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Short communication

Information content of dendritic spines after motor learning

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ABSTRACT

Dendritic spines, small protrusions emerging from the dendrites of most excitatory synapses in the mammalian brain, are highly dynamic structures and their shape and number is continuously modulated by memory formation and other adaptive changes of the brain.

In this study, using a behavioral paradigm of motor learning, we applied the non-linear analysis of dendritic spines to study spine complexity along dendrites of cortical and subcortical neural systems, such as the basal ganglia, that sustain important motor learning processes.

We show that, after learning, the spine organization has greater complexity, as indexed by the maximum Lyapunov exponent (LyE). The positive value of the exponent demonstrates that the system is chaotic, while recurrence plots show that the system is not simply composed by random noise, but displays quasi-periodic behavior. The increase in the maximum LyE and in the system entropy after learning was confirmed by the modification of the reconstructed trajectories in phase-space. Our results suggest that the remodeling of spines, as a result of a chaotic and non-random dynamical process along dendrites, may be a general feature associated with the structural plasticity underlying processes such as long-term memory maintenance.

Furthermore, this work indicates that the non-linear method is a very useful tool to allow the detection of subtle stimulus-induced changes in dendritic spine dynamics, giving a key contribution to the study of the relationship between structure and function of spines.

1. Introduction

Animal behavior depends on the ability of the brain to optimize responses to stimuli. Brain plasticity occurs through modulation of neural connectivity that, in turn, reflects functional changes of synaptic strength, possibly accompanied by structural modifications. The dendritic spines are continuously modulated in shape and size during synaptic plasticity [1]. The dynamic relationship between spine's structure and function indicates that spines may function as biochemical and electrical compartments at individual synapses [2]. Serving as synaptic points, the spines play a crucial role in further extending the circuit connectivity. Indeed, each neuron can integrate a large number of inputs, and each spine can independently integrate its own set of inputs, making each connection point independently plastic [3].

Traditional methods of describing dendritic spines involve the classification and counting of spines based on their different shape [4]. These classical approaches mostly examine the average density of spines in various conditions such as memory formation, sleep or pathologies. Under *in vitro* conditions or by *in vivo* high resolution imaging, some dynamical variables, such as the average rate of formation/disappearance of spines, have also been estimated [5]. Changes in spine densities or volume are interpreted as changes in synaptic

Abbreviations: DiI, 1,1 = − dioctadecyl-3,3,3 = ,3 = − tetramethylindocarbo-cyanine perchlorate crystals; ITI, inter-trial interval; LyE, , Lyapunov exponent; PBS, phosphate buffer saline

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strength and therefore it is generally assumed that experience-dependent memory formation and maintenance corresponds to an increase in synaptic density [6,7]. Although this approach has addressed many important questions related to memory formation, it should be considered that the behavior of a network is not simply a function of the number of connections/synapses, but derives also from the pattern of the connections between nodes/neurons. Thus, networks with exactly equal number of connections may have completely different behavior depending upon their architecture [8]. Therefore, most information on the system organization/structure would be lost using exclusively the spine count-based approaches.

In an attempt to extract this type of information from microscopy images, statistical indexes such as entropy (a measure of the amount of information; [9]), or fractal dimension (a measure of complexity; [10]) have been used. Moreover, an appropriate assessment of dendritic spines organization should take into consideration the process of spine formation. The latter could derive from a chaotic process [11], that occurs in a deterministic (not random) way, very sensitive to the initial conditions as well as to small fluctuations. This theoretical framework has been already adopted in other biological fields [12–14]. It has been demonstrated that dendritic arborization can be addressed by the synaptic contacts established on newly extended dendritic filopodia [15], suggesting that the position and shape of the spines may reflect the dynamic process occurring along a dendrite. This dynamic spatial structure may be efficiently analyzed by nonlinear dynamics measurements that make possible to test, in cellular images [13], whether the distribution of spines along dendrites is periodic, random or chaotic, and if the learning processes or neuropathological conditions may modify these dynamics.

In this framework, we analyzed the spine distribution along dendrites using the most accepted method for quantifying chaos in a data series, the maximum Lyapunov exponent (LyE; [12,16]), that is inversely proportional to the predictability of the series. We hypothesized that the formation of long-term memories would increase the complexity of the spine distribution along a dendrite. To test this hypothesis, we trained mice with the rotarod test, a simple protocol of long-term procedural memory in rodents. This type of memory is mediated by a circuit that includes the dorso-lateral striatum [7], a telencephalic area belonging to the basal ganglia, particularly rich in medium spiny neurons.

We measured the maximum LyE in the striatum of trained and control animals to assess whether this nonlinear measure could be used to quantify the modulation of dendritic spines complexity following motor learning.

