Effects of Cholinergic Modulation on Responses of Neocortical Neurons to Fluctuating Input

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Neocortical neurons *in vivo* are spontaneously active and intracellular recordings have revealed strongly fluctuating membrane potentials arising from the irregular arrival of excitatory and inhibitory synaptic potentials. In addition to these rapid fluctuations, more slowly varying influences from diffuse activation of neuromodulatory systems alter the excitability of cortical neurons by modulating a variety of potassium conductances. In particular, acetylcholine, which affects learning and memory, reduces the slow afterhyperpolarization, which contributes to spike frequency adaptation. We used whole-cell patch-clamp recordings of pyramidal neurons in neocortical slices and computational simulations to show, first, that when fluctuating inputs were added to a constant current pulse, spike frequency adaptation was reduced as the amplitude of the fluctuations was increased. High-frequency, high-amplitude fluctuating inputs that resembled *in vivo* conditions exhibited only weak spike frequency adaptation. Second, bath application of carbachol, a cholinergic agonist, significantly increased the firing rate in response to a fluctuating input but minimally displaced the spike times by <3 ms, comparable to the spike jitter observed when a visual stimulus is repeated under *in vivo* conditions. These results suggest that cholinergic modulation may preserve information encoded in precise spike timing, but not in interspike intervals, and that cholinergic mechanisms other than those involving adaptation may contribute significantly to cholinergic modulation of learning and memory.

Introduction

In the peripheral nervous system, sensory neurons in the auditory, somatosensory and electrosensory systems encode information in spike timing (Sejnowski, 1995), but in the cerebral cortex it has been generally thought that sensory information and motor commands are represented primarily by the firing rates of cortical neurons. Furthermore, the irregularity of spike timing in the cortex has led most investigators to conclude that the average number of spikes over a long time or over an ensemble of neurons is needed to accurately represent a sensory stimulus and that precise spike times are not important for coding sensory information (Shadlen and Newsome, 1994). It is not clear, however, whether the observed variability in spike timing might reflect a true difference in some aspects of the sensory environment or is simply noise that perceptual systems ignore.

Repeated stimulation of neocortical neurons in a neocortical slice preparation with a fluctuating current mimicking the bombardment of random synaptic inputs *in vivo* resulted in spike trains with a temporal jitter of ~1 ms (Mainen and Sejnowski, 1995). Similar results were recently obtained in ferret neocortical slices following injection of current designed to reproduce intracellular recordings from cortical neurons *in vivo* (Nowak *et al.*, 1997). Evidence is also accumulating that the temporal accuracy of spike timing in cortical circuits can be in the millisecond range *in vivo*. In awake, behaving monkeys, spike trains with a jitter of a few milliseconds were elicited from neurons in area MT during the repeated presentation of random dot motion stimuli *in vivo* (Bair and Koch, 1996). There is also evidence for the precise relative timing of neurons in visual cortex, which fire synchronously with millisecond time differences (Gray, 1994). Repeated patterns of spike firings with 1–3 ms jitter have been recorded from combinations of multiple neurons in the frontal cortex of the monkeys performing a behavioral task (Abeles *et al.*, 1993). It is possible that some of the remaining variability could be the result of small eye movements that occur at the rate of ~3/s during fixation (Beylin and Snodderly, 1997). Despite the increasing evidence for precision in spike timing a functional role for this precision has yet to be determined.

Recent *in vitro* recordings from the dendrites of cortical and hippocampal pyramidal cells provide direct evidence that the precise temporal relationship between presynaptic excitatory postsynaptic potentials and backpropagating action potentials can be critical for synaptic plasticity. Excitatory inputs on dendritic spines that coincide with backpropagating action potentials lead to supralinear calcium accumulation (Yuste and Denk, 1995) and induced either long-term depression (LTD) (Christie *et al.*, 1996) or long-term potentiation (LTP) (Magee and Johnston, 1997). The temporal order of the synaptic input and the backpropagating action potential determined whether LTP or LTD occurred in neocortical layer 5 pyramidal neurons (Markram *et al.*, 1997) with a shift occurring for spike timing differences as small as 10 ms. This suggests that at least one function for precise spike timing is to regulate synaptic plasticity (Sejnowski, 1997).

Cholinergic neurons in the basal forebrain, which innervate all cortical regions (Mesulam, 1995), modulate neuronal excitability (Nicoll, 1988; McCormick and Williamson, 1989; McCormick, 1993) and influence learning and memory (Hagan and Morris, 1989; Fibiger, 1991; Winkler *et al.*, 1995). Cholinergic neurons that innervate the cortex are silent during non-REM sleep but have tonic firing rates of ~20 Hz during the awake state and and REM sleep (Mitchell *et al.*, 1987). Cholinergic activity also varies transiently with the level of alertness and can occur in brief high-frequency bursts. Stimulation of cholinergic afferents to the cortex induces oscillatory cortical electroencephalographic (EEG) activity in the gamma range (30–70 Hz), which may be the result of synchronous spiking activity in cortical populations (Steriade, 1995).

The reduction of spike frequency adaptation by cholinergic activation in cortical neurons in response to a constant current pulse is considered an important mechanism for the control of neuronal excitability and the regulation of associative memory (Barkai et al., 1994). The release of acetylcholine in the cortex affects neuronal excitability by modulating potassium currents that underlie spike frequency adaptation (McCormick and

Williamson, 1989). Potassium conductances are important for homeostasis since they regulate the firing rate of a neuron over a wide range of time scales and can make neurons more sensitive to spike timing. If information is encoded in spike timing, how could this information be preserved despite the variability in the concentrations of acetylcholine and other neuromodulators in the neocortex?

