

# Paradoxical firing of thalamic neurons under neuropathic pain state in rats

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## Abstract

**OBJECTIVES:** A novel evaluative approach was used to determine single unit activities of non-bursting intralaminar thalamic nuclei under neuropathic pain state following dorsal rhizotomy.

**SETTINGS AND DESIGN:** Extensive dorsal rhizotomy at cervicothoracic level in rats was used as a model of central pain. After rhizotomy, rats were divided into two groups: rats without any signs of self-mutilation, and those presenting self-mutilation. Spontaneous single unit activities of neurons of intralaminar thalamic nuclei were recorded and interspike intervals (ISIs) of non-bursting cells were counted for both groups and compared with that of non-rhizotomized control rats. Chaodynamic methods were applied for the evaluation of the ISIs.

**RESULTS:** In control rats Lyapunov exponents, Shannon entropy and mutual information average values were significantly higher than those of rhizotomized rats without any signs of self-mutilation. Paradoxically, in animals presenting self-mutilation following rhizotomy the evaluated parameters were similar to those of controls. Further, Lyapunov exponents were positive values in all animals indicating chaotic pattern of the neuronal firing.

**MAIN FINDINGS:** 1. Neurons behave in chaotic way in all animals, 2. The most regular firing was found in non-mutilating rhizotomized animals, 3. Patterns of the firing in selfmutilating rats were similar to those in controls.

**CONCLUSIONS:** It is concluded that pain feeling is not executed neither by changes of chaotic dynamics of non-bursting intralaminar thalamic neurons. On the other hand, the paradoxical firing of the neurons under pathological brain matrix might participate in modification pain feeling.

## Abbreviations:

ANOVA analysis of variance  
ISIs interspike intervals  
SEM standard error of the mean

## 1. Introduction

Pain is a complex experience that involves not only the transduction of noxious environmental stimuli, but also cognitive and emotional processing by brain. The sensation of pain alerts the or-

ganism to the real or impending injury and triggers appropriate protective responses. Nearly a century ago, Sherrington [14] proposed the existence of the nociceptor, a primary sensory neuron that is activated by stimuli capable of causing tissue damage, and considered pain to be the sensory adjunct of an imperative protective reflex. Besides this kind of pain, namely nociceptive pain, clinically one also uses to speak about neuropathic pain caused by a primary lesion or dysfunction in the nervous system. Neuropathic pain is a chronic condition, and seems to have no purpose.

An extensive dorsal rhizotomy at the cervicothoracic level, i.e., the deafferentation in rats was used as a model of the central neuropathic pain [4]. Although there have been conflicting views on the correlation between self-mutilation and pain sensation in animal models [5,13], growing evidences would rather suggest the view of self-mutilation as a behavioral correlate of pain sensation [8,18]. In this context the self-mutilation in rats has been taken as a result of the development of chronic neuropathic pain. In our previous paper [17] it has been shown that approximately 60% of intralaminar neurones fire in bursts after the deafferentation. However, bursts correlated rather to the deafferentation than to self-mutilation. This finding is in good agreement with observation of Tasker et al. [16], who recorded single unit activity of thalamic neurons in man after deafferentation. In this case, bursting activity in man suffering from neuropathic pain as well as in man without chronic pain syndrome after the deafferentation was found. It seems that neuronal coding of pain states is to be executed in a different way.

As a consequence we decided to focus on single unit activity of non-bursting intralaminar thalamic neurons. The intralaminar nuclei are thought to be involved in motivational affective features of pain, which are likely to be of particular importance in chronic pain. Standard methods of analyzing a spontaneous single unit activity are based on the stochastic paradigm according to which it is assumed that timing of spikes is an ad hoc random process. However, a single

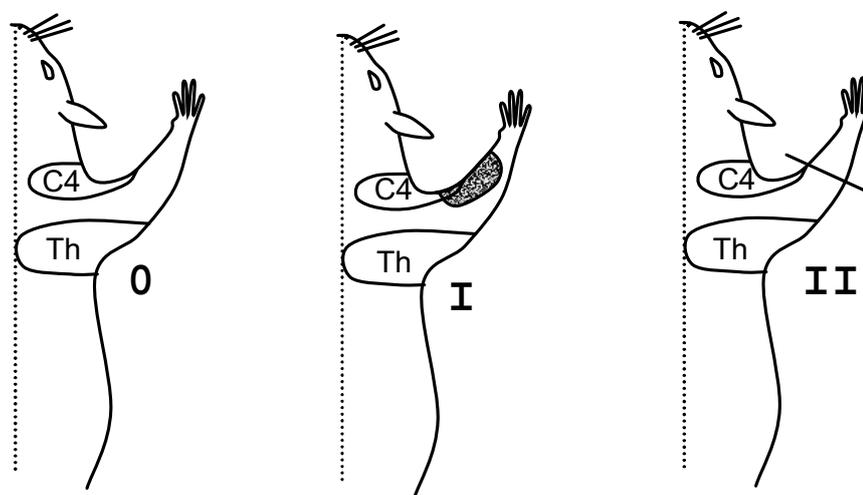
neuron has a potentiality to behave chaotically [3,6]. Therefore chaodynamic methods, namely Lyapunov exponents, Shannon entropy and mutual information, were applied for the evaluation of the interspike intervals (ISIs).

