Synchronization with mismatched synaptic delays: A unique role of elastic neuronal latency

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Abstract – We show that the unavoidable increase in neuronal response latency to ongoing stimulation serves as a nonuniform gradual stretching of neuronal circuit delay loops and emerges as an essential mechanism in the formation of various types of neuronal timers. Synchronization emerges as a transient phenomenon without predefined precise matched synaptic delays. These findings are described in an experimental procedure where conditioned stimulations were enforced on a circuit of neurons embedded within a large-scale network of cortical cells in vitro, and are corroborated by neuronal simulations. They evidence a new cortical time scale based on tens of µs stretching of neuronal circuit delay loops per spike, and with realistic delays of a few milliseconds, synchronization emerges for a finite fraction of neuronal circuit delays.

Introduction. – Psychological and physiological considerations entail that formation and functionality of neuronal cell assemblies [1–3] depend upon synchronized repeated activation such as zero-lag synchronization [4–6]. Several mechanisms for the emergence of this phenomenon have been suggested [7,8], including the global network quantity, the greatest common divisor of neuronal circuit delay loops [9,10]. However, they require strict biological prerequisites such as precise matched delays and connectivity, and synchronization is represented as a stationary mode of activity instead of a transient phenomenon [11,12].

Similarly, the harmonic activity of interconnected computational and communication devices requires accurate specifications, reliable units and precise wiring. Recently, these demands have been found to apply as well to the emergence of synchronous activity in other networks of threshold elements such as coupled laser networks [13–16]. Viewed from this perspective and given the compromised reliability of their building blocks, the capacity of biological neural networks to generate functional synchronizations on a millisecond time scale is puzzling.

We present in this letter theoretical evidence corroborated experimentally to show how the apparent variability in brain building blocks can turn into an advantage. We show that the unavoidable increase in neuronal response latency [17,18] to ongoing stimulation serves as a nonuniform gradual stretching of neuronal circuit delay loops. This apparent nuisance is revealed to be an essential mechanism in various types of neuronal timers since the emergence of synchronization emerges as a transient phenomenon and without predefined precise matched synaptic delays. These findings are described in an experimental procedure where conditioned stimulations [10] were enforced on a circuit of neurons embedded within a large-scale network of cortical cells in vitro [10,19–21], and are corroborated and extended by simulations of circuits composed of Hodgkin-Huxley (HH) neurons [22,23] with time-dependent latencies. An exhaustive enumeration of the space of a neuronal circuit with realistic delays of a few milliseconds indicates that synchronization is a common phenomenon that occurs for a finite fraction of neuronal circuit delays. These findings announce a
new cortical time scale based on tens of $\mu$s stretching of neuronal circuit delay loops per spike.

**Single-neuron level.** – At the single-neuron level, the most significant feature that appears to work against the formation of millisecond scale synchronies is the tendency of neurons when stimulated repeatedly to gradually change their stimulus-response delay over a few milliseconds [17,18] (see also the “Methods” section). To exemplify this neuronal feature, experiments were conducted on cultured cortical neurons that were functionally isolated from their network by a pharmacological block of both glutamatergic and GABAergic synapses [18,19]. Schematic of a neuron with 1:1 responses to a stimulation rate of 10 Hz (fig. 1(a)) and the experimental results for the neuronal response latency, time-lag between stimulation and evoked spike, are shown (fig. 1(b)). The results indicate that the latency increases by 4 ms in 100 s until critical latency is reached, where the neuron enters the intermittent phase [18]. The average increment of the latency per spike is 4 $\mu$s, which represents a new, finer time scale of cortical dynamics. The increase in the neuronal latency (internal dynamic) can be equivalently attributed to the extension of the self-feedback delay, whereas the neuronal response latency remains unchanged. For instance, the increase of 2 ms in the neuronal latency after 400 spikes is equivalent to the scenario where the neuronal response latency is unchanged and the self-feedback delay is extended to 100 + 2 ms (fig. 1(c)).

**Circuit-neuron level.** – While the precise underlying mechanisms might be system-specific (types of ionic channels and spatial considerations), it is generally agreed that the increase in latency reflects a decline in the exciting conductances and is fully reversible [17,18]. To analyze the impact of dynamic neuronal response latency at the circuit level, we artificially generated conditioned stimulations of a circuit of neurons embedded within a large-scale network of cortical cells in vitro (see the “Methods” section). Our first experimental design consisted of three neurons forming a heterogeneous neuronal circuit (fig. 2(a)), where the dynamics is initiated by an electrical stimulation to neuron A [10]. Conditioned stimulations were given according to the connectivity of the circuit; e.g., conditioned to an evoked spike from neuron B, neurons A and C are stimulated after $\tau$ ms. Since the circuit (fig. 2(a)) consists of 2$\tau$ (A → B → A) and 5$\tau$ loops (A → B → C → A) and the greatest common divisor GCD(2, 5) = 1, neuronal activity relaxes to zero-lag synchronization (ZLS) as was theoretically predicted [9,14]. Indeed standard simulations [9,10] of such a circuit indicate that after a short transient of 7$\tau$ ms ZLS is achieved (fig. 2(b)).

