Journal of Pharmacological Sciences 136 (2018) 172–176

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Short communication

Application of unfolding transformation in the random matrix theory to analyze *in vivo* neuronal spike firing during awake and anesthetized conditions

Risako Kato [a], Masanori Yamanaka [b, *], Masayuki Kobayashi [a, c, **]

[a] Department of Pharmacology, Nihon University School of Dentistry, 1-8-13 Kanda-Surugadai, Chiyoda-ku, Tokyo, Japan
[b] Department of Physics, Nihon University College of Science and Technology, 1-8-14 Kanda-Surugadai, Chiyoda-ku, Tokyo, Japan
[c] Molecular Dynamics Imaging Unit, RIKEN Center for Life Science Technologies, Kobe, Hyogo, Japan

ARTICLE INFO

Article history:
Received 27 December 2017
Received in revised form
11 January 2018
Accepted 18 January 2018
Available online 2 February 2018

Keywords:
Unfolding transformation
Spike-timing
Regularity

ABSTRACT

General anesthetics decrease the frequency and density of spike firing. This effect makes it difficult to detect spike regularity. To overcome this problem, we developed a method utilizing the unfolding transformation which analyzes the energy level statistics in the random matrix theory. We regarded the energy axis as time axis of neuron spike and analyzed the time series of cortical neural firing *in vivo*. Unfolding transformation detected regularities of neural firing while changes in firing densities were associated with pentobarbital. We found that unfolding transformation enables us to compare firing regularity between awake and anesthetic conditions on a universal scale.

Cortical neurons exhibit various temporal firing patterns (such as regular, burst, chattering, and random) and have wide variations in firing frequency depending on the neuronal subtype and the behavioral state.[1,2] The detection of regularity or randomness by autocorrelation and Fourier transformation[3,4] may be weakened in cases of firing with a mixture of high and low frequencies and/or variable firing patterns. Several studies have reported that general anesthetics decreased the neural and synaptic activities.[5] As a result, the density of spike firing is remarkably different between awake and anesthetic conditions.

Random matrix theory (RMT)[6–8] was introduced in physics in the 1950s to describe the energy spectrum of nuclei[9–12] and now is established as a methodology for analyzing randomly occurring events. Temporal features of action potentials in multiple types of neurons may be described as a simple temporal phenomenon by RMT: the unfolding transformation used in RMT could normalize each spike interval by the local average spike frequency and, similarly, generate spike firing on a universal axis. Here, we developed the unfolding transformation in RMT to study the time series of neuron firing under awake and anesthetized conditions by regarding the energy axis in physics systems as time axis of the neuron firing time series.

Eight-week-old male Wistar rats (n = 15, Japan SLC, Shizuoka, Japan) were attached with lightweight head attachments[13] (Narishige, Tokyo, Japan) to the skulls under 2–2.5% isoflurane anesthesia (Pfizer, Tokyo, Japan). Rats received analgesic (Carprofen, 5 mg/kg, s.c., Zoetis, Tokyo, Japan) and maintenance medium (10 ml, s.c., Sorita-T3, Ajinomoto, Tokyo, Japan). One week after surgery, the rats underwent a small craniotomy and an incision of the dura mater under 2.0–2.5% isoflurane anesthesia was made to insert the multi-channel recording electrode into the left side of the insular cortex (IC), which integrates nociception with limbic information.[14] Multiunit activities of IC were recorded using Plexon Recorder System (Plexon, Dallas, USA) under awake and pentobarbital-induced anesthetic conditions and were sorted into a single unit (1–2 units/channel) using Offline Sorter software (Plexon). All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of

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* Corresponding author. Department of Physics, Nihon University College of Science and Technology, 1-8-14 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8308, Japan.
** Corresponding author. Department of Pharmacology, Nihon University School of Dentistry, 1-8-13 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8310, Japan. Fax: +81 3 3219 8136.
E-mail addresses: yamanaka.masanori@nihon-u.ac.jp (M. Yamanaka), kobayashi.masayuki@nihon-u.ac.jp (M. Kobayashi).

Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2018.01.004

Laboratory Animals and likewise, approved by the Institutional Animal Care and Use Committee at Nihon University.

Statistical calculation of spike firing and the power spectral density of spike firing were performed using NeuroExplorer (ver. 4.110, Nex Technologies, USA). Bin width and time range of autocorrelograms were set to 50 ms and −10.0 to 10.0 s, respectively. The number of frequency values of Fourier analysis was 512 (bin width = 0.039 ms) with the maximum frequency set to 20 Hz.

