Estimating The Readily-Releasable Vesicle Pool Size at Layer 5 Pyramidal Connections in the Neocortex

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Introduction

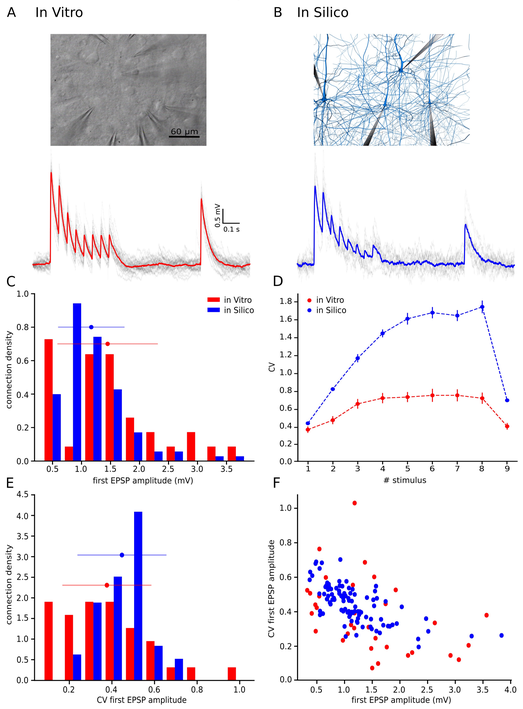
Previous studies based on the 'Quantal Model' for synaptic transmission suggested that neurotransmitter release is mediate by a single release site at individual synaptic contacts in the neocortex. However, recent studies seem to contradict this hypothesis, and indicate that multi-vesicular release (MVR) could better explain the synaptic response variability observed *in vitro*. In this study we present a novel method to estimate the number of release sites per synapse, also known as the size of the readily-releasable pool (N_{RRP}), from paired whole-cell recordings of layer 5 thick tufted pyramidal cell (L5-TTPC) connections in the somatosensory neocortex. Our approach extends the work of Loebel and colleges to take advantage of a recently reported data-driven biophysical model of neocortical tissue.

Materials and methods

Slice preparation and electrophysiology: multiple (6-12 cells) somatic whole cell patch-clamp recordings of layer 5 thick tuft pyramidal cells (L5_TTPC) from brain sagittal slices of 300 µm width from 14-16 days old Wistar rats (33 connections in total). The stimulation protocol consisted of eight electric pulses at 20 Hz followed by a single pulse 500 ms later (recovery test), at the sufficient current intensity to generate APs in the presynaptic neuron while the postsynaptic neuron responses were recorded. The protocol was repeated from 20 to 60 times. ***Deterministic model of synaptic short-term depression:*** this model is described in reference and assumes that a synaptic connection has a finite amount of resources and is based in three main parameters: the release probability (U), time constant of recovery from depression (D) and the time constant of recovery from facilitation (F). ***Fitting parameters to the model:*** we needed to extract the EPSP peaks from each averaged voltage trace. For this purpose we use a mathematical tool to remove the smoothing effect of the membrane cell low-pass filter. Then we introduce the peak values into a genetic algorithm to find the best U, D ,F match. ***In silico experiments: the cortical microcircuit***. We performed the same *in vitro* experiments in a somatosensory cortical microcircuit built into the frame of the Blue Brain Project. Is a data driven model with detailed anatomy and physiology. 31000 neurons, 8 million connections and 37 million synapses in a volume of 0.29 mm^3. ***Noise calibration:*** we implemented an Ornstein-Uhlenbeck process (OU-process), which is a stochastic process that allowed us to simulate random synaptic noise [8]. We defined σ and τ using the voltage values between the 8th and the 9th EPSPs, 400 ms in total, for each repetition in a connection and then we averaged the resulting values. By computing the standard deviation of these points, we got one σ per connection. Through the calculation of the autocorrelation of this section and fitting it to an exponential, we got one τ per connection. ***Computing the coefficient of variation (CV) profile:*** In order to compute the CV for the EPSP amplitudes in the case of the *in vitro* and the *in silico* experiments in the more accurate possible way, we implemented the Jack-Knife method (JKK). This method consists in excluding one observation at a time from a group of observations. From a set of single traces we computed the average of all but one of the traces each time, obtaining at the end a set of averaged-JKK traces. From each of this averaged-JKK traces we computed the amplitudes for the nine EPSPs. This computation is much more precise considering we removed the noise by averaging. Then we could compute the CV profiles for the *in vitro* and the *in silico* experiments.

