Chaotic and non-chaotic phases in experimental responses of a single neuron

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received 20 March 2014; accepted in final form 29 April 2014
published online 22 May 2014

PACS 64.60.aq – Networks
PACS 87.18.Sn – Neural networks and synaptic communication
PACS 05.45.Tp – Time series analysis

Abstract – Consistency and predictability of brain functionalities depend on the reproducible activity of a single neuron. We identify a reproducible non-chaotic neuronal phase where deviations between concave response latency profiles of a single neuron do not increase with the number of stimulations. A chaotic neuronal phase emerges at a transition to convex latency profiles which diverge exponentially, indicating irreproducible response timings. Our findings are supported by a quantitative mathematical framework and are found robust to periodic and random stimulation patterns. In addition, these results put a bound on the neuronal temporal resolution which can be enhanced below a millisecond using neuronal chains.

Introduction. – Neuronal chaotic dynamics were exhaustively examined on a network level [1–3], mainly using simulations [4–7], but never seen experimentally in the single neuron. The possible emergence of chaotic dynamics in a single neuron is a fundamental issue since it limits the reproducibility of neuronal responses, which is essential for achieving a desired level of predictability in human brain activity [8–10]. Thus, the quantitative examination of the intrinsic chaotic behavior of a single neuron, separated from its functional neural network, is required.

Three scenarios can be theoretically expected where reproducibility is quantitatively measured by the neuronal response timings for repeated identical sets of stimulations. First, unlimited reproducibility originated from neuronal deterministic responses is ideal, but unrealistic, due to noisy biological environments [11,12]. Second, neuronal response timings originate from an internal stochastic process [13], characterized by a small standard deviation around a biased value. Consequently, repeated stimulations of the neuron result in an additive noise which is expected to increase with the square root of the stimulation number. This minimal broadening source seems unavoidable and limits the reproducibility of the neuronal behavior. Last, very poor reproducibility originates from chaotic dynamics governing the responses of a single neuron. The difference in the neuronal response timings for repeated identical sets of stimulations is expected in such a chaotic dynamics to diverge exponentially with the stimulation number.

In this letter we examined the neuronal response latency, the time gap between stimulation and its corresponding evoked spike, of a neuron embedded within a large-scale network of cortical cells in vitro, but functionally separated from the network by synaptic blockers. The neuronal response latency is typically in the order of several milliseconds, and over few hundreds of periodic stimulations it shows a gradual increase which typically exceeds a millisecond [14,15] (fig. 1(a)). For each time step, the neuronal response latency is governed by a stochastic process characterized by an increase or decrease of tens of microseconds, \(\mu\)s, per stimulation (fig. 1(a), inset). The probability histogram of these local changes displays a small positive bias (fig. 1(b)), which over the course of stimulation leads to the overall accumulated increase of the response latency. The average and standard deviation of this histogram quantitatively change when different portions of the latency profile are taken into account; however qualitatively they remain in the same order as in fig. 1(b).

Non-chaotic phase. – The reproducibility of the neuronal responses can be quantified using local and global
This supreme reproducibility is a result of the concave av-
is seen here to accumulate to \( \sim L \) sampled from the probability histogram in fig. 1(b). For the simulated neurons (purple) compared to the experimental \( \sigma \) are less than 3 (fig. 1(c)). These variations are variations between its latency profiles under the same set of stimulation timings (fig. 1(c)). These variations are nearly constant over hundreds of periodic stimulations and are comparable with the standard deviation \( \sigma \) of local latency changes, e.g. for \( \sigma = 17 \mu s \) (fig. 1(b)) the variations are less than 3\( \sigma \) over 1800 stimulations (fig. 1(c)). This level of reproducibility is in contrast with a simple stochastic process, where local latency changes are independently sampled from the probability histogram in fig. 1(b). For such a stochastic process, \( \sigma \) among the latency profiles scales as the square root of the stimulation number, which is seen here to accumulate to \( \sim 0.7 \mu s \), in contrast with the experimentally recorded value of \( \sim 0.05 \mu s \) (fig. 1(d)). This supreme reproducibility is a result of the concave average latency profile, i.e. \( L'' = \frac{dL}{dStim^2} < 0 \) (fig. 1(c) and (e)), over several trials with similar stimulation profiles. For illustration, let us compare the time evolution of two initially close neuronal response latencies, \( L_1 \) and \( L_2 = L_1 + \gamma \), \( (L_2 - L_1 = \gamma) \). Following a stimulation, the latencies change to

\[
L_1 + L'(L_1)
\]

and

\[
L_2 = L_1 + \gamma + L'(L_2)
\]

in accordance with the first derivative of the latency profile (fig. 1(e), \( L' = \frac{dL}{dStim} \)). Now the difference between the two nearby latencies is

\[
\gamma + L'(L_2) - L'(L_1).
\]

