Central synapses release a resource-efficient amount of glutamate

Leonid P Savtchenko1,2, Sergiy Sylantyev1,2 & Dmitri A Rusakov1

Why synapses release a certain amount of neurotransmitter is poorly understood. We combined patch-clamp electrophysiology with computer simulations to estimate how much glutamate is discharged at two distinct central synapses of the rat. We found that, regardless of some uncertainty over synaptic microenvironment, synapses generate the maximal current per released glutamate molecule while maximizing signal information content. Our result suggests that synapses operate on a principle of resource optimization.

Information processing in the brain involves excitatory events generated by release of glutamate from a synaptic vesicle into the synaptic cleft. The amount of glutamate in each vesicle depends on the vesicle volume and activity of vesicular transporters. Small central synapses tend to release glutamate in single-vesicle mode, without saturating postsynaptic receptors1,2. This adds to the variability of transmitted signals, arguably reducing the computational certainty of brain circuits. The adaptive significance of this mode of operation is not known.

Figure 1 The amount of glutamate released at CMF-CGC synapses corresponds to the maximal current per released molecule. (a) Top: in situ configuration (GoC, Golgi cell axons). Bottom, one-cell example EPSC traces with the indicated γ-DGG concentrations. (b) Top: fast ligand-application system for patch probing: two streams ejected from two channels of a piezo-controlled theta-glass pipette (~0.2 ms pulse constant; solution in each channel can be exchanged within ~10 s (ref. 6)). Bottom: one-patch AMPAR responses (cultured CGC patch excision) to 1-ms pulses of 1 mM glutamate. (c) Summary data (mean ± s.e.m.) from experiments in a,b. O-o, outside-out. (d) Left: Monte Carlo model of AMPAR activation in patches5. Right: model outcomes (black and red) match experimental traces (gray, taken from b). (e) Top: CMF-CGC synapse model geometry (adapted from ref. 3); cuboid shapes represent cellular elements depicted in a (see Online Methods and ref. 9). Bottom: a model snapshot of diffusing glutamate molecules 2 ms after release (for clarity, every other molecule is depicted; red and gray, inside and outside the cleft, respectively). Right: simulated (colors) and experimental (gray, taken from a) EPSCs. (f) In black: matching simulated and experimental data through mean-square minimization (residuals combined for three conditions) predicts \( <N_{\text{glu}}^\gamma> = 2,001 ± 86 \) (mean ± 95% confidence limit, here and in g, arrow). In blue: simulated dependence between \( I_{\text{syn}} \) and \( N_{\text{glu}} \). (g) The maximum current-per-molecule ratio corresponds to \( N_{\text{glu}}^{\text{max}} = 1,970 ± 55 \) molecules (black, arrow), which coincides with \( N_{\text{glu}}^\gamma \) value for the maximal differential entropy \( H(\gamma) \). (h) Parametric map for \( <N_{\text{glu}}^\gamma> \) (color-coded) over a physiological range of the (unknown) intra-cleft glutamate diffusion coefficients and cleft heights. (i) Parametric map for \( N_{\text{glu}}^{\text{max}} \) which is virtually indistinguishable from that of \( N_{\text{glu}}^\gamma \) (Supplementary Fig. 2c).

We first sought to estimate the amount of released glutamate at synapses between cerebellar mossy fibers (CMFs) and granule cells (CGCs); CGCs are among the most electrically compact neurons in the brain, with negligible voltage-clamp errors in somatic recordings. Furthermore, functional features and the environment of CMF-CGC synapses have been explored in exhaustive detail3,4. To gauge how much glutamate is released there, we examined activation of AMPA receptors (AMPARs) using the fast-dissociating antagonist γ-DGG:glutamylglycine (γ-DGG); its inhibitory action is inversely related to the intra-cleft glutamate concentration2,5. γ-DGG at 0.5 mM and 2 mM produced stable reductions of AMPAR excitatory postsynaptic currents (EPSCs), by 52 ± 3% and 84 ± 1%, respectively (Fig. 1a). This reduction reflects the AMPAR kinetics plus the effects pertinent to diffusion and escape of glutamate. To isolate geometry and diffusion, we monitored AMPAR kinetics in outside-out patches of CGCs using 1-ms pulses of glutamate6, with and without γ-DGG.
Because AMPARs in CGCs in situ are almost exclusively intrasyaptic, we used cultured CGCs, in which AMPARs migrate to the soma4,7. γ-DGG (1 mM) reduced AMPAR responses recorded in the same patch (Online Methods) by 48 ± 3% (n = 6; Fig. 1b,c). These data incorporated into a Monte Carlo model of AMPAR activation by glutamate8,9 (Fig. 1d) gave us finely tuned kinetic constants for AMPAR interaction with γ-DGG (Online Methods), in accordance with ref. 5 (Supplementary Fig. 1).

