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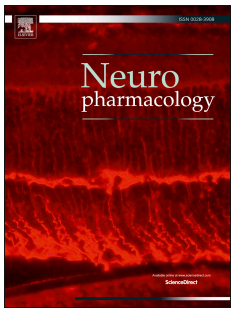


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# Optimising the energetic cost of the glutamatergic synapse

[1*]Jonathan Lezmy, [2*]Julia J. Harris & [1]David Attwell

[1]Department of Neuroscience, Physiology & Pharmacology

University College London, Gower Street, London, WC1E 6BT, UK

[2]The Crick Institute,

1 Midland Road, London, NW1 1AT, UK

*Equal contribution

Send correspondence to David Attwell, d.attwell@ucl.ac.uk

Key words: synapse, glutamate, energy, ATP, information

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**Abstract**

**As for electronic computation, neural information processing is energetically expensive. This is because information is coded in the brain as membrane voltage changes, which are generated largely by passive ion movements down electrochemical gradients, and these ion movements later need to be reversed by active ATP-dependent ion pumping. This article will review how much of the energetic cost of the brain reflects the activity of glutamatergic synapses, consider the relative amount of energy used pre- and postsynaptically, outline how evolution has energetically optimised synapse function by adjusting the presynaptic release probability and the postsynaptic number of glutamate receptors, and speculate on how energy use by synapses may be sensed and adjusted.**

## 1. Introduction: brain energy use

The brain is 2% of the human body's mass, but consumes about 20% of its resting energy production (Kety, 1957; Sokoloff, 1960; Rolfe and Brown, 1997). Energy is provided to the brain largely in the form of glucose and oxygen in the blood (although other energy sources, such as lactate and fatty acids may be used during exercise and starvation), through a plumbing system - the arterioles, capillaries and venules - that occupy only a few percent of the brain's volume (Tsai et al., 2009). Several analyses have been performed giving a broad outline of what brain energy is used on in the grey matter (Attwell & Laughlin, 2001; Lennie, 2003). These involve making simplifying assumptions, for example assuming that all neurons fire at the same rate and use the same amount of energy (but see Howarth et al. (2010, 2012) for a cerebellar analysis where each cell type is treated separately). Glutamatergic synapses are at the centre of these studies and of the present review as they dominate neuronal signaling in the grey matter: excitatory neurons outnumber inhibitory neurons by a factor of 9 to 1, and ~90% of synapses release glutamate (Abeles, 1991;

Braitenberg and Schüz, 1998). In combination, these works provide valuable insight into constraints on neuronal function imposed by the brain's high energy use.

Attwell & Laughlin (2001) used:

(i) anatomical measurements of cell area to calculate capacitance, and thus the charge entry needed to generate action potentials, the mean frequency of which was estimated from recordings from freely moving animals;

(ii) resting potential and input resistance data to evaluate the $Na^+$ leak into the cell at the resting potential;

(iii) patch-clamp data on presynaptic release probability, the number of postsynaptic glutamate-gated receptors and the current flowing through them, to estimate the charge entry underlying EPSCs; and

(iv) data on the number of molecules of glutamate in each vesicle and the stoichiometry of glutamate uptake, to estimate the ion movements underlying glutamate recycling at synapses.

All of these data were converted into ATP usage, by assuming a Na/K pump stoichiometry of 1 ATP used to pump 3 $Na^+$ ions out of the cell (and 2 $K^+$ ions into the cell). The results led to a crude energy budget for the brain, in which synaptic processes consumed 40% of the ATP used on signalling processes (Fig. 1; Attwell & Laughlin, 2001). Summing the different energy use components for the grey matter estimated as above, and adding on an estimated 25% for energy use on tasks not directly related to signalling such as protein synthesis (Engl et al., 2017), led to an estimate that the rat brain should use 40 μmol ATP/g/min, which is remarkably close to the measured value of 33-50 μmol ATP/g/min (Clarke & Sokoloff, 1999) given the rather general simplifying assumptions made in the analysis.

For the white matter, the energy use is significantly lower (Sokoloff et al., 1977). An energy budget for the white matter predicts that this partly reflects less energy use on action potentials (because myelination decreases the axon capacitance and hence the $Na^+$ entry needed to generate an action potential), but by far the dominant factor is the much lower

ATP use on synaptic transmission in the white matter due to the very low number of synapses present (Harris & Attwell, 2012).

In the following sections, we review findings from previous studies on synaptic energy use, highlighting different strategies that the brain uses to optimise energetic cost at the pre- and post-synaptic compartments. As we review the literature, we introduce new perspectives by comparing theoretical and experimental studies, and we conclude by presenting our hypotheses about how the brain might dynamically regulate its own energetic optimisation.

## 2. Assumptions affecting, and consequences of, the calculated synaptic energy use

Two interesting assumptions were made by Attwell & Laughlin (2001) when estimating energy use. First, the analysis was simplified by assuming that ATP usage can be quantified as 1/3 of the $Na^+$ load that needs to be pumped out of cells, which reduces the problem to merely calculating all the different sources of $Na^+$ entry into the cells. However, a detailed analysis, outlined in Attwell & Laughlin (2001) and developed in the Supplementary Material of Howarth et al. (2010), shows that there are actually two ways in which ion gradients are restored after $Na^+$ enters the cell. One is ion pumping via the $Na^+/K^+$-ATPase, with the stoichiometry mentioned above. The other is that the accumulation of $Na^+$ inside the cell raises $[Na^+]_i$, leading to a decrease of the passive influx of $Na^+$ into the cell. With the latter mechanism, there is no increase in energy use; instead the decrease in influx, with maintained $Na^+$ pumping out of the cell, leads to $[Na^+]_i$ returning to its normal level. The relative magnitude of these two mechanisms is determined by the dependence of the $Na^+$ pump rate on $[Na^+]_i$: a steep dependence increases the ATP use that is triggered by $Na^+$ influx (Howarth et al., 2010). But once the $Na^+$ pump is saturated by a sufficiently high $[Na^+]_i$ (as can occur in some neurons or during bursting activity; Shen & Johnson, 1998; Crambert et al., 2000), any extra $Na^+$ influx evoked by synaptic currents or action potentials will not further increase ATP consumption. The $Na^+$ pump will rather keep pumping ions at its maximal constant rate, and the reduction of the passive influx of $Na^+$ becomes the only mechanism available to deal with additional $Na^+$ influx from synaptic or spiking activity.

