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Abstract:

High-grade gliomas are lethal brain cancers whose progression is robustly regulated by neuronal activity. Activity-regulated growth factor release promotes glioma growth, but this alone is insufficient to explain the effect that activity exerts on glioma progression. Here, we use single-cell transcriptomics, electron microscopy, whole-cell patch-clamp electrophysiology and calcium imaging to demonstrate that neuron-glioma interactions include electrochemical communication through bona fide AMPA receptor-dependent neuron-glioma synapses. Neuronal activity also evokes non-synaptic activity-dependent potassium currents that are amplified through gap junction-mediated tumor interconnections forming an electrically-coupled network. Glioma membrane depolarization assessed with in vivo optogenetics promotes proliferation, while pharmacologically or genetically blocking electrochemical signaling inhibits glioma xenograft growth and extends mouse survival. Emphasizing positive feedback mechanisms by which gliomas increase neuronal excitability and thus activity-regulated glioma growth, human intraoperative electrocorticography demonstrates increased cortical excitability in glioma-infiltrated brain. Together, these findings indicate that synaptic and electrical integration in neural circuits promotes glioma progression.

Introduction

High-grade gliomas are the leading cause of central nervous system (CNS) cancer-related death in both children and adults. This clinical intractability indicates that current understanding of glioma pathophysiology is insufficient. Gliomas infiltrate extensively within the brain and spinal cord, but growth outside the CNS is exceedingly rare. Glioma progression is regulated not only by cell-intrinsic mechanisms, but also by important microenvironmental dependencies. Neurons are a critically important component of the glioma microenvironment and regulate malignant growth in an activity-dependent manner[1,2]. Activity-regulated release of neuroligin-3 (NLGN3)[1,2] is required for glioma progression[2], indicating a fundamental role in glioma pathophysiology incompletely explained by stimulation of classical oncogenic signaling pathways alone[2]. We previously found that neuroligin-3 induces glioma expression of numerous synaptic genes[2], raising the intriguing possibility that glioma may engage in synaptic communication. Synapses exist between neurons

and normal oligodendroglial precursor cells (OPCs)[3,4], and electrochemical signaling can regulate proliferation, differentiation or survival of OPCs and other neural precursor cells (NPCs)[5-9]. As cellular subpopulations within gliomas closely resemble OPCs[10,11], we hypothesized that gliomas may similarly engage in synaptic communication and that this integration into neural circuits may be fundamental to glioma progression.

Synaptic gene expression in glioma

To examine synaptic gene expression in primary human glioma, we analyzed single cell transcriptomic datasets generated from pre-treatment biopsy samples of the major classes of adult and pediatric high-grade gliomas, including adult IDH-mutant glioma[11], adult IDH-WT glioma[10], and pediatric histone-3 mutant (H3K27M) diffuse midline glioma[10]. We found broad expression of glutamate receptor genes and post-synaptic structural genes in malignant glioma cells (Fig. 1a, Extended Data Fig. 1a). Unsupervised principal component analysis revealed enrichment of synaptic gene expression within distinct malignant cellular subpopulations (Fig. 1b). Gliomas are comprised of cellular subpopulations that resemble various stages of astrocytic and oligodendrocytic differentiation[10,11]. Synaptic gene enrichment was chiefly found in glioma cells that resemble OPCs (Fig. 1b, Extended Data Fig. 2c), the only glial cell type that normally functions as a post-synaptic cell[3,4]. These observations are consistent with the principle that malignant cellular subpopulations assume distinct roles in the heterogeneous cancer ecosystem. Concordant with these findings from primary biopsy tissue (Fig. 1b), single cell transcriptomics of patient-derived H3K27M+ glioma xenografts demonstrated synaptic gene enrichment in the OPC-like subpopulation (Extended Data Fig. 1b, Extended Data Fig. 2a-b).

