ELSEVIER

Neuroscience Letters 300 (2001) 21–24

Neuroscience Letters

www.elsevier.com/locate/neulet

Frequency tuning of input-output relation in a rat cortical neuron in-vitro

Dror Tal, Eyal Jacobson, Vladimir Lyakhov, Shimon Marom*

The Bernard Katz Minerva Center for Cell Biophysics, Department of Physiology and Biophysics, Faculty of Medicine, Technion, Haifa 31096, Israel

Received 20 December 2000; received in revised form 9 January 2001; accepted 9 January 2001

Abstract

The input-output relation of a single neuron stands at the basis of every biologically oriented description of the brain. This report shows that the input-output relation of cultured cortical neurons is non-linearly tuned by the input frequency. Increasing the rate of stimulation results in the appearance of ordered temporal firing patterns, which are qualitatively different for different input frequencies. The experimental results of this study lead to the conclusion that frequency tuning of neuronal input-output relation arises from activity-dependent rates at the molecular level underlying the mechanism of excitability itself.

Keywords: Excitability; Input-output; Inactivation; Ionic-channel

Within a time frame of several milliseconds a single cortical neuron receives input from many other neurons. This input is non-linearly scaled according to synaptic strength and location, and integrated with other inputs that are received during the same time frame. The sum is compared to a threshold level. If above threshold, the nerve cell fires an action potential, which is received as a scaled input by other neurons. Normally, the spikes have a constant amplitude therefore a meaningful input must be represented by the output frequency or its temporal pattern [2–4,6,8–10]. An important postulate of this simplified picture is that, at least at some level of neural processing, the frequency or temporal pattern at which a significant input excites a nerve cell must be a parameter of the input-output relation of that cell. Here, this postulate is put to an experimental test at the single neuron level.

Cortical neurons were taken from rat newborns within 24 h from birth, following standard procedures that are in accordance with institutional guidelines. Briefly, the cortex tissue was digested with Trypsin and mechanically dissociated. The neurons were plated on 35 mm culture dishes that were pre-treated with poly-L-lysine. The cultures were bathed in a minimum essential medium (MEM) supplemented with 5% horse serum, glutamine, glucose, and antibiotics, and kept in the incubator (37°C, 5% CO_2). Experiments were performed on the fourth week after plating, allowing complete maturation of the neurons. During electrophysiological measurements the bath solution contained 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 20 mM glucose, 10 mM *N*-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), (pH 7.4), 0.005 mM bicuculin, 0.01 mM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 0.02 D(-)-2-amino-5-phosphonovaleric acid (APV). The recording pipet was filled with 10 mM KCl, 140 mM *K*-gluconate, 5 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, (pH 7.4), and 150 μg/ml Nystatin. The recordings were performed on morphologically identified pyramidal cells. Axopatch 200A amplifier (Axon Instruments, CA, USA) was used for voltage recordings in the Nystatin-induced perforated configuration (The Axon Guide, Axon Instruments, 1993). The membrane potential was free to develop spontaneously; currents were injected only during 2 ms stimulation pulses.

A neuron is stimulated by a 128 s-long series of above-threshold short current pulses. The input amplitude is set to a minimal, just-above threshold level that evokes a single action potential per stimulus when applied at a frequency of 1 Hz. By definition, under these conditions the neuron responds in a 1:1 mode. What should one expects if the stimulation amplitude and duration are kept fixed, but the stimulation rate is increased beyond 1 Hz (for which threshold level was determined)? Theory of excitability in biolo-

* Corresponding author. Tel./fax: +972-4-8529679.
E-mail address: marom@tx.technion.ac.il (S. Marom).