Secondly, in the original Attwell & Laughlin (2001) calculations, the $Na^+$ entry needed to generate the action potential was calculated as the minimum charge entry required (i.e. the capacitance of the cell multiplied by the voltage change of the action potential) multiplied by a correction factor of 4 (derived from Hodgkin & Huxley's work) which takes account of the fact that the outward $K^+$ current that repolarises the membrane at the end of the action potential overlaps considerably with the inward $Na^+$ current that generates it, resulting in a larger $Na^+$ influx being needed. However, at least in some neurons (depending on the temperature and the rate of $Na^+$ channel inactivation), there is much less overlap of the $Na^+$ and $K^+$ currents (Alle et al., 2009; Carter & Bean, 2009), so that the energy used on each action potential is ~3-fold less than that predicted by Attwell & Laughlin (2001). By lowering the percentage of signalling energy devoted to action potentials, this will increase the percentage of energy used on synapses. However, this high energetic efficiency of action potential initiation and propagation does not pertain to all neurons: more recently the ATP cost of an action potential in a tufted layer V cortical pyramidal cell *in vivo* (Hallermann et al., 2012) was calculated to be similar to that originally estimated by Attwell & Laughlin (2001).

Thus, the numbers provided by the simplified energy budget are only approximate. Nevertheless, even an approximate validity of the budget allows an interesting conclusion to be drawn. The two largest energy use components are synaptic currents and action potentials, both of which will increase roughly proportionally to spike rate. The energy use of these components thus imposes an upper limit to the time resolution of neural information encoding: raising the brain's frequency of action potentials and downstream synaptic transmission by a certain factor to increase this time resolution would require a corresponding increase in nutrient supply in the blood (Attwell & Gibb, 2005).

**3. Presynaptic versus postsynaptic energy use**

A synapse can be considered as an amplifier: presynaptic entry of ~12,000 $Ca^{2+}$ ions triggers the release of a vesicle containing ~4,000 neurotransmitter molecules, which evoke the entry into the postsynaptic cell of ~400,000 $Na^+$ and $Ca^{2+}$ ions (Attwell & Laughlin, 2001). Since ATP use is (loosely speaking) proportional to the number of ions or molecules being

moved, this suggests that a substantial fraction of the energy use of synapses will be post- rather than pre-synaptic (some processes on which energy is used at synapses are shown in Fig. 1A). Reflecting this, detailed analysis suggested that ATP use on presynaptic $Ca^{2+}$ entry and glutamate cycling at excitatory synapses were only about 15% of the total ATP use on synapses, which is dominated by ATP consumption on reversing the ion movements evoked by activation of postsynaptic receptors (Fig. 1B, Attwell & Laughlin, 2001; Harris et al., 2012). Rangaraju et al. (2014) presented data suggesting that vesicle cycling is the largest consumer of ATP presynaptically, although further calculations (Engl & Attwell, 2015) estimated that it is only 1% of the energy used postsynaptically.

**4. Optimising presynaptic release probability**

"Vesicle release probability" represents the likelihood that an action potential arriving at a presynaptic site triggers the release of a vesicle of neurotransmitter, and as such, is an indication of the presynaptic strength of a synapse. In the CNS it seems surprising that the vesicle release probability is often only ~0.25, apparently suggesting that the energy used to propagate action potentials to the terminal of a presynaptic neurons is often wasted, in the sense that no postsynaptic response is generated three quarters of the time. It has been suggested that a low release probability allows synapses to have a wide dynamic range, increases information transmission from correlated inputs, maximizes information storage or reflects matching of the rate at which information arrives from a presynaptic cell to the rate at which it can be transmitted down the axon of the postsynaptic cell (Zador, 1998; Goldman, 2004; Varshney et al., 2006; Levy & Baxter, 2002). However, in many parts of the CNS, including the neocortex, there is a convergence onto the same postsynaptic neuron of different release sites from a given presynaptic neuron (more than 6 release sites for the rat cortex: Deuchars & Thomson, 1995), perhaps in order to maintain stability of information transmission while synaptic components (such as dendritic spines) turn over. It turns out that this can have a profound effect on the reliability of information transmission, and on the energy needed to transmit information. If transmission is only needed at (say) 1 release site to generate a significant postsynaptic response, then there is no reason to have a release

probability of 1 at all (say) 6 of the synapses converging from one presynaptic neuron onto one postsynaptic neuron. A much lower release probability, p, would still give a reasonable chance of the presynaptic signal being detected postsynaptically, because the chance of all 6 release sites failing is $(1-p)^6$, which is only 0.015 for p=0.5 or 0.18 for p=0.25, implying a 98.5% or 82% chance of the information being transmitted. Given that most synaptic energy is used postsynaptically on reversing the ion movements generating postsynaptic currents, and the frequency of activation of postsynaptic receptors is governed (for a given presynaptic spike frequency) by the vesicle release probability, having a release probability substantially less than 1 will greatly decrease the energy used to transmit information. For example, for 6 release sites with p=0.5, the information transmitted is reduced very little, but the energy used postsynaptically is halved.

A detailed analysis of this was provided by Harris et al. (2012), who showed that, in the presence of spontaneous release of vesicles at the experimentally observed rate (1.2 Hz for a cortical pyramidal cell), for a mean firing rate (4 Hz) that is 1% of the assumed maximum rate (400 Hz) then, when the ratio of information transmitted to ATP used on postsynaptic currents is plotted against presynaptic release probability, the curve has a peak at which this ratio is maximised (Fig. 2A). The predicted optimal release probability decreases as the number of presynaptic synapses converging onto a postsynaptic cell increases. Remarkably, Hardingham et al. (2010) found just such a dependence of release probability on number of convergent synapses for cortical layer 2/3 to layer 2/3 connections (Fig. 2B: open circles and red line are their data; blue symbols are our theoretical predictions). The agreement of theory and experiment is not perfect, however, because the observed release probability is somewhat higher than that predicted by the theory (the blue points on Fig 2B for p<1 should be further to the right to agree perfectly with the experimental data). This may serve as a safety margin to allow the synapses to adapt somewhat to acute falls of energy level (during e.g. a sudden rise in the firing rate), as described below.