A slightly modified circuit is presented in fig. 2(c) with 2$\tau$ and 5$\tau$ + $\epsilon$ loops where ZLS is no longer a solution. Assume that the increase in the neuronal latency per spike, $\Delta$, is independent of the current latency (e.g., a linear fit up to 1100 spikes in fig. 1(b)). After $q$ evoked spikes per neuron, the effective delay loops are 2$\tau$ + 2$q\Delta$ and 5$\tau$ + $\epsilon$ + 3$q\Delta$, where the stretching of each unidirectional delay is illustrated by a red bar (fig. 2(d)). To verify whether the ratio 2 : 5 between the two loops is restored, the following equation is applied:

$$\frac{2\tau + 2q\Delta}{2} = \frac{5\tau + \epsilon + 3q\Delta}{5},$$

which indicates that after

$$q = \frac{\epsilon}{2\Delta}$$

spikes per neuron, ZLS is temporarily restored. Quantitative simulations of such a circuit model composed of HH neurons [9,10] with $\Delta =$ 0.004 $\mu$s, $\epsilon =$ 3 $\mu$s and artificially ignoring a second spike arriving at a neuron in a refractory period of 4 ms indicated that indeed the time-lag between the evoked spikes of neurons A and B decays linearly to zero after $q$ ≈ 3/(2 + 0.004) = 375 (eq. (2)) and is followed by desynchronization (fig. 2(e)). This phenomenon is robust for the case where all spikes are taken into account; however, when doubling the incoming coupling strength to neuron A, ZLS is achieved much faster (fig. 2(e)). Experimental results with $\epsilon =$ 1.5, 2.5 and 3 $\mu$s and $\tau =$ 80 ms indicated that the variable $\epsilon$ functions as a timer where the transient time to achieve synchronization increases with $\epsilon$ (fig. 2(f)–(h)). The noisy quantized behavior is an outcome of the experimental time resolution, 0.5 ms in the timing of stimulations and 1/16 ms in the identification of evoked spikes, as is evidenced by the large fluctuations around the edges of the latency stairs (fig. 2(f)–(h)).

The timer (fig. 2) depicts experimentally a synchronous activity with coordinated mismatches of 1–4 ms over synaptic delays of 80–100 ms, where such slightly relative imprecise delays still represent inflexible prerequisite biological conditions. Furthermore, transient periods to synchronous activity of a few hundreds of $\tau$ ms are also beyond the typical cortical response time scale. Producing much shorter delays which are relevant to cortical dynamics as well as monitoring circuits composed of much larger
numbers of neurons are currently beyond our experimental capabilities. Nevertheless, these types of neuronal circuits are scalable, where the stretching of synfire chains [5] (loops) increases linearly with the number of their relays. In addition, neuronal response latencies increase significantly faster (by one order of magnitude) in the initial spiking activity. Both of these ingredients are expected to significantly shorten the transient to synchronization.

Nonlinear stretching. – The quantity at the basis of this timer is the (entire loop latency stretching)/(unit delay \( \tau \)), which in our example (fig. 2) is \( \Delta (2\Delta/2) \) for the shorter loop (2\( \tau \)) and is only 3/5\( \Delta \) for the longer loop (5\( \tau \)) (eq. (1)). Hence, the relative stretching of the shorter loop is faster and compensates for the redundancy \( \epsilon \) of the longer loop and synchronization is achieved. The inverse scenario, leading to another type of timers, is shown by the heterogeneous circuit with 5 neurons and two loops, 4\( \tau \) and 6\( \tau \) (fig. 3(a)). Since GCD(4, 6) = 2, the neuronal activity relaxes into two neuronal clusters, (A, B, D) and (C, E), where each neuronal cluster is in ZLS. Simulations of such neuronal circuits confirm that after a transient of 9\( \tau \), two clusters are formed (fig. 3(b)). A modified circuit with 4\( \tau \) and 6\( \tau \) loops does not maintain ZLS between neurons A and B (fig. 3(c)). After a neuron evoked \( q \) spikes, its incoming unidirectional delays elongate by \( q\Delta \) as represented by the red bars (fig. 3(d)). The restored ratio 4 : 6 between the two loops is given by the equation