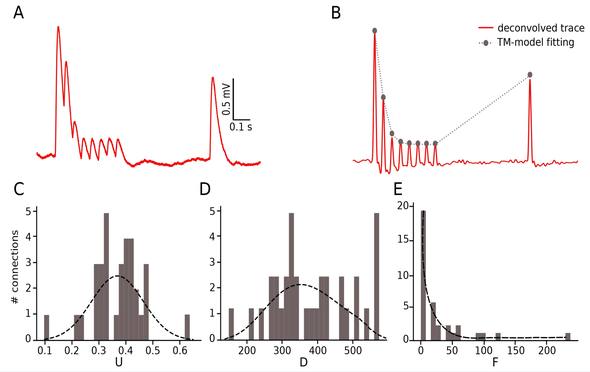
1. Motivation.

At the beginning all the synapses in the model were univesicular. As a result, our synapses were too variable when compared to *in vitro* data.



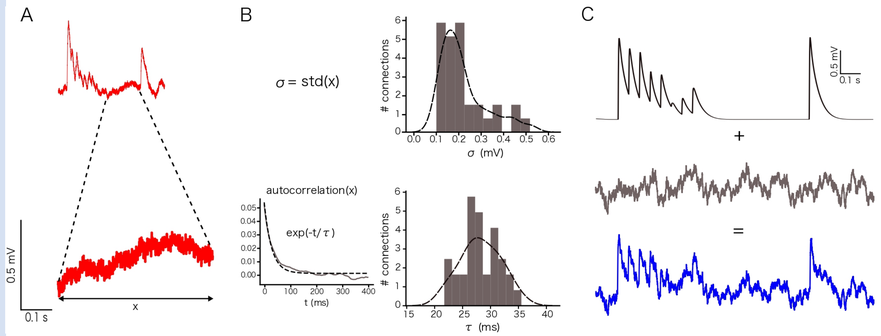
2. Extracting parameters for the TM-model using a deconvolution method.

U = 0.38 ± 0.02, D = 365.6 ± 17.91 ms and F = 25,71 ± 7.99 ms.



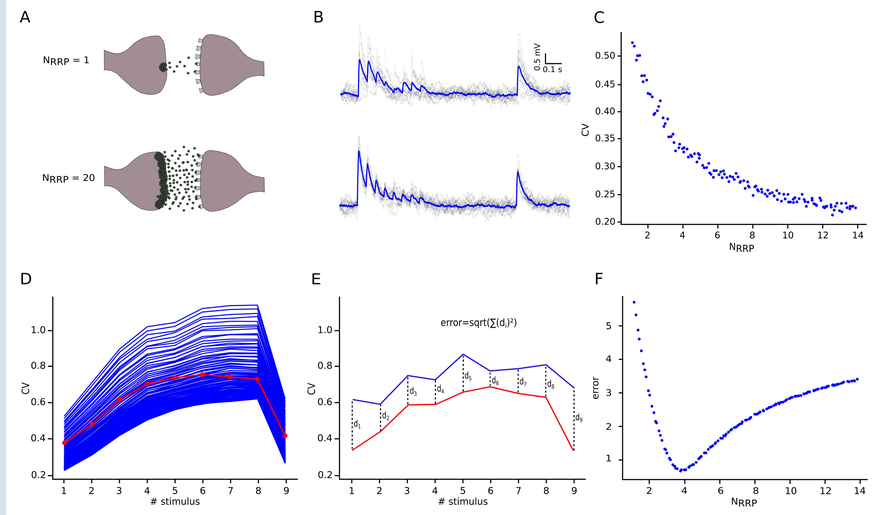
3. Noise calibration.

σ = 0.22 ± 0.10 and τ = 28.2 ± 3.5. With these parameters we capture the synaptic variability for this connection. Then we could add an OU-process to each of the simulated sweeps.

