

# Inferring the Dynamics of “Hidden” Neurons from Electrophysiological Recordings

Valeri A. Makarov, and Nazareth P. Castellanos

**Abstract**—Statistical analysis of electrophysiological recordings obtained under, e.g. tactile, stimulation frequently suggests participation in the network dynamics of experimentally unobserved “hidden” neurons. Such interneurons making synapses to experimentally recorded neurons may strongly alter their dynamical responses to the stimuli. We propose a mathematical method that formalizes this possibility and provides an algorithm for inferring on the presence and dynamics of hidden neurons based on fitting of the experimental data to spike trains generated by the network model. The model makes use of Integrate and Fire neurons “chemically” coupled through exponentially decaying synaptic currents. We test the method on simulated data and also provide an example of its application to the experimental recording from the Dorsal Column Nuclei neurons of the rat under tactile stimulation of a hind limb.

**Keywords**— Integrate and fire neuron, neural network models, spike trains.

## I. INTRODUCTION

Computational availability of the nervous system relies both on the properties of single neurons and importantly on their interactions, where a complex dynamical information processing unobservable on the single cell level emerges. Accordingly, the study of connectivity patterns and arising functional characteristics of the neural microcircuits is a must for understanding how the external world sensory information is processed in the brain [1]-[3]. Although any behavior of a neural network is based on the whole anatomical architecture, as a matter of fact, we usually have an access only to a small part of the network whose properties we aim capturing. Consequently, inferring the functionality of the whole from the study of the functional architecture (connectivity) and characteristics of sub-networks becomes fundamental for investigation of the information processing by the brain.

Common electrophysiological extracellular experimental data provide spike trains generated by a group of neurons, and no more direct information on the network structure, intrinsic neuron dynamics, type and characteristics of synaptic connections etc. is usually available. Accordingly, the use of indirect (model based) methods for inferring synaptic, single neuron and neural network properties frequently is only way of inferring the network dynamics. Thus we are obligated to build up, test and study mathematical models all the time standing on the basis of experimental data.

The authors thanks V. Bonacassa for providing experimental recordings from the DCN and useful discussions. This research has been supported in part by Universidad Complutense de Madrid under the grant PR1/06-14482-B and by the Spanish Ministerio de Educacion y Ciencia under a Ramon y Cajal grant (awarded to VAM).

Authors are with Escuela de Optica, Universidad Complutense de Madrid, Avda. Arcos de Jalon s/n, Madrid 28037, Spain (e-mail: vmakarov@opt.ucm.es).

To succeed in a biophysical study it is desirable to start with a relatively simply organized system, where the properties of individual cells are not extremely complex and the cell diversity is limited. A good candidate for studying the neural dynamics on the network level and its role in the stimulus processing is the Dorsal Column Nuclei (DCN) of the rat, the first relay station in the lemniscal pathway of the tactile information. Electrophysiological experiments show that most of the neurons in the DCN do not significantly differ in their electrical properties [4]-[6]; however, the neural assemblies they form do carry out different functional tasks, e.g. the DCN output depends strongly on the spatiotemporal and intensity characteristic of the tactile stimulus.

Despite of existing extensive electrophysiological and anatomical studies of the DCN little is known on exact functional mechanisms building the fundament of the precise and robust tactile information handling. Here we propose a different but complimentary approach. We assume that the DCN neuron ensembles perform like dedicated processing devices whose output is conditioned not by a complex intrinsic neural dynamics but by the sophisticated connectivity patterns made of individual afferent and efferent connections. Further on we shall refer to the functional (or effective) couplings among neurons rather than to anatomical connections. The former are given by the simplest circuit able to produce the same temporal relation between neurons in an ensemble [7]-[9]. Then we can use electrophysiological data and physical theory to develop a mathematical model of the system and shed light on the problem of processing of the tactile information.

