

## CHRONIC PAIN AS EXPRESSION OF NEURAL SUBSTRATES. ISSUES FROM THE NEURONAL DYNAMICS AND MUTUAL RELATIONS

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### INTRODUCTION

One of the lessons Professor Pompeiano taught me was the assiduous attentiveness to every neuronal behavior in the different experimental conditions and, meanwhile, the logic of their activity into the relational cascade of general neural activities leading to specific motor patterns. This conceptual approach to the study of the nervous system was a landmark to me and represented a crucial point in my future research. Though I later devoted to the sensory systems, the lesson was, and is, still valid and deep seeking, coloured with his style, brisk and unused to flamboyance.

Currently, my interests, devoted to sensory neurophysiology, are dedicated to central processing accompanying chronic pain.

Pain relies on central and peripheral anomalies that trigger and maintain diverse pathways of central networks. In the absence of peripheral triggers, however, chronic pain may be still evident. The phenomenon (it would be better using the plural) seems sustained by a network of activations and inactivations. This cohort of neural events is the result of an innate complexity of the central nervous system. In particular, this fact implies the role that higher central neural circuitries play in the construction of abnormal perceptual engrams in the painful syndromes, a sensory role often autonomous from actual inputs from the periphery (11).

On those accounts, a number of anomalies at different scales have been described, as markers of the central single neuron and circuitry irregularities concurrent to pain.

In our experimental setup, this double approach provides an account of the normal and anomalous dynamics of the unitary components, neurons and in addition it guarantees a quantitative description of the connectivity properties in the interaction among the units. This analytical choice tends to match up the neuronal dynamics to the behavioral data. The gap between the neural and the perceptual side of pain comes out unfilled; the two sides of the problem, perception and neural activity are however analyzed in detail.

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On these premises we set up experiments from diverse areas of the nervous system using different models of chronic pain in experimental animals. We shall show here results coming from electrophysiological experiments on neuropathic pain models.

Neuropathic pain is an excruciating condition generated by functional or structural damages to the peripheral nerve fibres. This condition brings to heavy consequences in the central circuits receiving the input from the damaged fibers. The enlargement of the receptive fields is a typical consequence of the damage and represents the peripheral counterpart of central rearrangements evident at the level of the single unit and of the networks. Single neurons show sensitization phenomena. Sensitization is a complex event due to resetting of the neuronal membrane excitability, exiting in neuronal hyperexcitability and hyperresponsiveness (11). The central rewiring is started and maintained by a peripheral damage, like compression and injuries. Chronic maintenance of painful perception needs no further peripheral input. Actually, once started by the anomalous peripheral conditions, cellular and network conversions may however be still observed once any peripheral anomaly disappeared.

In our experiments we try to understand the dynamic anomalies exhibited by single neurons in different districts of the somato-sensory pathway and, in addition, to elucidate the stable network irregularities commonly originated in models of chronic pain. In this paper we present data from simultaneous recordings from Ventro-Basal Nuclei of the Thalamus and the posterior paw cortical projection area in the first Somatosensory Cortical Area. The study of single neuron behavior was accompanied by the network dynamics analysis in the Thalamus and in the Cortex. The final target was the identification of specific neural dynamic profiles for chronic pain.

## METHODS

Adult male Sprague-Dawley rats were used. The neuropathic model was induced in the deeply anesthetized animal (Nembutal 40 mg/kg i.p.) by placing four loose ligatures around the femoral tract of the sciatic nerve along the recipes of the Bennett-Xie model or Chronic Constriction Injury (CCI) (1).

The electrophysiological experiments were performed 12-15 days after the induction of the neuropathy. The rats received a pre-anesthesia with Nembutal (40 mg/kg i.p. for induction), the cannulation of the jugular vein and of the trachea. Body temperature, arterial blood pressure and heart rate were monitored throughout the entire experiment; the stability of arterial pressure (values of 90-110 mm Hg) and heart rate (values of  $320 \pm 10$  beats per minute) signaled adequate anesthesia as the complementary signs of piloerection and lacrimation.

Rats were mounted in a metal stereotactical frame; two holes were drilled on the skull one for the access to the Somatosensory Cortex (stereotactic positioning 2.2 mm LL, 0.5-0.8 mm AP, 400-750  $\mu$ m depth with an orthogonal electrode), the other for the simultaneous access to the Thalamus (stereotactic positioning 2.5-2.7 mm LL, 5.5-5.7 mm AP, 5000-6000  $\mu$ m depth with a 25° anteriorly-slanted electrode). The Dura Mater was then carefully removed and the nervous tissue was then protected with the application of a warm saline soaked piece of surgical foam before the insertion of the electrodes. The animals were then paralyzed and maintained in complete paralysis with

gallamine triethiodide (20 mg/kg/h) and placed under gaseous anesthesia (Isoflurane 0.6-0.8 l/hr and Oxygen 0. 2 l/hr).