**5. Optimising the number of postsynaptic receptors**

Release of 4000 molecules of glutamate from a synaptic vesicle activates a relatively small number of postsynaptic receptors, e.g. probably less than 70 AMPA receptors (Hestrin, 1992; Silver et al., 1996; Spruston et al., 1995; Nusser et al., 1998). If the number of receptors activated is too low then the presynaptic neuron's contribution to postsynaptic signals may get lost in the noise of spontaneous vesicular release or postsynaptic ion channel opening and closing. However, if the function of the synaptic input is to evoke a postsynaptic action potential, then if more receptors are activated than are needed to achieve that aim, then energy will be wasted postsynaptically on the resulting synaptic currents. How might evolution optimise the number of postsynaptic receptors at a synapse?

The synapse between retinal ganglion cell axons and the lateral geniculate nucleus (LGN) neurons which project to visual cortex is normally thought of as a simple relay synapse. Harris et al. (2015) used dynamic clamp to adjust the effective number of postsynaptic glutamate receptors activated by a single retinal input axon, in order to examine the effect that this had on information passing through the synapse (the input action potential train mimicked that occurring for natural visual scenes, inhibition was blocked pharmacologically, and descending cortical input to the LGN was abolished by doing the experiments in brain slices with the cortex removed). Increasing the size of the synaptic current led to more information passing through the synapse, demonstrating that the number of postsynaptic receptors was not set in order to maximise information transfer (Fig. 3A). Instead, the ratio of information transmitted to the amount of ATP used on postsynaptic currents (or, indeed, on synaptic currents plus postsynaptic action potentials) was maximal at the observed magnitude of synaptic current (Fig. 3B). Thus, the number of postsynaptic receptors is appropriate for maximising the energy efficiency of the synapse, defined as bits of information transmitted per ATP used.

This approach was later extended to the far weaker synapse that exists between LGN neuron axons, and the spiny stellate cortical neurons that they send input to (Harris et al., 2019). Again, both in a theoretical model and experimentally, either increasing or

decreasing the number of postsynaptic channels activated by a presynaptic input led to a decrease in the amount of information transmitted per ATP used (Fig. 3C-D).

**6. How do neurons adapt their function according to energy use?**

The preceding review establishes that neural computation is energetically expensive, that much of the energy used is expended at synapses (especially on postsynaptic currents), but that there is an optimisation of the presynaptic release probability and the number of postsynaptic receptors - at least at some synapses - which maximises the amount of information transmitted per ATP used. In this section we speculate on how this optimisation might be achieved, and whether it might be a dynamic optimisation which changes with the prevailing intracellular ATP level.

During normal activity when animals are awake, synapses tend to get strengthened when coincident pre- and postsynaptic activity triggers long-term potentiation. This can be mediated by insertion of more glutamate-gated channels into the postsynaptic terminal or by an increase of presynaptic release probability, both of which would increase postsynaptic ATP use. The subsequent renormalisation of synapse strengths that occurs during sleep periods (Cirelli & Tononi, 2021) may serve to prevent a continuous increase of ATP use in the brain.

The energy status related molecules, ATP and adenosine, could play a role in regulating synaptic properties to match prevailing energetic conditions. ATP is released into the extracellular space during synaptic activity (Mishra et al., 2016) but the basal ATP concentration in the extracellular space is about $10^5$-$10^6$ fold lower than in the intracellular space (~10 nM vs 3-10 mM: Falzoni et al. 2013) because ecto-ATPases expressed by peri-synaptic glial cells (astrocytes, oligodendrocyte lineage cells and microglia) rapidly catabolize ATP to adenosine (Badimon et al. 2020, Pfeiffer and Attwell 2020). ATP and adenosine are interconverted within cells (in a direction determined by energetic state) and equilibrative nucleoside transporters release adenosine to the extracellular space (King et al., 2006), particularly when energy-generating substrates (such as glucose and oxygen) are insufficient to allow the successive phosphorylation of adenosine through AMP and ADP to

ATP. Extracellular adenosine levels are low under normal conditions (40-300 nM) (Zetterstrom et al. 1982, Ballarin et al., 1991), but rise significantly during energy deprivation (Hagberg et al., 1987). It is attractive to think that ATP and adenosine receptors evolved, at least partly, as sensors of energetic state, to adjust the function of the brain to cope with changes in energy level. The affinities of adenosine-activated and ATP-activated receptors for their native agonists (Fredholm et al., 2001, Jacobson, 2018) suggests they could efficiently sense ATP/adenosine fluctuations in the synaptic cleft or nearby.

Could released ATP and adenosine adjust synaptic release probability and the number of postsynaptic receptors to maximise the information transmitted per ATP used, in different energetic states? ATP-activated ionotropic P2X and metabotropic P2Y receptors found on presynaptic terminals can increase or decrease glutamate release, respectively (Gu and MacDermott 1997, Rodrigues et al. 2005) and postsynaptically can control the number of NMDA and AMPA receptors present in the plasma membrane (Khakh and North 2012, Pougnet et al. 2014). Adenosine-activated A1 and A2a receptors are also expressed presynaptically in glutamatergic synapses, where they oppose each other's activity via changes in cAMP levels (which are lowered by A1 and raised by A2a receptors) to regulate information transmission (Fowler, 1990; Mitchell et al., 1993; Manzoni et al. 1994; Ciruela et al. 2006; Rebola et al. 2008). In short-term energy deprivation conditions, when it would be desirable to reduce synaptic energy expenditure, it appears that adenosine release, and activation of presynaptic A1 receptors that reduce vesicle release probability, dominate the changes of synaptic function (Fowler, 1990). If synapses are sitting somewhat to the right of the optimal peak on the plots of information transmitted per ATP used versus release probability in Fig. 2A (see above), then conceivably this decrease of release probability will move them back towards the optimum value of information transmitted per ATP used postsynaptically. For long term decreases of energetic status, the role of presynaptic adenosine receptors may decline (Fowler, 1992), and we lack sufficient information on how changes in trafficking alter the number of postsynaptic receptors to understand how synapses adapt to the altered circumstances. Nevertheless, it is conceivable that, as energy

levels change, potentiation or depression of synaptic function may be driven by energy constraints rather than for (or in addition to) computational purposes. Similarly, computational changes in synaptic strength, driven by learning, for instance, may subsequently require population-level adjustments in order to maintain brain-wide energetic efficiency. Overall, ATP/adenosine signaling at glutamatergic synapses may contribute to adjusting the energetic efficiency of excitatory synapses, and this is an exciting line of enquiry for future research into how the brain maintains high computational power while concurrently optimising energy consumption throughout life.