Structural neuron-to-glioma synapses regulated by neuroligin-3

Having established that primary glioma cells express a repertoire of synaptic genes, we next assessed whether structural synapses form between glioma cells and neurons in the tumor microenvironment. Examination of primary glioblastoma tissue ultrastructure using electron microscopy (EM) revealed clear synaptic structures (Extended Data Fig. 2d). To confirm that glioma cells participate in such putative neuron-glioma synapses, we performed immuno-EM in GFP-labeled, patient-derived glioma xenografts. Immuno-EM analyses unambiguously identified GFP+ glioma cells on the post-synaptic side of synaptic structures, with synapses on ~10% of GFP+ glioma processes (Fig. 1c,d and Extended Data Fig. 2e-g). To test the contribution of microenvironmental NLGN3 to neuron-glioma synaptogenesis, we next co-cultured glioma cells expressing fluorescently-tagged PSD95 with WT or $NLGN3^{-/-}$ neurons. We found marked reduction in the co-localization of neuronal presynaptic puncta (synapsin) with glioma post-synaptic puncta (PSD95-RFP) in co-cultures with $NLGN3^{-/-}$ neurons compared to WT neurons (Fig.1e-f, Extended Data Fig. 2h). This further implicates NLGN3 in glioma synapse formation, a function distinct from its role stimulating classical oncogenic signaling pathways (Extended Data Fig.3a-c).

Electrophysiologically functional neuron-to-glioma synapses mediated by AMPA Receptors

Focusing on pediatric gliomas, we next tested if neurons and glioma cells establish electrophysiologically functional synapses using four distinct patient-derived orthotopic xenograft models (Extended Data Table 1). GFP-labeled glioma cells were stereotactically xenografted into the CA1 region of the hippocampal circuit. Following a period of engraftment and growth, acute hippocampal slices were prepared for whole cell patch clamp recordings of GFP+ glioma cells. Stimulation of Schaffer collateral/commissural afferent axons arising from CA3 while patch clamping CA1 region glioma cells enables measurement of the glioma response to axonal activity

(Fig. 2a-b). Voltage-clamp recordings revealed stimulation-evoked fast (<5 ms; 25-50 pA) inward currents consistent with excitatory postsynaptic currents (EPSCs, Fig. 2c). Current-clamp recordings demonstrated that these inward currents were depolarizing (Fig. 2d). Glioma EPSCs were blocked by the voltage-gated sodium channel blocker tetrodotoxin (TTX; Fig. 2e), illustrating dependence on neuronal action potentials. Measuring the current-voltage relationship (I-V curve) illustrated reversal at approximately 0 mV (Fig. 2f) and glioma EPSCs displayed facilitation in response to paired stimuli (Fig. 2g), electrophysiological characteristics suggesting synaptic communication through AMPA receptors (AMPAR, a type of ionotropic glutamate receptor). Concordantly, glioma EPSCs were blocked by NBQX, an AMPAR antagonist (Fig. 2g-i) and decreased by NASPM, an antagonist of calcium-permeable AMPARs (Extended Data Fig. 3d-e). AMPARs lacking GluA2 or containing GluA2 that has not undergone RNA editing of its Q/R site are calcium-permable[12,13]. GluA2 is broadly expressed in gliomas (Fig. 1a); examination of RNA editing of the Q/R site in pediatric glioma demonstrated GluA2 under-editing (~50-70% edited; Extended Data Fig. 3f-g). Taken together, these results indicate that axon stimulation-evoked, millisecond timescale glioma cell currents require action potentials and are mediated by AMPARs, properties consistent with the conclusion that subpopulations of glioma cells form bona fide synapses with neurons.

To test this hypothesis further, we replaced extracellular calcium with strontium, a manipulation that facilitates asynchronous presynaptic vesicle release[14] (Fig. 2j). In the presence of strontium we detected small, fast inward currents consistent with miniature EPSCs (mEPSCs), indicating quantal responses to synaptic vesicles[14] in glioma cells. Quantal glioma mEPSCs were similarly blocked by NBQX (Fig. 2j). No fast, large currents reminiscent of action potentials were observed in any of >640 glioma cell recordings. Taken together, these results indicate that synaptic

transmission occurs between neurons and a subset of xenografted human glioma cells, exhibiting properties similar to synapses formed with normal OPCs[3,4].