Since the latency profile is concave, \( L'(L_2) - L'(L_1) < 0 \), the difference between two nearby trajectories around the average latency decreases and can be expressed as

\[
\gamma + L'(L_1 + \gamma) - L'(L_1) = \gamma + \gamma L''(L_1) = \gamma (1 + L''(L_1)).
\]

where the negative constant \( L'' \) represents an effective intermediate second derivative in this concave region. Now, an iterative process leads to a multiplicative effect

\[
\Delta_{Stim} = \gamma (1 + L''(Stim)) = \gamma e^{ln(1 + L'')}Stim,
\]

where \( \Delta_0 = \gamma \). The negative Lyapunov exponent, \( ln(1 + L'') < 0 \), indicates an exponential convergence of two nearby trajectories, allowing to overcome the inherent broadening of a stochastic process. This non-chaotic phase, represented by a concave neuronal response latency profile, is generally found in our experiments to scale in the leading order as \( Stim^{0.5} \) (fig. 1(c)).

This process is very similar to a Bernoulli map [16,17], \( X_{n+1} = (aX_n) \ mod \ 1 \), which is non-chaotic for \( a < 1 \). The variable \( X_n \) stands not for the neuronal response latency \( L \), but for \( L'(L_n) \). The simplification of the
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Bernoulli map is that $a - 1$ (i.e. $L''$) is a constant, independent of $L_n$, whereas $L''$ for the neuronal response latency varies as a function of $L_n$.

The supreme reproducibility seen under periodic stimulations (fig. 1(c)) remains robust even under random stimulation patterns (fig. 1(f)). The same neuron, receiving different stimulation patterns sampled at random from a given distribution, shows, as expected, local variations between its latency profiles (fig. 1(f), inset). However, on the global scale these variations produce a nearly constant $\sigma$ over many hundreds of stimulations.

Chaotic phase. – The concave average latency profile is typically followed by a convex average latency profile preceding the intermittent period [14,18] (fig. 2(a)), a transition which substantially varies among neurons. Qualitatively, this transition is accompanied by a rapid increase in $\sigma$ among slightly perturbed latency profiles around the average profile (fig. 2(a)), seemingly stemming from the point of transition; however, a quantitative analysis is still demanded. Initially, and far from the transition to the convex latency profile, the neuronal response latency displays a concave profile which scales as $\text{Stim}^{0.5}$ (fig. 2(b)), similarly to fig. 1(c). The entire averaged increase of the neuronal response latency, excluding the initial concave-like profile, was found to be well approximated by a cubic polynomial (fig. 2(b)). The derivatives for this scaled fit quantitatively pinpoint the critical stimulation, $\text{Stim}_C$, at which the transition from concave to convex latency profiles occurs (fig. 2(c)). Since in the convex region ($\text{Stim} > \text{Stim}_C$), $L'' > 0$, the Lyapunov exponent is positive, $\ln(1 + L'') > 0$, and an exponential divergence between nearby latency profiles is expected. Experimentally, the latency difference between two nearby trajectories fits better to an exponential divergence (linear fit between $\ln(\Delta_{\text{Stim}}) \sim \ln(\sigma)$ and $\text{Stim} - \text{Stim}_C$) than to a power law (fig. 2(d), inset), demonstrating that this is a chaotic process. Both the theoretical arguments and the experimental data indicate that the convex profile represents a new chaotic neuronal phase.

The number of stimulations until the emergence of the chaotic phase depends on the stimulation rate (fig. 3(a)). Nevertheless, a support for a universal behavior is found where the transition to a chaotic phase is roughly a function of the latency increase, independent of the stimulation rate (fig. 3(b), blue line).

Neuronal temporal resolution. – We demonstrate here an extremely robust feature of reproducibility in the responses of a single neuron, which was experimentally verified under both periodic and random stimulation patterns. This supreme reproducibility hints at the temporal resolution of neuronal responses, where during the concave (non-chaotic) phase of the neuronal response latency, one can identify two nearby stimulation rates from the knowledge of their non-overlapping neuronal latency profiles. The neuronal temporal resolution is a much investigated topic [19–21], although fundamental questions still