Equipped with the receptor kinetics, we simulated AMPAR activation in the known average microenvironment of CMF-CGC synapses, which has been adapted for modeling3,4 (Fig. 1e, left). We used a previously validated Monte Carlo approach6,8,9 in which molecules are tracked every 0.1 μs (Online Methods). Varying the number of released molecules N_{glu} led to an excellent fit between simulated and recorded EPSCs (Fig. 1e, right). This optimization procedure was robust (clear single minimum for residuals; Fig. 1f), giving N_{glu} ≈ 2,001 ± 86 (mean ± 95% confidence limits; Fig. 1f). N_{glu} was broadly within the limits of previous estimates10, but what of its adaptive meaning? Our simulations indicated that although the EPSC amplitude I_{syn} depended on N_{glu} monotonically, the relationship was not linear (Fig. 1f). This nonlinearity robustly predicted that the value N^{max}_{glu} = 1,970 ± 55 corresponded to the maximal AMPAR current per molecule (Fig. 1g). Notably, this value was indistinguishable from N_{glu} (Fig. 1f). We also asked how the information content of the EPSC signal changes with N_{glu}; in Shannon theory, information content gauges the amount of uncertainty in the signal, which could be important for efficient neural code transfer11,12. We therefore calculated the differential entropy13 H of I_{syn} (Online Methods) for all simulated N_{glu} values and found that, again, H peaked at an entropy-optimal N_{glu} value (N^{E}_{glu}) indistinguishable from either N_{glu} or N^{max}_{glu} (Fig. 1g).

First, we calculated N_{glu} using the same γ-DGG experiments (Fig. 1a) while varying two poorly accessible features of the synaptic environment, the synaptic cleft height and the intra-cleft glutamate diffusion coefficient. This produced a parametric map for N_{glu} (Fig. 1h). Second, we carried out a similar exploration for N^{max}_{glu} and found that the parametric map for N^{max}_{glu} was virtually indistinguishable from that of N_{glu} (Fig. 1i). We carried out further map comparisons exploring the size of the postsynaptic density (PSD, populated with AMPARs) and the membrane apposition area; again, a correspondence between parametric maps for N^{max}_{glu} and N_{glu} was evident (Supplementary Fig. 2a). We repeated the parameter exploration for N^{E}_{glu} values and found little discrepancy (less than 50–80 glutamate molecules, or 3–4%) between N^{max}_{glu} and N^{E}_{glu} across the tested range (Supplementary Fig. 2b,c). Taken together, these results indicated that N_{glu} was close to both N^{max}_{glu} and N^{E}_{glu} regardless of the uncertainty about the exact architecture of CMF-CGC synapses.

To test whether the close association between N_{glu} and N^{max}_{glu} was a unique feature of these synapses, we also investigated hippocampal CA3-CA1 connections. Here, we examined the reduction of the AMPAR EPSC amplitude by four concentrations of γ-DGG (Fig. 2a) and tested AMPAR kinetics in outside-out patches from CA1 pyramidal cells with and without 1 mM γ-DGG (reduction to 48 ± 3% of control; n = 6; Fig. 2b). To account for voltage- and space-clamp errors in large CA1 pyramidal cells14, we conducted a separate investigation. Briefly, we documented the relationship between the EPSC amplitude and the effect of one γ-DGG concentration (0.5 mM) for n = 109 cells and then used a model of a CA1 pyramidal cell built in the software package NEURON to obtain corrections for the other three γ-DGG concentrations (Online Methods; Supplementary Fig. 3). The resulting data (Fig. 2c) provided several constraints to analyze γ-DGG effects in the synaptic cleft, gauging them against the effect of 1 mM γ-DGG in membrane patches. The best-fit kinetic constants, finely tuned to the CA1 pyramidal patch recordings (Fig. 2d, right), were undistinguishable from those for CGC AMPARs (Online Methods).
On the basis of these data, a detailed Monte Carlo model of the CA3-CA1 synapse, which has been extensively tested\(^6,9\), gave \(<N_{\text{glu}}> = 2,780 \pm 20\) molecules, with the experiment-theory match (Fig. 2c, right) obtained with robust optimization (Fig. 2f). Again, the value of \(<N_{\text{glu}}>\) for these synapses coincided with \(N_{\text{max,glu}}\) (2,690 \pm 95 molecules; Fig. 2g) and followed both \(N_{\text{max,glu}}\) and \(N_{\text{E,glu}}\) values over a wide range of synaptic cleft heights, glutamate diffusion coefficients (Fig. 2h–i), the postsynaptic density size or the membrane appositions areas (Supplementary Fig. 4).