The conditions typically found in slice preparations do not accurately reflect some aspects of cortical activity *in vivo*, such as ongoing spontaneous activity. In an attempt to bring slice experiments one step closer to *in vivo* conditions, we have examined the responses of cortical neurons to fluctuating inputs that mimic the fluctuating membrane potentials observed from intracellular recordings *in vivo* (Ferster and Jagadeesh, 1992; Nowak *et al.*, 1997). We report here that the responses of neurons to fluctuating current inputs are different from those elicited by constant current pulses in several important respects. First, spike frequency adaptation was much less pronounced with fluctuating inputs, and second, spike timing was preserved over a wide range of concentrations of a cholinergic agonist.

In addition to investigating the effects of neuromodulation on spike frequency adaptation and the spike timing of neocortical neurons in response to fluctuating inputs, we also developed compartmental models of neocortical pyramidal cells to explore some of the mechanisms that might account for our results. The models were matched to experimental recordings of the firing rate, spike frequency adaptation, spike jitter, and the effects of cholinergic modulation on responses to constant current pulses. We used the models to examine a broader range of parameter values than was possible during an experimental whole-cell patch recording of limited duration and to generate hypotheses about the mechanisms that may underlie unexplained observations.

Materials and Methods

Experiments

Coronal slices of 400 μm were prepared from 14–18 day old Long Evans rats. Whole-cell patch-clamp techniques were used to obtain somatic recordings under current clamp from 39 neocortical neurons in the rat visual cortex at both room temperature (23°C) and 34°C. Resting potential = –64.1 ± 1.07 mV (mean ± SEM); input resistance = 221 ± 30 MΩ (mean ± sem). The patch pipette contained (in mM): 100 potassium gluconate, 25 KCl, 5 NaCl, 0.2 EGTA, 10.0 HEPES, 4.0 ATP-Mg and 0.3 GTPLi. External solution contained (in mM): 126 NaCl, 1.25 NaH_2PO_4, 10.0 D-glucose, 2.50 KCl, 2.00 $MgCl_2$, 2.00 $CaCl_2$. Data were taken at the midrange of the neuron's total dynamic range to avoid ceiling effects.

Carbachol, a cholinergic agonist, at concentrations of 5, 7.5, 15 and 30 μM was delivered through bath perfusion (the perfusion time was between 1 and 20 min). For each cell, three sets of blocks were recorded before, during and after carbachol perfusion at a given concentration. This would correspond *in vivo* to a shift from deep sleep (where the cholinergic activity is near zero) to some level of arousal (see Discussion). Each block contained 20 trials of stimulation under identical experimental conditions.

To generate the fluctuating input, filtered Gaussian noise of zero mean was added to constant current pulses (Bryant and Segundo, 1976; Mainen and Sejnowski, 1995). The Gaussian noise signal was convolved with an alpha function with a time constant of 3 ms (Mainen and Sejnowski, 1995), chosen to reflect the time course of the synaptic events. Current fluctuation was no greater than 80% of the mean current input and resulted in a subthreshold membrane potential fluctuation comparable to those recorded under *in vivo* whole-cell patch-clamp (Ferster and Jagadeesh, 1992). This fluctuation to mean current ratio was consistent with the hypothesis that the intracellular membrane potential is the result of a large number of excitatory and inhibitory postsynaptic input currents of ~15 pA that are approximately in balance (Tsodyks and Sejnowski, 1995).

Simulations

Simulations were performed on a reduced eight-compartment model (Bush and Sejnowski, 1993), based on a reconstructed layer 2 pyramidal cell (Koch *et al.*, 1990) (data shown) and on a one-compartment model (data not shown) using the NEURON simulation program (Hines, 1993). An axon hillock and initial segment comprising two additional compartments taken from Mainen *et al.* (1995) were added to the eight-compartment model. The parameters of the models were constrained by the basic response characteristics of the experimentally recorded neocortical neurons shown in Figures 1 and 2.

Model Parameters

The implementation of the currents followed Mainen and Sejnowski (1996) with exceptions as noted. The calcium dependency of $g_{K(Ca)}$ and its channel density were chosen to reproduce the recorded spike trains under control and carbachol conditions. Temperature: 23°C. Fast sodium current, I_{Na}: half-activation voltages were shifted by –5 mV from Mainen and Sejnowski (1996); potassium delayed rectifier, $g_{K,v}$: as in Mainen and Sejnowski (1996); slow non-inactivating potassium current, $I_{K,M}$: $\alpha = 10^{-3}(v + 35)/(1 - e^{-(v+35)/9})$, $\beta = -10^{-3}(v + 35)/(1 - e^{(v+35)/9})$, modified from Mainen and Sejnowski (1996); originally based on Yamada *et al.* (1989) and Gutfreund *et al.* (1995); high voltage activated calcium current, I_{Ca} inactivation: $\alpha = 4.57 \times 10^{-4}e^{-(v+13)/50}$, $\beta = 0.001/(1 + e^{-(v+15)/28})$, modified from Mainen and Sejnowski (1996); originally based on Reuveni *et al.* (1993); slow calcium-dependent potassium current, I_{sAHP}: $\alpha([Ca^{2+}]_i) = 500([Ca^{2+}_i]^2 - [Ca^{2+}]^2_\infty)$, β = 0:001, see Sah (1996) for calcium-dependency; calcium removal: $d[Ca^{2+}_i]/dt = -10^5 I_{Ca}/2F - ([Ca^{2+}_i] - [Ca^{2+}_\infty])/\tau$, where τ = 127 ms, and $[Ca^{2+}_\infty]$ = 50 nM, modified from Helmchen *et al.* (1996). Passive membrane properties: specific capacitance C_m = 1 μF/cm^2; specific membrane resistance R_m = 40 kΩcm^2; specific axial resistance R_a = 200 Ωcm; resting membrane potential –70mV. Channel densities (in pS/μm^2) were as follows. Multicompartment model: dendrites: g_{Na} = 30; g_{Ca} = 0.1; $g_{K(Ca)}$ = 0.8, $g_{K,M}$ = 0.25, fast potassium delayed rectifier: $g_{K,v}$ = 0.5. Soma: except $g_{K,v}$ = 6 same as dendrites. Axon hillock and initial segment: g_{Na} = 30 000 and $g_{K,v}$ = 700. The dendritic passive and active properties except R_a were scaled by a factor of 3 to compensate for the reduction of surface area due to the reduction method (Bush and Sejnowski, 1993). One-compartment model: g_{Na} = 400, $g_{K,v}$ = 4, g_{Ca} = 0.1; $g_{K(Ca)}$ = 2.8, (no $g_{K,M}$). Other properties as in the multi- compartment model.

