mM MgCl₂) and running ACSF through the microdialysis tubing and probe at 0.8 μl/min. Microdialysis experiments were done in anaesthetized animals. In brief, mice were placed into a stereotaxic frame under isoflurane anaesthesia (4% induction, 1.75% sustained). The probe was inserted into the guide cannula, and dialysate was collected after 20 min. All collections were frozen at −80 °C immediately after collection.

**Liquid chromatography/mass spectrometry**

Adenosine was quantified using ultra pressure liquid chromatography/mass spectrometry (UPLC/MS) in the Vanderbilt University Neurochemistry Core. Analyses in 5 μl microdialysis fluid were derivatized by sequential dilution with 10 μl 100 mM NaCO₃ (aq) and 2% benzoyl chloride (BZC) in acetoneitrile. Following a 2-min incubation, the reaction was stopped by the addition of 10 μl internal standard solution (in 20% acetonitrile containing 3% sulfuric acid). The adenosine internal standard was prepared by derivatization with 1H3-sulfonylbenzoyl chloride as described. Standard stocks were frozen at −80 °C in aliquots to prevent multiple freeze–thaw cycles. A single internal standard stock aliquot...
was thawed the day of use and diluted 100-fold in 20% (v/v) acetonitrile containing 3% (v/v) sulfuric acid. BZC and NaCO₃ solutions were made fresh daily.

LC was performed on a 2.0 × 50 mm, 1.7 mm particle Acquity BEH C18 column (Waters Corporation, Milford, MA, USA) using a Waters Acquity UPLC® attached to a Waters Xevo TQ-XS triple quadrupole mass spectrometer. Mobile phase A was 15% aqueous formic acid and mobile phase B was acetonitrile. The flow rate was 200 μl/min and the elution gradient was as follows: initial, 1% B; 0.1 min, 7% B; 0.5 min, 15% B; 14 min, 55% B; 14.5 min, 70% B; 15 min, 99% B; and 19.1 min, 1% B. An additional 5 min of equilibration time was required for reproducible chromatography.

The adenosine internal standard was prepared by derivatization with 1C₆- benzoyl chloride as described previously. Standard stocks were frozen at −80 °C in aliquots to prevent multiple freeze–thaw cycles. A single internal standard stock aliquot was thawed on the day of use and diluted 100-fold in 20% (v/v) acetonitrile containing 3% (v/v) sulfuric acid.

High performance liquid chromatography

Microglia were isolated from neonatal pups as described above. Cultured microglia were pre-treated with CD39 inhibitor (ARL67156, 200 μM), CD73 inhibitor (APCP, 10 μM) or control (0.04% DMSO) 30 min before treatment with 100 μM ATP. 60 min after ATP treatment, 200 μl of medium was removed and chilled on ice for 10 min. Samples were spun at 400g for 10 min at 4 °C. The supernatant was transferred to a new tube and treated with 7.5 μl of 8 M perchloric acid (PCA), vortexed, flash frozen and stored at −80 °C until processing. PCA-treated samples were neutralized with 0.4 MKH₂PO₄ (Sigma-Aldrich, St. Louis, MO) and analysed with an Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a G1312B binary pump, a G1367C high-performance autosampler and a G1314C Variable Wavelength Detector VL+ set at 254 nm. Nucleotides were separated by ion-pair reversed-phase chromatography using an Atlantis dC18 column (3 mm × 150 mm, particle size 3 μm; Waters Corporation, Milford, MA). The samples were loaded on the column equilibrated with buffer A (0.1 M KH₂PO₄, 4 mM tetrabutylammonium hydrogen sulfate, Sigma-Aldrich; pH 6). The mobile phase developed linearly from 0% to 100% buffer B (70% buffer A/30% methanol) during the first 13 min and remained isocratic at 100% buffer B for 12 min. Subsequently, the column was re-equilibrated with buffer A for 7 min. The flow rate was 0.5 ml/min. ATP, ADP, AMP, and adenosine were identified by their retention times and concentrations were calculated using known standards run in parallel.

Telemetric EEG and EMG recording

Ten-week-old male mice were implanted with a wireless EEG device (HD-X02, Data Sciences International) into the subcutaneous pocket posterior to the neck and continuously recorded in the home cage. EEG leads were placed between the dura and skull, with one lead above the frontal cortex and one lead over the contralateral parietal cortex. EMG leads were placed in the neck muscle. Meloxicam (1 mg/kg) and ampicillin (20 mg/kg) were administered both during surgery and for 2 days after surgery. Data were acquired with a telemetry system (Data Sciences International) running Ponomah v6 software and analysed using Neuroscore v3.2.9 software (Data Sciences International). For comparison of control and microglia-deficient mice, transmitters were implanted and mice were placed on PLX or control chow and allowed to recover for at least one week. Seizures were visually identified behaviourally and from the EEG and EMG recordings.

Behaviour

All behaviour analyses were performed during the 0700–1900 light period. When possible, experimenters were blinded to the genotypes of the animals and genotypes were decoded after data had been processed and analysed. All subjects correspond to data points within 2 s.d. from the sample mean and no subjects were excluded from the behavioural analyses. For all behavioural experiments, sex- and age-matched corresponding littermate control animals were used. No randomization protocol was used. Animals were allocated to treatment groups to ensure uniform distribution of ages and sexes in each group. All procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the IACUC at Icahn School of Medicine at Mount Sinai.

Seizure induction and monitoring. For all seizure experiments, 14–20-week-old mice were used. Mice were injected i.p. with 10 × BW μl. SKF 81297 (Tocris) was diluted from stock (50 mg/ml in DMSO) with 0.9% saline to the indicated concentrations to induce seizures. Kainic acid (Sigma, K0250) was dissolved in saline just before use to the indicated concentrations for i.p. injection to induce seizures. Picrotoxin (Tocris) was diluted from stock (10 mg/ml in ethanol) with 0.9% saline to administer at 1 mg/kg to induce seizures. CPA (Tocris) was diluted from stock (50 mg/ml in DMSO) in 0.9% saline to 0.1 mg/kg. The A,R antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, Sigma-Aldrich) was diluted from stock (10 μg/ml in DMSO) to inject at 1 mg/ml. The A,R agonist CGS 21680 (Sigma) was diluted from stock (10 mg/ml in DMSO) with 0.9% saline to inject at a final dose of 0.1 mg/kg. Clodipogrel (Sanofi-Aventis, kindly provided by J.J. Badimon, as administered in Sipe et al.) was diluted from stock (50 mg/ml in DMSO) with 0.9% saline to inject at 100 mg/kg. Ticagrelor (Astra Zeneca, kindly provided by J. J. Badimon, as administered in Sipe et al. ) was dissolved in DMSO:PEG400:Triton80 5:35:10 and then diluted in saline. To assess the effects of inflammation on seizure susceptibility, mice were pretreated with lipopolysaccharide (LPS, Sigma, 0.2 mg/kg or 2 mg/kg) 24 h before D1 agonist administration.

Animals were observed for the time of onset of seizures and scored on the Racine scale. Seizure behaviour was monitored under a modified Racine scale as follows: (1) normal behaviour; (2) rigid posture with raised tail; (3) continuous head bobbing and forepaws shaking; (4) rearing, falling, and jumping; (5) loss of posture and generalized convulsion activity.

Open field analysis. Locomotion and exploratory behaviour was measured using the open field analysis in a new environment (clear plexiglass 40 × 40 × 30 cm open field arena) as previously described. Activity in the open field was quantified using Fusion Software (v5.0) (Omnitech Electronics). The distance travelled was recorded for each mouse. Data were collected at 5–10 min intervals over 30–90 min test sessions.

Accelerating rotarod. The motor function and balance of mice were analysed using the standard accelerated rotarod test (4–40 rpm over 5 min; Med Associates, St. Albans, VT) as previously described. The time taken for the mice to fall from the rod was measured in seconds. If a mouse, clinging on to the rod, completed two full passive rotations, the mouse was removed from the trial and the time was recorded as fallen from the rod. If a mouse stayed on the rod until the end of the 5-min trial, a time of 300 s was recorded. After one training trial, mice were subjected to three consecutive trials per day with 5-min inter-trial intervals for three consecutive days and measurements were taken from each trial.