The multiple electrodes were then inserted after planar stereotactic positioning. Two remotely controlled micro stepper engines, with 2  $\mu\text{m}$  step, drove the descent of the two electrodes. The electrodes were multiple platinum-iridium electrode structures with nine-channel 3 x 3 matrix configuration (FHC, Bowdoinham, CO, USA). The 3 x 3 electrode array has intertip distance of 150  $\mu\text{m}$ . In the TC configuration the recordings were from the granular lamina (lamina IV) of the Somatosensory Cortex and from the neural fields responding to the posterior paw in the Thalamic Ventrobasal Nucleus, contralateral to the injured paw. Histological control was performed for every experiment.

Recordings for three and eighteen channels were made by two diverse acquisition systems (Data-Wave and Neuralynx). Stereotactic correct positioning was then checked by analyzing online the responses to different peripheral stimuli, where neurons responded to non-noxious (brushing the skin with a cotton rod), and to noxious stimuli (calibrated pinching of ca 400 g on a contact area of 2 mm<sup>2</sup> for 10s) applied to the peripheral areas supplied by the sciatic nerve. Each of our single electrode had a tip impedance of 0.5 to 1.5 M $\Omega$  being able to record three to five neurons simultaneously. Thus a set of up to 90 neurons could be recorded. Off-line reconstruction and separation of the recorded spikes were made by a program set up in our laboratory, based on two-step selection criterion (multiple parameter and principal component, respectively). Signal analyses were made by mutual correlations of the recorded traces by Edit Distance and Temporal Series Homology (4, 17) and Gravity Method procedures<sup>6</sup>.

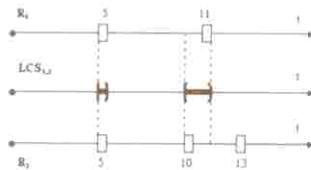
All the analyses were carried out on traces from ongoing, spontaneous non-stimulated activity.

#### *Edit Distance and Temporal Series Homology*

Given two spike-time sequences (taken as a description of the activity of two neurons in a time window) we can formalize the intuition of their quantity of similarity through the mathematical notion of *edit distance* separating them, as the amount of *efforts* needed to transform one in the other. According to the criterion of *maximum parsimony*, we choose the transformation involving the lowest quantity of efforts.

We decided upon a set of possible atomic transformations (ex: insertion, deletion, shift of a spike time) and of their costs (ex: the cost of an insertion/deletion and the cost of a unitary shift). Typically more than one series of atomic operations can transform a fixed spike train into another, we will consider the one minimizing the total sum of efforts.

Complementary, while defining the differences between two spike trains by means of edit operations, we state their similarities as the common subset of spikes acting as a pivot in the transformation.



It is exactly this subset of the distortion that we call Longest Common Subsequence (LCS) (4, 17); so that the length of the LCS between two spike trains is a measure of the degree of their similarity.

#### *Gravity Method*

The Gravity Method, (6), allows for the study of synchronies and discharge pattern similarities among N neuron discharge pattern. Neurons are held as electrically charged particles immersed in an electrical field. Given the bi-univocal correspondence  $i_{(n)}$  neuron- $i_{(n)}$  particle, all the particles are

placed in a N-dimensional hypercube in viscous medium. Each particle is under the influence of the other particles matched against the medium viscosity. With unitary mass 1 we have:

$$\frac{d^2 \vec{x}}{dt^2} = q\vec{f} - \frac{1}{\sigma} \vec{x}$$

$\vec{f}$  is the field acting on from the other particles independent of distance;  $q$  is the  $i_{th}$  particle charge;  $\sigma$  is proportional to the reciprocal of medium viscosity. There's a dependence of neurons on the global activity magnitude. Every time a neuron fires the corresponding particle it shows a  $\Delta q$  increases of its charge with  $\tau$  exponential decay. Without acceleration, at constant speed, if two particles have accordant higher or lower charge than the mean they attract, they repulse on the contrary

The equation for dynamics:  $\vec{x}(t + \Delta t) = \vec{x}(t) + \Delta t \sigma [q(t) - \bar{q}] \vec{f}(t) [\Delta t = 0.001s$  (temporal resolution)], where the decay constant  $\tau$  defines the degree of synchrony needed for attraction;  $\sigma$  is a fluidity factor ruling on particle coalescence speed (6).