Fig. 2: (Color online) (a) Response latencies of a single neuron, stimulated at 20 Hz over 15 trials, their average (black) and smoothed $\sigma$ (green) using a 50 $\text{Stim}$ sliding window. (b) The average of the neuronal response latencies seen in (a) (gray). The approximated fit for $\text{Stim} \in [50,150]$ (teal) indicates, after rescaling, a dominating behavior $L \sim 0.85 \cdot (\text{Stim}/100)^{1.5}$ ms. For $\text{Stim} \in [100,600]$ the latency is well approximated by a cubic polynomial fit (orange). (c) $L'$ (blue) and $L''$ (black) computed from the cubic polynomial fit in (b), where $L'' = 0$ at $\text{Stim}_C = 247$. (d) A linear fit (dashed red line) for $\ln(\sigma)$ vs. $\text{Stim} - \text{Stim}_C$ (green), indicating a chaotic behavior with a Lyapunov exponent of 0.0034. The inset, $\ln(\sigma)$ vs. $\ln(\text{Stim} - \text{Stim}_C)$, excludes a power-law fit.
Fig. 3: (Color online) (a) Response latencies of a single neuron, stimulated at various frequencies (colored dots) and their cubic polynomial fit for Stim > 100 (full lines). (b) \( L'' \) obtained from the fit in (a) for different stimulation frequencies at response latencies 1.00 (red), 2.35 (green) and 1.62 (blue) ms.

remain unanswered. In the auditory system, for example, a microsecond time resolution is needed while neuronal spiking resolution is in the millisecond range, raising the question as to whether such a highly precise neuronal temporal code is possible.

Using the neuronal response latency, the neuronal temporal resolution can be approximated by the ratio between the latency gap between two latency profiles and \( \sigma \). For illustration, we examine the ratio between the latency profiles of a neuron stimulated at 8 and 20 Hz at its non-chaotic phase, and their variability, which results in a temporal resolution of \( \sim 9 \) ms (fig. 4(a)). Typically, \( \sigma \) was found to be frequency independent and the latency gap maximized after a large number of stimulations in the non-chaotic phase (fig. 4(a)). Consequently, the temporal resolution is expected to be enhanced towards a millisecond with the increase of the stimulation frequency. This experimentally calculated temporal resolution fits well with past results [19–21], but still cannot clarify the feasibility of sub-millisecond time resolution.

In order to enhance the temporal resolution, a neuronal chain [22] can be suggested (as in fig. 4(b), top). The latency gap in such a chain resulting from a pair of stimulation rates accumulates and increases linearly with the number of neurons constituting the chain, \( N \) (fig. 4(b)).

Fig. 4: (Color online) (a) Average response latencies obtained from 5 trials of a single neuron stimulated at 20 Hz (blue) and 8 Hz (red), and their smoothed \( \sigma \) using a 50 Stim sliding window. The zoom-in (gray area) shows 2\( \sigma \) broadening. The ratio \( 16\sigma/2\sigma = 8 \) indicates that the neuronal temporal resolution between 8 Hz (125 ms) and 20 Hz (50 ms) is \( (125 – 50)/8 \sim 9 \) ms. (b) Schematic of a neuronal chain consisting of \( N = 9 \) neurons (top), and its accumulated response latency at 20 Hz (blue) and 8 Hz (red). The average response latency for \( N = 1 \) (dashed lines, from (a)) is shown for comparison. The zoom-in (gray area) shows a 150\( \sigma \) latency gap, whereas the estimated broadening is \( 2\sigma \cdot \sqrt{9} = 6\sigma \). Their ratio, 150\( \sigma \)/6\( \sigma \) = 25, indicates a \( (125 – 50)/25 = 3 \) ms chain temporal resolution.

We experimentally demonstrated the emergence of a chaotic phase in the dynamics of a single neuron, using the reproducibility of the neuronal responses to the same stimulation pattern. This phase is characterized by a single positive Lyapunov exponent, and the transition between the non-chaotic and chaotic phases may be governed by several universal features. However, future research is required in order to understand the cellular mechanisms.
underlying these phenomena, as well as a generalization to dynamics under cell assemblies.

Methods. – Culture preparation. Cortical neurons were obtained from newborn rats (Sprague-Dawley) within 48 h after birth using mechanical and enzymatic procedures [14,15,18]. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Bar-Ilan University Guidelines for the Use and Care of Laboratory Animals in Research and were approved and supervised by the Institutional Animal Care and Use Committee. The cortical tissue was digested enzymatically with 0.05% trypsin solution in phosphate-buffered saline (Dulbecco’s PBS) free of calcium and magnesium, and supplemented with 20 μM glucose, at 37 °C. The enzyme treatment was terminated using heat-inactivated horse serum, and cells were then mechanically dissociated. The neurons were plated directly onto substrate-integrated multi-electrode arrays (MEAs) and allowed to develop functionally and structurally mature networks over a time period of 2–3 weeks in vitro, prior to the experiments. Variability in the number of cultured days in this range had no effect on the observed results. The number of plated neurons in a typical network was in the order of 1300000, covering an area of about 380 mm². The preparations were bathed in minimal essential medium (MEM-Earle, Earle’s Salt Base without L-Glutamine) supplemented with heat-inactivated horse serum (5%), glutamine (0.5 μM), glucose (20 μM), and gentamicin (10 g/ml), and maintained in an atmosphere of 37 °C, 5% CO₂ and 95% air in an incubator as well as during the electrophysiological measurements.