Cholinergic modulation

The effects of carbachol were simulated by reducing three potassium conductances, $g_{K(Ca)}$, $g_{K,M}$ and $g_{K,leak}$, underlying the I_{sAHP}, I_M and $I_{K,leak}$ respectively (Madison and Nicoll, 1984; McCormick, 1993). The primary effects of carbachol were simulated by a 75% reduction in $g_{K(Ca)}$, a 12.5% reduction in $g_{K,M}$ and a 12.5% reduction in $g_{K,leak}$. The amount of reduction of $g_{K,M}$ was derived from the measured ratio of reduction of $g_{K(Ca)}/g_{K,M}$ in hippocampal pyramidal cells (Madison *et al.*, 1987). The reduction of $g_{K,leak}$ produced a depolarization of 2.5 mV.

Stimulus Parameters

Intrinsic noise was simulated by injecting fluctuating currents (standard deviation: 10 pA) to fit the spike jitter observed experimentally. The mean current injection in the simulations shown here was 150 pA, and the fluctuations had a standard deviation of 150 pA, leading to subthreshold membrane potential fluctuations comparable to those in intracellular recordings observed *in vivo* (Ferster and Jagadeesh, 1992).

Results

Effects of Cholinergic Modulation on Spike Frequency Adaptation

Neocortical neurons in a slice preparation of the rat visual cortex were stimulated with both conventional constant current pulses and fluctuating inputs that resembled the impact of synaptic

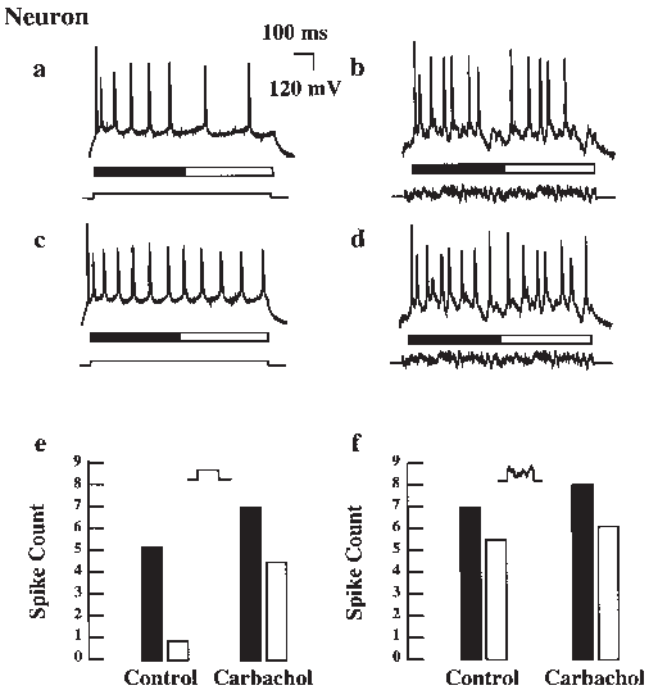

Figure 1. Cholinergic modulation of spike trains elicited by constant current pulses and fluctuating inputs in a neocortical neuron. The solid and white bars under the voltage traces mark the first and second halves of the total stimulus duration (900 ms). The bathing solution contained 5 μM carbachol in panels (*c*)–(*d*). (*a*) Strong spike frequency adaptation in response to 90 pA constant current pulse current injection (below). (*b*) Reduced spike frequency adaptation in response to the fluctuating current injection with a mean amplitude of 90 pA and a standard deviation of fluctuation of 72 pA. (*c*) Cholinergic reduction of adaptation and non-uniform increase of excitability in response to the same input as in (*a*). (*d*) Uniform increase of excitability due to cholinergic modulation in response to the same stimulus as in (*b*). (*e*, *f*) Effects of stimulus fluctuation on adaptation and effects of cholinergic modulation in neocortical neurons. For each condition shown in (*a*)–(*d*), the average spike count *C*1 during the first half (solid) and *C*2, the second half (white) of the stimulation (900 ms) are plotted in the bar graphs for a block of 20 trials. An adaptation index is defined as $A = (C1 - C2)/C1$. For the fluctuating input, the same random sequence was repeated during the first and second half of stimulus to insure fair comparison, and 20 different sequences were used. Carbachol = 5 μM. (*e*) Constant current pulse, control (mean ± SEM): A = 83 ± 4%. Carbachol: $A = 36 \pm 2\%$. (*f*) Fluctuating input, control: $A = 26 \pm 2\%$. Carbachol: $A = 22 \pm 3\%$.

inputs recorded *in vivo*. In previous studies using constant current pulses, acetylcholine significantly enhanced the firing rate by blocking the currents underlying spike frequency adaptation (Nicoll, 1988; McCormick and Williamson, 1989; McCormick, 1993). In contrast to the strong spike frequency adaptation evoked by a constant current pulse (Fig. 1*a*), injection of a fluctuating current into neocortical neurons reduced or completely eliminated pronounced spike frequency adaptation at room temperature (Fig. 1*b*).

