Normalized unitary synaptic signaling of the hippocampus and entorhinal cortex

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# Supplementary Discussions

## Synapse model selection

In our pipeline, we chose to simulate synaptic signals with a minimum number of data points to maximize the inclusion of studies from diverse sources. A parsimonious model also has a contained number of parameters allowing dimensionality reduction to improve machine learning performance. Tsodyks, Pawelzik, and Markram's (TPM) model only needs three data points per synaptic event to simulate signals and is fully defined by five parameters. Therefore, it was a suitable model for our study. However, experiments exclusively recording a single synaptic event only enable the estimation of g and τd. Traces including two successive events allow U to be also found, and with three or more events, all five constants can be computed. More synaptic events and the presence of a recovery event increase the accuracy of estimations.

There are various versions of the TPM model1, 2. A recent study investigating the dendritic impact of synaptic signals in CA1 used a complex version of the TPM model that simulates the synaptic activation time constant and the stochasticity of release1. Unfortunately, that version cannot analytically model the coupling of synaptic deactivation and recovery, which is required for accurate simulation of a burst of synaptic events. Therefore, we used a simplified TPM model, which also improved computational efficiency. For example, the original TPM solution has four differential equations3, 4, but we used a subsequent formulation reducing the problem to three3, 4. We solved the three differential equations with the exact integration method and further simplified the resulting analytical relation to reduce the evaluations of exponential function from four to three (see Supplementary Methods).

## Trace correction maximized the inclusion of studies

Background neuronal activity can change the subthreshold membrane potentials during synaptic recording. Membrane fluctuations can trigger HCN currents. Sufficient membrane depolarization can release the magnesium-block of NMDA channels. Repeated activation of GABAergic synapses may also activate GABAB receptors. Not all studies block GABAB, NMDA, and HCN channels before recording synaptic signals. Fortunately, these subthreshold membrane fluctuations are slower than AMPA and GABAA signals. We thus corrected the signal for the slower membrane fluctuations before and during signal simulation to allow the inclusion of more studies using a simpler synapse model.

## Limited impact of temperature, membrane potential, and late postnatal development on synaptic properties

Synaptic physiology is typically studied either at room temperature or body temperature, which in rodents differ by approximately 10°C. We account for the effect of temperature when computing Erev and Ej5. If the Q10 values of all TPM model parameters were known for all potential connections, it would be logical to normalize the temperature effect during parametric fitting. However, to the best of our knowledge such data does not exist. Consequently, we normalized temperature at the predictive modeling stage. The phenomenological impact of temperature on synaptic physiology was nominal compared to other covariates, but the change directions agreed with earlier studies, where higher recording temperature increased g and reduced τd6.

Postsynaptic membrane potential (Vm) changes not only the synaptic driving force, but also the membrane time constant by activating voltage-gated ion channels like hyperpolarization-activated cyclic nucleotide-gated cation (HCN), m-type potassium, and persistent sodium (NaP) currents7. Therefore, even though the synaptic properties are normalized for the driving force during parametric fitting, we still included Vm in the predictive modeling stage. Our results indicate that the remaining voltage-dependent factors have less than 10% impact on synaptic parameters for a 20-mV change in Vm.

Up to twelve postnatal days, the (juvenile) brain undergoes rapid circuit construction8. We found the change in synaptic properties with animal age after 14 days postnatal (adolescence) is less than 10%. Therefore, adolescent animal models are acceptable templates of synaptic properties in adulthood.

## Range and distribution of synaptic parameters are in line with their functions

The distribution of g and U were similar for GABAergic and glutamatergic synapses, suggesting optimal tuning of these synapse types to balance each other. Glutamatergic synapses had larger τf but smaller τd and τr values than GABAergic synapses. Smaller τd means faster synaptic kinetics and therefore more precise timing with respect to signal summation and long-term plasticity. Larger τf means slower decay of synaptic utilization (i.e., more synaptic facilitation), and smaller τr, a faster recovery of synaptic resources (i.e., less synaptic depression). This pattern suggests that glutamatergic synapses are more effective in handling multiple successive events. In contrast, GABAergic synapses are more depressing and less precise in timing so as to control network activity even at the expense of resource exhaustion.

Glutamatergic τd values were smallest in the DG (Fig. S6), raising the possibility that precise timing of excitatory inputs and coincidence detection may be important for this region’s role in pattern separation. The range of values in the EC was limited compared with the hippocampus, suggesting a more homogeneous synaptic electrophysiology in this region. However, since the availability of synaptic data is scarce for the EC relative to the hippocampus, this latter result deserves future experimental confirmation.