In our recent work [10] we have been able to show that spike trains simultaneously recorded from several neurons can be used for deducing the effective connectivity among them. Although this tool has been shown [11] to be very reliable and valuable in studies of the architecture of neural networks, in conditions of tactile stimulation the dynamics of experimentally observed neurons can be strongly altered by “hidden”, experimentally unobservable neurons. In this paper we propose and test a novel mathematical method allowing to infer on the presence and dynamics of experimentally unobservable neurons using experimentally recorded spike trains.

## II. EXPERIMENTAL PROCEDURE AND MOTIVATION

We study neural responses elicited by stimulation of the hind limbs of a rat and manifested in spiking activity in the DCN. Figure 1 sketches the experimental procedure.

### A. Stimulation Protocol

We mechanically stimulated the hind limb of a rat and recorded extracellularly the response activity in the corresponding Gracilis nucleus. The tactile stimuli lasting 200 ms were delivered by a thin metallic stick (tip area 1 mm<sup>2</sup>) driven forward and backward by a solenoid with 1.4 s inter-stimulus interval. The same stimulation has been repeated 30 times. Specialized skin receptors (with fast adaptation) are excited by that movement and then generated electrical signals travel along the sciatic nerve toward the brain stem where they bifurcate and synapse to neurons in the DCN.

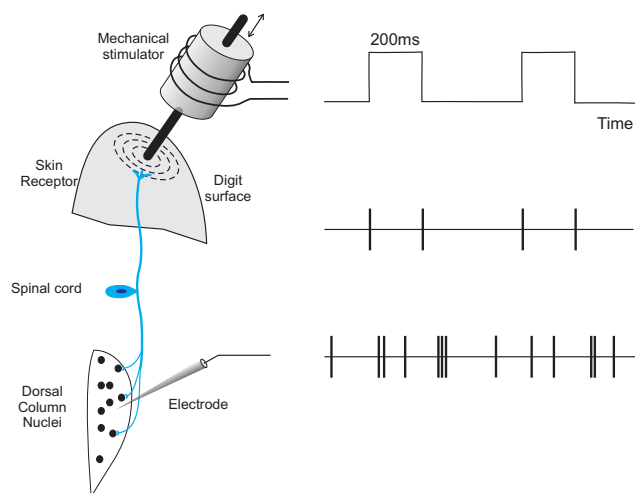


Fig. 1. Sketch of the experimental procedure. We stimulate mechanically the skin of a digit of hind limb of the rat. Touching events lasting 200 ms are repeated 30 times with 1.4 s intervals. The stimulus events excite skin receptors producing spike trains propagating over sensory fibers to the DCN where the fibers bifurcate and make synapses to neurons.

### B. Electrophysiological Recordings

Female adult Wistar rats (200 – 300 g) were anesthetized with urethane (1.6 g/kg, i.p.). Supplementary doses were administered when necessary during long surgical procedure and the body temperature was maintained at 37°C. Rats were placed in a stereotaxic frame and artificially ventilated under control of the end-tidal concentration of CO<sub>2</sub>. To get an access to the DCN we made a cut at the dorsal midline at the level of the neck, separated the musculature and opened the cisterna magna. During the experiments the DCN were covered with mineral oil. We record extracellularly spiking activity in the Gracilis nucleus using PCI-6071E E Series data acquisition card from National Instruments with multi-channel Michigan acute probes organized in 2x2 tetrodes (tip area 312 μm<sup>2</sup>, distance between tips 25 μm, distance between tetrodes and shanks 150 μm).

### C. Statistical Analysis

Recorded spikes have been sorted off-line with a custom package based on Principal Component and Wavelet Analyses [12]. The collected recordings provide spike trains of up to five simultaneously recorded neurons in different stimulation conditions (Fig. 2A).

