The single neuron as a probe for distributed synaptic activity evoked in *in vitro* neocortical slices through multi-site electrical stimulation

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Abstract

A stochastic extracellular stimulation paradigm, employing substrate micro-electrode arrays (MEAs), was used to evoke distributed irregular spiking activity in neocortical acute brain slices, while simultaneously recording the intracellular membrane voltage of a layer V pyramidal neuron. A data analysis method is proposed to extract from the recorded sub-threshold membrane potential, an estimate of the neuronal synaptic input resulting from the distributed activity in the slice. The spectral properties of such inputs are characterized, and a tentative interpretation is provided, by comparing the experimental data to the predictions of a dynamic mean-field theory approach, recently introduced for recurrent populations of interacting spiking neurons.

1 Introduction

The purpose of the present work is to show that a stochastic extracellular stimulation paradigm, employing substrate micro-electrode arrays (MEAs), can evoke a distributed and irregular spiking activity in an acute slice, and to demonstrate that a suitable analysis of the intracellularly recorded subthreshold membrane potential of a neuron in the slice carries enough information about the spiking activity impinging on it, to be semi-quantitatively compared with theoretical predictions.

2 Experimental Methods and Data Analysis

2.1 Substrate microelectrode arrays (MEAs) stimulation combined with whole-cell recordings

$300\mu m$-thick parasagittal acute slices of the rat somatosensory cortex were prepared according to standard procedures and coupled to MEAs (see Fig. 1).

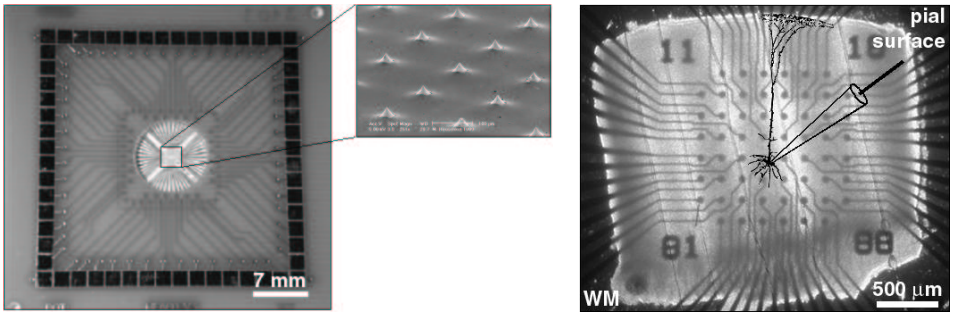

Fig. 1. MEAs consisting of 60 Pt tips electrodes, arranged over an area of $1.4 \times 1.4 mm^2$, were coupled to parasagittal neocortical slices and employed for distributed electrical stimulation. Simultaneous whole-cell recordings were performed as sketched in the right panel.

Whole-cell patch recordings were performed from DIC-visually identified pyramidal cells in layer V, while continuously perfusing the slices by artificial cerebrospinal fluid (ACSF) containing (in mM): 125 $NaCl$, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 20 $glucose$, balanced with $95\%O_2$ and $5\%CO_2$. Pipette solution contained (in mM): 115 $K-gluconate$, 20 KCl, 10 $HEPES$, 4 $ATP-Mg$, 0.3 Na_2-GTP, 10 $Na_2-Phosphocreatine$. At the end of a few recording sessions, Tetrodotoxin (TTX - 1.5 mM) was added to the bath solution, with the aim of isolating the electrical stimulation artifact properties, as it completely suppressed extracellularly evoked synaptic responses (see Fig. 2- lower right panel). Whole-cell recordings were filtered at $3kHz$, sampled at $\Delta t^{-1} = 5kHz$ and off-line analyzed. In the present work, glass substrate MEAs[1] were employed as the bottom of a standard recording submerged-chamber with the purpose of extracellularly evoking asynchronous synaptic responses by a spatially distributed electrical stimulation. Furthermore, as opposed to traditional *in vitro* extracellular stimulation paradigms consisting in brief charge-balanced voltage (or current) shocks, a recently proposed stochastic stimulation paradigm was exploited (see Fig. 2). It has been actually shown that a continuous randomly fluctuating electrical stimulation, delivered by individual MEA microelectrodes, evokes complex and irregular synaptic waveforms in intracellularly patched postsynaptic neurons (see Fig. 2- right panel). Such a novel technique should be regarded as a more physiological, controlled and repeatable alternative to modifying the extracellular ionic concentrations (e.g. $[K^+]_o$) with the aim of increasing the slice excitability and the spontaneous synaptic activity.