We describe the unfolding transformation[6−8] for spike train and denote the experimentally recorded spike train by $\{t_i\}$, where t_i is the i-th spike time, $i = 1, 2, \cdots, N$, and N is the total number of spikes. Using the delta function, $\delta(t)$, the spectral function of spike is defined by

$$\rho(t) = \sum_{i=1}^{N} \delta(t - t_i). \tag{1}$$

We define the cumulative spectral function of spike as

$$\eta(t) = \int_{-\infty}^{t} \rho(s)ds = \sum_{i=1}^{N} \Theta(t - t_i), \tag{2}$$

where $\Theta(t)$ is the step function. This function counts the number of spikes which fire before or equal to t and is also called as the staircase function or the number function for spike. The relation between $\eta(t)$ and $\rho(t)$ is

$$\rho(t) = \frac{d\eta(t)}{dt}. \tag{3}$$

An example of the cumulative spectral function of spike is shown in Fig. 1. It is decomposed into a smooth part, $x(t)$, and a fluctuating part,

$$\eta(t) = x(t) + f(t), \tag{4}$$

where the smooth part is defined by averaging the cumulative spectral function, wherein averaging is done to make the density of the spike spectral function of spike to be one,

$$\rho(t) = \frac{d\eta(t)}{dt} = 1. \tag{5}$$

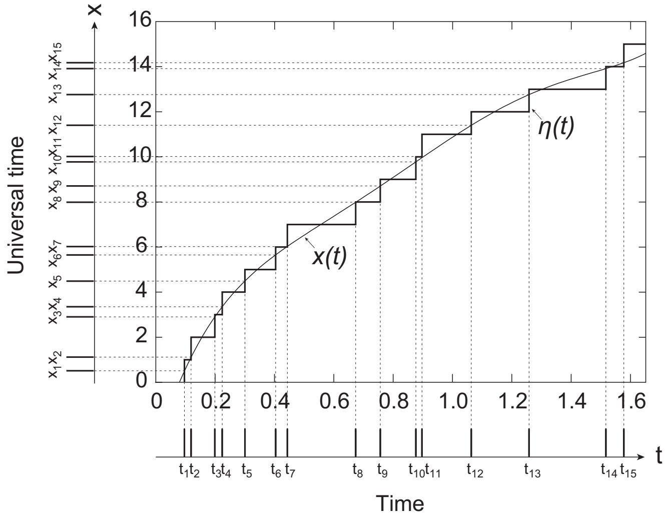

Fig. 1. Illustration of the actual unfolding procedure. Horizontal axis is the real time axis. Spike train obtained from the experiment is represented by t_i. Cumulative spectral function of spike, $\eta(t)$, is represented by the step function. Unfolding map, $x(t)$, is represented by a curve which is determined by the least-squares method. Vertical axis is the time after unfolding transformation which represents universal time.

In practice, the smooth part is approximated by a continuous function and is determined by fitting the cumulative spectral function to a higher-order polynomial using, for example, a least-square method. The unfolded spike train $\{x_i\}$ is obtained by

$$x_i = x(t_i), \quad i = 1, 2, \ldots, N. \tag{6}$$

In the unfolded variables, the cumulative spectral function of spike is $\tilde{\eta}(x) = x + \tilde{f}(x)$. We obtain unfolded ISI, $s = x_{i+1} - x_i$, and its distribution, $p(s)$, where it and its first moment are normalized to unity (Fig. 1).

We present a simulation that reveals a functional significance of the unfolding transformation. The dataset that includes Poisson distribution before and after unfolding transformation are shown in Fig. 2A and B. No peak is observed in a spike autocorrelogram or Fourier transformation (Fig. 2C). Unfolding transformation does not change this profile and both histograms of ISI show a Poisson distribution (Fig. 2C and D). The second dataset (Fig. 2E) is the distribution similar to that of regular neural firing (i.e., regular firing with some varied distribution). In autocorrelogram and Fourier transformation, some peaks before the unfolding transformation are detected, however, the peaks are ambiguous (Fig. 2G). Nevertheless, unfolding transformation makes the peaks clear (Fig. 2H). Comparing the ISI histogram, peaks are more conspicuous after unfolding transformation as compared to before (Fig. 2G and H). Thus, the detection rate of regularity can be improved by unfolding transformation.

Fig. 3 shows an example of the spike trains before and after the unfolding transformation during awake (top) and after pentobarbital injection (30 mg/kg, i.v.; bottom). The real time scale is stretched and diminished locally to make the spike density 1. High-density region in real time becomes sparse, whereas, a sparse region in real time becomes high-density on the universal time scale after unfolding transformation. For reference, ISI without unfolding transformation is shown in Fig. 3B and C. It is worth mentioning that we cannot distinguish whether data without unfolding transformation involve repulsion, which is observed in the case with spike correlation in a short time scale. However, unfolding transformation provides an advantage in solving this problem.

The plot in logarithmic graph (Fig. 3Dc) is well fitted with power function compared to prior unfolding transformation (Fig. 3Bc). Similarly, the plot in Fig. 3Ec is well fitted with exponential function compared to Fig. 3C. In addition, repulsion of the histogram is observed in Fig. 3Da. In cases of no correlation between two adjacent spike-timings, the distribution pattern of a plot is fitted by exponential decay without spike-timing repulsion as a Poisson process, whereas, deviation from the Poisson distribution implies a correlation between spikes. Except for the spike-timing repulsion, the results show that the detailed information for an individual neuron is inclusive of the short time part. For example, this sparse firing suggests power decay for $0 < s < 1$. This means this firing has a correlation in this short time, but has no correlation in the long time, $1 < s$.

RMT-based analysis is neither a simple rescaling of the firing rate nor a rescaling of partial sections of time but local rescaling throughout all arbitrary time. We summarized the advantage of the RMT-based analysis as follows: (i) quantitative decay rate evaluation of the long range part in ISI and spike-timing repulsion magnitude, and (ii) direct comparison of neural activities between awake and anesthetic conditions on a universal scale.