Elevated plus maze. The elevated plus maze test was used to determine the unconditioned response to a potentially dangerous environment. Anxiety-related behaviour was measured by the degree to which the rodent avoided the open arms of the maze. The mice were placed at the junction of an elevated four-arm maze in which two arms are open and two are enclosed. The number of times the animal entered each of the arms and the time spent in each arm was recorded for 5 min using the EthoVision video-tracking system (Noldus Information Technology Inc., Leesburg, VA). Total arm entries, percentage of open arm
entries, and percentage of time spent in the open arms were calculated as previously described\(^2\).

**Olfaction test (sniff test).** The sniff test was conducted as previously described.\(^7\) Olfaction was tested by exposing mice (n = 13–21) to a small amount of palatable food (Cinnamon Toast Crunch cereal; General Mills) once per day for 2 days. Mice were deprived of food overnight before the test. A clean cage was filled with roughly 3 inches of fresh bedding, and the stimulus food was buried in the bedding until it was not visible. Mice were then placed in the cage one at a time and allowed to freely explore. The latency to localize and retrieve the food was measured. All mice retrieved the food within 1 min. Bedding was mixed in between trials and tested mice were placed in a new holding cage until all cage-mates had been tested. After this, all mice were returned to their original cage and ad libitum food access was restored.

**Three-chamber social compatibility.** Social preference and social memory\(^1\) were tested as described\(^4\) using a Plexiglas chamber divided into three compartments. The two edge compartments contained an empty wire cup. Mice were habituated to the testing room for at least 1 h before the experiment. Stimulus mice were age- and sex-matched C67Bl/6 male mice that were housed in separate areas of the animal facility and had no prior contact with the test mice. Stimulus mice were habituated to the wire cup before testing. For the sociability test, the test mouse was introduced to the middle chamber and allowed to freely move and habituate to all three compartments for 10 min. Then, the mouse was restricted to the middle chamber using the dividers, while a novel object (Lego) was placed under the wire cup in one chamber and an unfamiliar mouse in the other. The test mouse was then allowed to investigate the whole apparatus for 10 min. Then, the mouse was again restricted to the middle chamber while the object was replaced by a second, unfamiliar mouse. The test mouse was allowed 10 min to investigate. Data were acquired using the Ethovision system (Noldus) to automatically track motion while manual scoring was used to quantify time spent sniffing the stimuli. Counterbalancing was used to control for potential left–right preferences.

**Statistical analysis**

Statistics were analysed using GraphPad Prism v7.0a, and significance was determined at P < 0.05. All statistical analyses were two-tailed. Normal distribution was assessed by the Shapiro–Wilk normality test. Grubbs’ test was used to identify and remove outliers. For two-group comparisons with equal variance as determined by the F-test, an unpaired two-tailed t-test was used. Normally distributed data with unequal variance were analysed with Welch’s correction. Non-normally distributed data were analysed by the Mann–Whitney U test. For analysis across three groups or more, a one-way ANOVA was used. For the analysis of seizure susceptibility, Fisher’s exact test was used for comparison between two groups. For the analysis of seizure susceptibility across three groups, a \(\chi^2\) test with Bonferroni post hoc adjustment was used to calculate adjusted \(P\) values to maintain an \(\alpha\) value of 0.05. For the analysis of accelerated rotarod data, a two-way ANOVA with repeated measures was used. For gene ontology analysis using ENRICHR, FDR values were calculated using the Benjamini–Hochberg test in ENRICHR and \(P\) values were calculated using Fisher’s exact test in ENRICHR\(^6\). All data are represented as vertical dot plots, vertical bar graphs, or \(X-Y\) plots showing mean ± s.e.m. Statistical methods were not used to predetermine the sample size, but our sample sizes are similar to those generally used for similar studies. All samples were randomly allocated into treatment groups. Experimenters were blind to genotype whenever possible.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**

The gene expression data related to this study are available at the NCBI Gene Expression Omnibus (GEO) under accession number GSE149897. Source data are provided with this paper.

**Code availability**

The code used for analysis of calcium transience in neurons to analyse event rates, magnitude, spatial correlation and synchrony can be found at https://github.com/GradinaruLab/striatum2P.
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97. Author contributions A.S. and A.B. conceived and designed the study. A.B. did molecular, behavioural, FACS and imaging experiments. H.I.S. did primary neuronal culture, microglia isolation, microglia culture, FACS and Axon microelectrode array experiments. P.A. did in vivo TRAP experiments. A.B., X.C., A.N., V.G. and A.S. designed two-photon imaging experiments, which were performed by X.C. and A.N. A.K. built the customized two-photon system. A.B., A.I., H.W. and A.S. designed the two-photon imaging of microglial protrusions, which was performed by A.I. A.T.C. and R.S. performed single-nucleus 10X sequencing. Y.-C.W. and H.W. performed single-nucleus 10X sequencing data. H.W. and A.S. performed microglia imaging experiments; and H.I.S. performed molecular and imaging experiments. C.L. and W.G.J. conducted the HIPP analysis. M.G.K. and E.S.C. conducted the microdissection experiments. A.B., J.O.U. and U.B.E. conducted seizure susceptibility experiments on P2ry12−/− mice. S.C.R. generated Cd39fl/fl mice. J.X.J. generated Csf1fl/fl mice. A.I., H.W. and A.S. designed and performed single-nucleus 10X sequencing. Y.-H.E.L. analysed bulk RNA-seq data from TRAP experiments. A.S., D.I.S. and S.M.G. designed experiments to measure neuronal excitability that were conducted by S.M.G. A.B., M.I., P.J.K. and A.S. designed experiments to measure sEPSCs that were conducted by M.I. A.S. and A.B. designed and performed molecular and imaging experiments; C.L. and W.G.J. conducted the HPLC analysis. M.G.K. and E.S.C. conducted the microdissection experiments. A.B., J.O.U. and U.B.E. conducted seizure susceptibility experiments on P2ry12−/− mice. S.C.R. generated Cd39fl/fl mice. J.X.J. generated Csf1fl/fl mice. M.C. generated 634mtm mice. M.A.W. and F.J.Q. generated Cd39fl/fl mice. M.A.W. and F.J.Q. generated Cd39fl/fl mice. M.A.W. and F.J.Q. generated Cd39fl/fl mice. M.A.W. and F.J.Q. generated Cd39fl/fl mice. A.B., M.A.W., F.J.Q. and A.S. designed and performed behavioural experiments. A.B. and A.S. wrote the manuscript. All authors discussed results, and provided input and edits on the manuscript.
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Extended Data Fig. 1 | DREADD-based mouse models to study microglia responses to neuronal activation and inhibition reveals distinct microglia responses. a, b, Neuron-specific activation (a) and inhibition (b) has been achieved by the expression of the Gq-coupled (activating) hM3Dq or Gi-coupled (inhibiting) hM4Di in CaMKII+ forebrain neurons. The CaMKII-tTa mice were bred to either tetO-CHRM3 or tetO-CHRM4 mice to generate CaMKII-tTa: tetO-CHRM3 or CaMKII-tTa: tetO-CHRM4 mice. hM3Dq or hM4Di were activated by i.p. injection of clozapine-N-oxide (CNO) to activate (0.25 mg kg–1) or inhibit (1 mg kg–1) CaMKII+ neuronal activity, respectively. c–e, Validation of CNO-mediated neuronal activation and inhibition: c, Heatmap (left) and violin plot (right) show RNA expression levels of 18 immediate early genes in total striatum 2 h after CNO-mediated neuronal inhibition (orange) or neuronal activation (blue) as compared with controls (n = 2 CaMKII-tTa; tetO-CHRM4, n = 5 control, and n = 3 CaMKII-tTa; tetO-CHRM3 mice) (right). P = 0.0001, One-way ANOVA (Kruskal–Wallis test) with Dunn’s multiple comparison test. d, Dot plot showing quantification of the average number of cFOS+ cells in the dorsal striatum of CaMKII-tTa: tetO-CHRM4 (orange, n = 4 mice), control (black, n = 6 mice), and CaMKII-tTa: tetO-CHRM3 (blue, n = 4 mice) one hour after treatment with CNO (P = 0.0004, One-way ANOVA with Tukey’s post hoc test). e, Representative images showing cFOS+ cells (green) in the striatum of CaMKII-tTa: tetO-CHRM4 (top), control (middle), and CaMKII-tTa: tetO-CHRM3 (bottom) mice in response to CNO, DAPI (blue). f, To allow for the microglia-specific analysis of changes in ribosome-associated RNA levels following neuron inhibition, the CaMKII-tTa: tetO-CHRM4 mice were bred to Cx3cr1CreERTE9; Eef1a1LSL.eGFP/L10a mice followed by tamoxifen-induced L10a-eGFP expression in microglia. g, Changes in ribosome-bound mRNA levels in striatal microglia were determined using the TRAP-sequencing approach. The heatmap shows the variation in the expression levels of 135 upregulated and 220 downregulated genes (z-scored log2(RPKM) at 2 h following CNO-mediated neuronal inhibition. h, Selected gene ontology (using GO) annotations for upregulated genes (using DESeq2) in striatal microglia in response to neuronal inhibition, GO analysis was performed using ENRICHR analysis69,70 (dotted line, P = 0.05). i, Venn diagrams comparing microglial genes up- and downregulated following CaMKII+ neuronal activation and inhibition reveals highly differential microglia response. j, qPCR confirmation of increased mRNA expression (lower ΔCT, normalized to Gapdh) in microglia upon neuronal activation (Ccl3, left, n = 3 mice, P = 0.059, unpaired two-tailed t-test) and neuronal inhibition (Cd74, right, n = 2 mice). k, Dot plots show lack of expression changes in selected genes in the striatum of wild type mice 2 h after saline, 0.25 mg kg–1 CNO injection, or 1 mg kg–1 CNO injection (n = 3, 3, and 4 mice; Kdm6b: P = 0.70 Adrb1: P = 0.22, Ccl24: P = 0.54, Ccl3: P = 0.43, Kcnk13: P = 0.37, Ikbkb, P = 0.62, One-way ANOVA with Tukey’s post hoc test). RPKM: reads per kilobase of transcript per million mapped reads, TRAP: translating ribosome affinity purification; Data shown as mean ± s.e.m.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Microglia deficient mice show normal baseline behaviours but exaggerated responses to neurostimulants. a, Dot plots show the average number of microglia per mm² in cortex, striatum, cerebellum and hippocampus in control and microglia deficient mice (n = 3 and 4 mice, cortex: P < 0.0001, striatum: P = 0.0003, cerebellum: P = 0.0001, DG: P < 0.0001, CA3: P < 0.0001, CA1: P < 0.0001, unpaired two-tailed t-test).