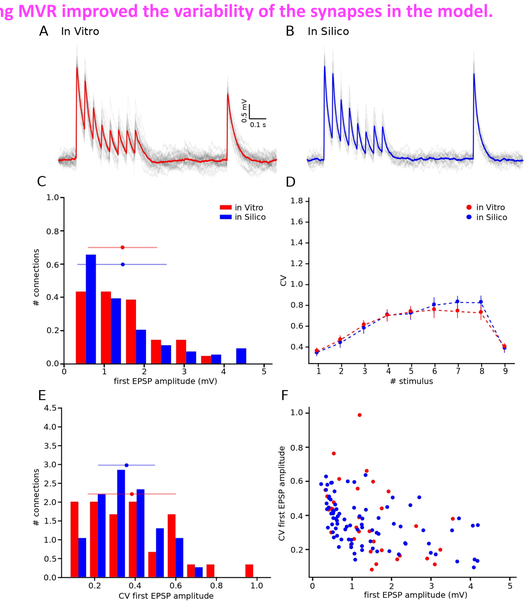


4. Optimizing NRRP for L5_TTPC connections.

we ran simulations with different N_{RRP} values. These values were shaped according to a Poisson distribution of 100 points shifted to the right one unit and with means varying from 0 to 12. We computed the CV profile with simulations run for each of the 100 different values. We iterated this procedure 50 times and then we provided the mean and the standard deviation for each N_{RRP}. We found that for this specific cell connection N_{RRP} had a value of 2.89 ± 1.37

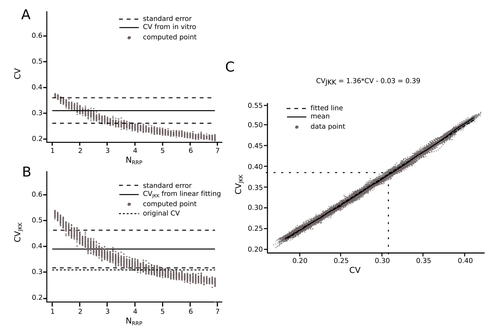


5. Implementing MVR improved the variability of the synapses in the model.



6. N_{RRP} prediction for other cell connections.

we extended this method to other cell connections for which we have models in our circuit and the CV for the first EPSP amplitude from literature. A transformation from no JKK CV to JKK CV was necessary. The average number of vesicles ready to be released at the presynaptic terminal is 2



Cell connection	Literature	Jack-Knife conversion	Prediction
	CV	CV	N_{RRP}
L23_NBC_LBC-L23_PC	0.40 ± 0.09 (Wang, 2002)	0.38 ± 0.21	1.96 ± 0.98
L23_PC-L23_PC	0.33 ± 0.18 (Feldmeyer, 2006)	0.48 ± 0.23	2.60 ± 1.28
L4_SSC-L23_PC	0.27 ± 0.13 (Feldmeyer, 2002)	0.37 ± 0.09	1.81 ± 0.37
L4_SSC-L5_TPC:C	0.33 ± 0.20 (Feldmeyer, 2005)	0.46 ± 0.15	1.26 ± 0.50
L5_TTPC-L5_SBC	0.32 ± 0.08 (Wang, 2002)	0.34 ± 0.16	1.82 ± 0.90
L5_TTPC-L5_TTPC	0.31 ± 0.14 (Our value)	0.39 ± 0.15	2.89 ± 1.37

- L23_NBC_LBC: layer 2 and 3 nest and large basket cells
- L23_PC: pyramidal cells in layer 2 and 3
- L4_SSC: layer 4 spiny stellate cells
- L5_TPC:C: thick tuft pyramidal cells that receive projections from thalamus
- L5_SBC: small basket cells from layer 5

Conclusion

In this work we provide a method to computed the N_{RRP} per active contact in a synapse. This method is an extension of Loebel and colleague's work from 2009. Through the comparison between a set of *in vitro* and *in silico* data of the EPSP amplitude CV, we were able to find the correspondent N_{RRP}. The results obtained by this method helped us to understand that MVR is a process that happens in some cortical areas of the rat brain and that is a mechanism important for the correct functioning of the information transmission through the brain.

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