Cholinergic modulation of phase response curves of cortical pyramidal neurons.

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Abstract:

When a periodically firing neurons is perturbed the spike is either advanced or delayed. The spike-time shift is the Phase Response Curve (PRC). Theory shows that neurons with strong spike frequency adaptation (SPA) should show type-II (biphasic) PRCs while non-adapting neurons have type-I (strictly positive). We tested the hypothesis that the PRC shape and/or type can be changed by acetylcholine modulating the SPA currents. We recorded from layer II/III pyramidal neurons in mouse visual cortex. Via current injection we depolarized the neurons above threshold and injected brief current pulses at random times during the inter-spike interval to determine the PRC. This was repeated after bath application of 20ìM of carbachol. Cholinergic neuromodulation transformed the phase-reset curve from type II to type I. Since the PRC shape has implications for neuronal synchronization behavior, such modulation can change the global behavior of cortical networks.

Introduction:

Neurons produce periodic action potentials due to dynamics of voltage gated cross membrane conductances. The analysis of neural excitability has established two mechanisms of spike generating dynamics. The mechanism in giant squid axons (Hodgkin-Huxley) has become known as Type II excitability, and is identified with a subcritical Hopf bifurcation *in computo*.

In vitro this mechanism implies action potentials of variable amplitude and a minimal firing frequency that is non-zero, determined by the frequency of the subthreshold oscillations.

Mechanisms of spike generation in most popularly used models of cortical pyramidal neurons are associated with a Saddle-Node bifurcation and are termed Type I. Such dynamics imply all-or-none action potentials, zero onset frequency and a general absence of subthreshold oscillations. In general such bifurcation are difficult to identify *in vitro*, yet one can observe certain quantities associated with the each. One such quantity is the Phase Response Curve (PRC).

The PRC measures how the phase of an oscillator is perturbed by transient inputs at different points in its firing cycle. For a neuron the PRC gives the change in the timing of the spike as function of when the perturbing stimulus occurs within the firing cycle measured in terms of the phase relative to the spike). When the amplitude of the perturbation is allowed to go to an infinitesimally small limit, we can obtain the infinitesimal PRC (here in referred to as {\it iPRC}) (see Hansel et al. 1995), which can be computed using numerical integration.

Ermentrout (1996) proved that Type I systems should have a strictly positive PRC, while type II systems have PRCs that are bi-phasic. We have hypothesized that slow Potassium currents, under modulatory control of Ach, can convert cortical pyramidal neurons from one type to another.

We measured PRCs of cortical pyramidal neurons in vitro under control conditions and under application of a cholinergic agonist carbachol. Our preliminary results show that neurons with initially biphasic PRCs transform into mono-phasic PRC under muscarinic modulation. The experimental results are backed up by simulations of conductance based models with I-M and a canonical model. Computational and experimental results together suggest that the dynamics of neural excitability and the bifurcation structure of spike generation are modulated by action of acetylcholine.

Methods:

We recorded with the patch-clamp technique from layer II/III pyramidal neurons in the slices of the mouse (P28 to P35) visual cortex. Tonic spiking was evoked by injecting DC current through the recording electrode. Short (40msec) depolarizing (20 to 40pA) current pulses were injected at regular intervals multiple times the length of the ISIs (typically 1 sec.).

At least 6 traces of 32 seconds of continuous spiking, perturbed by the short pulses, were recorded. Then the cholinergic agonist carbachol (20_M or 50_M) was bath applied and at least 6 more traces of continuous perturbed spiking were recorded. During the majority of experiments excitatory synaptic transmission was blocked by DNQX (20_M) and APV (50_M). The change of the duration of the ISI (Δt ISI) was determined as the difference of the ISI containing the pulse to the mean of the 5 previous ISIs. This difference was plotted as a function of phase or relative time (0... previous spike, 1... next spike) of the perturbing pulse (Fig.2, plots on top). These differences were then binned in regard to relative time, averaged within bins and represent the phase-reset curve (PRC).