To obtain a simple index to quantify adaptation under both constant current pulse and fluctuating inputs, we counted spikes during the first and second halves of the current injection interval, marked by the solid and white bars below the voltage traces in Figure 1. The adaptation index, *A*, defined as the normalized difference between the spike count during the early and late portions of the stimulation, was large for a constant current pulse, indicating strong adaptation, but was much smaller for fluctuating inputs (compare Fig. 1*e* and 1*f* control). For blocks of 20 trials in 10 neurons the difference was highly significant (paired *t*-test, $t = 4.923$, $P \leq 0.001$, $n = 10$). In contrast to the differential increase of excitability for the later period of

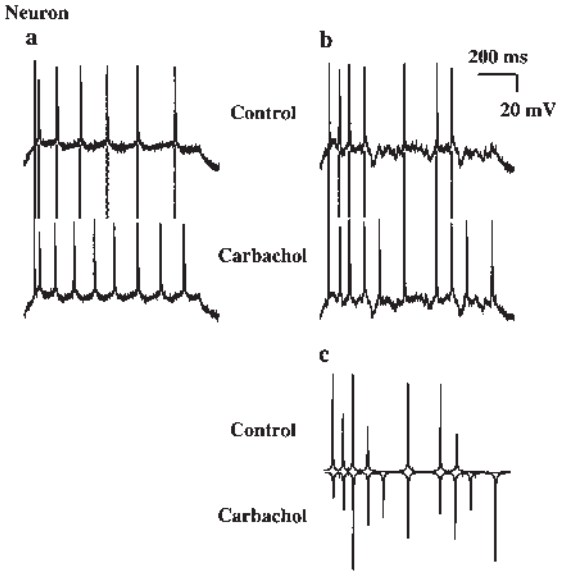

Figure 2. Cholinergic modulation preserved spike timing in response to the same fluctuating input. (*a*) Cholinergic reduction of spike frequency adaptation to constant input for a neocortical neuron. The vertical lines between the control and carbachol traces show the timing of spike initiation under the control condition. Misalignment to the carbachol condition indicates a modification of spike timing. (*b*) For the fluctuating input condition, cholinergic modulation preserved spike timing and enhanced excitability by inserting additional spikes. (*c*) Preservation of spike timing for fluctuating inputs shown in smoothed histograms (20 trials), comparing control (above) and carbachol (inverted) in the same neuron.

stimulation due to a blockade of adaptation for constant current pulses (Fig. 1*a*,*c*,*e*), cholinergic modulation increased the excitability more uniformly for the fluctuating input (Fig. 1*b*,*d*,*f*).

Effects of Cholinergic Modulation on Spike Timing

To examine the influence of cholinergic modulation on the accuracy of spike timing we directly compared the responses of neurons to both constant current pulses and fluctuating inputs before and after the bath application of carbachol. The results in Figure 2 show that large displacements of spikes occurred for constant current pulses, as expected from the large decrease in the spike frequency adaptation (Fig. 2*a*); however, for fluctuating inputs the spikes were preserved with relatively small displacements when carbachol was added and additional spikes were inserted between existing ones (Fig. 2*b*).

One way to assess the precision of the spike initiation mechanisms is to compute the average post stimulus time histogram, as shown in Figure 2*c*. The displacement in spike timing for each event in the histogram, d_i, is defined as the absolute value of the time difference between the nearest peak of the event under carbachol and control conditions (see Mainen and Sejnowski, 1995). The weight for each event, w_i, is determined by the height of the event (the greater the height, the less the spike jitter). Mean displacement, *D*, is defined by $D = \Sigma\, d_i w_i / \Sigma\, w_i$, where $i = 1, 2, \ldots$ is the event index in the control condition. For each cell, *D* was measured using identical fluctuating input. For 5 and 7.5 μM carbachol (mean ± SEM): 2.76 ± 0.38 ms, $n = 15$; for 15 μM carbachol: $D = 3.33$ ms, $n = 1$; for 30 μM carbachol: $D = 9.3$ ms, $n = 2$. For all 18 cells examined, the mean current injection ranged from 50 to 120 pA; the standard deviation of current fluctuation ranged from 50 to 100 pA. The spike jitter, measured as the mean half-width of the events, was not changed significantly by carbachol under

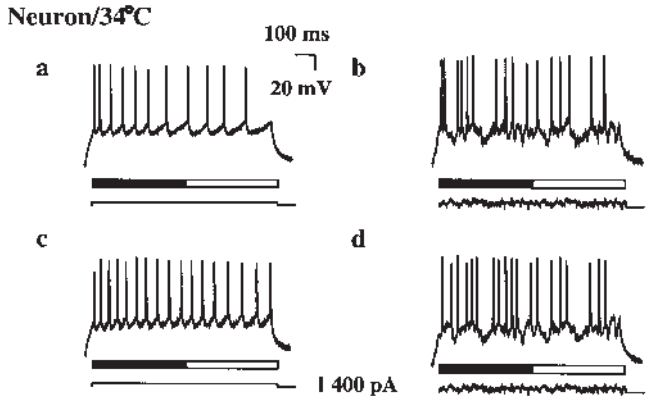

Figure 3. Cholinergic modulation of spike trains elicited by constant current pulses and fluctuating inputs in a neocortical neuron from a slice preparation kept at 34°C. The solid and white bars under the voltage traces mark the first and second halves of the total stimulus duration (900 ms). The bathing solution contained 10 μM carbachol in panels (*c*)–(*d*). (*a*) Strong spike frequency adaptation in response to 100 pA constant current pulse current injection (below). (*b*) Reduced spike frequency adaptation in response to the fluctuating current injection with a mean amplitude of 100 pA and a standard deviation of fluctuation of 65 pA. (*c*) Cholinergic reduction of adaptation and non-uniform increase of excitability in response to the same input as in (*a*). (*d*) Uniform increase of excitability due to cholinergic modulation in response to the same stimulus as in (*b*).