b–e, Behavioural characteristics of microglia deficient mice. b, Anxiety-like behaviour was measured by the ratio of time spent in the open arms/closed arms in the elevated plus maze (n = 10 mice, P = 0.65, unpaired two-tailed t-test).
c, Motor coordination was measured by latency to fall from the accelerating rotarod (n = 8 and 12 mice, interaction: P = 0.89, time: P = 0.13, treatment: P = 0.36; subjects: P < 0.0001, two way repeated measures ANOVA).
d, Olfactory behaviour was measured by the sniff test (n = 21 and 13 mice, P = 0.09, unpaired two-tailed t-test).
e, Social behaviour was measured by using the classic three-chamber sociability task (Social preference: mouse preference for sniffing another mouse over object, Control: n = 7 mice; P = 0.0002, microglia deficient: n = 9 mice, P < 0.0001; Social Memory: mouse preference for sniffing novel mouse over familiar mouse, Control: n = 7 mice, P = 0.0023, microglia deficient: n = 7 mice, P = 0.0009; paired two-tailed t-test).
f, Representative images show brain-wide gene expression patterns of receptors targeted by kainic acid (kainate and AMPA receptor), picrotoxin (GABA<sub>A</sub> receptor), and SKF81297 (D<sub>1</sub> receptor) (Allen Institute).
g, Number of stage IV-V seizures (Racine scale<sup>92</sup>) per mouse visually recorded within one hour in response to kainic acid (18 mg kg<sup>-1</sup>, i.p.) are shown as a dot plot (n = 9 and 10 mice, P = 0.0008, unpaired two-tailed t-test).
h, Dot plot showing distance travelled in response to D1 agonist in one hour in the open field (SKF81297, 3 mg kg<sup>-1</sup>, i.p.) (n = 14 and 8 mice, P = 0.025, unpaired two-tailed t-test).
i, Representative cortical EEG traces during a tonic-clonic seizure event in response to D1 agonist treatment (SKF81297, 5 mg kg<sup>-1</sup> i.p.) in control (top) and microglia deficient (bottom) mice showing high amplitude and rhythmic discharges followed by EEG depression. DG: dentate gyrus; Data shown as mean ± s.e.m.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Generation and characterization of Il34-deficient and Csf1-deficient mice. a, Violin plots show the expression levels of cell-type specific representative marker genes across the 10 identified cell types from striatum snRNA-seq data analysis. Black dots indicate mean expression of selected gene per cell type. b, In situ hybridization for Il34 (left) and Csf1 (right) mRNAs show differential, region-specific expression in cortex, striatum, CA1, dentate gyrus (DG), CA3, corpus callosum (CC), and cerebellum of wild-type mice (WM: white matter, GM: grey matter, ML: molecular layer, GCL: granule cell layer, scale bar = 100μm). c, h, The striatal grey matter-specific or white matter-specific microglia depletion was achieved by breeding Nestin Cre/+ mice to Il34 fl/fl mice or Csf1 fl/fl mice, respectively, to generate Il34 fl/fl ; Nestin Cre/+ (purple, c) and Csf1 fl/fl ; Nestin Cre/+ mice (blue, h). d, i, Dot plots showing relative expression levels of Il34 and Csf1 mRNA normalized to Gapdh in the striatum of Il34 fl/fl ; Nestin Cre/+ mice (d) or Csf1 fl/fl ; Nestin Cre/+ mice (i) compared with littermate controls (d, n = 4 mice each, Il34 P < 0.0001, Csf1 P = 0.69; i, n = 3 and 5 mice, Il34 P = 0.07, Csf1 P < 0.0001, unpaired two-tailed t-test). e, Dot plots show the average microglia density per mm² per mouse in cortex, striatum, cerebellum (cortex: n = 9, 12, and 10 mice, P < 0.0001, striatum: n = 9, 13, and 10 mice, P < 0.0001, cerebellum: n = 7, 7, and 8 mice, P = 0.34, One-way ANOVA with Tukey’s post hoc test). f, left, Dot plot shows levels of IL34 protein as determined by western blot analysis of striatal protein lysate from Il34 fl/fl, Il34 fl/+; Nestin Cre/+ or Il34 fl/fl; Nestin Cre/+ mice normalized to DARPP32 expression (n = 3 mice, P = 0.0077, One-way ANOVA with Tukey’s post hoc test). g, j, Bar graphs show the average percentage of white matter regions in striatal images (0.5mm × 0.5mm) used to count WM and GM microglia in control and mutant mice for the data shown in Fig. 2c and e. (g, P = 0.99, n = 4 and 3 mice, unpaired two-tailed t-test; j, n = 4 and 2 mice). For gel source data, see Supplementary Fig. 1. Data shown as mean ± s.e.m.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Generation of mice with striatum-specific microglia depletion. a, b, (left). The striatum-specific microglia depletion was achieved by breeding Il34<sup>fl/fl</sup> mice to Drd1<sup>aCre/+</sup> or Drd2<sup>Cre/+</sup> mice to generate Il34<sup>fl/fl;Drd1aCre/+</sup> (a, green) and Il34<sup>fl/fl;Drd2Cre/+</sup> mice (b, grey). Right, dot plots show relative expression of Il34 mRNA in the striatum normalized to Gapdh (a, n = 6 and 7 mice, P < 0.0001; b, n = 4 mice, P = 0.0004, unpaired two-tailed t-test).