## Choice of synaptic data for large-scale brain simulations

Estimating synaptic parameters in voltage-clamp and current-clamp have complementary advantages and disadvantages. The voltage-clamp method provides an electrically controlled environment, enabling a straightforward calculation of g. However, the recordings may suffer from space-clamp issues, due to Vm in dendritic spines and distal dendrites not following strict somatic control9. Even though the current-clamp method does not have the space-clamp issue, membrane fluctuation, due to synaptic activation or background noise, can activate subthreshold voltage-gated ion channels7. In distal dendrites, due to passive normalization, the local dendritic synaptic potential may be sufficient to activate ion channels involved in spike generation9.

It is possible to derive the dendritic synaptic parameters from somatic recordings using the morphological reconstruction of the postsynaptic neuron and a detailed account of all subthreshold-voltage-gated ionic channels1, 10. Yet, in most cases, such data are not available. Nevertheless, the estimation of g and τd using current-clamp should be closer to the local dendritic events since even a simple RC neuron model, as used in this study, can reduce the degree of dendritic filtering. Therefore, we suggest that synaptic inferences in current-clamp are suitable for network simulations that use complex neuron models incorporating the dendrites. Voltage-clamp, in contrast, better estimates the somatic impact of synaptic events since it includes dendritic filtering. Thus, we recommend use of voltage-clamp synaptic estimates for network simulations that use point-neuron models.

## Experimental validation of results

Until recently, *in vitro* multiple paired recording was the only method allowing reconstruction of recorded neurons, which is needed for identifying connected neuron types11. However, the advent of fast and highly sensitive voltage, calcium, and neurotransmitter indicators, along with continuous advances in microscope technology, might soon enable high-throughput gathering of synaptic signals *in vivo*. For example, the third-generation glutamate indicator iGluSnFR3 is ten times faster than its predecessors, which may allow the quantification of neurotransmitter release and reuptake dynamics12. The new SLAP2 two-photon microscope can record synaptic activity from a full-frame window up to 18 kHz sampling rate, sufficient to record most synapses innervating a single neuron with adequate temporal resolution13. Light-sheet fluorescence expansion microscopy has also solved the problem of post-hoc reconstruction of circuits at the level of individual synapses14. Therefore, combination of these new techniques may allow the direct experimental validation of synaptic physiology at the level of neuronal types in the foreseeable future.

# Supplementary Methods

## Derivation of a simplified Tsodyks, Pawelzik and Markram synapse model

Over the last 50 years, a large body of phenomenological synaptic plasticity models has been theorized3. One of the better-established models is that of Tsodyks, Pawelzik, and Markram (TPM)4. In this work, we adapted a simplified version of the TPM model3 and further streamlined the analytical solutions.

### Ordinary differential equations describing synaptic temporal dynamics

Short-term synaptic plasticity depends on the availability and utilization of synaptic resources (Fig. 2b), including the number of readily releasable synaptic vesicles and the concentration of calcium. Short-term synaptic facilitation begins with an increase of calcium ions within the presynaptic terminal resulting in an increase in synaptic resource utilization. Eq. 1 formulates the utilization dynamics:

|  |  |
| --- | --- |
|  | (1) |

where is the fractional degree of synaptic utilization at any moment , indicates the value of just before the synaptic event time , determines the increment proportion (between 0 and 1) with each presynaptic spike, and is Dirac’s delta function. Since () quantifies unutilized resources and synapses cannot use more than all the resources available to them, determines increment after each synaptic event. Whenever a synapse is not being stimulated, synaptic utilization exponentially decays to zero with the facilitation decay time constant .

Synaptic depression is due to the depletion of available synaptic resources. These resources can be partitioned into three portions, representing respectively the activated (A), deactivated (D), and recovered (R) states. After each presynaptic spike, an instantaneous shift occurs from recovered to activated state. The amount of shift is determined by . The active resources then decay to the deactivated state by the decay time constant . Since synaptic resources are limited, the more resources stay in the deactivated state, the more a synapse is depressed. In the TPM model, synaptic resources exponentially recover from depression with the recovery time constant *τr*. This process can be formulated by the following set of equations:

|  |  |
| --- | --- |
|  | (2) |
| (3) |
| (4) |

where is the value of just after synaptic event time, which can be determined using Eq. 1. is the value of R just before the synaptic event, which is determined by Eq. 2. The product represents the fraction of the synaptic resources being utilized after each synaptic event. This proportion is added to the already active resources () and taken from the readily usable resources (). Then, the change in at any moment is the difference between resources deactivating () and resources recovering ().