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**References**

Abeles, M., 1991. Corticonics: neural circuits of the cerebral cortex. Cambridge University Press.

Alle, H., Roth, A., Geiger, J.R.P., 2009. Energy-efficient action potentials in hippocampal mossy fibers. Science. 325, 1405–1408. https://doi.org/10.1126/science.1174331.

Attwell, D., Gibb, A., 2005. Neuroenergetics and the kinetic design of excitatory synapses. Nat. Rev. Neurosci. 6, 841–849. https://doi.org/10.1038/nrn1784.

Attwell, D., Laughlin, S.B., 2001. An energy budget for signaling in the grey matter of the brain. J. Cereb. Blood Flow Metab. 21, 1133–1145. https://doi.org/10.1097/00004647-200110000-00001.

Badimon, A., Strasburger, H.J., Ayata, P., Chen, X., Nair, A., Ikegami, A., Hwang, P., Chan, A.T., Graves, S.M., Uweru, J.O., Ledderose, C., Kutlu, M.G., Wheeler, M.A., Kahan, A., Ishikawa, M., Wang, Y.C., Loh, Y.E., Jiang, J.X., Surmeier, D.J., Robson, S.C., Junger, W.G., Sebra, R., Calipari, E.S., Kenny, P.J., Eyo, U.B., Colonna, M., Quintana. F.J., Wake, H., Gradinaru, V., Schaefer, A., 2020. Negative feedback control of neuronal activity by microglia. Nature. 586, 417-423. https://doi.org/10.1038/s41586-020-2777-8.

Ballarín, M., Fredholm, B.B., Ambrosio, S., Mahy, N., 1991. Extracellular levels of adenosine and its metabolites in the striatum of awake rats: inhibition of uptake and metabolism. Acta Physiol Scand. 142, 97-103. https://doi.org/10.1111/j.1748-1716.1991.tb09133.x.

Braitenberg, V., Schüz, A., 1998. Cortex: statistics and geometry of neuronal connectivity, 2nd ed. Springer-Verlag Berlin Heidelberg.

Carter, B.C., Bean, B.P., 2009. Sodium entry during action potentials of mammalian neurons: incomplete inactivation and reduced metabolic efficiency in fast-spiking neurons. Neuron. 64, 898-909. https://doi.org/10.1016/j.neuron.2009.12.011.

Cirelli, C., Tononi, G., 2021. The why and how of sleep-dependent synaptic down-selection. Semin Cell Dev Biol. 2021, S1084-9521(21)00031-8. https://doi.org/10.1016/j.semcdb.2021.02.007.

Ciruela, F., Casadó, V., Rodrigues, R.J., Luján, R., Burgueño, J., Canals, M., Borycz, J., Rebola, N., Goldberg, S.R., Mallol, J., Cortés, A., Canela, E.I., López-Giménez, J.F., Milligan, G., Lluis, C., Cunha, R.A., Ferré, S., Franco, R., 2006. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J. Neurosci. 26, 2080-7. https://doi.org/10.1523/JNEUROSCI.3574-05.2006.

Clarke, J.B., Sokoloff, L., 1999. Circulation and energy metabolism of the brain, in: Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., Uhler, M.D. (Eds.), Basic Neurochemistry, 6th ed. Lippincott- Raven, Philadelphia, pp 637–669.

Crambert, G., Hasler, U., Beggah, A.T., Yu, C., Modyanov, N.N., Horisberger, J.D., Lelièvre, L., Geering, K., 2000. Transport and pharmacological properties of nine different human Na,K- ATPase isozymes. J. Biol. Chem. 275, 1976–1986. https://doi.org/10.1074/jbc.275.3.1976.

Deuchars, J., Thomson, A.M., 1995. Innervation of burst firing spiny interneurons by pyramidal cells in deep layers of rat somatomotor cortex: Paired intracellular recordings with biocytin filling. Neuroscience 69, 739–755. https://doi.org/10.1016/0306-4522(95)00288-T.

Engl, E., Attwell, D., 2015. Non-signalling energy use in the brain. J. Physiol. 59, 3417-29. https://doi.org/10.1113/jphysiol.2014.282517.

Engl, E., Jolivet, R., Hall, C.N., Attwell, D., 2017. Non-signalling energy use in the developing rat brain. J. Cereb. Blood Flow Metab. 37, 951–966. https://doi.org/10.1177/0271678X16648710.

Falzoni, S., Donvito, G., Di Virgilio, F., 2013. Detecting adenosine triphosphate in the pericellular space. Interface Focus. 3, 20120101. https://doi.org/10.1098/rsfs.2012.0101.

Fowler, J.C., 1990. Adenosine antagonists alter the synaptic response to in vitro ischemia in the rat hippocampus. Brain Res. 509, 331-4. https://doi.org/10.1016/0006-8993(90)90560-x.

Fowler, J.C., 1992. Escape from inhibition of synaptic transmission during in vitro hypoxia and hypoglycemia in the hippocampus. Brain Res. 573, 169-73. https://doi.org/10.1016/0006-8993(92)90128-v .

Fredholm, B.B., Irenius, E., Kull, B., Schulte, G., 2001. Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. Biochem. Pharmacol. 61, 443-8. https://doi.org/10.1016/s0006-2952(00)00570-0.

Goldman, M.S., 2004. Enhancement of information transmission efficiency by synaptic failures. Neural Comput. 16, 1137–1162. https://doi.org/10.1162/089976604773717568.

Gu, J.G., MacDermott, A.B., 1997 Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. Nature. 389, 749-53. https://doi.org/10.1038/39639.

Hagberg, H., Andersson, P., Lacarewicz, J., Jacobson, I., Butcher, S., Sandberg, M., 1987 Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. J. Neurochem. 49, 227-31. https://doi.org/10.1111/j.1471-4159.1987.tb03419.x.

Hallermann, S., de Kock, C.P., Stuart, G.J., Kole, M.H., 2012. State and location dependence of action potential metabolic cost in cortical pyramidal neurons. Nat. Neurosci. 15, 1007-14. https://doi.org/10.1038/nn.3132.