Repeated realizations of a stationary noisy MEA stimulation, lasting 10 seconds each, were delivered while simultaneously recording the intracellular membrane voltage of a pyramidal neuron (see Fig. 2 - left). The reported observations are based on data collected from 4 different acute slices.

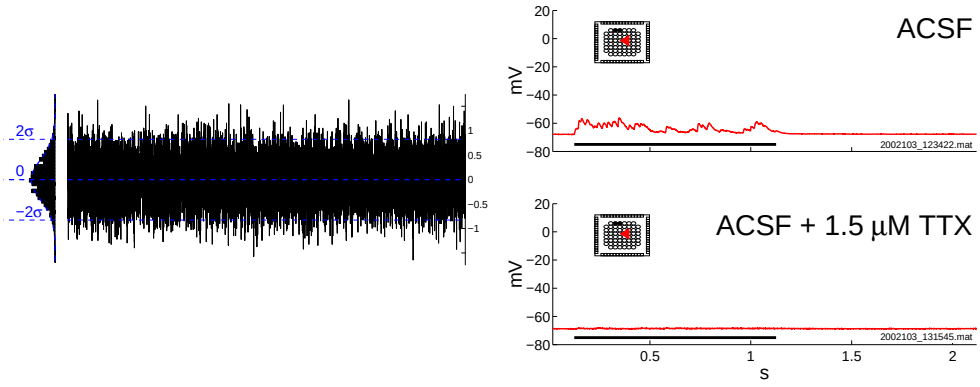

Fig. 2. Independent realizations of the Ornstein-Uhlenbeck stochastic process $dW(t) = -W(t)\,dt/\tau + \sigma/\sqrt{\tau}dZ(t)$ ($\tau = 0.5ms$, $0 \leq \sigma \leq 1V$) are numerically generated (left panel). Z(t) is a Gaussian white noise with zero mean and unit variance. $W(t)$ is delivered to the MEA microelectrodes through a multi-channel voltage stimulus-isolator, evoking irregular and complex TTX-sensitive synaptic responses (right panel).

2.2 Neuronal Frequency filter properties upon MEA extracellular noisy stimulation: a linear system?

In most of the stimulation sessions, the membrane potential $V(t)$ of the patched neurons kept fluctuating not far from the resting potential and well below the threshold for spike emission, as a result of an evoked synaptic barrage. Under such conditions, the nonlinearities due to voltage-dependent ionic conductances and synaptic reversal potentials were assumed to be negligible. On a first approximation, this led to model the postsynaptic potentials generation, integration and dendritic propagation as linear processes.

As expected from traditional extracellular stimulation techniques, the prolonged noisy stimulation induced a stationary artifactual measured component, spread over the entire time interval of the stimulation. Under complete pharmacological suppression of presynaptic activity, by adding TTX to the bath, such a stimulation artifact was identified (see Fig. 3) and an appropriate signal analysis strategy was devised to extract the purely synaptic component.