\[
\frac{4\tau + 2q\Delta}{4} = \frac{6\tau - \epsilon + 5q\Delta}{6}
\]

which results in

\[
q = \frac{\epsilon}{2\Delta}
\]

and the restored duration is 2\( \tau \), as the firing period is GCD \( \times \) \( \tau = 2\tau \) (fig. 3(b)). Simulations with \( \Delta = 0.004 \) ms, \( \epsilon = 3 \) ms and ignoring spikes in a refractory period of 4 ms to a neuron indicate that \( q \sim 3/(2 + 0.004) = 375 \) (fig. 3(e)), whereas including all spikes in the dynamics results in the same phenomenon but a shorter transient (fig. 3(e)). Experimental results with \( \epsilon = 3 \) ms and \( \tau = 60 \) ms indicate that ZLS is achieved after \( q \sim 200 \) spikes (fig. 3(f)), and exemplify the robustness of the proposed mechanism to nonidentical profiles (mostly concave) of the 5 neuronal response latencies (not shown here). Actually, the emergence of ZLS is controlled by the relative stretching of the two loops \( \Delta L_{4,6} = (L_A + L_B + L_C + L_D + L_E)/6 - (L_A + L_B)/4 \) (fig. 3(g)). The prominent feature of this timer is that after a transient trajectory, synchronization appears as a stationary phenomenon (fig. 3(e)–(f)), unlike the momentary synchronous activity of the first timer (fig. 2(e)–(h)).

These two types of timers are not an outcome of their different GCD, but rather originated from their intrinsic structures. The first moment where two neuronal clusters emerge (fig. 3(a), (b)), neuron A receives two
evoked spikes via two identical delay routes: \((A \rightarrow B \rightarrow A)\) and \((B \rightarrow C \rightarrow D \rightarrow E \rightarrow A)\). As the dynamics evolves, the first route becomes relatively shorter since its expansion comprises only two latencies (fig. 3(d)). Hence, a spike absorbed via the second route falls into the refractory period of the spike that arrived earlier via the first route. Practically, the circuit degenerates into two face-to-face \((A \leftrightarrow B)\) neurons. Similarly, for the first timer when ZLS first emerges (fig. 2(a)), neuron A receives two evoked spikes via two identical delay routes: \((B \rightarrow C \rightarrow A)\) and \((A \rightarrow B \rightarrow A \rightarrow B \rightarrow A)\). The expansion of the second route is relatively larger, since it comprises more latencies and consequently its spike falls into the refractory period of the first route. Practically the circuit degenerates into a directed heterogeneous loop, \((A \rightarrow B \rightarrow C \rightarrow A)\), where synchronous activity cannot be maintained.

**Phenomenological approach.** – A phenomenological approach to characterizing the emergence of neuronal timers requires a mapping between actual circuit delays (figs. 2(d) and 3(d)) and the dynamics of each neuronal response latency (fig. 1(b)). The key element is the increase in neuronal response latency with stimulation frequency (fig. 4(a)), where it can be approximated by a linear fit before arriving at an intermittent phase (fig. 4(b)). For sufficient high frequencies the increase in the neuronal response latency per spike saturates and converges to \(\Delta L_0\) (fig. 4(b)). The latency now has two main components; an abrupt increase per spike and slow recovery, fading (fig. 4(c)). Although neuronal response latencies are highly varied, in principle for each delay the equation below can be written

\[
d\tau_{ij}/dt = \Delta L_0 \delta(t - t_i) - dF(t - t_i)/dt, \quad (5)
\]

where \(\tau_{ij}\) is the delay from neuron \(j\) to \(i\), \(t_i\) is the timing of the last spike of neuron \(i\) and the last term stands for the derivative of latency fading (fig. 4(c)) with respect to the elapsed time since the last spike. This set of circuit delay equations together with the HH circuit equations [9,10] comprise the entire network dynamical behavior. Since the number of delays is superior to the number of neurons some of these additional equations are redundant; however, an update of each neuronal latency by its internal parameters requires an additional physiological insight [24].