Hardingham, N.R., Read, J.C.A., Trevelyan, A.J., Nelson, J.C., Jack, J.J.B., Bannister, N.J., 2010. Quantal analysis reveals a functional correlation between presynaptic and postsynaptic efficacy in excitatory connections from rat neocortex. J. Neurosci. 30, 1441–1451. https://doi.org/10.1523/JNEUROSCI.3244-09.2010.

Harris, J.J., Attwell, D., 2012. The energetics of CNS white matter. J. Neurosci. 32, 356–371. https://doi.org/10.1523/JNEUROSCI.3430-11.2012.

Harris, J.J., Engl, E., Attwell, D., Jolivet, R.B., 2019. Energy-efficient information transfer at thalamocortical synapses. PLoS Comput. Biol. 15, e1007226. https://doi.org/10.1371/journal.pcbi.1007226.

Harris, J.J., Jolivet, R., Attwell, D., 2012. Synaptic Energy Use and Supply. Neuron. 75, 762-777. https://doi.org/10.1016/j.neuron.2012.08.019.

Harris, J.J., Jolivet, R., Engl, E., Attwell, D., 2015. Energy-Efficient Information Transfer by Visual Pathway Synapses. Curr. Biol. 25, 3151–3160. https://doi.org/10.1016/j.cub.2015.10.063.

Hestrin, S., 1992. Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. Nature. 357, 686–689. https://doi.org/10.1038/357686a0.

Howarth, C., Gleeson, P., Attwell, D., 2012. Updated energy budgets for neural computation in the neocortex and cerebellum. J. Cereb. Blood Flow Metab. 32, 1222–1232. https://doi.org/10.1038/jcbfm.2012.35.

Howarth, C., Peppiatt-Wildman, C.M., Attwell, D., 2010. The energy use associated with neural computation in the cerebellum. J. Cereb. Blood Flow Metab. 30, 403–414. https://doi.org/10.1038/jcbfm.2009.231.

Jacobson, K.A., 2018. P2X and P2Y Receptors. Tocris Scientific Review Series. https://resources.tocris.com/pdfs/literature/reviews/purinergic-receptors-review-edition-2-web.pdf

Kety, S.S., 1957. The general metabolism of the brain in vivo, in: Richter, D. (Ed), Metabolism of the nervous system. Pergamon, London, pp. 221–237.

Khakh, B.S., North, R.A., 2012. Neuromodulation by extracellular ATP and P2X receptors in the CNS. Neuron. 76, 51-69. https://doi.org/10.1016/j.neuron.2012.09.024.

King, A.E., Ackley, M.A., Cass, C.E., Young, J.D., Baldwin, S.A., 2006 Nucleoside transporters: from scavengers to novel therapeutic targets. Trends Pharmacol Sci. 27, 416-25. https://doi.org/10.1016/j.tips.2006.06.004.

Lennie, P., 2003. The cost of cortical computation. Curr. Biol. 13, 493–497. https://doi.org/10.1016/S0960-9822(03)00135-0.

Levy, W.B., Baxter, R.A., 2002. Energy-efficient neuronal computation via quantal synaptic failures. J. Neurosci. 22, 4746-55. https://doi.org/10.1523/JNEUROSCI.22-11-04746.2002.

Manzoni, O.J., Manabe, T., Nicoll, R.A., 1994. Release of adenosine by activation of NMDA receptors in the hippocampus. Science. 265, 2098-101. https://doi.org/10.1126/science.7916485.

Mishra, A., Reynolds, J.P., Chen, Y., Gourine, A.V., Rusakov, D.A., Attwell, D., 2016. Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. Nat. Neurosci. 19, 1619-1627. https://doi.org/10.1038/nn.4428.

Mitchell, J.B., Lupica, C.R., Dunwiddie, T.V., 1993. Activity-dependent release of endogenous adenosine modulates synaptic responses in the rat hippocampus. J. Neurosci. 13, 3439-47. https://doi.org/10.1523/JNEUROSCI.13-08-03439.1993.

Nusser, Z., Lujan, R., Laube, G., Roberts, J.D.B., Molnar, E., Somogyi, P., 1998. Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. Neuron. 21, 545–559. https://doi.org/10.1016/S0896-6273(00)80565-6.

Pfeiffer, T., Attwell, D., 2020. Brain's immune cells put the brakes on neurons. Nature. 586, 366-367. https://doi.org/10.1038/d41586-020-02713-7.

Pougnet, J.T., Toulme, E., Martinez, A., Choquet, D., Hosy, E., Boué-Grabot, E., 2014. ATP P2X receptors downregulate AMPA receptor trafficking and postsynaptic efficacy in hippocampal neurons. Neuron. 83, 417-430. https://doi.org/10.1016/j.neuron.2014.06.005.

Rangaraju, V., Calloway, N., Ryan, T.A., 2014. Activity-driven local ATP synthesis is required for synaptic function. Cell. 156, 825-35. https://doi.org/10.1016/j.cell.2013.12.042.

Rebola, N., Lujan, R., Cunha, R.A., Mulle, C., 2008. Adenosine A2A receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. Neuron. 57, 121-34. https://doi.org/10.1016/j.neuron.2007.11.023.

Rodrigues, R.J., Almeida, T., Richardson, P.J., Oliveira, C.R., Cunha, R.A., 2005. Dual presynaptic control by ATP of glutamate release via facilitatory P2X1, P2X2/3, and P2X3 and inhibitory P2Y1, P2Y2, and/or P2Y4 receptors in the rat hippocampus. J. Neurosci. 25, 6286-95. https://doi.org/10.1523/JNEUROSCI.0628-05.2005.

Rolfe, D.F.S., Brown, G.C., 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol. Rev. 77, 731–758. https://doi.org/10.1152/physrev.1997.77.3.731.

Shen, K.Z., Johnson, S.W., 1998. Sodium pump evokes high density pump currents in rat midbrain dopamine neurons. J. Physiol. 512, 449–457. https://doi.org/10.1111/j.1469-7793.1998.449be.x.

Silver, R.A., Cull-Candy, S.G., Takahashi, T., 1996. Non-NMDA glutamate receptor occupancy and open probability at a rat cerebellar synapse with single and multiple release sites. J. Physiol. 494, 231–250. https://doi.org/10.1113/jphysiol.1996.sp021487.