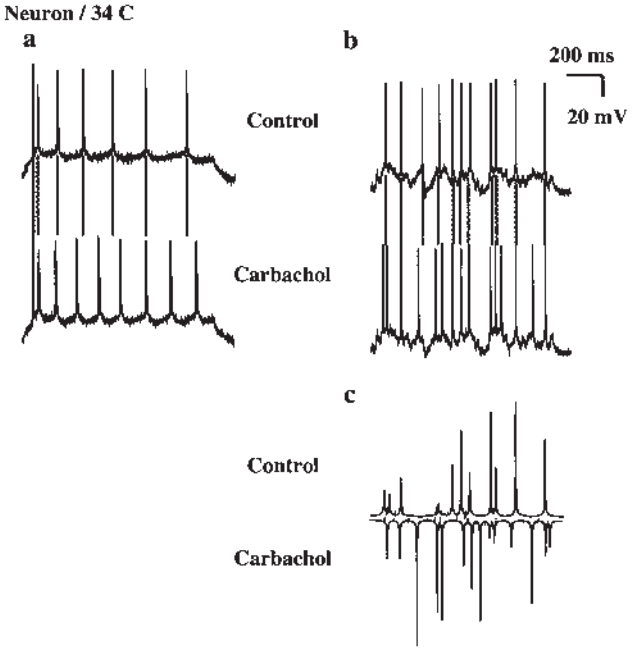

Figure 4. Cholinergic modulation preserved spike timing in response to the same fluctuating input in a slice preparation maintained at 34°C. (*a*) Cholinergic reduction of spike frequency adaptation to constant input for a neocortical neuron. The vertical lines between the control and carbachol traces show the timing of spike initiation under the control condition. Misalignment to the carbachol condition indicates a modification of spike timing. (*b*) Cholinergic modulation of spike timing to fluctuating inputs for a neocortical neuron. Cholinergic modulation preserved spike timing and enhanced excitability by inserting additional spikes. (*c*) Preservation of spike timing for fluctuating inputs shown in smoothed histograms (20 trials), comparing control (above) and carbachol (inverted) in the same neuron.

repeated identical stimulation [standard deviation for control (mean ± SEM) was 0.9 ± 0.3 ms and for the carbachol condition was 0.9 ± 0.4 ms].

Effects of Temperature on Spike Frequency Adaptation and Spike Timing Precision

The results described above were obtained at room temperature, which may introduce artifacts that are not present at body temperature. In particular, the magnitude of the calcium-dependent potassium current, I_{sAHP}, a primary current underlying spike frequency adaptation, decreases with increasing temperature (Thompson *et al.*, 1985). Thus, the adaptation reported here may be an overestimate of adaptation *in vivo*. We performed several experiments at 34°C to examine the temperature dependence of adaptation and found that a much lower percentage of neurons (~20%, n = 9) showed a large adaptation to constant current pulses compared with previous experiments performed at room temperature (90%, n = 69).

Figure 3 shows examples of spike trains elicited by a constant current pulse and a fluctuating input recorded at 34°C. In this cell, adaptation was reduced from 50% to 19% by increasing the standard deviation of the current fluctuation from 0 to 65% of the mean current injection (Fig. 3*a*,*b*). For constant pulse inputs, cholinergic reduction of adaptation (50% to 27%) resulted in a 50% increase in excitability (Fig. 3*a*,*c*). However, for fluctuating inputs, a 32% increase in excitability (Fig. 3*b*,*d*) was induced with little change in adaptation (19% to 20%). Preservation of spike timing following bath application of carbachol (with a displacement of 2.4 ms) from another cell is shown in Figure 4. This result is also consistent with the reduced I_{AHP} at 37°C (Thompson *et al.*, 1985).

Simulations of the Cholinergic Modulation of Spike Frequency Adaptation

To show that the reduced adaptation due to increased stimulus fluctuation observed in the slice experiments is a general property of cortical neurons over a wide range of conditions, we constructed a model neuron that exhibited the basic response characteristics of neocortical neurons and varied the parameters in the model to determine which were essential for reproducing the observed responses from the cortical neurons *in vitro*. Noise was added to the membrane potential to make the variability of the responses of the computer model quantitatively match the variability observed in recordings from slice preparations. Details of the compartmental model are given in Materials and Methods.

The model neocortical neuron was stimulated with the same conventional constant current pulses and fluctuating inputs used in the *in vitro* studies reported above. Compare the results of the experiments in Figure 1 with those of the model in Figure 5. As in the experiments, pronounced spike frequency adaptation was evoked in model neurons by a constant current pulse (Fig. 5*a*), but injection of a fluctuating current reduced spike frequency adaptation (Fig. 5*b*). Cholinergic modulation was simulated by reducing three potassium conductances in the model: $g_{K(Ca)}$, $g_{K,M}$ and $g_{K,leak}$. Cholinergic modulation increased excitability for the later period of stimulation due to a blockade of adaptation (Fig. 5*c*,*e*), but cholinergic modulation increased the excitability more uniformly for the fluctuating input (Fig. 5*d*,*f*).