c, Representative striatal images of sagittal brain slices from Il34<sup>fl/fl</sup>, Il34<sup>fl/fl;Drd1aCre/+</sup> and Il34<sup>fl/fl;Drd2Cre/+</sup> mice following immunofluorescent staining for P2RY12 (microglia, green) and DAPI (nuclei, blue) (scale bar = 50μm).

d, e, Dot plots show the average microglia density per mm<sup>2</sup> per mouse per specific region in the hippocampus of Il34<sup>fl/fl</sup>; Drd1<sup>aCre/+</sup> (d) and Il34<sup>fl/fl</sup>; Drd2<sup>Cre/+</sup> mice (e) compared to littermate controls (d, n = 3 mice, DG: P = 0.88, CA3: P = 0.85, CA1: P = 0.1; e, n = 3 mice, DG: P = 0.69, CA3: P = 0.56, CA1: P = 0.72; unpaired two-tailed t-test).

f, g, Dot plots showing total distance travelled in response to D1 agonist (SKF81297, 3 mg kg<sup>−1</sup>, i.p.) in one hour in the open field for Il34<sup>fl/fl</sup>; Drd1<sup>aCre/+</sup> (f) and Il34<sup>fl/fl</sup>; Drd2<sup>Cre/+</sup> mice (g) compared with littermate controls (f: n = 8 and 9 mice, P = 0.034; g: n = 8 mice, P = 0.0087, unpaired two-tailed t-test).

h, Percentage of mice seizing 30 min after administration of picrotoxin (1 mg kg<sup>−1</sup>, i.p.) shown as a bar graph (n = 21, 9, and 8 mice; P = 0.80, Chi-squared test). DG: dentate gyrus. i, Microglia-neuron ratio defines the threshold of D1 neuron activation by D1 agonist. Bar graph shows the percentage of mice with stage IV-V seizures in response to D1 agonist (4 mg kg<sup>−1</sup>, i.p.) in control, Il34<sup>fl/fl</sup>; Drd1<sup>aCre/+</sup>, and microglia deficient mice (n = 11, 13, and 9 mice; right, P = 0.0005, Chi-squared test). While all mice display an increased seizure response to 5 mg kg<sup>−1</sup> D1 agonist treatment, only microglia deficient (99% reduction of microglia), but not Il34<sup>fl/fl;Drd1aCre/+</sup> (60% reduction of microglia in the striatum) display an increased seizure response at 4 mg kg<sup>−1</sup> D1 agonist treatment. Data shown as mean ± s.e.m.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Striatum-specific microglia reduction has no overall effects on striatal cellular composition, D1/D2 neuronal morphology, D1/D2 MSN characteristic electrophysiological and molecular phenotypes, and glial phenotypes. a, Dot plots show average number of D1 neurons (left, dark green, GFP+, DARPP32+) and D2 neurons (right, light green, GFP, DARPP32+) per mouse in the striatum of Il34fl/fl Drd1aCre/+ and Il34fl/fl Drd1aeGFPL10a Drd1aCre/+ mice. Mice expressing eGFP-tagged ribosomal subunit L10a under the Drd1a promoter were used to identify GFP+ D1 neurons and GFP D2 neurons in control Il34fl/fl Drd1aCre/+ and mutant Il34fl/fl Drd1aeGFPL10a Drd1aCre+ mice (n = 2 mice). b, c, D1 or D2 neuron cell morphology was determined by the number of primary dendrites (b), total dendritic length (c, left), and sholl analysis (c, right); b, D1 neurons: n = 11 and 15 D1 neurons, P = 0.33; D2 neurons: n = 15 and 11 D2 neurons, P = 0.59; unpaired two-tailed t-test; c, D1 neurons, n = 11 and 15 D1 neurons, dendritic length: P = 0.83, unpaired two-tailed t-test; sholl, interaction: P = 0.99; genotype: P = 0.069; distance: P = 0.0001, two-way ANOVA; D2 neurons, n = 15 and 10 D2 neurons, dendritic length: P = 0.80, unpaired two-tailed t-test; sholl, interaction: P = 0.051; genotype: P = 0.67; distance: P < 0.0001, two-way ANOVA. d, Intrinsic excitability of D1 neurons (left) and D2 neurons (right) in ex vivo slices as measured by current-evoked action potentials (AP, left) and equilibrium potentials as voltage-current (VC) plots (right). D1: n = 11 and 15 D1 neurons, AP: interaction: P = 1.0; genotype: P = 0.98; pA: P = 0.0001, subjects: P < 0.0001; VC: interaction: P = 1.0; genotype: P = 0.48; distance: P = 0.0001, subjects: P < 0.0001; D2: n = 16 and 10 D2 neurons; AP: interaction: P = 1.0; genotype: P = 0.5; distance: P = 0.0001, subjects: P < 0.0001; VC: interaction: P = 0.99; genotype: P = 0.7; distance: P < 0.0001, subjects: P < 0.0001; two-way ANOVA. e, Dendritic excitability of D1 neurons (left) and D2 neurons (right) in ex vivo slices as determined by back-propagating action potentials as measured by Ca+2-sensitive fluorescence (D1: n = 12 and 15 D1 neurons, dendrites: P = 0.90, spines: P = 0.85; D2: n = 16 and 10 D2 neurons, dendrites, P = 0.27, spines, P = 0.61; two-way ANOVA). f, Frequency (Hz) and amplitude (pA) of sEPSPs in D1 neurons from ex vivo slices shown as box and whisker plots (Frequency: n = 19 cells from 5 mice and 16 cells from 5 mice, P = 0.23, unpaired two-tailed t-test; amplitude: n = 19 cells from 5 mice and 16 cells from 5 mice, P = 0.796, unpaired two-tailed t-test with Welch's correction). g, Membrane bound DRD1 protein expression normalized to total DRD1 expression as determined by ex vivo brain slice biotinylation assay shown as a dot plot (n = 6 mice, P = 0.21). h, Generation of Il34fl/fl Drd1aCre/+ Drd1eGFPL10a mice for D1 neuron specific TRAP sequencing analysis. i, Volcano plot shows lack of any major gene expression changes in D1 neurons in 3 month old Il34fl/fl Drd1aCre/+ Drd1eGFPL10a mice and littermate controls as determined by differential expression analysis (DESeq2, n = 3 mice each, P < 0.05, fold change >1.5, red: upregulated, blue: downregulated). j–k, Total striatal RNA expression analysis from control and Il34fl/fl Drd1aCre/+ mice reveals unperturbed striatum cell-type specific gene expression pattern except the expected ~50% reduction in the expression of microglia-enriched genes. j, RPKM, normalized to controls, showing pan-medium spiny neuron (MSN), D1 neuron (D1), D2 neuron (D2), interneuron (IN), astrocyte (astro), oligodendrocyte (oligo), and microglia specific genes in Il34fl/fl Drd1aCre/+ and Il34fl/fl Drd1aCre/+ Drd1eGFPL10a mice (n = 4 mice each. P2ry12: P = 0.0033, Siglech: P = 0.001, Cx3cr1: P = 0.01, Csf1r: P = 0.007, Tmem119: P = 0.005, Fcrls: P = 0.03, unpaired two-tailed t-test). k, RPKM, normalized to controls, showing unperturbed expression of astrocyte-specific activation markers (n = 4 mice each, unpaired two-tailed t-test). The experiments shown in h–k have been independently repeated in a second cohort (n = 3 mice) with identical results. For gel source data, see Supplementary Fig. 1. Box and whisker plots in b, c, e, and f are shown with arithmetic median (middle line), box shows upper and lower quartile, whiskers show min-max range. Data shown as means ± s.e.m.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Microglia regulate striatal neuron synchrony and responses to D1 agonist treatment in an ADO/A1R dependent fashion.