Synaptic blockers. All experiments were conducted on cultured cortical neurons that were functionally isolated from their network by a pharmacological block of glutamatergic and GABAergic synapses. For each culture 20 μl of a cocktail of synaptic blockers was used, consisting of 10 μM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), 80 μM APV (alpha-5-phosphonovalaric acid) and 5 μM bicuculline. This cocktail did not block the spontaneous network activity completely, but rather made it sparse. At least one hour was allowed for stabilization of the effect.

Stimulation and recording. An array of 60 Ti/Au/TiN extracellular electrodes, 30 μm in diameter, and spaced either 200 or 500 μm from each other (Multi-ChannelSystems, Reutlingen, Germany) was used. The insulation layer (silicon nitride) was pre-treated with polyethyleneimine (0.01% in 0.1 M borate buffer solution). A commercial setup (MEA2100-2×60-headstage, MEA2100-interface board, MCS, Reutlingen, Germany) for recording and analyzing data from two 60-electrode MEAs was used, with integrated data acquisition from 120 MEA electrodes and 8 additional analog channels, integrated filter amplifier and 3-channel current or voltage stimulus generator (for each 60-electrode array). Monophasic square voltage pulses (−800 mV, 200 μs) were applied through extracellular electrodes. Each channel was sampled at a frequency of 50k samples/s, thus the changes in the neuronal response latency were measured at a resolution of 20 μs.

Cell selection. Each node was represented by a stimulation source (source electrode) and a target for the stimulation of the recording electrode (target electrode). These electrodes (source and target) were selected as the ones that evoked well-isolated, well-formed spikes and a reliable response with a high signal-to-noise ratio. This examination was done with a stimulus intensity of −800 mV using 30 repetitions at a rate of 5 Hz followed by 1200 repetitions at a rate of 10 Hz.

Stimulation control. A node response was defined as a spike occurring within a typical time window of 2–10 ms following the electrical stimulation. The activity of the source and target electrodes of each node in the feed-forward neuronal chain was collected. Conditioned stimulations were enforced on the chain neurons, embedded within a large-scale network of cortical cells in vitro, following the chain connectivity. The timings of conditioned stimulations to the subsequent node in the feed-forward neuronal chain were computed off-line according to the timings of evoked spikes of the former node.

Data analysis. Analyses were performed in a Matlab environment (MathWorks, Natwick, MA, USA). Action potentials were detected off-line by voltage minima estimation. The reported results were confirmed based on at least eight experiments each, using different sets of neurons and several tissue cultures.

Spikes detection and response latency calculation by estimating voltage minima with interpolation. Recordings from selected electrodes were analyzed off-line, using a detection window of typically 2–10 ms following the beginning of a stimulation. In order to surpass the 20 μs timescale of the recording device, the following interpolation method was used. We fit a parabola to the local voltage minima (v2) and the two nearby voltage recordings (v1 and v3). The three coefficients (a, b, c) of the interpolated parabola v = at² + bt + c are determined using the three points (t1, v1), (t2, v2), (t3, v3) where t1 = t2 − 20 μs and t3 = t2 + 20 μs. One can verify that t̂min = 0.5(−4v2 + 3v1 + v3)/(v3 − 2v2 + v1). The neuronal response latency was then calculated as the duration from the beginning of a stimulation to t̂min.

Histogram of local neuronal response latency changes. A neuron was stimulated 1800 times at 10 Hz, over 5 trials. For each trial, the difference in the neuronal response latency per step was computed (ΔLi = Li − Li−1, where Li is the latency at the i-th stimulation). This data was used to generate a histogram of ΔLi consisting of 40 bins.

Neuronal response latency simulation. One thousand neurons were simulated for 1800 steps such that each ΔLi was sampled from the experimental ΔLi histogram. For each simulated step, a random number was selected from a
uniform distribution, \( U \sim [0, 1] \). The accumulated probabilities of the histogram bins were computed, and \( \Delta L_i \) was chosen from the bin with the largest accumulated probability smaller than the random number. For each trial, the simulated \( \Delta L_i \) were summed to show the accumulated latency. The first 50 simulated neurons are displayed in the inset of fig. 1(d).

** We thank Moshe Abeles for stimulating discussions. Invaluable computational assistance by Yair Sahar, Amir Goldental and technical assistance by Hana Arnon are acknowledged. This research was supported by the Ministry of Science and Technology, Israel.

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