A simplified version of the four-state TPM model3 eliminated Eq. 4 which is possible since the total amount of synaptic resources is fixed:

|  |  |
| --- | --- |
|  | (5) |

Substituting , the four-state TPM model can be reduced to the following three-state model:

|  |  |
| --- | --- |
|  | (6) |

### Analytical solution of the model

This three-state model can be solved using the technique of exact integration15. If is a time varying function of S(t),

is:

|  |  |
| --- | --- |
|  | (7) |

Applying this formula to solve Eq. 1:

Since , we will have:

|  |  |
| --- | --- |
|  | (8) |

Similarly, the solution for is:

|  |  |
| --- | --- |
|  | (9) |

The solution for is:

Substituting A from Eq. 9 and expanding the integral, yields:

Since *,*

Which simplifies to the following equation assuming :

|  |  |
| --- | --- |
|  | (10) |

### Summary of the analytical solution

Since is independent of the rest of the equations, the simulation of synaptic *amplitude* after each synaptic event only requires the calculation of Eq. 9. When a synaptic event occurs, the value of each of the states should be calculated just before the synaptic event.

|  |  |
| --- | --- |
|  | (11) |

Note that only three exponential function evaluations are required if is calculated just before the calculation of . Once pre-event values have been calculated, the following set of equations are used to update , , and :

|  |  |
| --- | --- |
|  | (12) |

We emphasize that the order of equations is important: since is the value of u just after a synaptic event, A and R must be updated after .

### The first synaptic event

Ohm’s law is used to calculate the synaptic currents ():

In the TPM model, is calculated with the following equation:

Therefore,

Before any synaptic event, all resources are readily usable, and there is no utilization and activation. Therefore,

For the first synaptic event, the value of A is easily calculatable.

Therefore,

|  |  |
| --- | --- |
|  | (13) |

### Distinction between short-term plasticity measures

The distinction between the paired-pulse ratio () and paired-pulse ratio from the baseline is formulated with the following equations:

|  |  |
| --- | --- |
|  | (14) |
|  | (15) |

These equations indicate that the measures the evolution of A state but the evolution of .

### Convergence of numerical and analytical solutions

We implemented the numerical and analytical solutions in the NEURON simulation environment and compared them to the original four-state model to confirm the convergence of all the formalisms (Fig. S9). The simulation files are available to download from the ModelDB portal (Accession: 266934 - password: hippo).

# Supplementary Figure Legends

**Figure S1: Signal correction methods.** We removed any membrane fluctuations with slow kinetics superposed on the recorded synaptic signals using two methods. **(a)** We extracted the amplitude (A) and deactivation time constant (τd) of the first synaptic event from a digitized trace to reconstruct the signal by estimating the initiation points. **(b)** We used a simulated signal to approximate the correction amount at the initiation points of synaptic events (e.g., Δai and Δai+1). Triangulation was then used to linearly approximate the correction amounts of intermediate points (Δax).

**Figure S2: Impact of covariates on synaptic properties data availability. (a)** Synaptic traces from two studies16, 17 recording GABAergic signals from CA1 Axo-axonic to CA1 Pyramidal cells in different species and with different intracellular solutions. Higher chloride concentration ([Cl]i) increased the recorded signal via enhanced driving force (i.e., Vm - Erev). Note large differences in g, τd, and U. **(b)** GABAergic signals from DG HICAP to DG Granule cells recorded at two different temperatures18. All other covariates were identical. **(c)** Voltage- and current-clamp recording of glutamatergic synaptic signals between CA3 Pyramidal cells at two different membrane potentials19. Even though the estimated synaptic conductances are almost equal, the rest of the parameter estimates differ substantially. **(d)** Glutamatergic synaptic currents recorded from CA1 Basket CCK+ neurons using three different stimulation paradigms20. Evoked currents were smaller than miniature and spontaneous ones, because of the minimal stimulation protocol. Note that researchers only reported one synaptic event, preventing the estimation of τr, τf, and U. **(e-f)** Heatmap representations of the number of data points available for each of 3,120 potential connections among 122 neuron types (rows: presynaptic, columns: postsynaptic). Light pink entries are entries with missing synaptic data (19.7% for τd and 38.5% for ST-P parameters). Black entries mark absence of potential connection. In all panels (a-f), blue and yellow colors represent GABAergic and glutamatergic synapses, respectively.