Our results thus suggest that glutamate discharges at small excitatory synapses tend to provide both the highest ‘signal-to-molecule’ ratio and the highest information content of synaptic signals. Indeed, vesicle-stored glutamate is a precious resource: metabolic recycling and transporting glutamate into the vesicle lumen is a highly energy-consuming process. Providing the strongest synaptic signal per released molecule thus suggests the principle of energy resource optimization. Similarly, preserving as much information as possible during signal processing in the brain has been an important notion of theoretical studies into the machinery of neural coding\(^11,12\). How could such optimization affect synaptic structure and function? One possibility is that formation of synaptic connections may involve structural adaptations leading to the optimal configuration. To test the plausibility of this scenario, we asked whether immature CMF-CGC synapses are ‘suboptimal’. We therefore repeated our tests in CMF-CGC synapses (as in Fig. 1) using postnatal day 6 preparations: at this early age, synaptic architecture is distinctly different from that of mature CMF-CGC connections\(^4\) (Online Methods and Supplementary Fig. 5a,b). We found that \(<N_{\text{glu}}>\) and \(N_{\text{max,glu}}\) diverged significantly at postnatal day 6 (Supplementary Fig. 5c), thus lending support to the hypothesis that resource optimization may result from developmental adaptation of synaptic configuration. Notably, CMF-CGC synapses showed substantially larger values of the maximum current per molecule and information entropy compared with CA3-CA1 synapses (Figs. 1g and 2g). Whether this can be attributed to the fact that CGCs receive only four CMF inputs, compared to thousands of CA3-CA1 connections per cell, remains to be ascertained.

Will resource optimization hold during use-dependent plasticity? First, our samples are likely to contain synapses expressing various degrees of potentiation or depression. Second, we have observed the same principle at two different synapses, with distinct architectures and numbers of released molecules. Finally, it appears that varying principal features of the synaptic environment within the expected physiological range does not impinge on the correspondence between \(<N_{\text{glu}}>, N_{\text{max,glu}}\) and \(N_{\text{E,glu}}\), which, however, breaks down for immature synapses. It is therefore reasonable to hypothesize that, during homeostatic or use-dependent plasticity, the amount of released glutamate or the synaptic architecture, or both, could be adjusted in accord with the minimum resource/maximum information transfer requirement. Intriguingly, the synaptic cleft height also appears optimized for boosting the synaptic current\(^8\), and energy resource optimization has been suggested to underlie spike generation in central neurons\(^13\), failures of presynaptic release\(^16\) and dendritic integration of synaptic inputs\(^17\). It remains an open question whether such observations represent elements of a free energy minimization regime that has recently been proposed to govern the brain machinery of perception and learning\(^18\).

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. ModelDB: 2796 and 7509.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

L.P.S. conducted theoretical studies and simulations; S.S. carried out experiments and analyzes; D.A.R., L.P.S. and S.S. designed the study; D.A.R. wrote the paper, which was further edited by all authors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS
Electrophysiology in situ: acute slices from cerebellum and hippocampus. Animal experimentation met all relevant UK and European Union regulations. We cut 250-µm parasagittal slices from the cerebellar vermis, or transverse 300-µm hippocampal slices, from 3- to 4-week-old male Sprague-Dawley or Wistar rats (or P6 pups where specified) and incubated for 1 h in a solution containing (in mM) 124 NaCl, 3 KCl, 1 CaCl2, 3 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, and 10 D-glucose, pH 7.4, and bubbled with 95%:5% O2:CO2. Slices were next transferred to a recording chamber superfused with an external solution that was similar to the incubation solution plus 2 mM CaCl2 and 2 mM MgCl2. AMPAR EPSCs were isolated by adding 1 µM GPPS545 and 100 µM D-APV, 250 µM M-CPG, 1 µM strychnine and 100 µM picrotoxin. The intracellular solution for voltage-clamp recordings contained (mM) 117.5 cesium gluconate, 17.5 NaCl, 10 HEPES, pH 7.2 (adjusted with KOH), 10o BAPTA, 8 NaCl, 5 QX-314, 2 Mg-ATP, 0.3 GTP (295 mOsm). Patch-clamp recordings were performed at 33–35 °C using a Multiclamp-700B amplifier; signals were digitized at 10 kHz. The pipette resistance was 7–9 MΩ for CGCs and 3–6 MΩ for CA1 pyramids.