Further exploring the consequences of activity-dependent currents in glioma, we performed *in situ* two-photon calcium imaging of xenografted glioma expressing the genetically encoded calcium indicator GCaMP6s. Glioma-specific expression of the calcium indicator was validated by co-staining for human nuclear antigen (Extended Data Fig. 4a). Spontaneous calcium transients were consistently observed (Extended Data Fig. 4b-d). Stimulation of Schaffer collateral/commissural afferents elicited calcium transients in glioma cells located in the CA1 target area of the stimulated axons (Fig. 2k-l), providing additional evidence that endogenous circuit activity may exert functionally relevant effects on glioma cells. These evoked calcium transients were blocked by TTX (Fig. 2m, Extended Data Fig. 4e).

Neuronal activity-dependent potassium currents in glioma cells

A longer duration electrophysiological response to neuronal activity was found in a subset of glioma cells (Fig. 3a). Distinct from the classical EPSCs (<5ms) described above, these prolonged currents (>1 sec) exhibited kinetics inconsistent with a synaptic response and are instead reminiscent of the neuronal activity-evoked currents observed in normal astrocytes. Supporting the idea that these prolonged currents are distinct from the synaptic responses described above, the calcium-permeable AMPAR inhibitor NASPM had no effect (Extended data Fig. 5a-b). These prolonged glioma currents were blocked by TTX (Fig. 3b-c). Further illustrating a response coupled to neuronal population firing, the morphology of the prolonged currents revealed spike-like waveforms phase-locked to neuronal field potential waveforms that scaled with increased axonal stimulation intensity (Extended Data Fig. 5c-d). Simultaneous whole cell current clamp and field potential recordings reveal that the prolonged current amplitude scaled directly with field

potential, meaning that prolonged glioma current amplitude increases with increasing neuronal activity (Extended Data Fig. 5e-f). In normal astrocytes, activity-dependent currents are attributable to glutamate transporter currents and inward potassium currents due to a rise in extracellular potassium from neurons[15-17]. Consistent with a direct role for increases in extracellular potassium in generating these prolonged currents, similar prolonged glioma currents were elicited by application of potassium alone with neuronal activity pharmacologically blocked (Fig. 3d, Extended Data Fig. 5g). Further, activity-dependent prolonged currents were largely blocked by barium, an ion that occludes potassium pores (Fig.3e-f). In contrast, the glutamate transporter antagonist TBOA had a negligible effect (Extended Data Fig. 5h). Taken together, these results support the interpretation that non-synaptic, prolonged glioma currents chiefly reflect potassium flux attributable to a rise in extracellular potassium with neuronal activity.

Gap junction coupling amplifies potassium currents in glioma

Cells with prolonged currents exhibit strikingly low input resistance (Extended Data Fig. 6a), reminiscent of astrocytes. Extensive gap-junctional coupling is partially responsible for low membrane resistance in astrocytes[18]. Gap junctions couple adult glioma cells through long processes called tumor microtubes[19], which we demonstrated in primary pediatric glioma tissue (Extended Data Fig. 5j-o). Following biocytin dye-filling of single cells exhibiting prolonged currents, biocytin diffused to a network of glioma cells (Extended Data Fig. 6b), supporting the existence of a gap junction-coupled network. To test this conclusion, we applied the gap junction blockers carbenoxolone (CBX) or meclofenamate, which reduced the amplitude of prolonged glioma currents (Fig. 3g, Extended Data Fig. 6c-e, 6h-i) while simultaneously increasing glioma input resistance (Extended Data Fig. 6f-g, 6j-k). Together, these observations strongly suggest that activity-regulated increases in extracellular potassium concentrations cause glioma depolarization

and that a gap junction-coupled glioma network amplifies the consequences of activity-induced changes in the extracellular ionic environment.