For numerical simulations used a single compartment model for a cortical pyramidal neuron due to Golomb and Amitai (1997). Parameters are as in Golomb and Amitai (1997), with the exception of the maximal conductance for the muscarinic potassium current conductance, g_M, which was varied as a free parameter. The background firing rate of the neuron was controlled with a DC injection. The infinitesimal PRCs were computed using averaging techniques in XPPAUT software. 4th order Runge-Kutta method was used.

Results:

We recorded from 8 layer II/III cortical pyramidal cells. These cells displayed regular spiking with a mean frequency ranging from 2.6 to 8.6 Hz in response to DC current injection (Fig.1). The return map in Fig.1D shows that there is no apparent temporal structure in the variance of the ISIs. When short depolarizing pulses were injected during the ISIs, two types of PRCs were observed: purely positive and biphasic.

In 6 neurons the PRCs were biphasic, showing a delay of spiking when the perturbing pulse was given in the initial phase of the ISI and an acceleration of spiking when the perturbing pulse was given towards the end of the ISI (Fig.2A, B). Cholinergic neuromodulation qualitatively changed these PRCs, making them purely negative. Figure 3 shows an average of the PRCs of these cells with and without cholinergic neuromodulation.

In 2 neurons the PRCs were purely negative, showing an acceleration of spiking at all relative pulse times, albeit to a different degree (Fig.2C). These PRCs were not qualitatively changed by cholinergic neuromodulation. However we did observe that the left-ward skew in such PRCs decreased in the presence of carbachol.

We have confirmed with simulations and direct computation of the PRC from the biophysical model that the methods used to measure the PRC experimentally do indeed approximate the theoretically computed PRC (simulations not shown). Simulations with the biophysical model indicate that blocking the muscarinic sensitive potassium current decreases the negative portion of the PRC. At sufficiently low levels of I-M the PRC becomes purely positive, and with the IM completely blocked the PRC develops a rightward skew. In fact, the negative portion of the PRC is present when the adapting neuron is firing at relatively low rates, while at higher firing rates the negative portion is reduced (simulations not shown). These simulations support our hypothesis that cholinergic modulation of the slow potassium currents controls the shape of the PRC.

Summary and Conclusions:

Our combined experimental and theoretical analysis shows that the modulatory influence of Ach induces changes in the shape of the Phase Response Curves in cortical pyramidal neurons. We believe this is the first experimental measurement the influence exerted by a neuromodulator on phase response functions in cortical neurons. While the PRC gives information about how a neural oscillator reacts to differently time transient inputs, its shape is largely determined by the way in which the neuron makes the transition from rest to repetitive firing (Hansel et al. (1995), Ermentrout (1996), and Izhikevich (1999)). The type II dynamics lead to biphasic PRCs while type I dynamics imply positive PRCs. We show experimentally that type II PRC are converted into Type I PRCs by the action of carbachol. Furthermore, computational results confirm the hypothesis that the change in the PRC type is due to a block of the muscarinic sensitive K-current. The change in the PRC is compatible with a change in the bifurcation structure of neural excitability: due to the M-current like adaptation neuron changes from a saddlenode oscillator (Type I) to a subcritical Hopf oscillator (Type II). Thus the measurements we present here in hint at a change in the bifurcations underlying neuronal excitability induced by acetylcholine.

Previously it was shown theoretically that type I neurons synchronize only with inhibitory coupling, while type II synchronize by excitation. Also type II neurons have strong resonant frequencies, while type I neurons can generate a continuum of firing rates. Thus our experimental measurements of the cholinergic modulations of the PRCs in cortical neurons may

explain the desynchronization of neural activity and the concomitant increase in the mean firing rates, seen in awake states when ach is present in the cortex.

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References:

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Figure Legends.

Figure 1. Example recording. A: 32 seconds of continuous spiking perturbed by brief current pulses every second. B: Detail of A. C. Time course of ISIs during the spike train. D. Return map for consecutive ISIs preceding a current pulse. Note that lack of structure indicates that there was no systematic drift in the last 5 ISIs and we can use the average in calculating the PRC.