2. Materials and methods

Young male C57BL/6 mice (40 days old) were used for this study. All procedures conformed to the guidelines EU Directive 2010/63/EU for animal experiments. Animals were housed in group on standard 12:12 light:dark cycle with food and water *ad libitum*.

Mice were divided into two experimental groups and subjected to the rotarod test (Ugo Basile, Italy). One group of animals (trained mice, n = 5) was given 10 trials [cut off time = 300 s, Inter Trial Interval (ITI) = 300 s] on the accelerating rotarod (from 4 to 40 rpm in 5 min) for two consecutive days. The latency to fall down from the rotating rod was recorded. The animals of the second group (untrained mice, n = 9) were paired with the trained mice (one to one) and allowed to stay on the rod at a fixed speed of 4 rpm for the same time spent by the corresponding mouse during each of the 10 trials. Nine days after training, animals were tested on the rotating rod for 2 trials with an ITI of 300 s, to verify long-term memory maintenance. We selected nine days as retention interval since it was previously determined as the optimal time to assess the dynamic changes associated to long-term memory maintenance [17].

Immediately after the rotarod test, mice were intraperitoneally anesthetized with avertine and intracardially perfused with PBS, followed by fresh 4% paraformaldehyde in PBS. Brains were post-fixed in the same solution for 24 h at 4°, washed in PBS, and coronally sectioned in 100 μm thick slices with a vibratome. The sections were collected in PBS and exposed to solid 1,1 = −dioctadecyl-3,3,3=,3 = −tetramethylindocarbo- cyanine perchlorate crystals (DiI), a fluorescent lipophilic dye that exhibits enhanced fluorescence when inserted into the cell's membrane. Using the tip of a thin rod, crystals were evenly placed on the dorso-lateral surface of the striatum under stereomicroscopic inspection. Sections were then kept at 4 °C in a cell plate with PBS for 3–4 weeks.

Fluorescence images of DiI-labeled dendrites of the striatal medium spiny neurons were acquired using a Leica SP2 confocal microscope and analyzed according to the protocol described in [8].

The maximum LyE of profile plots was used to measure the structure of the variability occurring in a data series, in our case in the position and size of spines along dendrites [8].

To evaluate how many state variables (or number of embedding dimensions) were generating the dynamical process, we used the false nearest neighborhood algorithm (G).

Using the above described parameters [time lag 100 steps (1 μM, Fig. 1E), three embedding dimensions], we estimated the maximum LyE using the function in the t series Chaos library of R, based on the Kantz algorithm [18]. The other parameters used were: ref = 300 (number of points taken into account), k = 2 (number of considered neighbors), s = 100 (number of iterations along which follow the neighbors). The same experimental data were also used to calculate the entropy of the sequence using the maximum likelihood method. We also used recurrence plots to visualize recurrences (periodicities) of dynamical systems [19], in order to further validate the existence of a chaotic system and modifications of the attractors.

For statistical analyses, latency to fall off the rod was analyzed by two-way ANOVA for repeated measures to assess learning across days and trials in the trained group; testing day latency was analyzed using a two-way repeated measures ANOVA, mixed design (with group as a factor between subjects, and trial as a factor within subject). Data were first tested for sphericity (Mauchly's test), and then for equality in error variance (Levene's test). Morphological data (LyE, entropy) were subjected to *t*-test for non-paired data. Significance was set at $p \leq 0.05$.

3. Results

As shown in Fig. 2A, trained mice (two days of rotarod experience before the test, see methods) performed significantly better than untrained mice, when tested on the rotarod. Indeed, trained mice remained on the rod for slightly less than 300 s (maximum time for the trial), while untrained mice fell off the rod at about 220 s.

To verify if the improved rotarod performance of the trained mice was accompanied by morphological modifications of dendritic spines, we analyzed a total of 4182 DiI-labeled spines (2288 in striatum and 1894 in the cerebral cortex) of trained and untrained animals. Neurons labelled with DiI display a clear visualization of dendrites and dendritic spines (Fig. 1B). A count of the spine density along dendrites of striatal medium spiny neurons and cortical neurons did not reveal significant difference between the trained and untrained group (Fig. 2B). Since the spine density measurement does not give information on the spatial structure and/or organization of spines along the dendrite itself, we extended our analysis applying measures from nonlinear dynamics. Thus, we used the maximum LyE, that describes the divergence of the data trajectories in the state-space. A negative value indicates that trajectories do not diverge, and the system is not chaotic, while a positive value indicates that the system is chaotic or random. As shown in Fig. 2C this is the case of our data on dendritic spines, for both brain regions of trained and untrained mice. These results were confirmed by positive values of entropy for all the samples analyzed (Fig. 2D). Interestingly, the nonlinear dynamics analysis of our data showed