**Figure S3: Deep learning model architecture.** The deep learning model in this study is a five-layer encoder-decoder perceptron regularized with different techniques. We attained the best results with 128 nodes in the encoder (third) layer. For model regularization, we set a 50% dropout rate for all layers except for the encoder layer, which was set to 5%. Prediction accuracy improved by coupling dropout with unitary max-norm weight constraint and batch normalization.

**Figure S4:** **Performance of the deep learning model for all types of stimulation methods. (a)** Prediction error of the model monitored after each training epoch using k-fold cross-validation. The prediction error was close to the training error and the difference between the two decreased after each training epoch. **(b-c)** Comparison of training and prediction accuracies with target variability for all types of synaptic stimulations. These results, including prediction reliability (PR), are comparable to those obtained when only considering unitary stimulation (Fig. 3). **(d)** Trimmed-mean and interquartile range of target variability and training and prediction accuracies (in SMAPE) for different parameters.

**Figure S5: Comparison of synaptic parameters with existing sparse estimates in CA1.** The human brain project (HBP) has recently estimated synaptic parameters of 16 potential connections in CA1 (Ecker et al., 2020). A pairwise comparison by potential connections and synaptic parameter with our estimates to be included in Hippocampome.org (HCO) detects no statistically significant difference.

**Figure S6: Range and distribution of synaptic parameters in different anatomical regions. (a)** Probability density functions of the synaptic parameter in standard conditions globally normalized with the min-max method across different regions and synapse types. Filled circles denote median parameter values. **(b)** Distributions of min-max normalized GABAergic and glutamatergic parameter inferences. All synaptic parameters except U are right-tailed. The three time constants, but not g and U, differ by neurotransmitter: relative to GABAergic, glutamatergic synapses have smaller τd and τr, but larger τf.

**Figure S7: Influence of axonal targeting patterns on synaptic input.** We compared synaptic properties grouped by the axonal morphology of the postsynaptic neuron: the OR group had axons in strata oriens and radiatum; OPR group was similar to OR but also had axons in stratum pyramidale; SLM group had axons in stratum lacunosum moleculare; SP group had axons only in stratum pyramidale. We also extended the study to CA2, CA3, and DG with similar grouping, if the regions had equivalent neuron types. SG and SMo in DG are homologous to SP and SLM in CA1. **(a)** We measured the average difference of synaptic parameters among groups using symmetric percentage distance (SPD). Bold values indicate statistical significance. A positive (negative) value indicates the synaptic parameter is larger (smaller) for G1 than for G2. **(b)** Simulated signals using averaged synaptic parameters in each group (Vh = -60 mV, Erev = 0 mV, and ISI = 10 ms). **(c)** To visualize the distance of the groups **(Left)**, we normalized the trimmed-mean measures of g, τd, and AB3:A1 by dividing values to the maximum among all synapses. To compare the similarities within each group **(Right)**, we calculated the CV of every group and across all synapses (gray) in each region.

**Figure S8: Inter-run variability of the deep learning model predictions.** Since deep learning models depend on the (stochastic) order in which the training dataset is presented, we expected a certain degree of variation in inferences among the 100 trained models. In all analyses we reported the mean over the 100 values, but here we report the coefficient of variation (CV) of the model predictions for each synaptic parameter and potential connection. The potential connections and the synaptic parameters varied with respect to the CV. On average, τf had a higher CV than the other parameters.

**Figure S9: Equivalence of numerical and analytical solutions.** We used the NEURON simulation environment to simulate the synaptic current of a typical signal both by solving the differential equations numerically and by using the analytical equations. Moreover, we compared the results to the original ModelDB implementation of the four-state TPM model. All three simulations produced identical results, confirming the accuracy of our solution.

# Supplementary Video

**Synapses with smaller and larger amplitudes tend to undergo short-term facilitation and depression, respectively.** We sorted all entorhinal-hippocampal synapses by conductance (high-amplitude to low-amplitude) and simulated each synapse separately in voltage-clamp and standard condition with ISI = 20 ms. We animated the evolution of synaptic currents as a function of conductance, which revealed the transition from depression to facilitation.

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