Two-photon calcium imaging further revealed distinct synchronous network calcium transients that both occur spontaneously (Fig. 3h, Extended Data Fig. 5i, Supplementary Video 1) and are elicited by afferent stimulation (Fig. 3i, Supplementary Video 2). This synchronicity could be explained by gap-junction coupling, and accordingly was blocked by application of CBX (Extended Data Fig. 6l-o, Supplementary Video 3), further indicating a functional glioma network through which depolarizing currents propagate. Demonstration of glutamatergic chemical synapses (Fig. 2) and activity-dependent, non-synaptic potassium currents (Fig. 3) build upon early work illustrating glutamate-dependent currents in glioblastoma[20] to underscore the surprising observation that this cancer is an electrically active tissue (see Supplementary Videos 1, 2, and 3).

Intratumoral electrophysiological heterogeneity

Gliomas exhibit intratumoral and intertumoral cellular heterogeneity, with subpopulations of cancer cells assuming particular roles and even very small cellular fractions proving essential for cancer progression[21]. Considering all pediatric glioma cells examined (n=643), we find that ~5–10% of glioma cells exhibit synaptic EPSCs, ~40% exhibit prolonged currents in response to neuronal activity. While all four patient-derived xenograft models exhibited neuronal activity-evoked inward currents, the proportion of cells displaying fast EPSCs or prolonged currents varies between patient-derived models. This intertumoral heterogeneity is evident even within a molecularly-defined subtype such as H3K27M+ glioma and predicted by the varied composition of OPC-like and astrocyte-like compartments in individual tumors (Extended Data Fig. 2b, 7a).

Membrane depolarization promotes glioma proliferation

Depolarization can profoundly affect cellular behavior[5-9], and we have found two distinct mechanisms by which neuronal activity induces glioma cell membrane depolarization. To test if glioma cell membrane depolarization promotes proliferation, we used *in vivo* optogenetic techniques to depolarize xenografted glioma cells expressing the blue light-sensitive cation channel channelrhodopsin-2 (ChR2; Extended Data Fig. 7b). Glioma cells expressing ChR2-YFP were xenografted to the cortex, and after a period of engraftment and growth blue light was delivered to depolarize the glioma xenograft. Compared to mock-stimulated control groups, we found that glioma depolarization robustly promoted glioma xenograft proliferation (Fig. 4a-d). Blue light exposure alone had no effect on proliferation nor apoptosis in control glioma xenografts (Extended Data Fig. 7c-e).

Targeting mechanisms of electrochemical communication reduces glioma growth

As membrane depolarization promotes glioma proliferation, we next tested the relative functional contributions of each mechanism of electrochemical communication, beginning with AMPAR-mediated EPSCs. We over-expressed either WT-GluA2 subunit fused to GFP, GFP alone, or a dominant-negative GluA2 (GluA2-DN-GFP) in glioma and confirmed decreased conductance in GluA2-DN-GFP-expressing glioma cells (Extended Data Fig. 7f-g). Mice bearing xenografts overexpressing WT-GluA2-GFP survived a shorter time than GFP-only xenografts, while mice bearing GluA2-DN-GFP xenografts exhibited improved survival and decreased tumor burden compared to GFP-only controls (Fig. 4e-j, Extended Data Fig. 8a-b). To test for a possible *in vivo* growth advantage of GluA2-expression, we xenografted a mixture of 80% GluA2-DN-GFP construct-expressing:20% non-expressing cells and found that tumors were composed almost entirely of non-GluA2-DN-GFP expressing cells at the survival endpoint (Fig. 4g-h).