0304-3940/01/$ - see front matter
PII: S0304-3940(01)01534-8

gical membranes predicts that as the input frequency increases the neuron will start to fail responding to stimuli. The reason being that stimulation to just-above threshold level dictates dependencies of response upon the availability of a small number of ionic channels. The expected randomly distributed failures would then reflect the underlying stochastic machinery of excitability (see Refs. [1,7]). The first row of Fig. 1 shows that this is what happens when the stimulation frequency is increased from 1 to 3 Hz. The ordered 1:1 mode, which was defined at 1 Hz stimulation rate, disappears, and the responses become randomly distributed. This is seen as a gradual appearance of a seemingly random walk in the plot of inter-spike-interval (ISI) evolution (Fig. 1, second column), and as a homogenous filling of the first-return map at this frequency (Fig. 1, fourth column). The first-return map is constructed by plotting each ISI, normalized to the stimulation cycle-length, against the normalized ISI that preceded it. However, not as predicted by the theory of excitable membranes, when the input frequency is increased further, order emerges out of the seemingly random response. In the case of the neuron of Fig. 1, this emergent order is most evident at the 30 Hz stimulation frequency (second row). Note the appearance of an additional time interval in the plot of the ISI evolution, and the well-defined pattern in the first-return map. The results of Fig. 1 suggest that the input-output relations of a single neuron are non-linearly tuned by the input frequency.

Fig. 2 demonstrates the same phenomenon in the responses of two other pyramidal neurons, for several tested input frequencies. The instantaneous ratio of input to output frequency (depicted as Response Mode) was chosen as a variable that reflects the order of the neuronal response. At 1 Hz stimulation rate, the responses of all the cells are 1:1 (the 'baseline' condition). As the stimulation frequency is increased, the neuronal response qualitatively changes. The distributions of the response modes, which are shown at three different input frequencies (other then 1 Hz), suggest that different neurons are tuned to respond orderly at different ranges of frequencies. Overall, out of 14 neurons that were put through the range of input frequencies as described in Fig. 2, eight neurons demonstrated frequency tuning of input-output relations; i.e., qualitatively different firing patterns were evoked as the input frequency was changed. Six out of fourteen neurons did not demonstrate frequency tuning; instead, these neurons responded in a 1:1 mode or slowly adapted to the input (Fig. 3).