It is seen that for low frequencies (up to $\omega/2\pi \simeq 200 - 300$ Hz) most of the power of $V(t)$ is contained in the purely synaptic part, and the contribution of the stimulation artifact is negligible, while for high frequencies the power spectrum is essentially due to the artifact. The peaks at 50 Hz, 150 Hz and 250 Hz in Fig. 3 are due to the line power noise. The right panel shows the spectrogram $P_V(\omega, t)$ of the stimulation artifact, which confirms the hypothesis of an essentially time independent power spectrum. Under the above mentioned hypothesis of linearity, and incoherent and linear superposition of the synaptically induced signal and the stimulation artifact, the power spectrum of the recorded membrane voltage dynamics can be seen as $P_V(\omega, t) = P_{\mathrm{MEA}}(\omega, t) + P_{\mathrm{syn}}(\omega, t) = P_{\mathrm{MEA}}(\omega, t) + |\mathrm{T}(\omega)|^2 P_\nu(\omega, t)$. T($\omega$) is

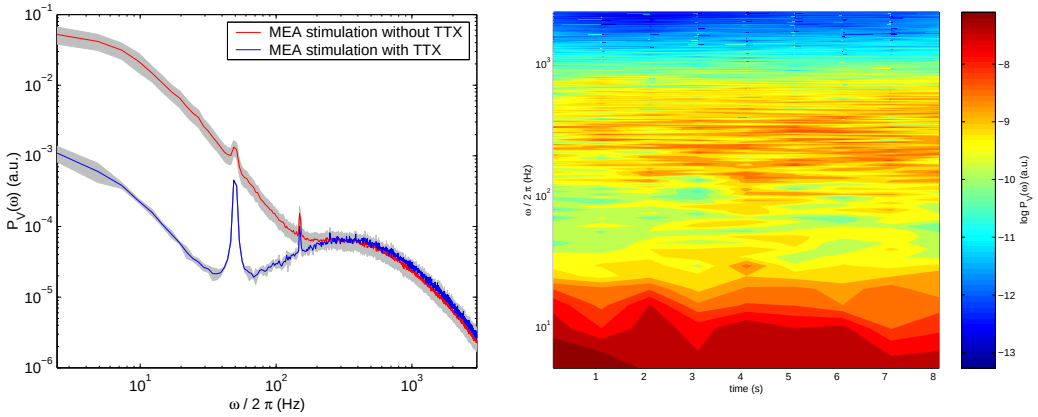

Fig. 3. The measured power spectral density has been estimated under control conditions ($P_V(\omega)$ - left panel) and under TTX ($P_{\mathrm{MEA}}(\omega)$), under stationary conditions.

the (unknown) transfer function which embodies the whole chain of processes, activated by the pre-synaptic bombardment at frequency ν (with power spectrum $P_\nu(\omega, t)$), resulting in fluctuations of $V(t)$. To compensate for the lack of knowledge of $\mathrm{T}(\omega)$, we defined an alternative observable eliminating the dependence on $\mathrm{T}(\omega)$: we computed the "asymptotic" power spectrum $P_V(\omega, \infty)$ as the average of the power spectral density in the time interval in which it has reached a steady state, and we took the ratio: $R(\omega, t) = \frac{P_V(\omega,t)}{P_V(\omega,\infty)}$. Under the above assumptions, this leads to: $R(\omega, t) \simeq \frac{P_{\mathrm{syn}}(\omega,t)}{P_{\mathrm{syn}}(\omega,\infty)} + \frac{P_{\mathrm{MEA}}(\omega,t)}{P_{\mathrm{MEA}}(\omega,\infty)}$. As a consequence, $R(\omega, t)$ was estimated as

$$R(\omega, t) \simeq \frac{P_{\mathrm{syn}}(\omega, t)}{P_{\mathrm{syn}}(\omega, \infty)} + 1 = \frac{P_\nu(\omega, t)}{P_\nu(\omega, \infty)} + 1$$

3 Preliminary experimental results and tentative interpretation

Repeated MEA stimulations lasting $10s$ (the inter-stimulus time is $50s$), with constant variance σ^2, were applied to the MEAs. During the stimulation the membrane potential of the patched neuron showed, after a sharp onset, a slow transient well above the resting conditions (see the top panel in Fig. 4, left). The corresponding $R(\omega, t)$ (bottom panel in Fig. 4, left) exposes non stationary features present in $P_\nu(\omega, t)$, sensed by the neuron through its synaptic inputs. Fig. 4, right, shows the recorded $V(t)$ obtained under TTX (top panel) and the corresponding $R(\omega, t)$ (bottom panel).