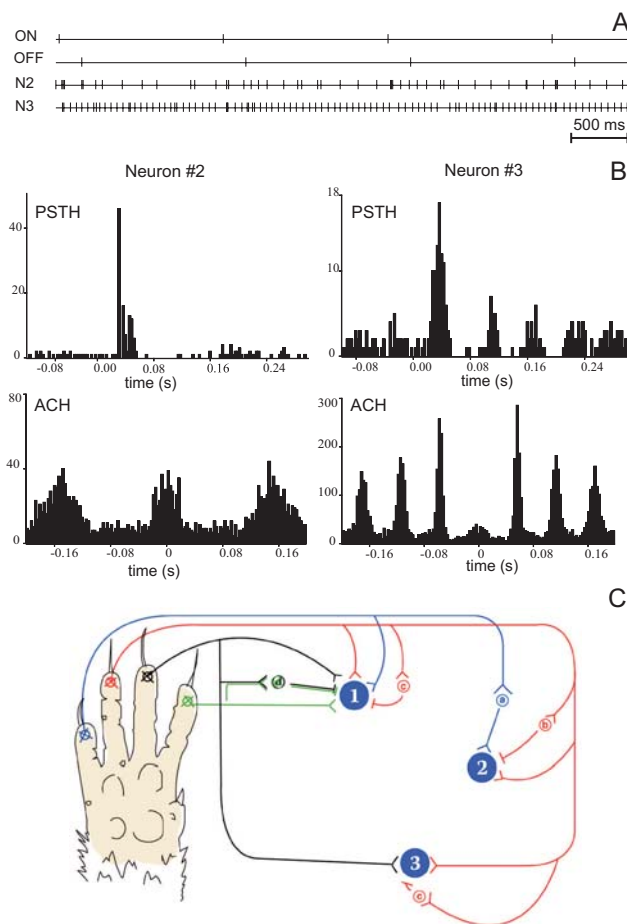


Fig. 2. A representative example of statistical analysis of experimental data. A) Spike trains corresponding to stimulus “On” and “Off” events and response of two neurons. B) Peristimulus and auto-correlation histograms obtained for neurons N2 and N3 under mechanical stimulation of digit #2. C) Hypothesized network constructed on the basis of the PSTHs and ACHs. Neurons numbered by 1, 2, and 3 are experimentally observed (recorded). Neurons marked by letters a – d are experimentally unobserved but essential to explain the properties showing up in PSTHs and ACHs of the observed neurons.

The experimental spike events were compiled into Peri-Stimulus Time Histograms (PSTHs) and Auto-Correlation Histograms (ACHs) in order to study statistical properties of the spike trains. Figure 2B shows a representative example of the histograms. Both neurons strongly respond to the stimulation with latencies about 25ms and 19ms for N2 and N3, respectively, after which the neurons suffer an inhibition. Neuron #2 also has rhythmic component practically uncoupled from the stimulus phase. Neuron #3 exhibits even more prominent rhythmic firing with phase resetting provoked by stimulation events.

Careful analysis of the structure of firing activity and histograms suggests that the dynamics of the neurons can be influenced by other experimentally unobserved neurons. However a rigorous prove of this statement based solely on the histograms is impossible. Nevertheless, on the basis of our experience we drew a possible inter-neuron connections shown in Fig. 2C. The obtained hypothetical network incorporates observable and unobservable neurons. For instance we suppose

that neuron number 3 can receive a direct excitation and indirect inhibition mediated by the hidden neuron “c”.

In the next section we present a mathematical method that formalizes the above described possibility of the presence of hidden neurons and provides an algorithm for their identification.

### III. INFERRING MATHEMATICAL NEURAL NETWORK MODELS FROM EXPERIMENTAL DATA

#### A. Original INCAM Method

Let us first briefly recall the main points of INCAM method [10]. It has been shown [13] that a physiologically detailed conductance-based neuron model (Hodgkin-Huxley type) can be well fitted by single-variable integrate-and-fire models. Accordingly, to describe the neuron dynamics we use the single-compartment leaky integrate-and-fire model (e.g. [14], [15]):