Similar to *in vivo* experiments, co-culture of glioma cells with neurons markedly increased proliferation. NBQX partially reduced this effect (Extended Data Fig. 8c-d); neuronal secreted factors such as NLGN3 accounts for residual elevated proliferation[1]. Given the stark effect of AMPAR function on glioma growth *in vivo* and in co-culture, we next tested the relative contributions of cell-intrinsic glutamate signaling mechanisms. While paracrine/autocrine AMPA signaling may promote adult glioblastoma growth[22,23], NBQX had no effect on pediatric glioma proliferation in the absence of neurons (Extended Data Fig. 8c-d). Testing cell-intrinsic effects of GluA2-DN expression, we similarly found no difference in growth rate or apoptosis in pediatric glioma cells outside of the neuronal microenvironment (Extended Data Fig. 8e-f). In contrast, pediatric glioma cell migration and invasion were influenced by GluA2-DN expression in a cell-intrinsic manner (Extended Data Fig. 8g-j), consistent with previous reports in adult gliomas[24]. Taken together, these findings indicate that glioma AMPAR activation promotes pediatric glioma growth chiefly through microenvironmental interactions such as neuron-to-glioma synaptic transmission.

Next, we sought to therapeutically target glioma currents using existing drugs. Using an AMPAR-blocking anti-epileptic drug (perampanel), we found a ~50% decrease in pediatric glioma proliferation in perampanel-treated mice compared to vehicle-treated controls (Fig. 4k, Extended Data Fig. 8k). We then targeted gap junction-mediated amplification of neuronal activity-dependent potassium currents using the brain-penetrant gap-junction blocker meclofenamate[25]. Meclofenamate treatment similarly decreased pediatric glioma xenograft proliferation and growth (Fig. 4l, Extended Data Fig. 8l).

Gliomas increase neuronal excitability in humans

Neuron-glioma interactions are bidirectional; neuronal activity increases glioma growth[1], and gliomas are thought to increase neuronal activity. In preclinical adult glioblastoma models, glioma cells induce neuronal hyperexcitability and seizures[26,27] through non-synaptic glutamate secretion[26,28], and through secretion of synaptogenic factors[27]. To assess neuronal hyperexcitability in primary human glioblastoma, we performed intraoperative electrocorticography in three awake adult human subjects with cortical high-grade gliomas (IDH WT) prior to surgical resection (Fig. 5). High gamma frequency (70-110 Hz) power, a measure that correlates with neuronal firing rate and local field potential[29] and that is elevated by cortical hyperexcitability[30], was sampled over a 3-minute period while the subjects were in a resting state. Outside of the necrotic nodular core of the tumor, we found markedly increased high gamma power in tumor-infiltrated brain compared to normal-appearing brain (Fig. 5a-b, Extended Data Fig. 9a). Concordantly, we found neuronal hyperexcitability in the microenvironment of pediatric glioma xenografts (Fig. 5c). These findings support the concept[26,27] of hyperexcitable neurons in the glioma microenvironment, which would potentiate mechanisms of activity-regulated glioma progression. As neuronal action potentials result in extracellular potassium rise[31,32], neuronal hyperexcitability in the glioma microenvironment would promote non-synaptic prolonged glioma potassium currents, as well as synaptic neuron-to-glioma EPSCs (Extended Data Fig. 9b).

Discussion

Membrane depolarization and depolarization-induced calcium transients promote normal NPC development, in part through voltage-gated calcium channel signaling[5-9]. Non-synaptic neurotransmitter release mediates synchronous calcium transients in gap-junction-coupled NPCs in many contexts[33-35], but synapses between presynaptic neurons and post-synaptic NPCs are also well-described, including transient synapses onto migrating neuroblasts during corticogenesis[36]

and synapses onto OPCs in the developing and adult brain[3,4]. High-grade gliomas integrate into electrical networks and that depolarizing current promotes glioma progression similarly to the well-established effect in normal NPCs. In glioma, we have demonstrated bona fide neuron-to-glioma synapses, reminiscent of the synapses found on normal OPCs. As well, we have shown neuronal activity-evoked potassium currents in glioma cells, reminiscent of activity-dependent currents in normal astrocytes[37]. Neuronal activity-induced glioma membrane depolarization by either mechanism promotes glioma proliferation and growth, through voltage-sensitive signaling pathway(s) to be fully elucidated in future work.