a, Representative tile scan of coronal brain slice showing implantation of GRIN lens and AAV9.hSyn.GCaMP6s expression in the dorsal striatum. b, Increased synchrony in the dorsal medial striatum of microglia deficient mice (n = 9 mice) at baseline compared with controls (n = 7 mice) (treatment: P < 0.0001, distance: P < 0.0001, interaction: P < 0.0001; Two-way ANOVA with Sidak’s multiple comparisons test). c, Bar graphs show magnitude of Ca²⁺ events (ΔF/F) recorded in control (black) and microglia deficient mice (grey) at baseline (left) and in response to D1 agonist (SKF81297, 3 mg kg⁻¹, right) (baseline: control, n = 824 cells from 7 mice; microglia deficient, n = 775 cells from 9 mice, P = 0.87; D1 agonist: control, n = 995 cells from 7 mice; microglia deficient, n = 1021 cells from 9 mice; P = 0.89, unpaired two-tailed t-test). d, e, Co-administration of A1R agonist (CPA, 0.1 mg kg⁻¹) with D1 agonist (SKF81297, 3 mg kg⁻¹) normalizes increased neuronal activity in microglia deficient mice. Bar graphs show wild type-like frequency (per mouse, d) and magnitude (ΔF/F, e) of Ca²⁺ events per neuron per minute in control (black) and microglia deficient (grey) (control, n = 7 mice; microglia deficient, n = 9 mice, P = 0.82, unpaired two-tailed t-test; e, control, n = 387 cells from 7 mice; microglia deficient, n = 305 cells from 9 mice; P = 0.69, unpaired two-tailed t-test). f, Spatiotemporal coding of neuronal activity (baseline shown in Fig. 3c) is disrupted by D1 agonist administration (dotted line) and largely normalized by co-administration with an A1R agonist (blue line) in control (top, n = 7 mice) and microglia deficient mice (left, n = 9 mice). For better visualization, the distance axis was logarithmically scaled. (Control, n = 7 mice: interaction: P = 0.0012, distance: P < 0.0001, treatment: P < 0.0001; Microglia deficient, n = 9 mice: interaction: P = 0.0014, distance: P < 0.0001, treatment: P < 0.0001; Two-way ANOVA with Sidak’s multiple comparisons test). g, Bar graphs show the frequency of Ca²⁺ events per neuron per minute in control (left) and microglia deficient (right) mice at baseline, in response to D1 agonist (SKF81297, 3 mg kg⁻¹, i.p.) alone, or in response to D1 agonist and A1R agonist treatment (CPA, 0.1 mg kg⁻¹, i.p.) treatment (Control: n = 332-995 cells from 7 mice, P < 0.0001; Microglia deficient: n = 243-1021 cells from 9 mice, P < 0.0001; One-way ANOVA with Bonferroni post hoc test). h, Confirmation of CNO-mediated neuronal activation for data shown in Fig. 3h. The neuron-specific expression of GCaMP6s and hM3Dq was achieved by injecting the indicated viruses. Virally labelled thalamocortical projection neurons were identified (mCherry expression) and calcium transients were recorded at baseline, after saline injection, and after CNO injection. (Control, n = 3 mice: interaction: P = 0.0012, distance: P < 0.0001, treatment: P < 0.0001; Microglia deficient, n = 3 mice: interaction: P = 0.0009, distance: P < 0.0001, treatment: P < 0.0001; Two-way ANOVA with Bonferroni post hoc test). i, Representative traces (left) and quantification of the area under the curve (AUC) (right) of calcium transients per mouse in virally labelled neurons pre-injection, after saline injection, and after CNO injection (n = 3 mice, P = 0.0009, One-way ANOVA with Tukey’s post hoc test). j, Microglia baseline process velocity (left) and contact with synaptic boutons (right) is not affected by either the expression of the DREADD virus (red bars) or by CNO injection (5 mg kg⁻¹, black bars) alone (n = 3 mice, left: P = 0.96, right, P = 0.25, unpaired two-tailed t-test). The experiments shown in a-g are data combined from two independent imaging cohorts of mice. Box and whisker plots in c, e, and g are shown with arithmetic median (middle line), box shows upper and lower quartile, whiskers show 1.5x interquartile range. CNO: clozapine-N-oxide; Data shown as mean ± s.e.m.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Microglial expression of $Entpd1$/CD39 and $Nt5e$/CD73 in vitro and in vivo. a, Dot plots show normalized, ribosome-associated mRNA levels (RPKM) for $Entpd1$ (left) and $Nt5e$ (right) in astrocytes, neurons, and microglia from distinct brain regions of adult mice using cell-type specific TRAP sequencing ($n = 2, 2, 3, 6, 4, 5, 19$ and $15$ mice). b, CD39 surface protein expression on ex vivo isolated forebrain cells of Cx3cr1$^{CreERT2/1}$ (Litt) mice (mice express cytosolic YFP in Cx3cr1$^+$ microglia). Percoll-purified cells were incubated with anti-CD39-AlexaFluor700 followed by FACS analysis. The histogram shows expression levels of CD39, which is almost exclusively restricted to YFP$^+$ microglia (red) and is not found on YFP$^-$ non-microglia cells (grey) as shown previously (data are representative of three independent experiments). c, Scheme shows ex vivo isolation procedure of CD11b$^+$ microglia following neonatal mouse forebrain tissue dissociation and Percoll enrichment for live cells. d, e, Ex vivo CD11b$^+$ microglia isolation procedure from neonatal pups yields highly pure microglia population. d, Microglia were positively selected for by using CD11b$^+$ magnetic bead purification and were incubated with anti-CD39-AlexaFluor700 followed by FACS analysis to assess the purity of the population. The numbers show the percentage of live (DAPI$^-$) cells with distinct pattern of CD39 expression levels ($>$98% CD39$^+$; data are representative of two independent experiments). e, Immunofluorescent analysis of purity of CD11b$^+$ microglia isolation. Left, cells were plated on cover slips and stained for cell-type specific protein expression using antibodies specific for IBA1 (microglia), GFAP (astrocyte), OLIG2 (oligodendrocytes) or NEUN (neurons) to identify and quantify different cells within the populations in order to assess microglia purity ($n = 6$ GFAP/IBA1 images and $6$ OLIG2/NEUN/IBA1 images). Right, representative image of cover slip containing 99% pure microglia following CD11b$^+$ isolation procedure with anti-IBA1 (green; DAPI, blue). f, Cell lysates of increasing numbers of CD11b$^+$ bead-purified microglia cells have been analysed for CD39, CD73, P2RY12, and IBA1 protein expression by Western Blot analysis as indicated. Sn$^g$ of total striatal lysate from control or $Nt5e^{-/-}$ (CD73-deficient) mice have been used to verify CD73 antibody specificity. H3 protein expression has been used as a loading control (k = thousand, M = million; SuperSignal ECL substrate was used to visualize CD73 expression, regular ECL was used for all other proteins) Right, Whole striatal tissue lysates of control and $Nt5e^{-/-}$ (CD73-deficient) striatal tissue were loaded at low (Sn$^g$) and high (30ng) concentrations and analysed for microglia-specific protein expression (CD39, CD73, P2RY12, and IBA1) by Western Blot analysis as indicated. Whole striatal tissue lysates of control and microglia deficient mice have been used to verify antibody specificity. H3 protein expression has been used as a loading control. (SuperSignal ECL substrate was used to visualize P2RY12 expression, regular ECL was used for all other proteins). Blots are representative from two independent experiments. For gel source data, see Supplementary Fig. 1. Data shown as mean ± s.e.m.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Microglia suppress neuronal activation via an ATP/AMP/ADO/A1R-dependent feedback mechanism. a, Scheme for generation of mice with microglia-specific CD39 depletion by breeding Cd39\textsuperscript{fl/fl} mice to Cd39\textsuperscript{fl/fl};Cx3cr1\textsuperscript{CreER\textsubscript{2}/+(Jung)} mice followed by tamoxifen-mediated Cre induction at 4–6 weeks of age. b, Dot plots show relative expression of Entpd1, Il34, and Csf1 mRNA in the striatum of Cd39\textsuperscript{fl/fl};Cx3cr1\textsuperscript{CreER\textsubscript{2}/+(Jung)} mice and littermate controls normalized to Gapdh (n = 5 and 6 mice, Entpd1: P = 0.0012, Il34: P = 0.38, Csf1: P = 0.22, unpaired two-tailed t-test). c, Dot plots show relative expression of Entpd1, Il34, and Csf1 mRNA in the striatum of Cd39\textsuperscript{fl/fl};Cx3cr1\textsuperscript{CreER\textsubscript{2}/+(Jung)} mice and littermate controls normalized to Gapdh (n = 5 and 6 mice, Entpd1: P = 0.0012, Il34: P = 0.38, Csf1: P = 0.22, unpaired two-tailed t-test). d, Left, Representative images of striatal sections from Cd39\textsuperscript{fl/fl} and Cd39\textsuperscript{fl/fl};Cx3cr1\textsuperscript{CreER\textsubscript{2}/+(Jung)} mice stained for IBA1 (microglia, green) and DAPI (nuclei, blue) (scale bar:100μm); right, dot plots show the average number of microglia per mm\textsuperscript{2} per mouse in the striatum of Cd39\textsuperscript{fl/fl} and Cd39\textsuperscript{fl/fl};Cx3cr1\textsuperscript{CreER\textsubscript{2}/+(Jung)} mice (n = 4 mice, P = 0.33, unpaired two-tailed t-test with Welch’s correction for variance). e, Microglia-specific CD39 ablation leads to increased levels of neuronal PKA activity in the striatum as measured by phosphorylation levels of GLUR1 at Ser845 in striatal protein lysate from Cd39\textsuperscript{fl/fl};Cx3cr1\textsuperscript{CreER\textsubscript{2}/+(Jung)} and littermate controls, pGLUR1 levels have been normalized to total GLUR1 in each sample (n = 8 and 6 mice, P = 0.029, two-tailed Mann–Whitney Test). e, f, Increased seizure response in Cd39\textsuperscript{fl/fl};Cx3cr1\textsuperscript{CreER\textsubscript{2}/+(Jung)} mice as compared to littermate controls (n = 5 and 8 mice; left, P = 0.17, Fisher’s exact test with Yates correction, right, P = 0.032, unpaired two-tailed t-test). g, Left, Scheme for the generation of mice with a D1 neuron-specific Adora1 depletion by breeding Adora1\textsuperscript{fl/fl} mice to Drd1a\textsuperscript{Cre/+(Jung)} mice; right, dot plots show relative expression of Adora1 mRNA in the striatum of Adora1\textsuperscript{fl/fl};Drd1a\textsuperscript{Cre/+(Jung)} mice and littermate controls normalized to Gapdh (n = 5 and 4 mice, P = 0.002, unpaired two-tailed t-test). h, Co-administration of A\textsubscript{r} agonist (CPA, 0.1 mg kg\textsuperscript{-1}) and D1 agonist (SKF81297, 5 mg kg\textsuperscript{-1}) does not prevent the increased seizure susceptibility in Adora1\textsuperscript{fl/fl};Drd1a\textsuperscript{Cre/+(Jung)} mice (n = 12 and 6 mice, P = 0.009, Fisher’s exact test with Yates correction). i, Bar graph shows percentage of microglia deficient mice with seizures in response to D1 agonist alone (SKF81297, 5 mg kg\textsuperscript{-1}, i.p.) or co-administered with an A\textsubscript{2r} agonist (CGS21680, 0.1 mg kg\textsuperscript{-1}, i.p.) or an A1R agonist (CPA, 0.1 mg kg\textsuperscript{-1}, i.p.) (n = 9-10 mice, P = 0.005, Chi-squared test with Bonferroni post hoc adjustment). j, A1R agonist administration (CPA, 0.1 mg kg\textsuperscript{-1}) normalizes increased PKA activity in Il34\textsuperscript{fl/fl};Drd1a\textsuperscript{Cre/+(Jung)} mice but does not affect PKA activity in control Il34\textsuperscript{fl/fl} mice as measured by phosphorylation levels of GLUR1 at Ser845 in striatal protein lysate. pGLUR1 levels have been normalized to total GLUR1 in each sample (Il34\textsuperscript{fl/fl} mice, n = 5 mice, P = 0.62, Il34\textsuperscript{fl/fl};Drd1a\textsuperscript{Cre/+(Jung)} mice, n = 5 mice, P = 0.06, unpaired two-tailed t-test). All statistical tests are two-tailed; Data shown as mean ± s.e.m.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Microglia can suppress glutamate-induced cortical neuron activation in a CD39/ADO/A₁R-dependent fashion in vitro.