## 2. Materials and methods

**Animals and surgery:** Section of the dorsal roots of C5 – Th1 spinal nerves proximal to the spinal ganglion was performed in 8 Wistar male rats weighing between 250 and 340 g on the left side after the hemilaminectomy under pentobarbital (40 mg/kg) anaesthesia. After the surgery, animals were caged separately, observed daily for a maximum of 80 days, and they underwent electrophysiological recordings by 2 days after the onset of self-mutilation. The self-mutilation was evaluated at the three-grade scale based on qualitative pattern (Figure 1). Five rats exhibited self-mutilation degree I or II, and were considered to be taken into the first group (selfmutilating). Three rats without any signs of self-mutilation (degree 0) underwent the recordings on the postoperative day 80. Three control animals were sham-operated and the activities were recorded 80 days after the surgery.

The experiments were permitted by the Expert Committee for Animal Care and Use of 3rd Faculty of Medicine, Charles University, Prague and conducted according to the guidelines of the Ethics Committee of the International Association for the Study of Pain [19].

**Single unit recording:** For the recordings, animals were anaesthetized by intramuscular injection of a combination of ketamine (100 mg/kg) and xylazine (16 mg/kg). According to the Swanson atlas, the stereotaxic coordinates for the intralaminar thalamic nuclei were: AP: -4.2 mm, LL 1.5 mm and DV 3.6 – 6.0 mm to the bregma. At the end of the recording, the recording sites were iontophoretically signed for further histological verification. Extracellular single neurons activities were recorded by means of standard tech-



**Figure 1:** 3-grade scale for evaluation of self-mutilation: 0 – no self-mutilation; I – superficial wounds mostly located in the partially deafferentated area; II – total amputation of distal anesthetic part of the limb.

niques [17]. The data were digitized at a sampling rate of 6000 Hz. Software prepared under Microcal Origin 6.0 Labtalk was used for a calculation of interspike intervals.

**Chaodynamics:** Records containing bursts were excluded from the study. Presence of bursts were considered if ISIs lower than 2,5 ms were present in more than 1% of all ISIs. ISIs of non-bursting cells were analyzed using chaodynamic methods. The ISIs were used as a new time mapping to characterize the dynamics of firing neurons.

For the calculations of chaodynamic parameters the software package for the methods of chaodynamics worked out as M-tool-boxes under MATLAB at the Institute of Computer Science, Academy of Sciences, Prague and partly also TISEAN Package of Max Planck Institute for Physics of Complex System, Dresden were used.

Data in figure represent the mean  $\pm$  SEM. The chaodynamic parameters were calculated for every neurons, then the means were calculated according to the groups. To determine the statistical significance when comparing the means of the chaodynamical parameters between the groups, the ANOVA test (Statistica 6.0, StatSoft Inc.) was used. Differences between means were considered statistically significant if  $p < 0.05$ .

### 3. Results

Only few evidences of change in rats' behavior after the rhizotomy could be seen when surveilling rats freely moving in their cages. Programmed behavior (grooming) was identical to that of control animals. Only occasional misplacement of the deafferentated limb was noticeable in their movements. Sitting down on the hind legs, the rats kept the deafferentated fore-

limb in extension, but when the forelimb was to be used, again, almost no visible differences could be noticed.