$$\frac{du}{dt} = -\frac{u}{\tau} + i^0 + i_{syn}(t), \quad (1)$$

where  $u$  accounts for the neural membrane potential,  $\tau$  and  $i^0$  are constants defining the intrinsic neural dynamics and  $i_{syn}(t)$  is the synaptic current induced by the spikes from the other neurons and/or sensory fibers. A rather accurate model of the synaptic current is given by:

$$i_x^{syn}(t) = \frac{w_{xy}}{\lambda_{xy}} \sum_i H(t - s_i) \exp\left(-\frac{t - s_i}{\lambda_{xy}}\right). \quad (2)$$

where  $H(\cdot)$  is the Heaviside function,  $s_i$  are the time instances of incoming spikes. The pair  $(w_{xy}, \lambda_{xy})$  describes strength and time scale of the synapse with the subindex defining the post (x) and pre (y) – synaptic element (e.g. “es” means the synapse to the Experimental neuron from the Sensory fiber, see below). In the case of multiple synapses the total synaptic current is given by a sum of individual currents (2).

The method relies on the spiking activity of experimentally observed neurons. Then the connectivity pattern and the basic characteristics of the neural microcircuit are deduced by fitting the model (1), (2) into the available data (Fig. 3).

The original direct INCAM algorithm performs by searching, in the parameter space  $P_{direct} = \{i^0, \tau, w_{xy}, \lambda_{xy}\}$ , the set of parameters minimizing the cost function given by:

$$C(P) = \sum_k \left( ISI_k^{exp} - ISI_k^{mod}(P) \right)^2, \quad (3)$$

where  $ISI_k^{exp, mod}$  are experimentally observed and model predicted  $k$ -th Inter-Spike-Interval. The parameter set  $P^*$  minimizing the cost function provides the best fit of the model to the experimental data and gives all basic characteristics of the network (e.g. type and relative strength of synapses).

#### B. INCAMh Method Including the Dynamics of Hidden Neurons

Let us now assume that a sensory fibre leaves collaterals on both observed and hidden neurons and excites both of them (Fig. 4; see also Fig. 2C). In turn the hidden neuron excites or inhibits the experimentally observed neuron. Then to provide a

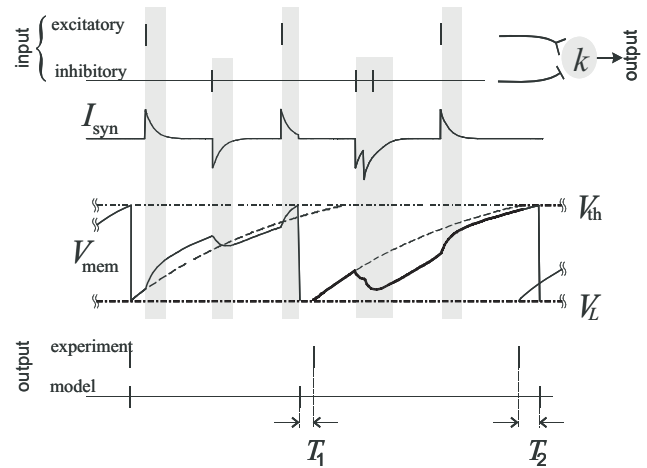


Fig. 3. Example illustrating the dynamics of a neuron under simultaneous inhibition and excitation. The parameter values of the model (1), (2) are adjusted to minimize the sum of the squared differences between the experimentally observed firing (“experimental output”) and the firing predicted by the model (“model output”).

correct mathematical model of the tactile information processing accomplished by this small network we should take into account the dynamics of experimentally hidden neuron. Thus our objective is to deduce the presence, type, characteristics of synaptic couplings in the network and also provide insight on the dynamics of the hidden neuron relying solely on the input and output spike trains.