a–d, Experimental approaches for the assessment of adenosine-mediated regulation of cortical neuron activity in vitro. Embryonic cortical neurons were cultured on Axion microelectrode array (MEA) plates which allow for continuous electrical field recordings. a, A₁Rs modulate cortical neuronal activity at baseline and in response to glutamate. On day in vitro (DIV) 14, neuronal cultures were treated with vehicle, glutamate (10μM), A₁R agonist (CPA, 100nM), A₁R antagonist (DCPCX, 100nM), glutamate and A₁R agonist, or glutamate and A₁R antagonist. Dot plot shows the percentage change in mean firing rate of neurons 1 h after treatment compared to their baseline before drug treatment. (n = 7 wells, P < 0.0001, One-way ANOVA with Tukey’s post hoc test).

b, Adenosine suppresses neuronal activity via A₁R activation. On DIV 14, cultures were treated with vehicle, adenosine (10μM), A₁R antagonist (DCPCX, 100nM), or co-treated with adenosine and A₁R antagonist. Dot plot shows percentage change in mean firing rate of neurons 1 h after treatment compared to their baseline before drug treatment. (n = 8 wells, P < 0.0001, One-way ANOVA with Tukey’s post hoc test).

c, Microglia suppress neuronal activity in response to glutamate-induced activation in an A₁R-dependent manner. Microglia were isolated from neonatal pups, plated onto the neuronal culture on DIV 14, and allowed to settle for 48 h. Mixed cultures were treated with vehicle and/or glutamate (10μM) and/or A₁R antagonist (100nM) on DIV 16. Dot plot shows percentage change in mean firing rate of neurons 1 h after treatment compared to their baseline before drug treatment. (left, n = 12 wells, P < 0.0001, right, n = 4, 6, 9, and 7 wells, P = 0.001, One-way ANOVA with Tukey’s post hoc test).