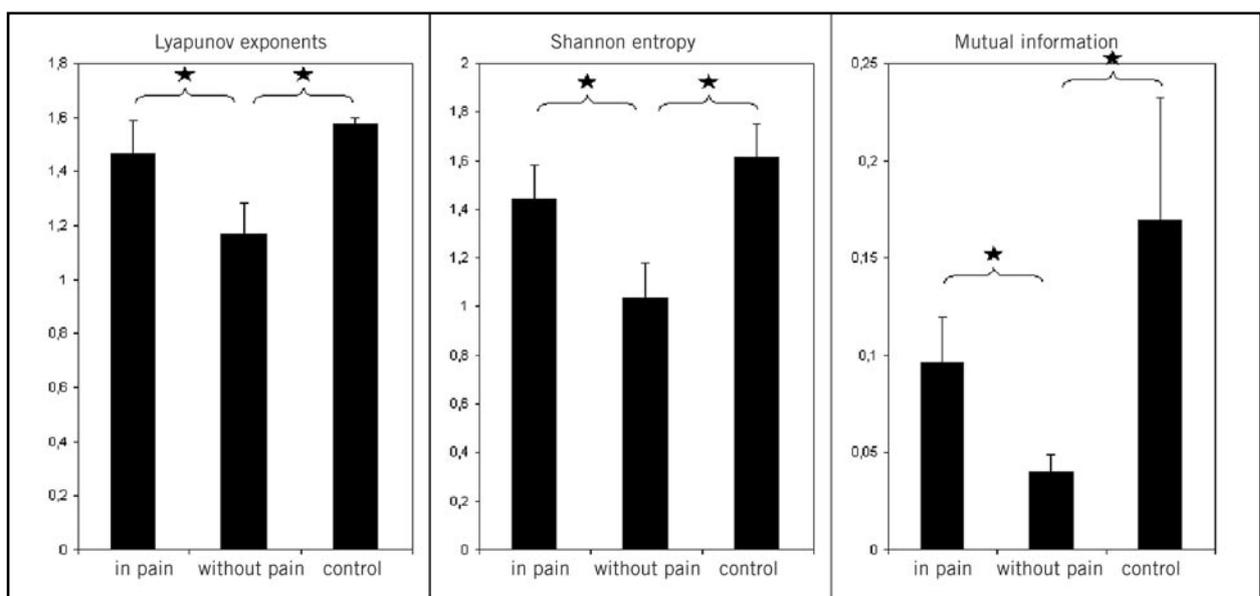
Light pinching with surgical forceps was applied to both forelimbs to evaluate the sensitivity in the rhizotomized forelimb. Touching the intact extremity became impossible whereas pinching in the deafferentated paw did not evoke any response.

The self-mutilation appeared in 63% of animals. The self-mutilation mostly consisted of superficial wounds on the dorsal surface of the forearm. Scratching appeared mostly in a partially deafferentated area corresponding to the distribution and to the overlapping of neighboring receptive fields in rats.

In the selfmutilating group, nine neurons were investigated. In the non-selfmutilating group and in the control group 10 and 8 neurons, respectively, were included in the study.

Lyapunov exponents calculated from ISIs series were positive values in all animals. Such a finding would indicate some level of chaotic manner in the neuronal firing. In the control animals mean Lyapunov exponent was  $1.576 \pm 0.020$ . In rhizotomized animals without any signs of self-mutilation mean Lyapunov exponent was significantly ( $p < 0.05$ ) lower:  $1.168 \pm 0.116$ . Interestingly, in animals presenting self-mutilation, mean Lyapunov exponent was similar to that of control group:  $1.466 \pm 0.120$ .

Shannon entropy and mutual information average values of control animals were  $1.611 \pm 0.141$ , and  $0.169 \pm 0.062$  respectively. In rhizotomized animals without any signs of self-mutilation the means were again significantly ( $p < 0.05$ ) lower:  $1.034 \pm 0.144$  and  $0.040 \pm 0.008$ , respectively. In animals presenting self-mutilation, means of Shannon entropy and mutual information were similar to that of control group:  $1.440 \pm 0.144$ , and  $0.096 \pm 0.023$ , respectively.



**Figure 2:** Comparison of Lyapunov exponents, Shannon entropy and mutual information in two experimental groups (rhizotomy with and without pain-related behaviour) and in control animals (mean  $\pm$  SEM;  $n = 5-6$ ). \*  $p < 0.05$ , ANOVA test.

#### 4. Discussion

To characterize a degree of chaoticity of observed time series data – ISIs, dynamical measures, as Lyapunov exponents, mutual information and Shannon entropy, were used [7,15].

The degree of instability of given dynamics is characterized by Lyapunov exponents. If such exponent is negative then one has a stable equilibrium state. For its positive value the dynamics is chaotic. With increasing value the process is more random. In the case the exponent equals to infinity it characterizes an ad hoc random process. Here the maximal Lyapunov exponent was calculated from the ISIs.

The Shannon entropy is the well-known measure of information contained in a series of symbols generated by a random source. It is measured in bits. In our case such a series of symbols is generated by a chaotic dynamics on a chaotic strange attractor reconstructed from the experimental ISI maps.

The other characteristic of chaotic dynamics is the mutual information. Here the time delayed mutual information suggested by Fraser and Swinney [7] was used. The mutual information of two in any way bound random processes is the information generated by the source of first process related to the information generated by the source of second process. In our case the first process is represented by a series of spikes generated by the given firing neuron and the second process is represented by the same series shifted in time by some time constant. Processes with a fixed point dynamics or limit cycles have the mutual information equal to zero. For the chaotic dynamics the mutual information is finite and with a growing value of mutual information also an informational entanglement (dependence) of both processes is increasing.