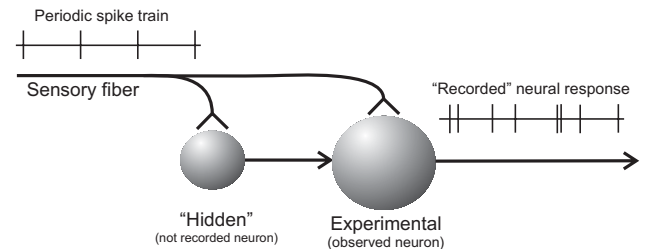


Fig. 4. Graphical representation of the model of experimental recording. Sensory fiber bifurcates and conveys input periodic spike train to two (in general) reciprocally connected neurons. We “record” spike train only from the Experimental neuron, while the firing dynamics of the Hidden neuron is unavailable. Our objective is inferring the presence of the interneuron and characteristics of the network.

In the problem statement shown in Fig. 4, when the dynamics of the hidden neuron plays an important role, an application of INCAM method, i.e. minimization of (3) over the input and output spike trains, will provide a “suboptimal” solution for the dynamics of the experimentally observed neuron. By suboptimal here we mean that the obtained solution will fail to explain accidental properties in the dynamics of the observed neuron provoked by the spikes received from the hidden neuron. Signs of such suboptimality can be observed in Fig. 2 in the form of “erratic” inhibition. To overcome this problem of missing knowledge on the spike trains of the hidden neuron and considerably improve modeling results we modify the original INCAM procedure.

First, we include corresponding equations of type (1), (2)

for the hidden neuron in the model of the network:

$$\begin{aligned} \frac{du_e}{dt} &= -u_e/\tau_e + i_e^0 + i_e^{syn}(t) \\ \frac{du_h}{dt} &= -u_h/\tau_h + i_h^0 + i_h^{syn}(t) \\ i_e^{syn}(t) &= \frac{w_{es}}{\lambda_{es}} \sum_i H(t - s_i) \exp\left(-\frac{t-s_i}{\lambda_{es}}\right) + \\ &+ \frac{w_{eh}}{\lambda_{eh}} \sum_j H(t - z_j) \exp\left(-\frac{t-z_j}{\lambda_{eh}}\right) \\ i_h^{syn}(t) &= \frac{w_{hs}}{\lambda_{hs}} \sum_i H(t - s_i) \exp\left(-\frac{t-s_i}{\lambda_{hs}}\right), \end{aligned} \quad (4)$$

where subindexes  $e$  and  $h$  stay for Experimental and Hidden neurons, respectively; and  $s_i, z_j$  are the spike trains coming from the sensory fiber and the hidden neuron, respectively. Then the parameter set describing the dynamics of the experimental neuron extends to:

$$P_{exp} = \{i_h^0, \tau_e, w_{es}, \lambda_{es}, w_{eh}, \lambda_{eh}\}.$$

We also get a similar set for the hidden neuron

$$P_{hid} = \{i_h^0, \tau_h, w_{hs}, \lambda_{hs}\}.$$

Second, for a given parameter set  $P_{hid}$  we can predict the spike train generated by this neuron under input from the sensory fiber simply integrating the corresponding part of Eq. (4). Once the spike train of the hidden neuron is inferred (for the given parameter values) we can evaluate the cost function (3) for the experimental neuron by ordinary INCAM but now incorporating the synaptic coupling from the hidden neuron.

Third, we search over the extended parameter space  $P_{extended} = \{P_{hid}, P_{exp}\}$  for the minimum of the cost function (3). Note that the extended parameter space includes the direct parameter space, hence the minimum of the cost function will be smaller or equal to the best prediction by the direct method. This particularly means that the new approach will provide a better or at least the same good solution as the direct INCAM method. Further we shall refer to the new method as INCAMh.

### C. Decision Criterion on the Presence of Hidden Neuron

As we noted above INCAMh always provides the same or better solution to the problem than the direct INCAM does. This is due to the fact that ICAMh uses the more complex model with ten fitting parameters, whereas direct INCAM uses only four parameters.