d, Microglia suppress neuronal activity in a CD39-dependent manner in response to glutamate-induced activation. Microglia were isolated from neonatal pups, plated onto the neuronal culture on DIV 14, and allowed to settle for 48 h. Mixed cultures were pretreated with CD39 inhibitor (ARL67156, 200μM) or vehicle (30 min) and then treated with glutamate (10μM). Dot plot shows percentage change in mean firing rate of neurons 1 h after treatment compared to the corresponding baseline neuronal activity levels before their baseline before drug treatment. (n = 12, 12, 11, and 11 wells, P = 0.0045, One-way ANOVA with Tukey’s post hoc test). Data shown as mean ± s.e.m. and representative of 2–3 independent experiments.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Reactive microglia in different neuroinflammatory and neurodegenerative conditions show a reduction in $\text{Entpd1}$ and $\text{P2ry12}$ expression that is associated with an $\text{A}_1\text{R}$-dependent increase in D1 neuron responses. a–g, Changes in $\text{Entpd1}$ and $\text{P2ry12}$ gene expression are shown in: a, RNA extracted from whole striatum of 6-month old control mice and Q175 (Huntington’s disease) mice$^{94}$ ($\text{Entpd1}: P = 0.0001; \text{P2ry12}: P = 0.004; n = 8$ mice, fold change and $P$-value provided in publication). b, RNA from FACS-sorted CD11b+/F4/80+ cortical and hippocampal microglia from 8.5-month old control and 5xfAD mouse model of Alzheimer’s Disease$^{95}$ ($\text{Entpd1}: P = 0.009; \text{P2ry12}: P = 0.0035; n = 5$ mice, fold change and $P$-value provided in publication). c, RNA from FACS-sorted forebrain microglia from 10-month old control and APP/PS1 Alzheimer’s disease mouse model$^{39}$ ($n = 3$ mice, $\text{Entpd1}: P = 0.038; \text{P2ry12}: P = 0.023$, unpaired two-tailed $t$-test). d, RNA from FACS-sorted FCRLS+ phagocytic and non-phagocytic microglia isolated after stereotaxic injection of apoptotic neurons$^{39}$ ($n = 4$ mice, $\text{Entpd1}: P < 0.0001; \text{P2ry12}: P < 0.0001$, unpaired two-tailed $t$-test). e, FACS-sorted FCRLS+ microglia in 24-month old control mice or APP/PS1 Alzheimer’s disease mouse model. Plaque associated microglia were identified and sorted based on CLEC7A expression$^{39}$ ($n = 6$ mice, $\text{Entpd1}: P = 0.01; \text{P2ry12}: P < 0.0001$, One-way ANOVA with Tukey’s post hoc test). f, Massively parallel single-cell RNA-seq (MARS-seq) from isolated homeostatic microglia and disease associated microglia (DAM) in 5xfAD mice$^{96}$ ($\text{Entpd1}: P < 0.0001; \text{P2ry12}: P < 0.0001; n = 893$ single microglia, fold change and $P$-value provided in publication). g, FACS-sorted CD11b+/CD45− single microglia in control and LPS-injected mice (4 mg kg$^{-1}$) ($\text{Entpd1}: P = 0.0001; \text{P2ry12}: P < 0.0001; n = 477$ microglia from saline injected mice and 770 microglia from LPS injected mice, fold change and $P$-value provided in publication). h, i, Bar graphs show increased seizure susceptibility to D1 agonist administration (SKF81297, 5 mg kg$^{-1}$, i.p.) in LPS-injected (indicated doses, i.p.) (h) and 6-8-month old 5xfAD Alzheimer’s mice (i) that is prevented by co-administration of an $\text{A}_1\text{R}$ agonist (CPA, 0.1 mg kg$^{-1}$, i.p.) (h, $n = 10-22$ male mice, $P = 0.032$, Chi-squared test; i, $n = 5-10$ mice per genotype, left, $P = 0.031$, Fisher’s exact test with Yates correction; right, $P = 0.49$, Fisher’s exact test with Yates correction). j, Scheme illustrating the model of microglia-mediated adenosine-controlled regulation of D1 neuron responses in the healthy striatum (left) and its potential dysfunction upon microglia activation during inflammatory and/or neurodegenerative diseases (right). All statistical tests are two-tailed; Data shown as mean ± s.e.m.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a  Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

RNA/DNA quality check: 2100 Expert Software (v8.02.07.S1532)
RNA/DNA sequencing: NextSeq System Suite, Illumina bcl2fastq2 Conversion Software v2.17
qPCR: StepOne Software (v2.3)
Imaging: Zen 2012 software (v6.1)
Microglia imaging: NIS-Elements AR (Ver4.40.00)
Mouse behavior: EthoVision (v9), Fusion (v5.0)
FACS Analysis: AttuneNXT software v4.2
Software and script usage is described in the method section.

Data analysis

Bulk RNA seq bioinformatics: TopHat2 (v2.1.0), HTSeq-count (v0.6.0), SPEctRA (v1.0), DESeq2 package (v1.20.0), R (v3.1.1), Enrichr
10X single nuclei sequencing: 10X Cell Ranger (v2.1.0), Loupe Browser (v2.0.0), ggplot2 (v3.2.1)
Data representation: Multiple Experiment Viewer 4.8 (v10.2), GraphPad Prism (v7.0a)
Imaging analysis: ImageJ (v1.48 and v1.52), Zen2011 (v8.1), NeuroLucida (v11)
Calcium imaging analysis: NoRMCorre (CalAmin v1.6.3), Suite2P (v0.6.16), MatLab (R2018a and R2019b)
Microglia imaging analysis: HyperStackReg (v5.6), Color2 (v3.0.0), Manual Tracking (v2.1.1)
sEPSCs analysis: Clampfit10.3
FACS analysis: FCS Express 7 Plus Software
The code used for analysis of calcium transience in neurons to analyze event rates, magnitude, spatial correlation and synchrony can be found at https://github.com/GradinaruLab/striatum2P
Software and script usage is described in the method section.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The accession number for gene expression analysis in the paper is available at NCBI Gene Expression Omnibus (GEO) under the accession number GSE149897. Raw data is associated with Figure 1, Extended Data Figure 1, and Extended Data Figure 5.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
We do not include a justification of sample size for this study. We used the minimum number of animals needed to reliably detect the expected effect size with an alpha rate set at 0.05 in a standard powered experiment and based on extensive laboratory experience and literature in the field.

Data exclusions
Data were only formally excluded by Grubbs’ outlier test (one instance). For Axion MEA experiments, wells were excluded from the analysis, based on pre-established criteria, if baseline firing rate was <1 Hz or if <16 electrodes were active.

Replication
All attempts of replications were successful. Each experiment was reproduced with similar results. Reproducibility has been either indicated in the Figure Legends, or shown as a quantification.

Randomization
For all experiments, animals were randomly assigned to groups.

Blinding
Experimenters were blinded during imaging and behavioral experiments. Tissue collections for gene expression and protein analyses were not performed blind to the conditions of the experiments as genotypes and/or drug treatments were determined beforehand and mice were selected in order to acquire sufficient n per group. All subsequent sample processing was done blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | n/a     |
| Involved in the study           | Involved in the study |
| Antibodies                      | ChIP-seq |
| Eukaryotic cell lines           | Flow cytometry |
| Palaeontology and archaeology   | MRI-based neuroimaging |
| Animals and other organisms     |         |
| Human research participants     |         |
| Clinical data                   |         |
| Dual use research of concern    |         |

Antibodies

Primary antibodies: IBA1 (1:500) Cat#019-19741, Waco; CD11b, clone 5C6 (1:500) Cat#MCA711, Biorad; GFP (1:2000) Cat#ab6556, Abcam; GFP (1:500) Cat#ab13970, Abcam; NEUN clone A60 (1:500) Cat#MAB377, Millipore; cFOS (1:1000) Cat#ab190289, Abcam; GFAP clone G-A-S (1:500) G3893, Sigma; Olig2 clone 1G11 (1:500) sc-293163, Santa Cruz; P2RY12 (1:1000) Cat#AS-55043A, AnaSpec; Secondary antibodies: Alexa Fluor 488-labeled goat anti-mouse IgGs (H+L) (1:500, A32723, Life Technologies), Alexa Fluor 488-goat anti-rat IgGs (H+L) (1:500, A-11006, Life Technologies), Alexa Fluor 488-goat anti-chicken IgGs (H+L) (1:500, A-11039, Life Technologies), Alexa Fluor 488-goat anti-rabbit IgGs (H+L) (1:500, Life Technologies, A-11008), Alexa Fluor 568-labeled goat anti-
mouse IgGs (H+L) (1:500, Life Technologies, A-11004), Alexa Fluor 568-goat anti-rat IgGs (H+L) (1:500, Life Technologies, A-11077), Alexa Fluor 568-goat anti-rabbit IgGs (H+L) (1:500, Life Technologies, A-11011).