Figure 2. Example of PRCs determined in 3 different pyramidal neurons. Note that carbachol changes the shape of the ISI, in some cases negative portions disappear. Left column: PRC under control condition. Right column: PRC during the bath application of carbachol. Upper plots: y-axis gives changes in in ISI duration for each trial (each dot); x-axis gives time of the pulse relative to the control ISI duration. Lower panel: y-axis average values for ISI change; x-axis: ISI was divided into 10 bins.

Figure 3. Results of the Biophysical Model: blocking the I-M converts a bi-phasic PRC into a purely positive PRC, confirming the hypothesis that the effects of carbachol on the PRC is mediated though blocking the spike frequency adaptation. X-axis is in %ISI, Y-axis is arbitrary.
A. Left: Control PRC computed for the model with full level of adaptation, Right: PRC with adaptation current blocked.
B: Progressively blocking the I-M increases the firing rate as well as changing the shape of the PRC: g_M 4; Fr ~10 Hz (solid), 3, Fr ~35Hz (dashed), 1 Fr ~80 (dash-dot).

Figure 1.

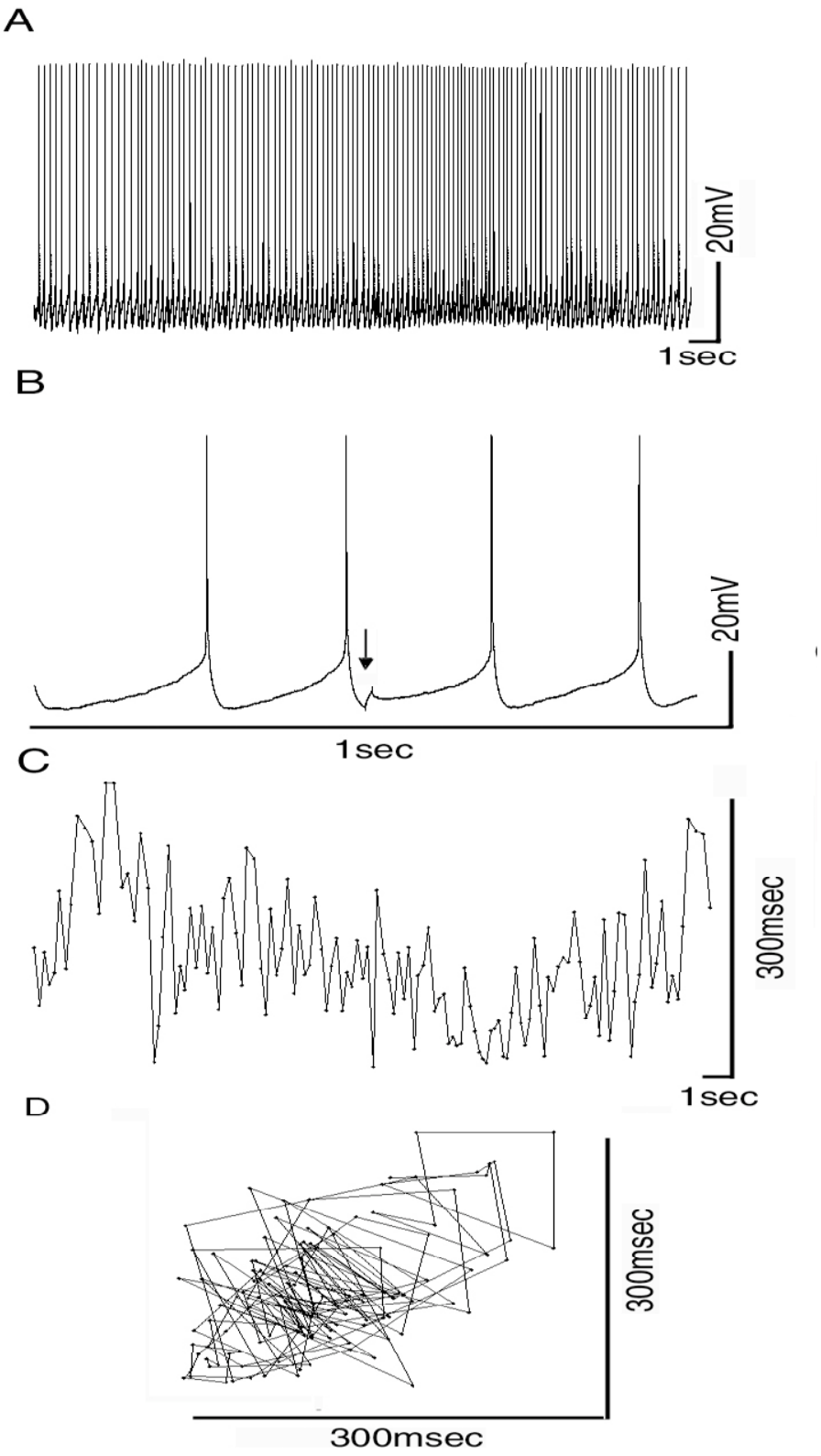


Figure 2.