When fitting two different models to the same experimental data we should take into account the model orders. For comparing results of fitting of the INCAM and INCAMh models to the spike trains we use a second order Akaike Information Criterion (AIC) given by [16]:

$$AIC = N \log \left( \frac{1}{N} \sum_{i=1}^N r_i^2 \right) + 2K + \frac{2K(K+1)}{N-K-1}, \quad (5)$$

where  $r$  are the residuals after the model fitting and  $K$  is the number of model parameters (we assume a Gaussian distribution of points around nonlinear curve). Then the more complex model (INCAMh) is more probable than the simpler

one (INCAM) when its AIC is lower. Accordingly, we positively confirm the presence of a hidden neuron when AIC of INCAMh is lower than AIC of the direct method:

$$AIC^{INCAMh} < AIC^{INCAM}. \quad (6)$$

Thus when (6) is fulfilled, the network includes the hidden neuron with corresponding characteristics. Otherwise the simpler model with the direct connection going from the sensory fibre to the observed neuron is preferable.

## IV. METHOD ASSESSMENT ON SIMULATION DATA AND APPLICATION TO EXPERIMENTAL RECORDINGS

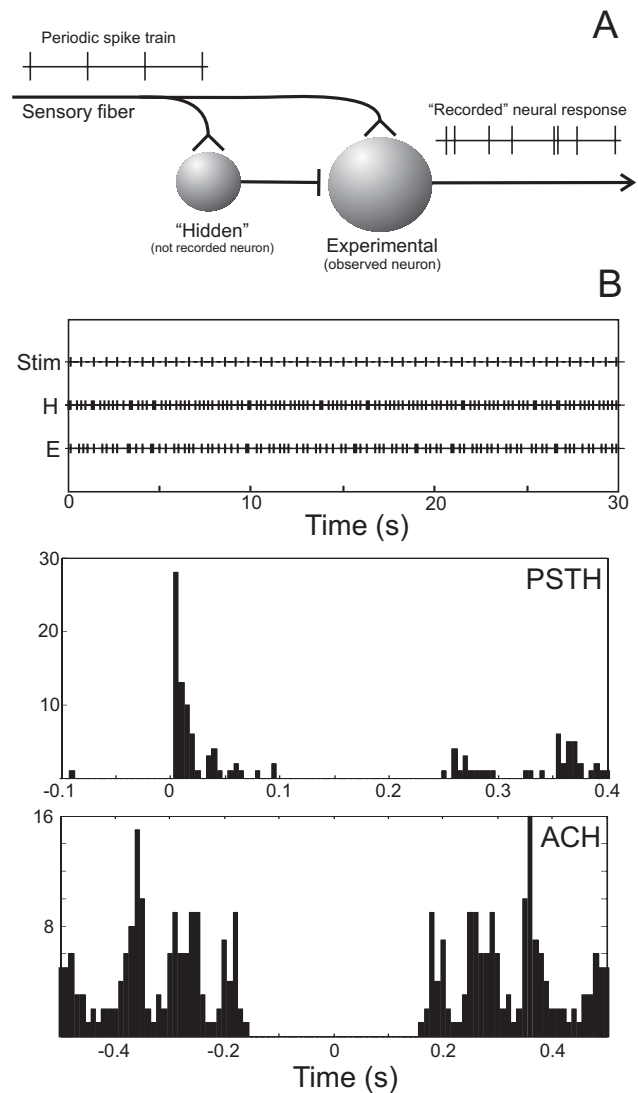


Fig. 5. Method assessment on simulated recordings. A) Sketch of the neuron connectivity. Sensory fiber conveys a periodic spike train that excites two neurons: hidden (H) and experimental (E). Hidden neuron in turn inhibits the experimental neuron. B) "Recorded" spike trains, PeriStimulus Time Histogram and Auto Correlation function for the experimental neuron.

First, we assess the performance of our method on simulated data. To reproduce the above discussed experimental data we use the neural network shown in Fig. 5. We model two neurons labeled as experimental and hidden under excitation by a

periodic sensory input. The hidden neuron sends inhibitory synapsis to the experimental neuron.