Sokoloff, L., 1960. The metabolism of the central nervous system in vivo, in: Field, J., Magoun, H.W., Hall, V.E. (Eds.), Handbook of Physiology, Section I, Neurophysiology, vol. 3. American Physiological Society, Washington D.C., pp 1843–1864.

Sokoloff, L., Reivich, M., Kennedy, C., Rosiers, M.H.D., Patlak, C.S., Pettigrew, K.D., Sakurada, O., Shinohara, M., 1977. The [14C] deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. 28, 897–916. https://doi.org/10.1111/j.1471-4159.1977.tb10649.x.

Spruston, N., Jonas, P., Sakmann, B., 1995. Dendritic glutamate receptor channels in rat hippocampal CA3 and CA1 pyramidal neurons. J. Physiol. 482, 325–352. https://doi.org/10.1113/jphysiol.1995.sp020521.

Tsai, P.S., Kaufhold, J.P., Blinder, P., Friedman, B., Drew, P.J., Karten, H.J., Lyden, P.D., Kleinfeld, D., 2009. Correlations of neuronal and microvascular densities in murine cortex revealed by direct counting and colocalization of nuclei and vessels. J. Neurosci. 29, 14553–14570. https://doi.org/10.1523/JNEUROSCI.3287-09.2009.

Varshney, L.R., Sjöström, P.J., Chklovskii, D.B.B., 2006. Optimal information storage in noisy synapses under resource constraints. Neuron. 52, 409–423. https://doi.org/10.1016/j.neuron.2006.10.017.

Zador, A., 1998. Impact of synaptic unreliability on the information transmitted by spiking neurons. J. Neurophysiol. 79, 1219–1229. https://doi.org/10.1152/jn.1998.79.3.1219.

Zetterström, T., Vernet, L., Ungerstedt, U., Tossman, U., Jonzon, B., Fredholm, B.B., 1982, Purine levels in the intact rat brain. Studies with an implanted perfused hollow fibre. Neurosci. Lett. 29, 111-5. https://doi.org/10.1016/0304-3940(82)90338-x.

## Figure Legends

**Figure 1. Energy use at synapses.**

**A**. Schematic showing major processes on which energy is used at synapses. Presynaptically, after an action potential arrives at the terminal (not shown), $Ca^{2+}$ that enters through voltage-gated channels needs to be pumped out, consuming ATP either on the plasma membrane $Ca^{2+}$-ATPase or on extruding the $Na^{+}$ that powers Na/Ca exchange (not shown). Synaptic vesicle trafficking to and from the membrane also consumes ATP, as does accumulation of glutamate into vesicles. Postsynaptically, $Na^{+}$ and $Ca^{2+}$ ions entering through glutamate-gated channels and $Ca^{2+}$ released from intracellular stores need to be pumped back. ATP is also used on recycling glutamate via astrocytes, on the accumulation of glutamate up its electrochemical gradient into astrocytes and on its conversion to glutamine. **B**. ATP usage on synaptic processes per vesicle of glutamate released, as predicted by Attwell & Laughlin (2001) for the processes in A. Note that vesicle recycling is predicted to use less than 1% of the total synaptic energy use, and that the great majority of ATP is used to restore ion gradients after activation of postsynaptic receptors.

**Figure 2. Optimal presynaptic release probability.**

**A**. Plot of information transmitted per ATP used on postsynaptic currents as a function of vesicle release probability, for a postsynaptic cell receiving N convergent synapses from one presynaptic cell which fires action potentials at 4 Hz, and with spontaneous vesicle release from all N synapses onto the postsynaptic cell at 1.2 Hz (originally published as part of Harris et al., 2012, Fig. 3E). **B**. Open symbols and red trend line show dependence of release probability (abscissa) on the number of convergent synapses mediating single cortical layer 2/3 neuron to cortical layer 2/3 neuron connections (from Hardingham et al., 2010, Fig. 7C; n = 50 L2/3 to L2/3 connections, $r^2 = 0.36$, $p < 0.001$). Superimposed blue symbols show the predicted relationship from the theoretical curves in A.

**Figure 3. Optimal number of postsynaptic glutamate receptors.**

**A-B**. Retinal ganglion cell - LGN synapse (from Harris et al., 2015, Fig. 5A, E). **C-D**. Thalamocortical synapse from LGN axon to spiny stellate cell in area V1 (from Harris et al.,

2019, Figs. 2D & 6D). **A**. Increasing the effective number of postsynaptic receptors (quantified as the effective synaptic conductance generated using dynamic clamp, divided by the physiological value of the synaptic conductance, $g_{syn}$) increases information transmission at the retina-LGN synapse. Black points, here and in panel B, are from dynamic clamp experiments, while white diamond is from optic tract stimulation. **B**. There is an optimum number of receptors similar to that found experimentally ($g_{syn}$/(normal $g_{syn}$)=1) that maximises the ratio of information transmitted to ATP used on reversing postsynaptic ion fluxes. **C**. In a computational model of the thalamocortical synapse the ratio of information transmitted to ATP used on postsynaptic currents (blue and red curves show slightly different measures of information: see original paper for details) has a maximum with the synaptic conductance close to (but slightly lower than) the experimental value. **D**. Experimental data for the thalamocortical synapse show a maximum ratio of information transmitted to ATP used on postsynaptic currents with a postsynaptic conductance of the magnitude found experimentally.