Chaotic behavior has been found in all investigated neurons. This is confirmed by positive values of Lyapunov exponents calculated from all ISIs series. It corresponds well with previous findings *in vivo*, e.g., the chaotic repetitive firing of action potentials in sinusoidal current stimulated squid giant axon [11], chaos in the spontaneous activity of some simian cortical neurons in the precentral gyri of anesthetized squirrel monkeys [12] or in measurements on sciatic nerve of adult male rats of ISIs and chaos in neural information coding. This is also in good agreement with *in vitro* recordings [9]. Furthermore, a chaotic dynamics has been shown to exist in many theoretical models of single neurons, e.g., in the refractory function model [1], in the Hodgkin-Huxley model with a periodic stimulation [10], what is in agreement with expectations resulting from mathematical analysis [2].

When comparing dynamics of chaotic firings of recorded units by means of ISIs maps of mentioned three groups (Figure 2), it was found out that deafferentation without any signs of self-mutilation results in significantly more regular firing than that in control animals. Surprisingly, when self-mutilation occurs after the deafferentation, the chaodynamic patterns of the intralaminar thalamic non-bursting neurons do not

differ from those of the controls. From the chaotic dynamics point of view, neurons paradoxically fire under neuropathic pain state in the almost similar way as under normal conditions. It might suggest potential protective effect of pain sensation in terms of single neuron activity. On the other hand, according to the pain matrix scheme, this paradoxical firing might be under new condition evaluated as painful. The possible role of non-bursting thalamic neurons either in the suppression of pain, or, on the contrary, in genesis of pain feeling is suggested.

#### Acknowledgements

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#### REFERENCES

- Aihara K, Takabe T, Toyoda M. Chaotic Neural Networks. *Phys Lett A* 1990; **144**:333–40.
- Andrey L. Potentiality of Dynamic Chaos in a Single Neurons with the Sigmoidal Transfer Function. In: Proceedings of the ICMP'97. Brisbane: International Congress of Mathematical Physics; 1997.
- Andrey L. Potentiality of Complex Behaviour in Single Neurons. In: Barbi M, Chillemi S, editors. *Chaos and Noise in Biology and Medicine*. Singapore: World Scientific; 1998. p.288–93.
- Basbaum AI. Effects of central lesions on disorders produced by multiple dorsal rhizotomy in rats. *Exp Neurol* 1974; **42**:490–501.
- Coderre TJ, Grimes RW, Melzack R. Deafferentation and chronic pain in animals: an evaluation of evidence suggesting autotomy is related to pain. *Pain* 1986; **26**:61–84.
- Judd KT, Aihara K. The role of dynamics in information processing by the brain. *Concepts Neurosci* 1993; **3**:123–33.
- Kantz H, Schreiber T. *Nonlinear Time Series Analysis*. Cambridge University Press; 2000.
- Kaupplila T. Correlation between autotomy-behavior and current theories of neuropathic pain. *Neurosci Biobehav Rev* 1998; **23**: 111–29.
- Matsumoto G, Aihara K, Hanyu Y, Takahashi N, Yoshizawa S, Nagumo J. Chaos and Phase Locking in Normal Squid Axons. *Phys Lett A* 1987; **123**:162–6.
- Matsumoto G, Aihara K, Ichikawa M, Tasaki A. Periodic and non-periodic responses of membrane potentials in squid giant axons during sinusoidal current stimulation. *J Theor Neurobiol* 1984; **3**: 1–14.
- Matsumoto G, Aihara K, Utsunomiya T. A Spatially-Ordered Pacemaker Observed in Squid Giant-Axons. *J Phys Soc Jpn* 1982; **51**: 942–50.
- Rapp PE, Zimmerman ID, Albano AM, Deguzman GC, Greenbaun NN. Dynamics of Spontaneous Neural Activity in the Simian Motor Cortex - the Dimension of Chaotic Neurons. *Phys Lett A* 1985; **110**: 335–8.
- Rodin BE, Kruger L. Deafferentation in animals as a model for the study of pain: an alternative hypothesis. *Brain Res* 1984; **319**: 213–28.
- Sherrington CS. *Textbook of Physiology*. Edinburg: Pentland; 1900.
- Sprott JC. *Chaos and Time-Series Analysis*. Oxford University Press; 2003.
- Tasker RR, Gorecki J, Lenz FA, Hirayama T, Dostrovsky JO. Thalamic microelectrode recording and microstimulation in central and deafferentation pain. *Appl Neurophysiol* 1987; **50**:414–7.
- Vaculin S, Franek M, Rokyta R. Dorsal rhizotomy changes the spontaneous neuronal activity of nuclei in the medial thalamus. *Physiol Res* 2000; **49**:279–83.
- Vaculin S, Rokyta R. Effects of anesthesia and nociceptive stimulation in an experimental model of brachial plexus avulsion. *Physiol Res* 2004; **53**:209–14.
- Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983; **16**:109–10.