Frequency tuning of single neuron input-output relations

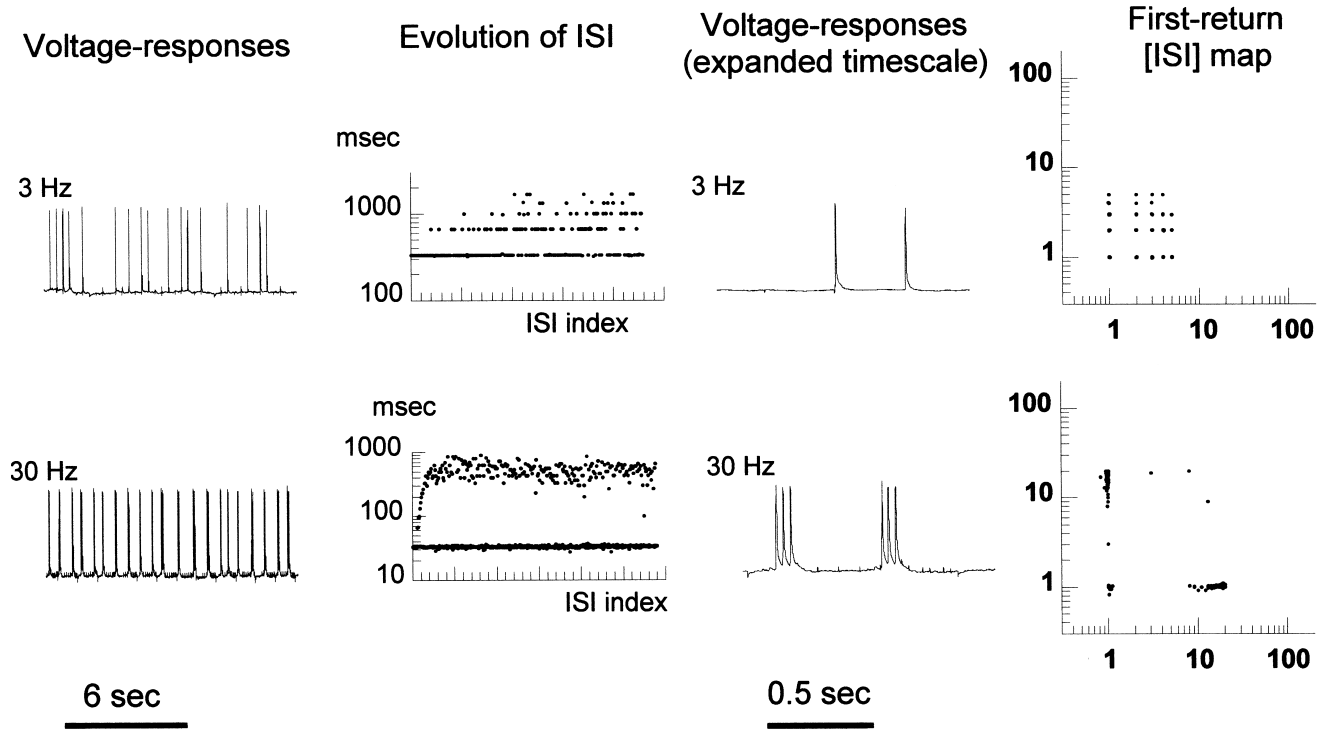

Fig. 1. Voltage responses of a cortical neuron in a network to repetitive, just-above threshold current pulses. The network was bathed in a mixture of synaptic blockers in order to eliminate spontaneous activation of the recorded neuron by other neurons. The neuron was exposed to 128 s-long series of pulses. Each row in this figure presents the results obtained by a particular value of stimulation frequency (f_{in}), 3 Hz (first row) and 30 Hz (second row). A $\approx$ 12 s-long exemplary response is shown on the left column (in this figure, the average passive response was subtracted digitally). In the second column, all the interspike intervals (ISI, ms) are plotted according to the order of their appearance (ISI index). The third column is an expanded section of the first column. The first-return plot of the normalized ISI ($(ISI) = ISI_{sec} \times f_{in}$) is shown at the right column for each stimulation frequency. Note the disorganizing effect of increasing f_{in} from 1 to 3 Hz, and the organizing effect of further increasing f_{in} from 3 to 30 Hz.

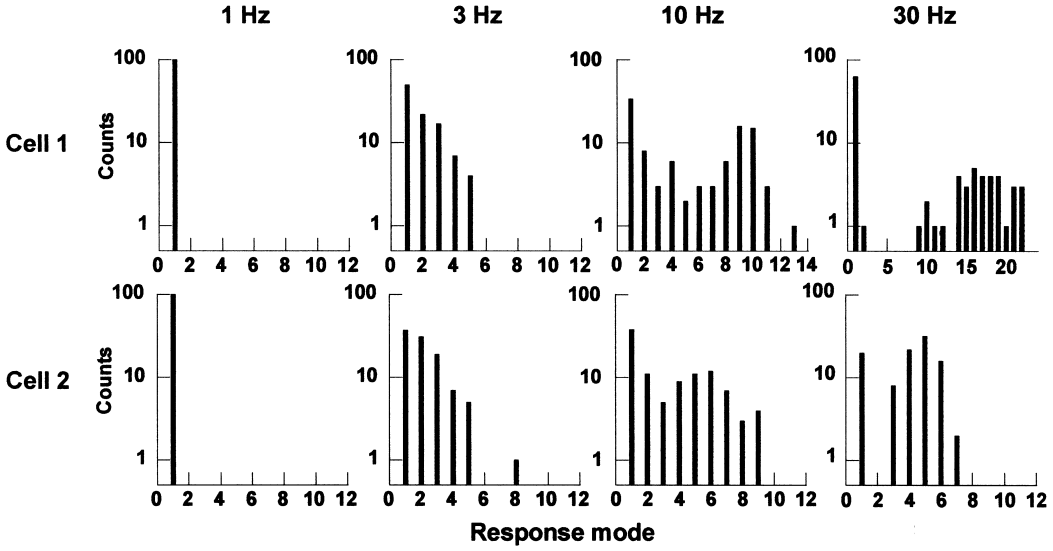

Fig. 2. Histograms of normalized ISIs (depicted as response mode) at four stimulation frequencies (f_{in} = 1, 3, 10 and 30 Hz) for two different neurons. Counts are presented using logarithmic scale. Note the variability between neurons.