Interestingly, some features consistently appeared throughout the experiments: 1) The noisy stimulation protocol evokes irregular synaptic waveforms in the recorded pyramidal cells; 2) there is a prominent spectral peak in $P_\nu(\omega, t)$, whose position is around 90 Hz and approximately independent of time, though its amplitude and width change in time; 3) the position of the above peak is approximately independent from the strength of stimulation (represented by the control parameter σ^2); 4) the high-ω, asymptotic value of $P_\nu(\omega, t)$ is very

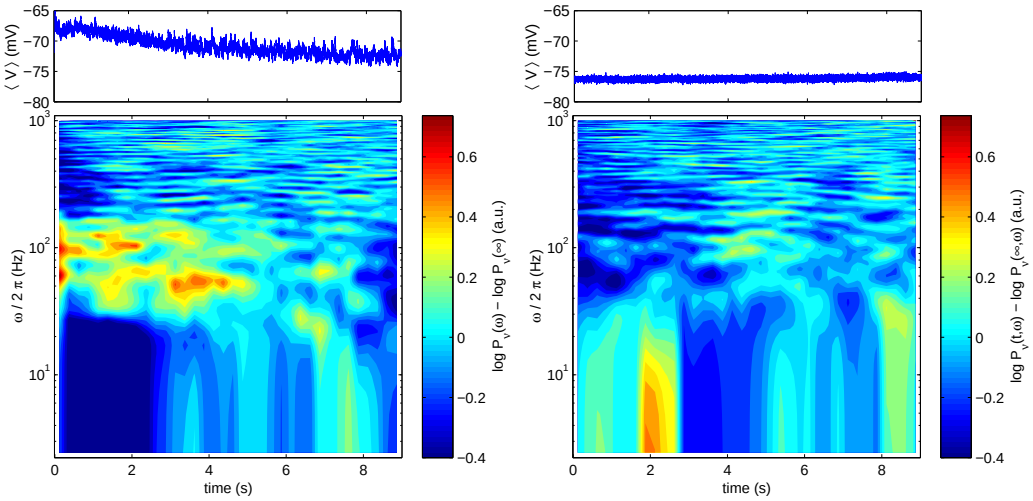

Fig. 4. Left panels: Time course of the recorded membrane potential $V(t)$ (top) and the measured quantity $R(\omega, t)$ (bottom) during MEA stimulation. Right panels: $V(t)$ (top) and $R(\omega, t)$ (bottom) in the same stimulation conditions, under TTX.

weakly dependent on time; 5) there is a significant trough in $P_\nu(\omega, t)$ for low ω, and deepest at the onset, where the peak is highest ; 6) under stronger MEA stimulation, irregular firing activity was elicited in the patched layer V pyramidal neurons, with low rate depending on the stimulation power; while those supra-threshold recordings were not used for the data analysis, this evidence led us to suppose that also the neurons contributing to the synaptic input of the patched neuron (when the latter is subthreshold) share the same properties; 7) Under TTX, the spectral peak disappears, and the $R(\omega, t)$ is approximately flat and time independent.

To get an insight into the meaning of the above results, we examine in Fig. 5 some candidate network scenarios, in the light of theoretical predictions derived via a dynamic mean field approach ([2]). The theory predicts specific features to be observed in the power spectrum of the collective spiking activity ν, under the hypotheses of asynchronous regimes with noisy, stationary background inputs. Two kinds of peaks in the $P_\nu(\omega, t)$ are predicted: 'diffusion' peaks, at frequencies multiple of the population spike emission rate ν, and 'transmission' peaks, located at frequencies which are determined by the effective transmission delays, including synaptic integration times, and do not depend on ν.