In the model, spike frequency adaptation decreased as a function of increasing amplitude of stimulus fluctuation (Fig. 6*a*). Similar results were also obtained for different levels of mean synaptic inputs (mean current injections from 100 to 300 pA; 150 pA shown in Fig. 6*a*), a range of initial degrees of adaptation ($g_{K(Ca)}$ = 0.2–1.6 pS/μm^2, shown for 0.8 pS/μm^2 in Fig. 6*a*), and a variety of model geometries (1- and 10-compartment

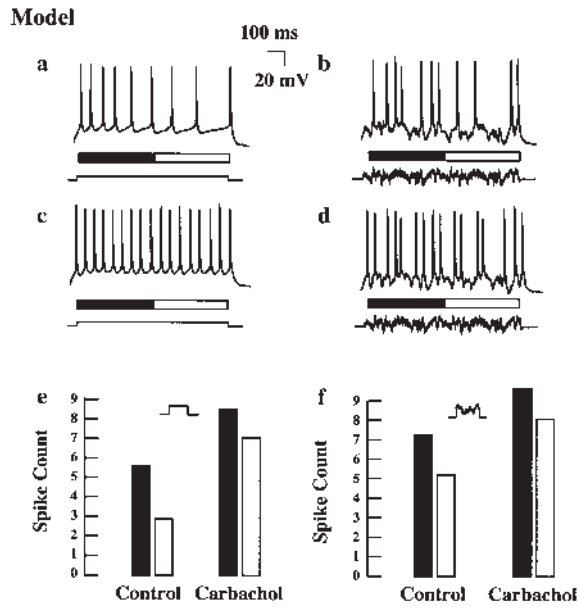

Figure 5. Simulations of cholinergic modulation of spike trains elicited by constant current pulse and fluctuating inputs in a neocortical neuron. The solid and white bars under the voltage traces mark the first and second halves of the total stimulus duration (900 ms). (*a*) Strong spike frequency adaptation in response to 150 pA constant current pulse current injection (below). (*b*) Reduced spike frequency adaptation in response to the fluctuating current injection (standard deviation of fluctuation: 150 pA, mean amplitude: 150 pA). (*c*) Simulated cholinergic reduction of adaptation and non-uniform increase of excitability in response to the same input as in (*a*). (*d*) Uniform cholinergic increase of excitability in response to the same stimulus as in (*b*). (*e*, *f*) Simulated effects of stimulus fluctuation on adaptation and effects of cholinergic modulation of adaptation and excitability in neocortical neurons. The adaptation index *A* is defined as in Figure 2. (*e*) Constant current pulses, control: $A = 47 \pm 3\%$. Carbachol: $A = 16 \pm 2\%$. (*f*) Fluctuating inputs, control: $A = 27 \pm 3\%$. Carbachol: $A = 13 \pm 2\%$.

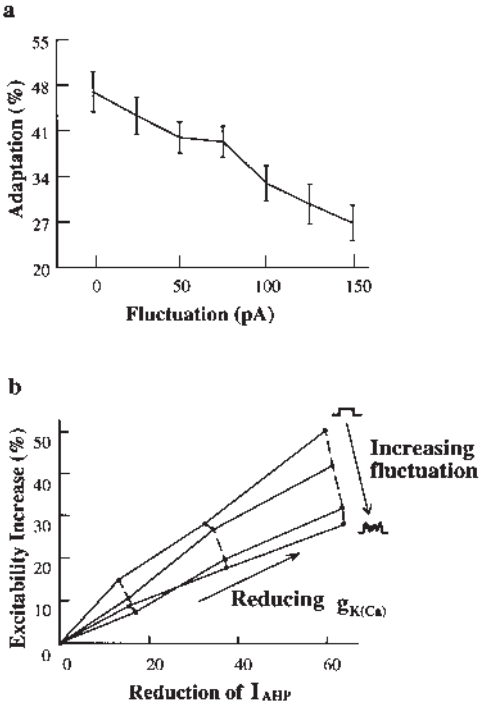

Figure 6. Simulations of adaptation and cholinergic control of excitability in a model cortical neuron with 10 compartments. (*a*) Adaptation decreased as a function of the standard deviation of the stimulus fluctuation. The mean current injection was 150 pA. $g_{K(Ca)} = 0.8$ pS/μm^2. $n = 20$ trials (with 20 different seeds) per condition. (*b*) Increased excitability as a function of reduction in I_{sAHP}. The solid lines represent different levels of stimulus fluctuation (from 0, 50, 100 to 150 pA). the reduction in $g_{K(Ca)}$ was set to 0,8, 0.6, 0.4 and 0.2 pS/μm^2 respectively for control (origin) and increasing carbachol concentrations (successive points on the solid line).

layer 2/3 pyramidal cell models, shown in Fig. 6*a* for the 10-compartment model).

I_{sAHP}, which is generated by $g_{K(Ca)}$, is the primary current underlying adaptation in cortical neurons *in vitro* (Madison *et al.*, 1987). If reduction of adaptation contributes less to cholinergic control of excitability *in vivo*, then $g_{K(Ca)}$ should have correspondingly less influence with increasing excitability. Consistent with this hypothesis, simulations at multiple levels of cholinergic reduction of $g_{K(Ca)}$ produced less increase in neuronal excitability when the stimulus fluctuation was increased (Fig. 6*b*), indicating that reducing I_{sAHP} became less effective in enhancing excitability as input fluctuation increased.

The relatively uniform increase of excitability throughout the stimulation interval during fluctuating inputs in Figure 5 suggests an important role for potassium currents other than $g_{K(Ca)}$. This observation is supported by simulations showing that modulation of I_{leak} and I_M significantly potentiated the effects of cholinergic modulation of I_{sAHP} on excitability (Fig. 7). Reducing I_M or I_{leak} has only a weak effect on the modulation of the firing rate when $g_{K(Ca)}$ is at its maximum value. When $g_{K(Ca)}$ has been reduced, reducing these other potassium currents can significantly boost the firing rate.

Simulations of the Effects of Cholinergic Modulation on Spike Timing

In recordings from neocortical neurons, the cholinergic modulation had a much greater effect on the accuracy of spike timing for constant current pulses compared with fluctuating inputs. The experimental results in Figure 2 showing that carbachol induced large displacements of spikes for constant current pulses but relatively small displacements for fluctuating inputs was replicated in the model pyramidal neuron without changing any of the parameters that were determined from the previous simulations.