To stimulate the bulk of Schaffer collaterals in hippocampal slices, a bipolar stimulating electrode was placed in stratum radiatum approximately 200 µm from stratum pyramidale. In cerebellar slices, mossy fiber axons were stimulated with a bipolar tungsten electrode placed in the cerebellar white matter near the gyrus cistern to stimulate fibers entering the granule cell layer. Electrical stimuli (100 µA) were applied to afferent fibers evoking EPSCs. Individual recording sweeps were collected at 15-s intervals. Other receptor and transporter blockers were added as indicated. Data were routinely represented as mean ± s.e.m.; Student’s unpaired or paired t-test (or nonparametric Wilcoxon paired tests when distribution was non-Gaussian) was used for statistical hypothesis testing.

Electrophysiology: fast glutamate application in outside-out patches. Patches were excised from cerebellar granule cells or CA1 pyramidal cells held in whole-cell mode in the respective acute slices. The fast ligand application method was adapted from ref. 19. We used a 0.2-mm glass application pipette pulled to a 200-µm tip diameter. The pipette was fixed in a microclamp, which was glued directly to a piezo bending actuator mounted on an electrode holder. Patch pipettes were filled with the bath solution or bath solution containing different pharmacological agents (Fig. 1b). Three separate microcapillaries inserted into each of two channels provided application solution supply; solutions in each channel could be replaced within ~10 s by toggling the pressure pump circuit between the supplying microcapillaries. Pressure in the application pipette channels was adjusted using the two-channel PDE5-02DX pneumatic micro injector (npi electronic GmbH) using compressed nitrogen. The ~1-ms electric pulses were applied via a constant voltage stimulus isolator; stimulus duration and amplitude were adjusted using a control test in which one pipette channel was filled with distilled water and the current was recorded by an open patch pipette. The characteristic time constant of the rapid switch response in these control experiments was 150–250 µs, as documented earlier6.

Kinetic model: AMPA receptors. We used a kinetic scheme published earlier7 that included state transitions dealing with effective concentrations of local glutamate and γ-DGG (Supplementary Fig. 1). To accurately reproduce the kinetics of native AMPARs in our experiments, we adjusted some of the above kinetic constants to match the experimental AMPAR kinetics in well-controlled conditions of ligand application (1-ms pulse of 1 mM glutamate, with and without 1 mM γ-DGG) to outside-out patches. For fine-tuning purposes, we introduced proportionality factors PGLu and PDDG to scale the constants dealing with receptor interaction with glutamate and γ-DGG, respectively, as indicated above. We obtained values of PGLu = 0.851 ± 0.012 for CA1-CA3 synapses and PGLu = 0.898 ± 0.078 for CMF-CGC synapses through accurate fitting of outside-out AMPAR responses (n = 5); with these values and best-fit PDDG = 0.98 ± 0.02 (n = 5), the kinetic scheme provided an excellent match with the AMPAR activation time course in patches, including the 48% amplitude reduction by 2 mM γ-DGG (Figs. 1d and 2d).

Monte-Carlo model: main notations and symbols. R, radius of the synaptic apposition zone; δ, synaptic cleft height; Q, the number of released neurotransmitter molecules; D, effective diffusion coefficient of glutamate in the cleft; t, time variable; r, radial distance from the cleft center; N, total number of receptors (AMPA) within the active zone; rPDG, radius of the postsynaptic density; P(r), fraction of open receptors; Iapp, total peak synaptic current through open receptors; γ conductivity of a single receptor-channel; Vc, the postsynaptic resting membrane potential outside the cleft; Vr, the receptor reversal potential of AMPAR; C(r,r), local glutamate concentration.