Neuronal activity is emerging as a critical regulator of glioma progression. The electrochemical communication described here joins activity-regulated secretion of growth factors[1,2] as mechanisms that mediate this important microenvironmental interaction. Appreciating the crosstalk between neurons and the glioma cells invading and integrating into neural circuitry elucidates promising therapeutic targets, including activity-regulated secreted growth factors[1,2], neuron-to-glioma neurotransmission, ion channels and gap-junction coupling. Modulating the influence of glioma on neuronal excitability represents an important opportunity to dampen the magnitude of activity-regulated glioma growth. Taken together, the findings presented here identify synaptic neurotransmission and activity-dependent potassium currents as mechanisms driving glioma growth and elucidate the previously unexplored potential to target glioma circuit dynamics for therapy of these lethal cancers.

References:

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1 Venkatesh, H. S. *et al.* Neuronal Activity Promotes Glioma Growth through Neuroligin-3 Secretion. *Cell* **161**, 803-816, doi:10.1016/j.cell.2015.04.012 (2015).

2 Venkatesh, H. S. *et al.* Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma. *Nature* **549**, 533-537, doi:10.1038/nature24014 (2017).

3 Bergles, D. E., Roberts, J. D., Somogyi, P. & Jahr, C. E. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* **405**, 187-191, doi:10.1038/35012083 (2000).

4 Karadottir, R., Cavelier, P., Bergersen, L. & Attwell, D. NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. *Nature* **438**, 1162-1166, doi:10.1038/nature04302 (2005).

5 LoTurco, J. J., Owens, D. F., Heath, M. J., Davis, M. B. & Kriegstein, A. R. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* **15**, 1287-1298 (1995).

6 Luk, K. C. & Sadikot, A. F. Glutamate and regulation of proliferation in the developing mammalian telencephalon. *Dev Neurosci* **26**, 218-228, doi:10.1159/000082139 (2004).

7 Liu, X., Wang, Q., Haydar, T. F. & Bordey, A. Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat Neurosci* **8**, 1179-1187, doi:10.1038/nn1522 (2005).

8 Deisseroth, K. *et al.* Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* **42**, 535-552, doi:S0896627304002661 [pii] (2004).
9 Kougioumtzidou, E. *et al.* Signalling through AMPA receptors on oligodendrocyte precursors promotes myelination by enhancing oligodendrocyte survival. *Elife* **6**, doi:10.7554/eLife.28080 (2017).
10 Filbin, M. G. *et al.* Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. *Science* **360**, 331-335, doi:10.1126/science.aao4750 (2018).
11 Venteicher, A. S. *et al.* Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq. *Science* **355**, doi:10.1126/science.aai8478 (2017).
12 Sommer, B., Kohler, M., Sprengel, R. & Seeburg, P. H. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell* **67**, 11-19 (1991).
13 Hollmann, M., Hartley, M. & Heinemann, S. Ca2+ permeability of KA-AMPA--gated glutamate receptor channels depends on subunit composition. *Science* **252**, 851-853 (1991).
14 Oliet, S. H., Malenka, R. C. & Nicoll, R. A. Bidirectional control of quantal size by synaptic activity in the hippocampus. *Science* **271**, 1294-1297 (1996).
15 Bergles, D. E. & Jahr, C. E. Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* **19**, 1297-1308 (1997).
16 Luscher, C., Malenka, R. C. & Nicoll, R. A. Monitoring glutamate release during LTP with glial transporter currents. *Neuron* **21**, 435-441 (1998).
17 Sibille, J., Pannasch, U. & Rouach, N. Astroglial potassium clearance contributes to short-term plasticity of synaptically evoked currents at the tripartite synapse. *J Physiol* **592**, 87-102, doi:10.1113/jphysiol.2013.261735 (2014).
18 McKhann, G. M., 2nd, D'Ambrosio, R. & Janigro, D. Heterogeneity of astrocyte resting membrane potentials and intercellular coupling revealed by whole-cell and gramicidin-perforated patch recordings from cultured neocortical and hippocampal slice astrocytes. *J Neurosci* **17**, 6850-6863 (1997).
19 Osswald, M. *et al.* Brain tumour cells interconnect to a functional and resistant network. *Nature* **528**, 93-98, doi:10.1038/nature16071 (2015).
20 Labrakakis, C., Patt, S., Hartmann, J. & Kettenmann, H. Glutamate receptor activation can trigger electrical activity in human glioma cells. *Eur J Neurosci* **10**, 2153-2162 (1998).
21 Singh, S. K. *et al.* Identification of human brain tumour initiating cells. *Nature* **432**, 396-401, doi:nature03128 [pii]
10.1038/nature03128 (2004).
22 Ishiuchi, S. *et al.* Ca2+-permeable AMPA receptors regulate growth of human glioblastoma via Akt activation. *J Neurosci* **27**, 7987-8001, doi:10.1523/JNEUROSCI.2180-07.2007 (2007).
23 Sontheimer, H. A role for glutamate in growth and invasion of primary brain tumors. *J Neurochem* **105**, 287-295, doi:10.1111/j.1471-4159.2008.05301.x (2008).
24 Lyons, S. A., Chung, W. J., Weaver, A. K., Ogunrinu, T. & Sontheimer, H. Autocrine glutamate signaling promotes glioma cell invasion. *Cancer Res* **67**, 9463-9471, doi:10.1158/0008-5472.CAN-07-2034 (2007).
25 Chen, Q. *et al.* Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. *Nature* **533**, 493-498, doi:10.1038/nature18268 (2016).