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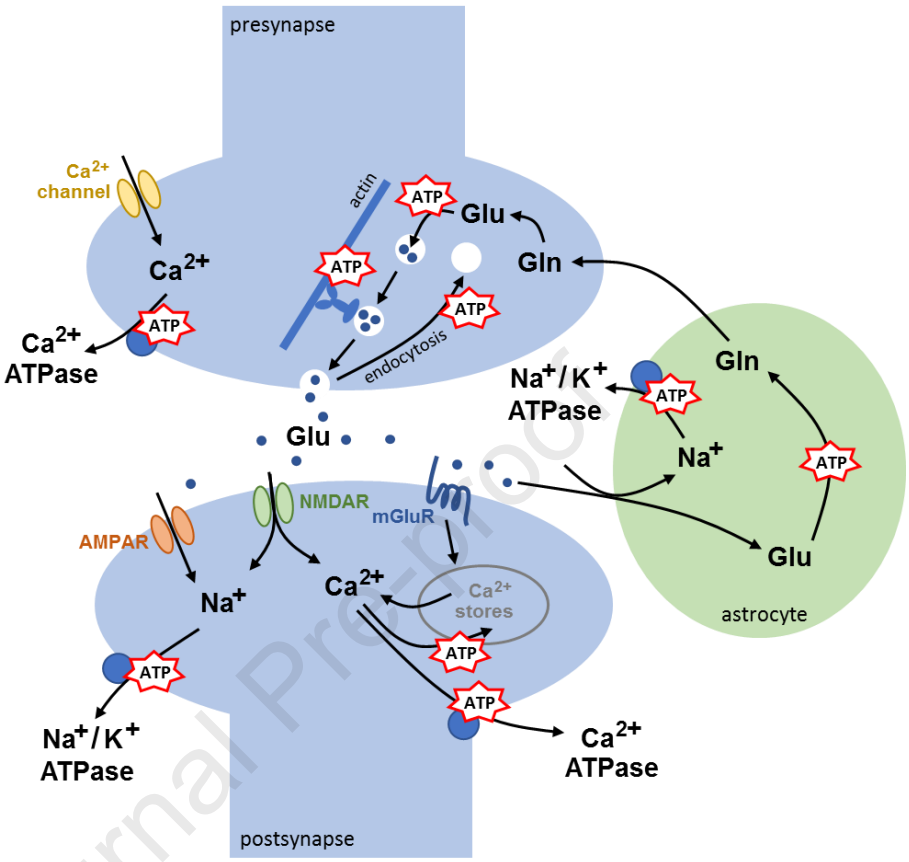
A




B

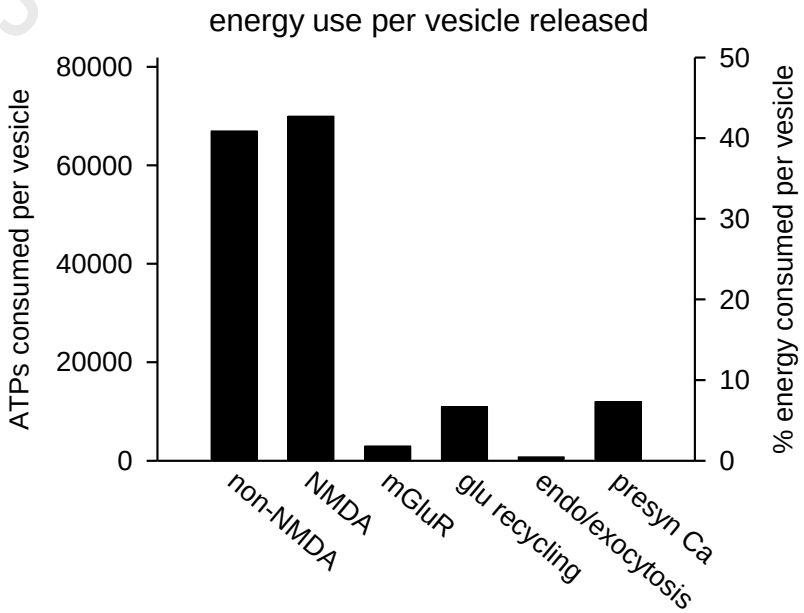
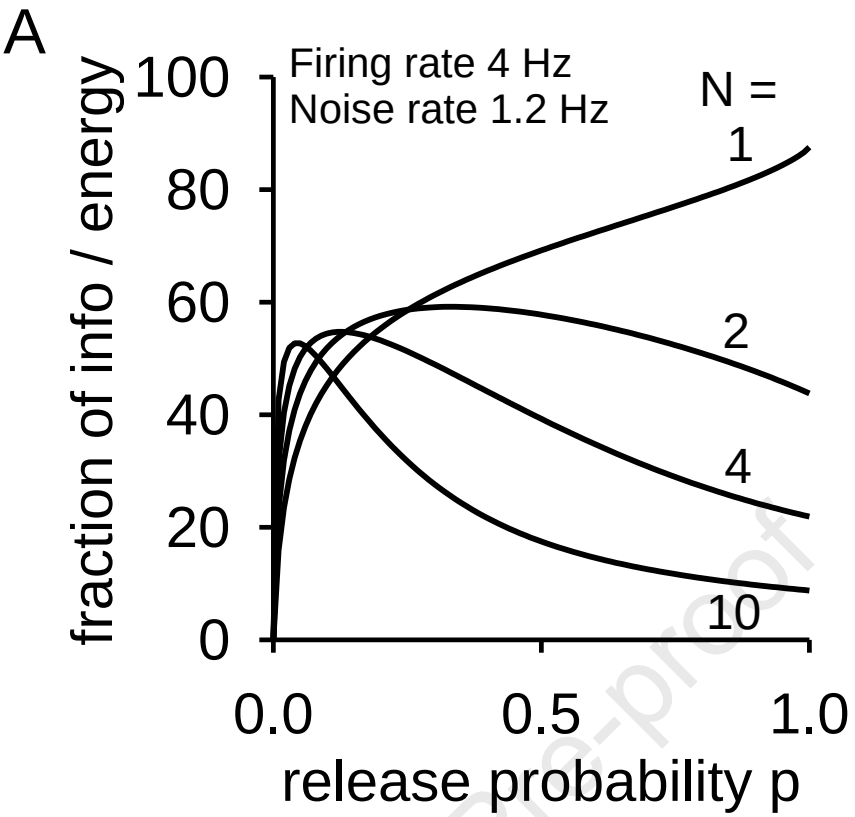
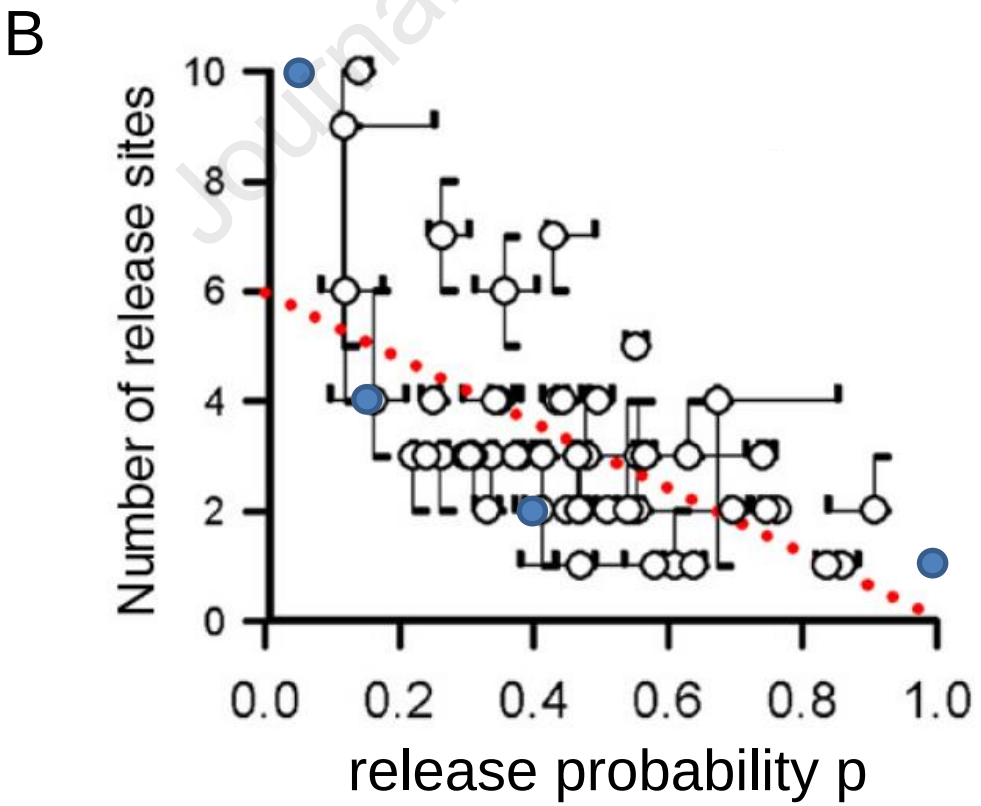