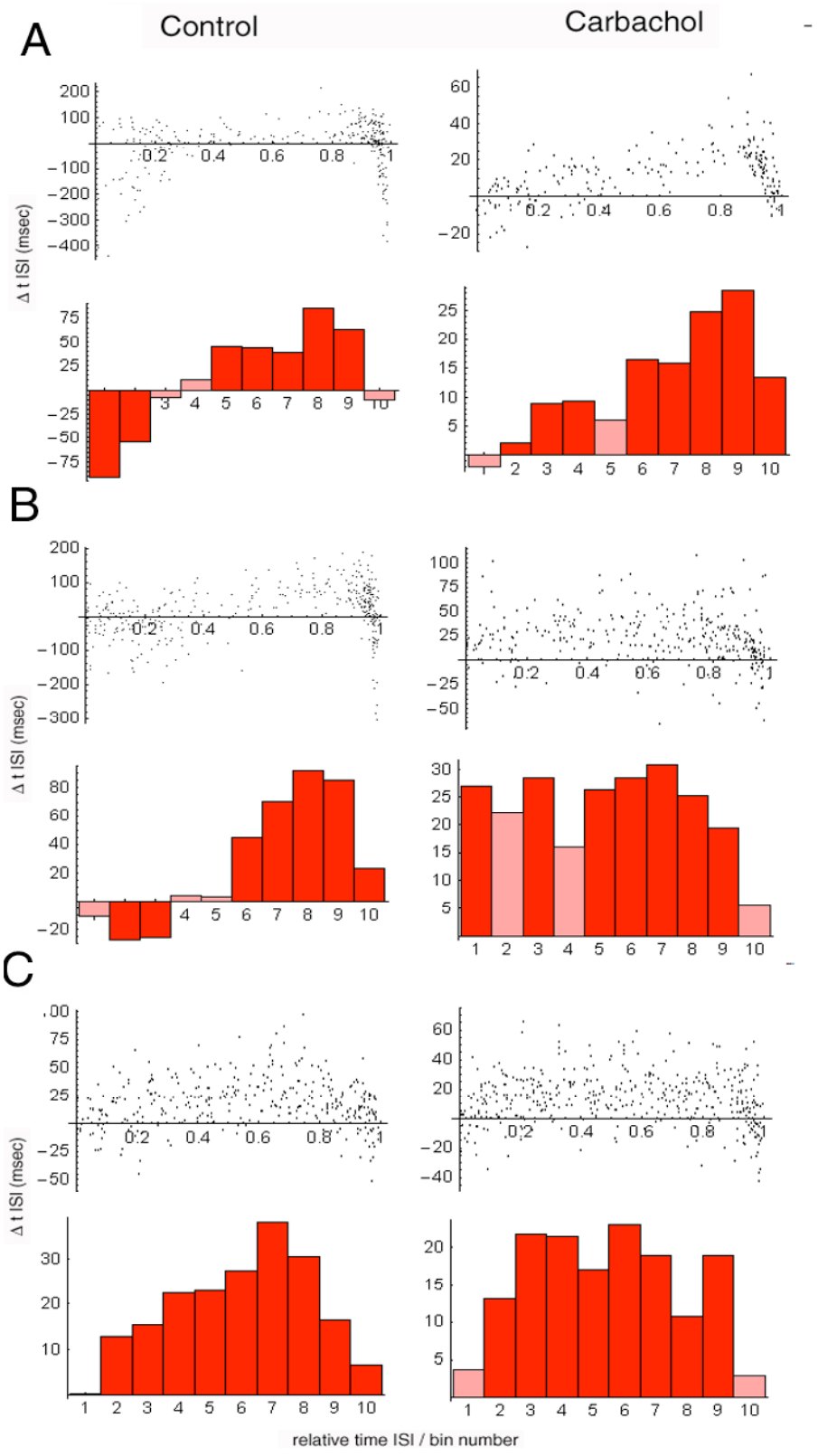
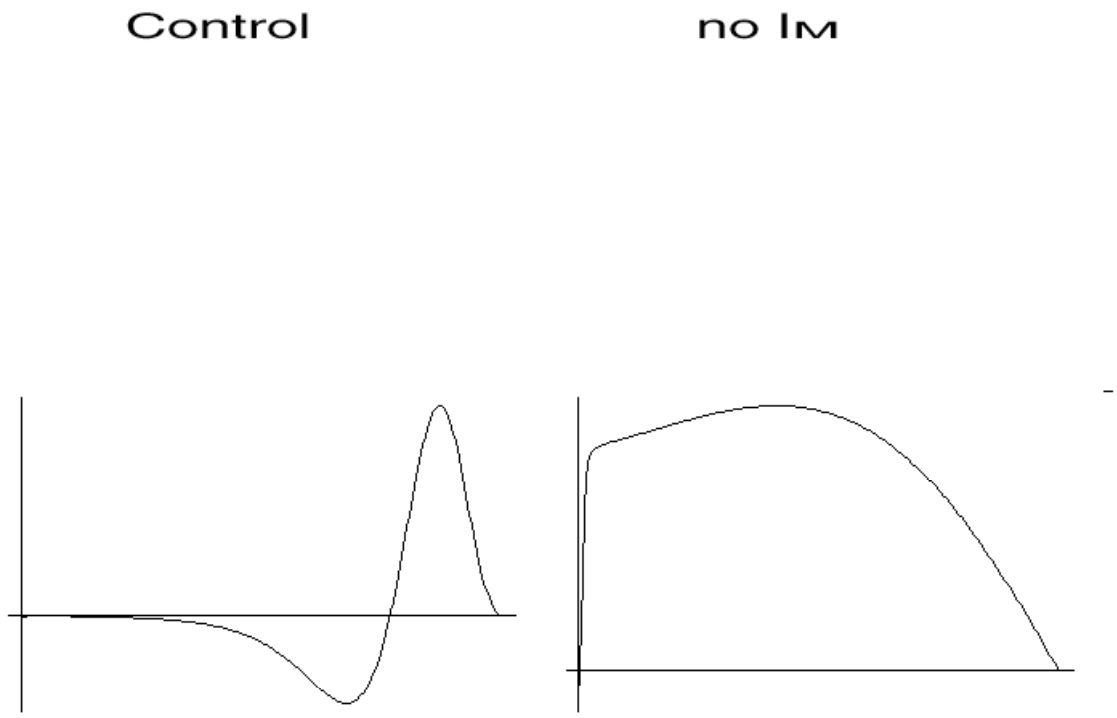

A
Control
Carbachol
B
C
Δ t ISI (msec)
relative time ISI / bin number

Figure 3

A.



B.