This study adhered to the Ethical Guidelines of the IASP (18).

## RESULTS

In the graphics below we plotted the matrices of the sizes of the LCS between couples of neurons in the Thalamus and in the Cortex, considering the two areas as independent systems, and as a unique Thalamo-Cortical-Thalamic system (Fig. 1).

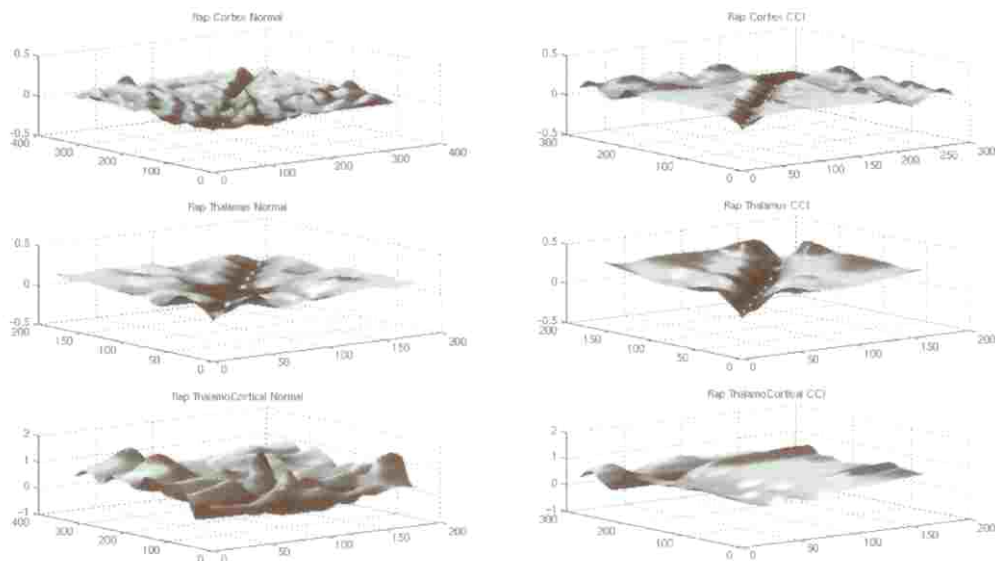


Fig. 1. - Matrices of the dimensions of the LCS between several couples of neurons in the thalamus and in the cortex.

The left and right items at the bottom row allow us to observe the smoother variation of the LCS dimensions between pair of neurons from chronic pain models compared to the higher variability that can be observed in normal subjects.



As for the Thalamo-Cortical unit and population dynamics, the LCS shows a reduction of the similarity sequences in the various dynamic profiles of the recorded neurons. Experimentally it was tested the capability of the LCS measure in discriminating among the neural (Thalamo-Cortical) population activity of our neuropathy models and of normal subjects. Strong and significant ( $p < 0,001$ ) reductions of the similarity degree in Thalamic, Cortical and particularly in the Thalamo-Cortical circuits of neuropathic animals is observable.