might be attributed to spatial factors, especially in light of the complex structure of the neuron [5]. This possibility, however, is not consistent with the relatively slow relaxation towards a particular response mode. For example, the relaxation at 30 Hz (Fig. 1, second column second row) occurs at a time scale, which is far beyond the milliseconds time scale of conduction along the axo-dendritic tree [11]. An alternative source for frequency tuning of single nerve cell input-output relations, is the non-linearity at the level of ion channels, the proteins that underlie the machinery of excitability itself. This possibility is further supported by the results summarized in Fig. 4 in which a comparison is made between action potentials of frequency tuned (left panel) and slowly adapting (middle panel) cells. Action potentials from frequency tuned neurons are higher and shorter (84 ± 8 mV, 5.2 ± 1.1 ms, eight neurons), compared to slowly adapting neurons (69 ± 9 mV, 9.7 ± 2.1 ms, six neurons). These differences are statistically significant ($P < 0.01$, two-tailed t-test). Judged from the averaged resting potentials and initial membrane responses to a stimulating pulse, the two groups of cells

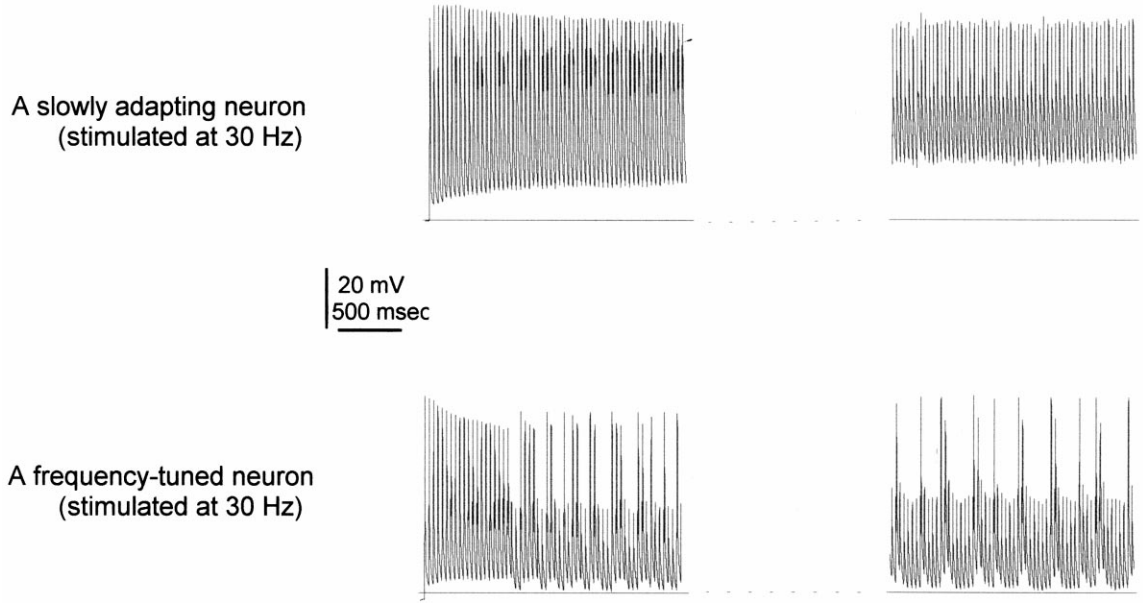

Fig. 3. Example of an adaptation process in a slowly adapting neuron (top) stimulated at 30 Hz. The left section shows the beginning of the adaptation process; the right section shows the steady-state behavior. Bottom: An example of a frequency-tuned neuron under the same stimulation protocol. Horizontal lines depict −60 mV.