The cholinergic reduction of spike frequency adaptation to constant current pulses for a model neocortical neuron is significant and the absolute timing of spikes as well as interspike intervals is altered (Fig. 8*a*). However, for fluctuating inputs, cholinergic modulation preserved spike timing (Fig. 8*b*). Excitability was enhanced by the insertion of additional spikes. The average spike timing displacement due to cholinergic modulation was 2.34 ± 0.73 ms (mean ± SD; n = 10 seeds). The average spike jitter for the control condition was not significantly changed between the control (0.91 ± 0.27 ms) and carbachol (0.96 ± 0.14 ms) conditions.

These simulations are similar to the recordings from neocortical neurons in Figure 2. The smoothed histogram (20 trials) in Figure 8*c* showing control (above) and the carbachol condition (below) can be compared with a similar histogram in Figure 2*c*.

In the model it was possible to vary independently all of the potassium conductances and to determine how each of them affects the jitter and the displacement of spikes in response to carbachol. Intrinsic noise was added to produce the same amount of spike jitter observed in neocortical neurons under the control condition. Reduction of $g_{K(Ca)}$ to the maximal amount estimated from the recordings caused a 1–3 ms spike displacement (Fig. 9*a*), similar to that measured in the carbachol experiments. For $g_{K,M}$

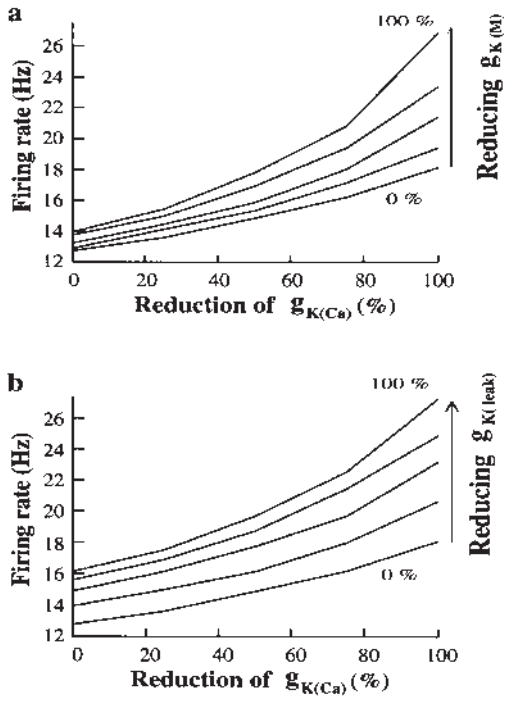

Figure 7. Simulation of a model cortical neuron showing that other potassium currents potentiate the effect of I_{sAHP} modulation on cholinergic control of excitability. In the absence of I_M and I_{leak} modulation, complete blockade of I_{sAHP} increased the firing rate from 13 to 18 Hz. (*a*) Accompanied by a complete I_M blockade, the effect of I_{sAHP} modulation almost doubled (14 to 27 Hz). (b) Accompanied by a modulation of I_{leak}, associated with an increase in resting membrane potential by 10 mV, the effects of I_{sAHP} modulation increased by 69% (16 to 27 Hz).

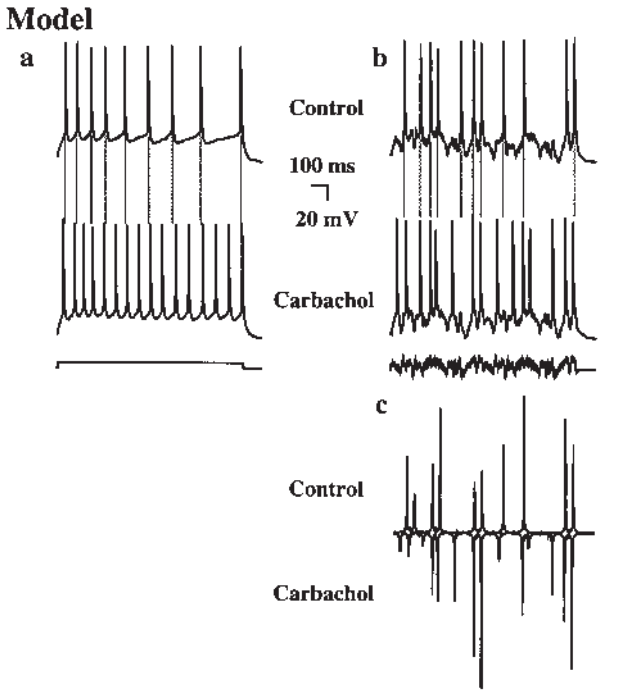

Figure 8. Simulations of the effects of cholinergic modulation on spike timing in response to the same fluctuating input. (*a*) Cholinergic reduction of spike frequency adaptation to constant input for a model neocortical neuron. The vertical lines between the control and carbachol traces show the timing of spike initiation under the control condition. Misalignment to the carbachol condition indicates a modification of spike timing. (*b*) For the fluctuating input condition, cholinergic modulation preserved spike timing and enhanced excitability by inserting additional spikes. (c) Preservation of spike timing for fluctuating inputs shown in smoothed histograms (20 trials), comparing control (above) and carbachol (inverted) in the same model neuron.

and $g_{K,leak}$ the displacements were smaller, in the range 0.5–1 ms (Fig. 9*b–c*). Spike jitter did not vary with changes in any of the potassium conductances (bottom panels in Fig. 9).

Discussion

Spontaneous activity characterized by ongoing spike trains and membrane potential fluctuations occurs throughout the cerebral cortex in the awake state. Alertness is also characterized by high-frequency, low-amplitude activity in the gamma band (30–70 Hz) of the EEG (Steriade, 1995), suggesting that the spontaneous activity is not random but may be coordinated among populations of cortical neurons (Gray, 1994; Buzsaki and Chrobak, 1995). In this paper we explored the responses of neurons in neocortical slices to inputs that resembled the ongoing spontaneous activity and the effects of cholinergic modulation on these responses. In particular, we examined the influence of a range of concentrations of bath-applied carbachol on the spike timing of neocortical pyramidal neurons in response to fluctuating current injection designed to resemble the fluctuations observed *in vivo* (Ferster and Jagadeesh, 1992; Nowak *et al.*, 1997). Most of the experiments reported here were performed at room temperature, but several experiments were performed at 34°C to confirm the main findings. Additional experiments are needed to assess fully the effects of temperature on cholinergic modulation of spike frequency adaptation and its role in spike timing *in vivo*.