In simulation we used the following parameter values:  $i_h^0 = 3.5$ ,  $i_e^0 = 1.57$ ,  $\tau_h = 40$ ,  $\tau_e = 20$ , and the coupling and synaptic scales matrices are given by:

$$w = \begin{pmatrix} 0 & -0.6 & 0.5 \\ 0 & 0 & 0.3 \\ 0 & 0 & 0 \end{pmatrix}, \lambda = \begin{pmatrix} 0 & 0.2 & 0.01 \\ 0 & 0 & 0.01 \\ 0 & 0 & 0 \end{pmatrix}. \quad (7)$$

First we use the direct INCAM method applied to all the three spike trains. As expected it recovers the initial parameter values practically with no errors. The AIC coefficients (5) for the “E” (experimental) and “H” (hidden) neurons are:

$$AIC_E = -1386.9, \quad AIC_H = -1811.4. \quad (8)$$

These values give the absolute minima for the AIC criterion.

Now using the direct INCAM applied to the experimental spike train only we obtain correct identification of the synaptic coupling made by the sensory fiber on the “E” neuron:  $w_{es} = 0.5$ . However, we also observe a strong increase in the criterion coefficient  $AIC_E^{INCAM} = -849.7$ . This means that the model excluding the dynamics of the hidden neuron describes quite poorly the dynamics of the experimentally observed neuron.

Application of INCAMh gives correct identification of synaptic types of all neurons and reduces AIC to  $AIC_E^{INCAMh} = -1273.5$ , which is close enough to the absolute minimum (8). Accordingly, the new method strongly improves prediction of the direct INCAM method and provides correct picture of the neural network behind the experimentally observed spike trains.

Let us now illustrate the method application on the real experimental recordings shown in Fig. 2. As an input to the algorithm we use the spike train of the second neuron and the train of stimulus onsets. The results of prediction indeed confirm the presence of the hidden inhibitory inter-neuron labeled by “b” in Fig. 2C.

Figure 6 shows experimental and simulated PSTHs obtained over spike trains generated by two models. The results provided by INCAMh method are much closer to the experimental, thus confirming the strength of the method.

## V. CONCLUSION

In this paper we have provided a mathematical method of inferring the presence of hidden (inter) neuron and its firing dynamics influencing the output spike train of the experimentally observed neuron under sensory stimulation. The method allows to deduce the strength and type of the synapse sent by the hidden neuron to the experimentally observed one. We also can predict the dynamics of the hidden neuron and its importance for the spike train of the experimental neuron.

Using simulated data resembling experimental conditions we have demonstrated the method validity. Finally we have presented a method application for identification of the hidden neuron dynamics in the experimental recordings made in the dorsal column nuclei of the rat under tactile stimulation.

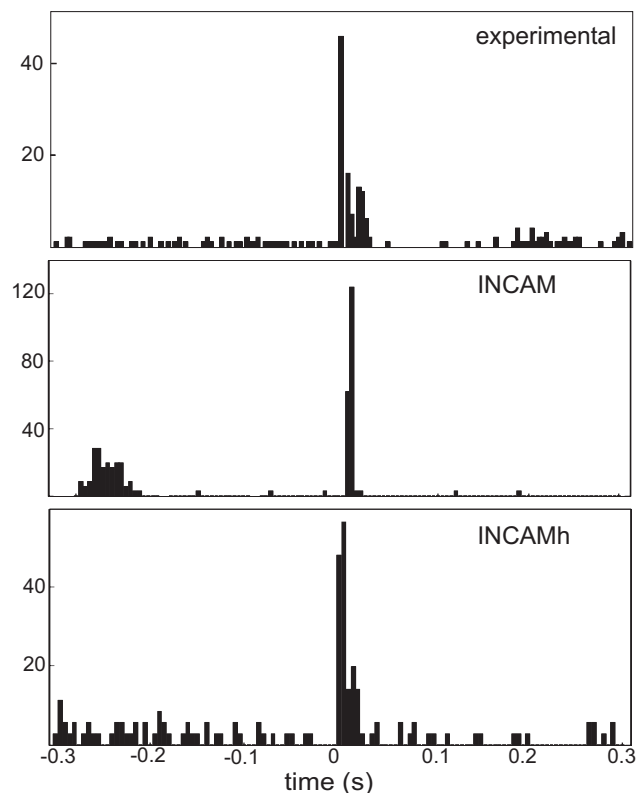


Fig. 6. Method application to experimental data. PSTHs for N2 obtained over experimental recordings (up); after application of the direct INCAM (middle); and after new ICAMh (bottom).