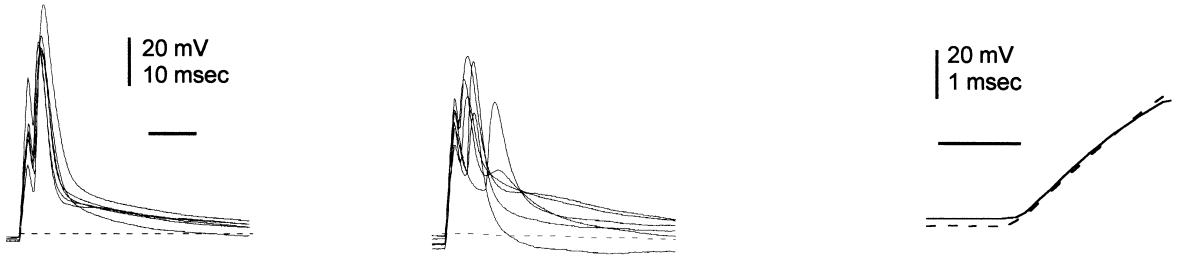

Fig. 4. A comparison between the action potential shapes of frequency tuned neurons (left) and slowly adapting neurons (middle). The right panel shows the average initial passive response of slowly adapting (broken line, $n = 6$) and frequency tuned (continuous line, $n = 8$) neurons to the stimulating current.

(frequency tuned and slowly adapting neurons) have similar passive electrical properties (Fig. 4, right panel), suggesting that the two groups of cells differ in their active electrical properties.

In summary, this study shows that the input-output relations of a neuron are dynamic. These relations change as a function of activity in a non-linear manner over a wide range of time scales. As a result, neurons are capable of actively generating history-determined complex firing patterns from simple input patterns, a capability that is usually attributed to networks of cells.

While these capabilities of single neurons might be important for theories about the dynamics of single neurons and neural networks, one must bear in mind the artificial situation in which the above experiments were conducted; the neurons are in cell culture, the stimulating input is precise in terms of time and amplitude, and the synapses are blocked. It remains to be seen how much of the single neuron dynamics presented here is expressed at more complicated situations.

The authors are grateful to Drs M. Frank, G. Shahaf, E. Braun, D. Dagan, I. Perlman and N. Ziv, for comments and discussions. The research was partially supported by grants from the Israel Science Foundation, and the Bernard Katz Minerva Center for Cell Biophysics.

[1] DeFelice, L.J. and Isaac, A., Chaotic states in a random world: relationship between the nonlinear differential equations of excitability and the stochastic properties of ion channels, J. Stat. Phys., 70 (1992) 339–354.
[2] Ferster, D. and Spruston, N., Cracking the neuronal code , Science, 270 (1995) 756–757 (Review) (20 refs.).
[3] Konig, P., Engel, A.K. and Singer, W., Integrator or coincidence detector? The role of the cortical neuron revisited. (see comments), Trends Neurosci., 19 (1996) 130–137 (Review) (45 refs.).
[4] Mainen, Z.F. and Sejnowski, T.J., Reliability of spike timing in neocortical neurons, Science, 268 (1995) 1503–1506.
[5] Mainen, Z.F. and Sejnowski, T.J., Influence of dendritic structure on firing pattern in model neocortical neurons, Nature, 382 (1996) 363–366.
[6] Nowak, L.G., Sanchez-Vives, M.V. and McCormick, D.A., Influence of low and high frequency inputs on spike timing in visual cortical neurons, Cereb. Cortex, 7 (1997) 487–501.
[7] Rubinstein, J.T., Threshold fluctuations in an N sodium channel model of the node of Ranvier, Biophys. J., 68 (1995) 779–785.
[8] Sejnowski, T.J., Pattern recognition. Time for a new neural code? (comment), Nature, 376 (1995) 21–22.
[9] Shadlen, M.N. and Newsome, W.T., Is there a signal in the noise? (comment), Curr. Opin. Neurobiol., 5 (1995) 248–250.
[10] Softky, W.R., Simple codes versus efficient codes (see comments), Curr. Opin. Neurobiol., 5 (1995) 239–247 (Review) (47 refs.).
[11] Stuart, G.J. and Sakmann, B., Active propagation of somatic action potentials into neocortical pyramidal cell dendrites, Nature, 367 (1994) 69–72.