We propose the following tentative interpretation of the experimental findings (see Fig. 5): i) Feature 7 strongly suggests that the observed spectral structure is due to synaptic input; ii) the spectral features of the synaptic input could be due in principle to network properties reflected in the population activity $\nu(t)$ (first three scheme and power spectrum pairs in Fig. 5, top to bottom), and/or to intrinsic, spiking spectral properties of single pre-synaptic neurons (bottom panels in Fig. 5). The "?" in the figure recognizes that such single neuron effects cannot at present be ruled out; however, both observations of

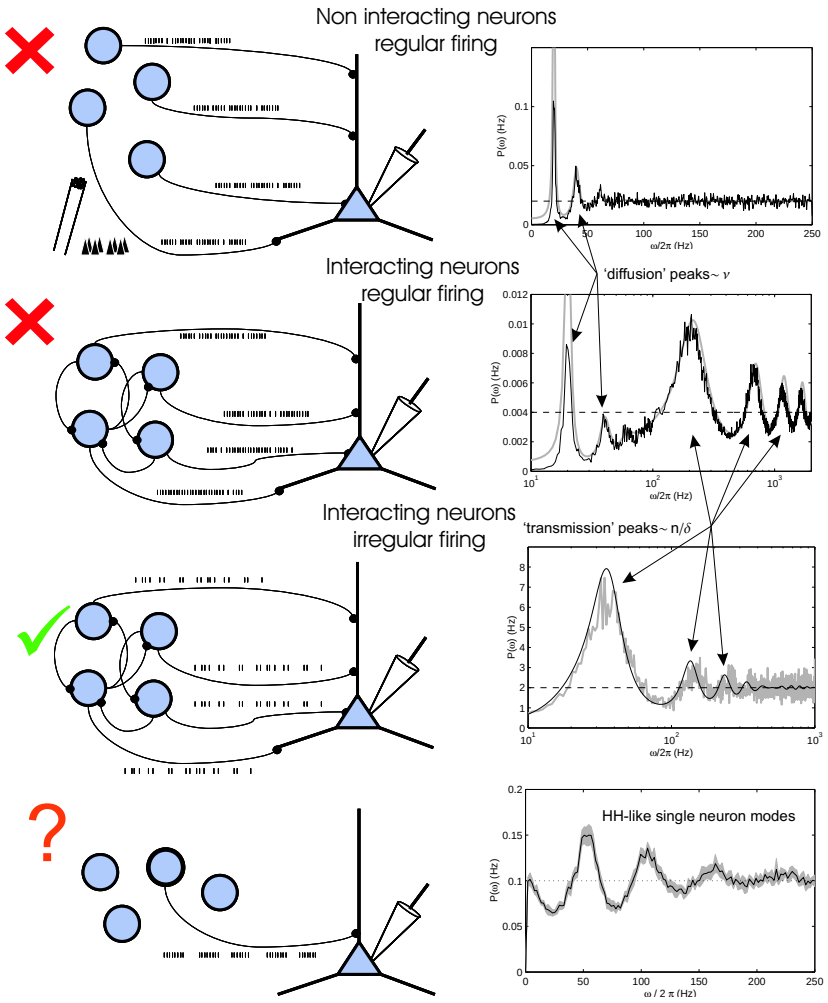

Fig. 5. Possible network-level interpretations of the experimental findings. See text.

the ISI distributions for a few pyramidal neurons, and feature 2 above, tend to favor the network interpretation; iii) red crosses express the conclusion that it is unlikely that the peak in $P_\nu(\omega, t)$ is due to a diffusion' peak, because of feature 1, 3 and 6 (the 'uncoupled network' scenario is ruled out for similar reasons, besides point i)); iv) features 2, 3 and 5 are compatible with the interpretation of the peak in $P_\nu(\omega)$ as a 'transmission' peak; v) the modulation in the height and width of the spectral peak is consistent with the time course of the recorded membrane potential, plausibly reflecting a transient in the activity induced in the slice. The latter in turn could be due to adaptation effects.

References

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