Delays of a few ms are still beyond our experimental capabilities. Nevertheless, we theoretically analyzed a six-neuron circuit consisting of \(X\) and \(Y\) delay loops \((Y \geq X\) \) with a maximal 5 ms latency increase (fig. 4(d)). The condition to achieve ZLS (in nontrivial locations) is given by

\[
\frac{x + 3\Delta}{m} = \frac{Y + 5\Delta}{n}, \quad (6)
\]

where \(\Delta\) represents the current latency increase, \(n > m\) and \(\text{GCD}(m, n) = 1\). One can verify that the larger loop is bounded by

\[
y_1 = \frac{nx}{m} \quad \text{and} \quad y_2 = \frac{nx}{m} + 5\frac{(3n - 5m)}{m}, \quad (7)
\]
Fig. 4: (Color online) (a) Neuronal response latency as a function of spikes for various stimulation frequencies in the range of 5–35Hz. (b) The average increase in the neuronal response latency per spike, before arriving at an intermittent phase, is obtained using a linear fit for each stimulation frequency in (a). (c) Fading in the neuronal response latency as a function of the elapsed time since the last spike. Results are estimated from (b) where 35Hz is taken to be the asymptotic value for high stimulation frequencies. (d) Schematic of a heterogeneous neuronal circuit consisting of two delay loops, X and Y (Y > X), where delays among the six neurons are chosen arbitrarily. (e) Initial conditions for 5 ≤ X ≤ 10 ms and 5 ≤ Y ≤ 20 ms leading to ZLS under the assumption of a maximal 5 ms increase in the response latency of each neuron. Initial (X, Y) delay loops leading to delay loops obeying the ratios 1 : 2 or 2 : 3 are marked by the two bounded gray regions. The fraction of the initial (X, Y) leading to ZLS is 50%.

and X > 5(5m – 3n)/n. For 5 ≤ X ≤ 10 ms and 5 ≤ Y ≤ 20 ms, ZLS is achieved in at least 50% of the possible initial X and Y delays (fig. 4(e)).

**Conclusion.** – Synchronous flashes and epochs are expected to be a common phenomenon in neuronal brain activity. Their probability of occurrence is anticipated to be enhanced by much shorter biological delays and by long synfire chains along the circuit loops which enhance the stretchability of the neuronal circuit. In a set of simulation studies at the population dynamics (cell assembly) level we demonstrated that the experimentally exemplified new cortical mechanism remains valid (fig. 5), and the circuit activity becomes less sensitive to background fluctuations [9]. This feature is especially crucial to the realization of shorter neuronal loops, where the activity of a single neuron will stop spontaneously due to the relative neuronal refractory period or synaptic fatigue. It is also evident that the variety of possible timers is much larger, in that sub-neuronal circuits might consecutively pass through a few types of synchronous activities during the entire latency increase and also in the intermittent phase where the neuronal response latency fluctuates by few ms [18].

**Methods.** – Cell preparation. Cortical neurons were obtained from newborn rats (Sprague-Dawley) within 48 h after birth using mechanical and enzymatic procedures described in earlier studies [10,18,19]. Rats were euthanized by CO2 treatment according to protocols approved by the National Institutes of Health. The neurons were plated directly onto substrate-integrated multi-electrode arrays and allowed to develop functionally and structurally mature networks over a time period of 2–3 weeks. The number of plated neurons in a typical network is on the order of 1300000, covering an area of about 380 mm2. The preparations were bathed in MEM supplemented with heat-inactivated horse serum (5%), glutamine (0.5 mM), glucose (20 µM), and gentamicin (10 g/ml), and maintained in an atmosphere of 37°C, 5% CO2, and 95% N2.
and 95% air in an incubator as well as during the recording phases. All experiments were conducted in the standard growth medium, supplemented with 25 μM Bicuculline, 50.26 μM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and 401.6 μM APV (amino-5-phosphonovaleric acid); this cocktail of synaptic blockers made the spontaneous network activity sparse. At least an hour was allowed for stabilization of the effect.

**Measurements and stimulation.** An array of 60 Ti/Au/TiN extracellular electrodes, 30 μm in diameter, and spaced either 500 or 200 μm from each other (Multi-Channel Systems, Reutlingen, Germany) was used. The insulation layer (silicon nitride) was pre-treated with polyethyleneimine (Sigma, 0.01% in 0.1 M Borate buffer solution). A commercial amplifier (MEA-1060-inv-BC, 3M/12H, UEI, Walpole, MA, USA). Each channel was digitized using a data acquisition board (PD2-MF-64-1024 was used. Mono-phasic square voltage pulses (100–500 μs, 100–900 mV) were applied through extracellular electrodes. The data were detected online by threshold crossing. Data processing and conditioned stimulation were performed using Simulink (The Mathworks, Natick, MA, USA) based xPC target application.

**Cell selection.** Each circuit node was represented by a stimulation source (source electrode) and a target for the stimulation the recorded electrode (target electrode). The stimulation electrodes (source and target) were selected as the ones that evoked well-isolated and 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, after 30 repetitions at a frequency of 5 Hz.

**Stimulation control.** The activity of all target electrodes for each stimulation was collected and entailed stimuli were delivered in accordance to the adjacency matrix.

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