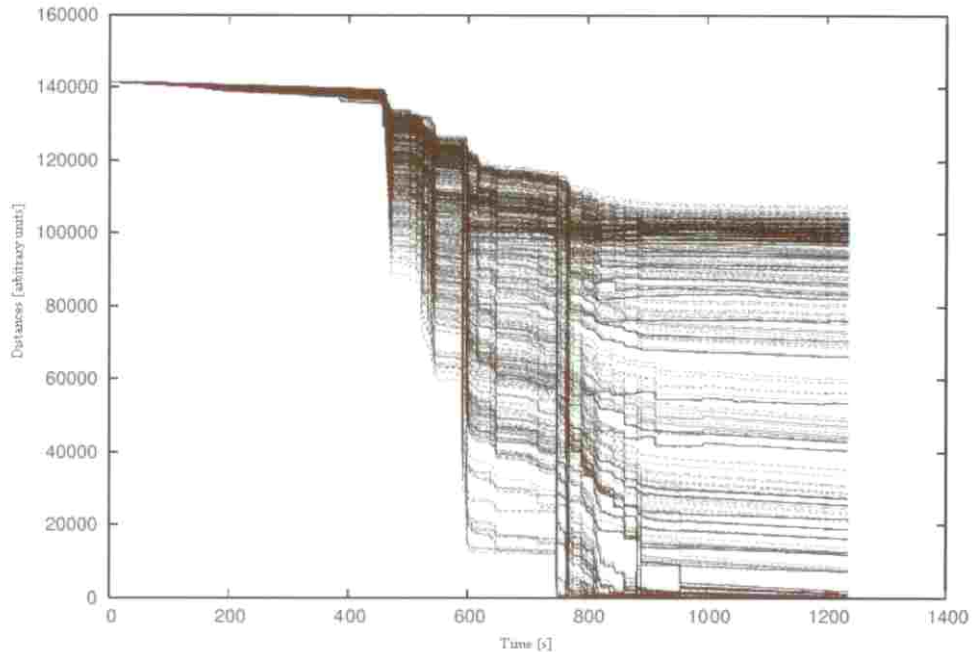


Fig. 2. - *Neural dynamics of the Thalamo-Cortical system in normal rats exhibits a 'fan' of correlations distributed on the neuronal whole population set.*

Sudden dynamic changes are evident (for instance at  $t = 420$ s).

The analyses with Gravity Method (Figs. 2 and 3) show two examples of collective neuronal behavior in the different experimental conditions in normal and neuropathic animals. Figure 2 shows the description of the system activity by the collection of Thalamic and Cortical neurons of a normal animal (treated as they were taken from a definite single population). A diffusely distributed fan of dynamic profiles is shown, indicating that no special correlative behavior (no segregative selection of neuronal groups) is present. Correlations are variable and endow the whole network in richly variable forms. Figure 3 shows a recording from a neuropathic animal where segregated clusters iteratively isolate functionally and groups in stable strong correlations.

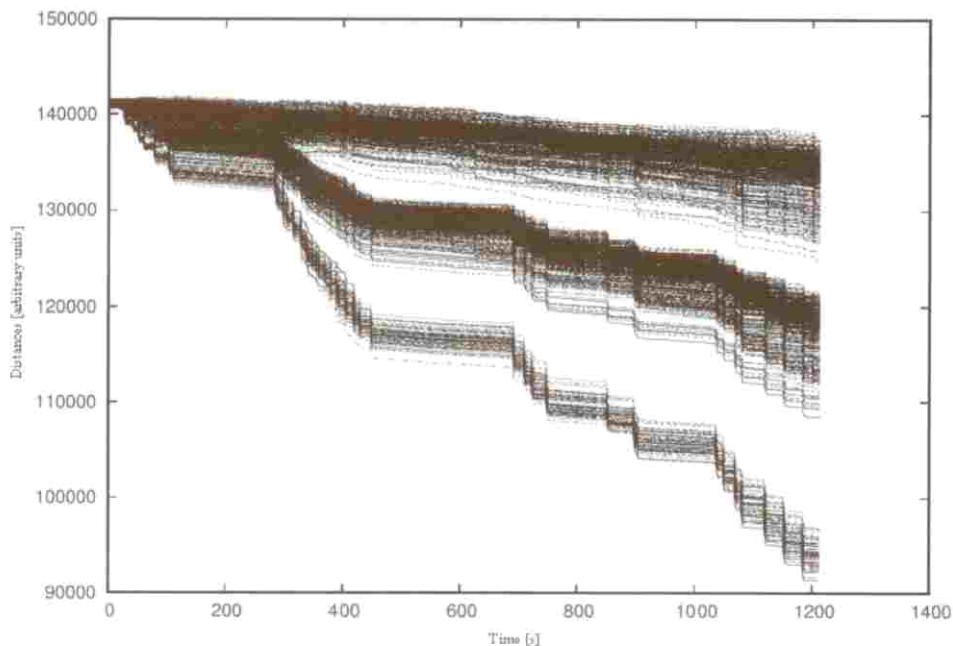


Fig. 3. - *The behavior of the Thalamo-Cortical system in neuropathic rats shows families of highly correlated neurons.*

## DISCUSSION

In this paper we show a results obtained from normal and neuropathic rat models by multiple simultaneous recordings of neuronal activity in the Ventrobasal Thalamus and in the Somatosensory Cortex. The results showed that well identifiable differences are recognizable both in the single cell behavior and in the connectivity properties of neuronal population in the networks recorded. We observed a reduction in the 'variability' of the discharge patterns of the Thalamic and Cortical neurons and a strong dynamic segregation into monotonic, functionally co-varying neuronal subsets.