26 Campbell, S. L., Buckingham, S. C. & Sontheimer, H. Human glioma cells induce hyperexcitability in cortical networks. *Epilepsia* **53**, 1360-1370, doi:10.1111/j.1528-1167.2012.03557.x (2012).
27 John Lin, C. C. *et al.* Identification of diverse astrocyte populations and their malignant analogs. *Nat Neurosci* **20**, 396-405, doi:10.1038/nn.4493 (2017).
28 Buckingham, S. C. *et al.* Glutamate release by primary brain tumors induces epileptic activity. *Nat Med* **17**, 1269-1274, doi:10.1038/nm.2453 (2011).
29 Ray, S., Crone, N. E., Niebur, E., Franaszczuk, P. J. & Hsiao, S. S. Neural correlates of high-gamma oscillations (60-200 Hz) in macaque local field potentials and their potential implications in electrocorticography. *J Neurosci* **28**, 11526-11536, doi:10.1523/JNEUROSCI.2848-08.2008 (2008).
30 Yizhar, O. *et al.* Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* **477**, 171-178, doi:10.1038/nature10360 (2011).
31 Hodgkin, A. L. & Katz, B. The effect of sodium ions on the electrical activity of giant axon of the squid. *J Physiol* **108**, 37-77 (1949).
32 Ransom, B. R. & Goldring, S. Ionic determinants of membrane potential of cells presumed to be glia in cerebral cortex of cat. *J Neurophysiol* **36**, 855-868, doi:10.1152/jn.1973.36.5.855 (1973).
33 Bittman, K. S. & LoTurco, J. J. Differential regulation of connexin 26 and 43 in murine neocortical precursors. *Cereb Cortex* **9**, 188-195 (1999).
34 LoTurco, J. J., Blanton, M. G. & Kriegstein, A. R. Initial expression and endogenous activation of NMDA channels in early neocortical development. *J Neurosci* **11**, 792-799 (1991).
35 Marins, M. *et al.* Gap junctions are involved in cell migration in the early postnatal subventricular zone. *Dev Neurobiol* **69**, 715-730, doi:10.1002/dneu.20737 (2009).
36 Ohtaka-Maruyama, C. *et al.* Synaptic transmission from subplate neurons controls radial migration of neocortical neurons. *Science* **360**, 313-317, doi:10.1126/science.aar2866 (2018).
37 Kuffler, S. W. Neuroglial cells: physiological properties and a potassium mediated effect of neuronal activity on the glial membrane potential. *Proc R Soc Lond B Biol Sci* **168**, 1-21, doi:10.1098/rspb.1967.0047 (1967).