## REFERENCES

- [1] G. P. Moore, D. P. Perkel, and J. P. Segundo, “Statistical analysis and functional interpretation of neural spike data”. *Annu Rev Physiol*, vol. 28, pp. 493-522, 1966.
- [2] D. H. Perkel, G. L. Gerstein, and G. P. Moore, “Neuronal spike trains and stochastic point processes. II Simultaneous spike train”. *Biophys J*, vol. 7, pp. 419-440, 1967.
- [3] E. R. Kandel, J. H. Schwartz, and T. M. Jessell, *Principles of neural science*. 4th ed. New York: McGraw-Hill, 2000.
- [4] F. Panetsos, A. Nunez, and C. Avendano, “Electrophysiological effects of temporary differentiation on two characterized cell types in the nucleus gracilis of the rat”. *Eur J Neurosci*, vol. 9, pp. 563-572, 1997
- [5] F. Panetsos, A. Nunez, C. Avendano, “Sensory information processing in the dorsal column nuclei by neuronal oscillators”. *Neurosci*, vol. 84, pp. 635-639, 1998.
- [6] A. Nunez, F. Panetsos, and C. Avendao, “Rhythmic neuronal interactions and synchronization in the rat dorsal column nuclei”. *Neurosci*, vol. 100, pp. 599-609, 2000.
- [7] G. Gerstein, P. Bedenbaugh, and M. Aertse, “Neuronal assemblies”. *IEEE Trans biomed engineering*, vol. 36, no. 1, pp. 4-14, 1989.
- [8] A. Aertsen and H. Preissl, *Dynamics of activity and connectivity in physiological neural networks*. In: Schuster H.G. Ed., New York: VCH. Nonlinear dynamics and neuronal networks, pp. 281-302, 1991.
- [9] O. Sporns, G. Tonino, and G. M. Edelman, “Connectivity and complexity: the relationship between neuroanatomy and brain dynamics”. *Neural Network*, vol. 13, pp. 909-922, 2000.
- [10] V. A. Makarov, F. Panetsos, and O. De Feo, “A method for determining neural connectivity and inferring the underlying network dynamics using extracellular spike recordings”. *J Neurosci Methods*, vol. 144, pp. 265-279, 2005.
- [11] N. Castellanos, V. A. Makarov, O. de Feo, A. Perez de Vargas, and F. Panetsos, “Identification of functional neural circuits from extracellular recordings using a novel mathematical method”. *Proc. of FENS*, 2004.
- [12] A. Pavlov, V. A. Makarov, I. Makarova, and F. Panetsos, “Sorting of neural spikes: When wavelet based methods outperform principal

- components analysis”. *Natural Computing*, DOI 10.1007/s11047-006-9014-8 (in press), 2006.
- [13] R. Jolivet, T.J. Lewis, and W. Gerstner, “Generalized Integrate-and-Fire models of neuronal activity approximate spike trains of a detailed model to a high degree of accuracy”. *J Neurophysiol*, vol. 92, pp. 959-976, 2004.
- [14] R. B. Stein, “Some models of neuronal variability”. *Biophys J* vol. 7, pp. 37-68, 1967.
- [15] C. Koch and I. Segev, *Methods in neuronal modelling: From ions to networks*. MIT Press: Cambridge, Massachusetts, 1998.
- [16] H. Motulsky and A. Christopoulos, *Fitting models to biological data using linear and nonlinear regression: A practical guide to curve fitting*.