Fig. 1


A
Firing rate 4 Hz
Noise rate 1.2 Hz
N =
1
2
4
10
fraction of info / energy
100
80
60
40
20
0
0.0
0.5
1.0
release probability p


B
Number of release sites
10
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6
4
2
0
0.0
0.2
0.4
0.6
0.8
1.0
release probability p

Fig. 2

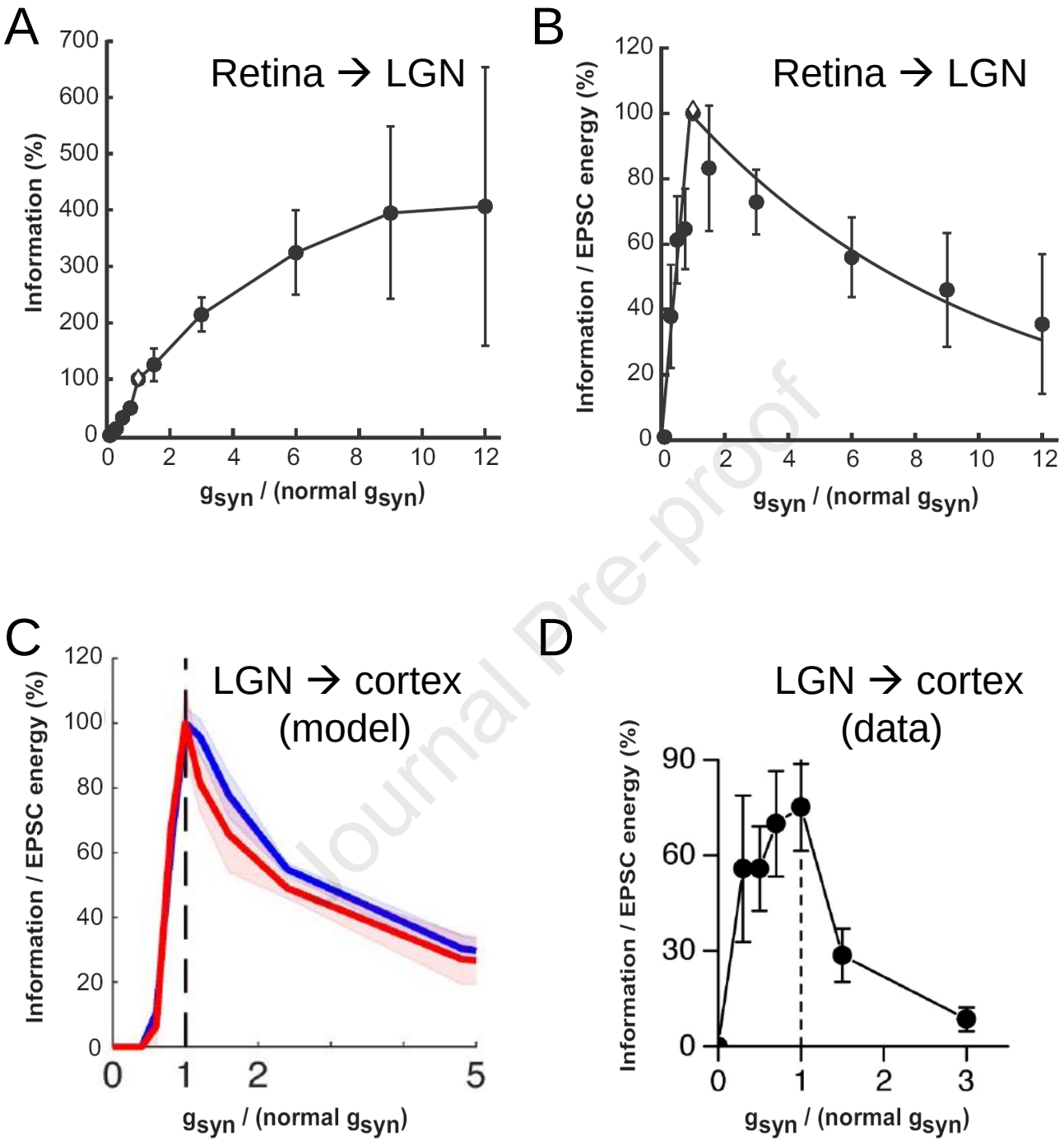




Fig. 3

**Highlights**

- Energy use is a significant constraint on brain function
- Most brain energy is used postsynaptically
- Presynaptic release probability is low to maximise information transmitted/ATP used
- Postsynaptic AMPA receptor number maximises information transmitted/ATP used
- Dynamic regulation of the brain's energetic efficiency may occur