**Immunoblotting:**
Primary Antibodies: DARPP32, Novus, Cat#NB300-304, (1:1000); DARPP32-THR34, clone D27A4, Cell Signaling, Cat#12438, (1:1000); DARPP32-THR75, clone cc911, (1:1000) kindly provided by A Nairn and P Greengard (not commercially available); DARPP32, clone 6a, (1:5000) kindly provided by A Nairn and P Greengard (not commercially available); IL34, R&amp;D Systems, Cat#AF5195, (1:1000); GLUR1-SER845, clone D10G5, Cell Signaling, Cat#0804, (1:1000); GLUR1, clone RH95, Millipore, Cat#AB2263, (1:1000); DRD1, Abcam, Cat#ab20066, (1:1000); CD39, R&amp;D Systems, Cat#AF4398, (1:1000); CD73, clone D7F9A, Cell Signaling, Cat#13160, (1:500); H3, Abcam, Cat#ab1791, (1:5000); IBA1, Wako, Cat#016-20001, (1:1000); P2Y12, Anaspec, Cat#AS-55043A, (1:1000);
Secondary antibodies: horseradish-peroxidase-conjugated anti- mouse (Cat#31438, 1:10,000, Life Technologies), horseradish-peroxidase-conjugated anti-rabbit IgG secondary antibody (NA934V, 1:10,000, GE), horseradish-peroxidase-conjugated anti-sheep IgG secondary antibody (A16041, 1:10,000, Invitrogen), horseradish-peroxidase-conjugated anti-rat IgG secondary antibody (31470, 1:10,000, Invitrogen).

TRAP: mouse monoclonal anti-GFP (clone 19F7 (cat# HtzGFP19F7) and clone 19C8 (cat# HtxGFP-19C8), Antibody & Bioresource Core Facility Memorial Sloan Kettering Cancer Center)

FACS: CD39-A700 (clone 24DMS1, Cat#M6-0391-82, ThermoFisher), CD73-PE (clone eBioTY/11.8, 12-0731-82, ThermoFisher)

**Validation Immunostaining antibodies:** IBA1 (019-19741) CD11B (MCA711), P2RY12 (AS-55043A) are validated for immunostaining of microglia in mouse on the manufacturers website.

NEUN (MAB377), cFOS (ab190289), OLIG2 (sc-293163), have been validated on the manufacturers website for immunostaining in mouse.

Both GFP (ab6556) and GFP (ab13970) are validated on the manufacturers website for immunostaining against GFP.

GFAP (G3893) is validated on the company website for immunostaining. It has been further validating in multiple references for use in mouse including Yenari et al, 2006, Ayata et al, 2018, Lathia et al, 2008 and Zhang et al, 2014.

**Immunoblotting antibodies:** H3 (ab1791), CD39 (AF4398), CD73 (13160), P2RY12 (AS-55043A), IBA1 (016-20001), DARPP32 (NB300-324), GLUR1 (MAB8263), IL34 (AF5195), DRD1 (ab20066), GLUR1ser845 (8084), and DARPP32-THR34 (12438), are verified for immunoblotting in mouse on the company website. CD39 and CD73 were additionally validated in house with knock out mice.

DARPP32-THR75, cc911 and DARPP32, 6a were validated for immunoblotting in Rapanelli et al, 2016 and Nishi et al, 2017.

Mouse monoclonal anti-GFP (19F7 and 19C8, Antibody & Bioresource Core Facility Memorial Sloan Kettering Cancer Center) were verified for TRAP in Heiman et al. 2008 and Doyle et al. 2008.

CD39A700 (24DMS1) and CD73-PE (eBioTY/11.8) are verified for FACS on the company website. Specificity of CD39A700 (24DMS1) was confirmed in mouse spleenocytes on the company website. CD39-A700 was further validated in house on Cx3cr1Cre Ert2/+ (Litt) mice, that express cytosolic YFP in microglia, to confirm selective labeling of microglia by flow cytometry (see Extended Data Fig7b).

Specifics on manufacturer validation can be accessed on the company website using the product catalogue numbers listed above.

**Animals and other organisms**

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**

For lines that were generated in other strains, lines were backcrossed for ≥5 generations to C57Bl/6j mice. Male and female mice ages 8 weeks-9 months were used for this study. Whenever male and female mice were used together, the ratio in control and treatment groups were equal. Whenever possible, littermate controls were used. For microglia depletion experiments, live imaging of neuronal calcium transience, and pharmacological seizure experiments, male C57Bl/6j mice (Jackson Laboratory, stock number 000664) were used. For FACS experiments and microglia isolation experiments, neonatal pups (post natal day 7) were used.

- The following lines were used in this study: Il34fl/fl mice (PMID: 22729249), Csf1fl/fl mice (PMID: 21958845), Entpd1/Cd39fl/fl mice (PMID: 31243614), Adora1fl/fl mice (PMID: 12843280), Drd1aCre/+ mice (CP73, Gensat, PMID: 19013281), Drd2eGFPL10a/+ mice (CP101, Gensat, PMID: 19013281), Aldh1l1eGFPL10a/+ mice (Jd130, Gensat), CaMKII-tTa mice (Jackson Laboratory, stock number 003010), TetO-CHRM3 mice (Jackson Laboratory, stock number 014093), TetO-CHRM4 mice (Jackson Laboratory, stock number 024114), SfxFAD mice (Jackson Laboratory/MMRRC, stock number 034840-JAX), Eef1a1LSLeGFPL10a/+ (PMID: 24093682), Drd1aTdToma10a/+ mice (Jackson Laboratory, stock number 016204), Nr5e/- mice (Jackson Laboratory, stock number 018986), P2ry12/- mice (PMID: 12897207).

**Wild animals**

This study did not involve wild animals.

**Field-collected samples**

This study did not involve samples collected from the field.
All protocols were approved by IACUC at Icahn School of Medicine at Mount Sinai and were in accordance with NIH guidelines. Note that full information on the approval of the study protocol must also be provided in the manuscript.

**Flow Cytometry**

**Plots**

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

| Sample preparation | Tissue was rapidly dissected and mechanically dissociated in HBSS and centrifuged over 20% Percoll (GE Healthcare) and mononuclear cells were collected. Cells were resuspended in FACS buffer (500mL sterile PBS, .5% BSA, 2ml 0.5M EDTA), blocked in FC blocker (BioRad) as per manufacturer’s instructions and then stained on ice for 15 minutes with a combination of the following antibodies: CD73-PE (12-0731-82, ThermoFisher), CD39-Alexafluor700 (24DMS1, ThermoFisher). The samples were stained with DAPI on the final resuspension. For FACS analysis of CD11b+ isolated neonatal microglia, forebrain was rapidly dissected and mechanically dissociated in HBSS and centrifuged over 20% Percoll (GE Healthcare) and mononuclear cells were collected. Microglia were positively selected for using CD11b+ magnetic beads. Cells were resuspended in FACS buffer (500mL sterile PBS, .5% BSA, 2ml 0.5M EDTA), blocked in FC blocker (BioRad) as per manufacturer’s instructions and then stained on ice for 15 minutes with a combination of the following antibodies: CD73-PE (12-0731-82, ThermoFisher), CD39-Alexafluor700 (24DMS1, ThermoFisher). The samples were stained with DAPI on the final resuspension. |
| Instrument | 2020 Attune NxT Flow Cytometer (ThermoFisher) |
| Software | Data was collected using Attune NxT software (ThermoFisher). Data was analyzed using FCS Express Plus Software (De Novo Software) |
| Cell population abundance | In samples prepared from adult Cx3cr1CreErt2/+ (Litt), 6% of cells were microglia based on gating on live single cells. 100% of YFP+ cells were CD39+. 0% of cells were YFP-/CD39+. In samples prepared from adult control (CD73+/+) and CD73-/- mice, 7% of cells were microglia based on gating on live single cells and CD39+ expression. 0% of cells were CD73+ in Nt5e/-/- mice. In samples prepared from CD11b+ isolated neonatal microglia, 98.2% of cells were microglia based on gating on live single cells and CD39+ expression. |
| Gating strategy | Compensation was performed on single-stained samples of UltraComp eBeads (ThermoFisher), unstained beads, and unstained cells. Forward and side scatter was used to gate on a defined population of cells to exclude debris and also select single cells. Live cells were determined as DAPI negative. Gates to define positive and negative cells were determined using unstained samples, fluorescence minus one (FMO) controls, in which one antibody was omitted per sample, YFP unstained brain (YFP only), isotype control for the CD73 antibody, and CD73 +/- sample. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.