These results imply that gross rearrangements in the overall connectivity and dynamic properties of the Thalamo-Cortico-Thalamic loop are observable in models of chronic pain. The anatomical and functional disarray that we recorded could represent the outcome of a disordered input inscribed on a developing local structural damage. More than a few parallel cooperating systems make up the Thalamo-Cortical connecting system. Neurons acting in concert into task-generated populations or ensembles work together in potentially definite hierarchies. They, yet, can work in likely or different ways into the execution of other tasks, changing hierarchical position or preferred connection on the basis of neurochemical and activity dependent cues (9). By the way, neurochemical observations put into light the

exclusive or preferential abnormal involvement of specific neurotransmitters or neuro-modulators in the build-up and in the maintenance of chronic pain conditions (10,11,12). However, neither clear relationship between the perceptual issue and the transmitter change is factual, nor the causal-effect relation is straightly obvious in chronic pain. Back to the issue, the freedom of rearrangement of the central circuits can obviously not be unlimited. It is basically assumed that neuronal interactions are still preferentially established among subsets of interconnected neurons (16). Still, it is known that a matrix of neurons, immunoreactive for calbindin, extend throughout the Thalamus projecting over wide cortical areas well beyond the limits of classical somatotopical anatomical areas (7). In the meantime, the exact Thalamo-Cortical map projection is signed by co-extant Thalamic core neurons (7). As additional anatomic issue, a principle of reciprocity is a most stable feature of the brain circuits (5). Reciprocity entails neurons in the Thalamus and the Cortex to act, functionally, as potential ensembles in sensory processes (3). Wide stages of neuronal synchronies are, on the other hand, hypothesized to act as a signature for binding areas and perceptual state definition (15). Additionally, several membrane electrodynamic features of the Thalamo-Cortical circuit exhibit non-linear properties, an important feature at the cellular level can involve diffuse effects like those quoted, at higher scales (14). In fact, it has been shown, on other systems, that the process of transformation of input activations into sparse coding, a plausible functional background for exploiting sensory processing, based on connectivity properties discussed before, is guaranteed by the membrane non-linearity (14). Taking into account the amount of data, we could show that the disrupted relationship between Thalamus and Cortex, potentially instantiated thanks to non-linear properties of cellular membranes, could stably change the spatio-temporal features of the system itself reconfiguring the dynamic profiles both of single neurons and of the loop per se. These facts, matched to our preliminary data, induce to think that potentially specific neural signs could endorse a perceptual condition like chronic pain, and that the collective properties of neuronal population behavior can show sign. The shift from sparse coding to local coding in chronic pain models, perhaps evident in the dynamic profile segregation of gravity method analyses, shows reduced variability and richness of the sensory message to few items. It is interesting that the precise mapping from the Thalamus to the Cortex and back tends to fade in chronic pain. It is difficult to understand the logical connection between reduced plasticity and precision of mapping beyond the known synaptic plasticity events (3) or the potential relation to the calbindin population matrix functions in pathologic conditions.

Also, modeling of single cell dynamics in the Thalamus and in the Cortex shows that a disruption of finely timed input arrival times can severely reduce the input estimate ability of a circuit (8, 13). The reduced capability of the input estimates by the circuits is strongly associated to the single neuron integrating properties as leaky integrator or coincidence detector (8). Extending our previous observation in the neuropathic model of a disordered inhibitory control and a reduced degree of coordination of the local networks (2) potentially influencing the supraspinal centers, we

present here parallel coordinated data. The frequency means (not reported here) show no significant differences between the two experimental conditions. Still, strong differences are reported in the overall dynamic profile of the two networks and in their interrelationship. The LCS shows a reduction in message variability associated to the segregation of few functionally uniform neuronal subsets. The low frequency dependence degree of the results implies a deeper code for neural pain estimates.

The understanding of these conditions seems a most promising issue to be able to intervene on pain mechanism for a proper and efficacious therapeutic intervention.

#### SUMMARY

The Thalamo-Cortical somatosensory loop shows important synaptic re-organization in cases of chronic pain. Animal models exhibit severe functional distortions, potentially related to the anatomic rearrangements. Connectivity and information theoretic measurement represent important tools to quantify the functional disarrays. We performed electrophysiological experiments with multisite, multielectrode simultaneous recordings in the Thalamus and in the Somatosensory Cortex. The recurrent anomalies in the analytic estimates induce to hypothesize a potential neurodynamical explanation of the sensory context.

*Acknowledgements:* - The authors are grateful to Mr. Pasquale Petta for technical assistance

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