Monte-Carlo model: receptor activation. The model duty cycle following glutamate release event was as follows. At each time step (Δt = 0.1 µs), the model first updated the coordinates of all individual glutamate molecules following Brownian movement. Next, it calculated the concentration profile of glutamate C(r,r) in the cleft based on all molecular positions. In conditions of approximate rotational symmetry (again, rectangular shapes of synaptic elements at 250–300 nm from the center had a negligible effect on these calculations), this corresponded to C(r,r) = Q (2πrδR)−2, where Q is the number of glutamate molecules occurring at time point t inside the flat cylindrical ring of height δ and width R and radius r. The average occurrence (concentration) of open receptors (O(r)/r) in the PSD was then calculated at the same time point from the multi-stage AMPAR kinetic scheme, in accordance with the local glutamate concentration C(r,r). When the fast-dissociating antagonist γ-DGG was present in the extracellular medium, the AMPA receptor activation kinetics were computed accordingly. These calculations gave the total synaptic current in the discrete form as

Iσ = 2π ∫ 0 PDDG/Δr ∑ |i| iνijτα(Δr)O(r)

where rPDG/Δr was rounded to the nearest integer. We routinely verified that reducing the time step did not change the outcome of simulations.

Monte Carlo model: synaptic environment. Computations were carried using an ad hoc built-in house 64-node PC cluster optimized for parallel computing9. The modeling methodology and computational Monte Carlo algorithms adapted our approach, which was outlined in detail previously6,8. Geometric features of mossy fiber (CMF)-cerebellar granule cell (CGC) synapses were approximated by the pre- and postsynaptic rectangular elements representing the structure of cerebella glomeruli, as described in a previously published model1. Glutamate molecules (NGLu = 200 to 6,000) were released in the center of the 600-nm-wide apposition area separated by a 50-nm space from neighboring structures (Fig. 1e); the average synaptic cleft height was 19 nm (varied between 15 and 25 nm), and the PSD width was 160 nm (varied between 140 and 300 nm). AMPARs (30–300) were scattered inside the postsynaptic density, with a channel conductance of 10 pS. In the trials focusing on immature P6 synapses, synaptic geometry was amended, in accordance with 3D microscopy data documented for P8 animals (Supplementary Fig. 5b): notably, the PSD was expanded to 400 nm, with the synaptic cleft having a simple 2D geometry (as opposed to the 3D structure depicted in Fig. 1e) characteristic of immature CMF-CGC connections4. The default glutamate diffusion coefficient was 0.3 µm2/ms, as estimated earlier1, and varied between 0.2 and 0.6 µm2/ms in parameter exploration tests.

CA3-CA1 synapses were modeled by two cylindrical elements (diameter 150–600 nm, average value 170 nm; PSD diameter 120–360 nm, average value 140 nm) separated by the apposition cleft (varied between 15 and 25 nm), as detailed earlier6,8. Movements of individual glutamate and γ-DGG molecules, their binding to individual receptor molecules, and receptor state transitions were computed with a time step of 0.1 µs (further reduction of the time step by an order of magnitude improved computation accuracy by only <1%). Because electro-diffusion phenomena in the cleft could only manifest themselves as a 15–20% deceleration of the EPSC decay upon reversal of the synaptic current, with no effect on the EPSC amplitude6, they were not considered in the present model.

NEURON model. To correct for space-clamp errors, a NEURON20 library model of a CA1 pyramidal cell was used incorporating known distributed membrane ion channel kinetics11-12 (accession codes 2796 and 7509, Supplementary Fig. 3).

Information content: differential entropy. To gauge the information content of the EPSC amplitude Iapp(NGLu) at each vesicular content NGLu we used differential entropy, a version of Shannon entropy extended for continuous distributions13:

H(x) = − ∫ x∞ x f(x) log f(x)dx

where x(f) stands for the probability density function of stochastically generated Iapp(NGLu) at each value of NGLu. In evaluating H(x), we observed that, across the
explored range of synaptic parameters, stochastic fluctuation of \( I_{\text{syn}}(N_{\text{glu}}) \) produced a distribution indistinguishable from a Gaussian distribution. Therefore, we could calculate \( H(I_{\text{syn}}) \) as
\[
H(I_{\text{syn}}) = \log_2(\sigma \sqrt{2\pi}),
\]
where \( \sigma \) is the s.d. of \( I_{\text{syn}}(N_{\text{glu}}) \) values.

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