Cholinergic Modulation of Spike Timing

The responses of neurons to two different types of current injection were compared in this study: constant current pulses and fluctuating current inputs that were repeated under

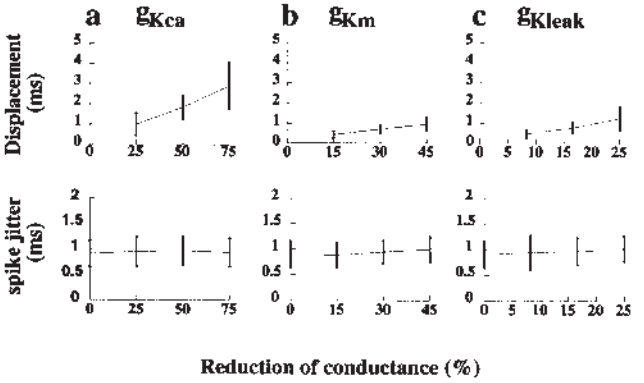

Figure 9. Simulations of spike displacement as a function of potassium conductance modulation in spike trains elicited with fluctuating inputs with simulated intrinsic noise. Standard deviation of fluctuation: 130 pA, mean amplitude: 150 pA. Spike displacement and spike jitter were measured in spike trains of 900 ms duration. Top: Reduction of all conductances by the maximal amount estimated from the recordings caused only a small increase in spike displacement. Bottom: Spike jitter did not vary with changes in any of the potassium conductances. (*a*) $g_{K(Ca)}$; (*b*) $g_{K,M}$; (c) $g_{K,leak}$.

stationary conditions in a slice preparation. Previous studies have shown that the precision in the timing of spikes depends on the amplitude and frequency of the fluctuations, with higher accuracy at higher amplitudes and higher frequencies (Mainen and Sejnowski, 1995; Nowak *et al.*, 1997). For amplitudes and frequencies of fluctuations similar to those observed intracellularly *in vivo* (Ferster and Jagadeesh, 1992), the jitter in the spike timing was ~1–3 ms.

The effects of cholinergic modulation on spike timing depended strongly on the temporal structure of the current input. The greater the amplitude of the stimulus fluctuation, the more resistant was the precision of spike timing to neuromodulation. For stimulus fluctuations with properties similar to *in vivo* conditions, the spike time displacement over a wide range of cholinergic excitation was of the order of a few milliseconds, comparable to spike jitter observed *in vivo* (Bair and Koch, 1996; Berry *et al.*, 1997). However, there are many other factors that differ between the slice preparation and *in vivo* conditions. Direct measurement of spike timing needs to be made *in vivo* to verify that cholinergic modulation indeed preserves temporal structures in spike trains.

The range of concentrations of acetylcholine in the cortex during alertness is uncertain, so we cannot be sure whether the range of concentrations of bath-applied carbachol was comparable. Nonetheless, the relative insensitivity of spike timing over the wide range of concentrations of carbachol tested (5–30 μM) suggests that cholinergic neuromodulation should not be as large a factor as had been thought based on current pulse experiments. Additional experiments with other neuromodulators such as norepinephrine, serotonin and histamine need to be undertaken to determine the generality of the results obtained here with a cholinergic agonist. The other neuromodulators also affect postsynaptic potassium currents and are likely to have a postsynaptic influence similar to that of acetylcholine on spike timing to fluctuating current inputs.

We have not performed pharmacological experiments to dissect the contributions of the various potassium currents that are affected by cholinergic modulation (Nicoll, 1988; McCormick, 1993). However, computational models were used to compare the influence of three different potassium currents known to be involved. We found that the modulation of the calcium-dependent potassium conductance, $g_{K(Ca)}$, could account for the major effects of cholinergic modulation of neocortical cells *in vitro*, although the modulation of other potassium conductances also had an influence. Modulation of $g_{K,M}$ or $g_{K,leak}$ alone had relatively small effects on the firing rate when the $g_{K(Ca)}$ was fully activated. However, when $g_{K(Ca)}$ was modulated simultaneously, a reduction of $g_{K,M}$ or $g_{K,leak}$ significantly potentiated the effects of $g_{K(Ca)}$ modulation, resulting in a non-linear increase of the firing rate (Fig. 8). Additional experiments and modeling studies need to be carried out to explore these interactions further.

Spike frequency adaptation induced by a constant current pulse has been considered a mechanism for mediating cholinergic enhancement of neuronal excitability which also results in apparent changes in spike timing; however, the reduction of adaptation observed with fluctuating inputs suggests that the contribution of spike frequency adaptation to cholinergic control of excitability *in vivo* needs to be reconsidered. The decreased impact of I_{AHP} and the strong interaction among multiple potassium currents with fluctuating inputs suggests that other cholinergic mechanisms, such as a reduction in leak potassium or M currents (Krnjevic *et al.*, 1971; Madison *et al.*, 1987; Nicoll, 1988; McCormick, 1993), or select- ive suppression of recurrent synaptic transmission (Hasselmo and Bower, 1992) should be included in models of learning and memory and targeted for drug intervention to alleviate cognitive deficits.

Information Conveyed by Spike Timing

Recent empirical and theoretical studies have raised the possibility that spike timing could be used in the cerebral cortex under some conditions to encode sensory information in addition to the average firing rate (Abeles *et al.*, 1993; Hopfield, 1995; Mainen and Sejnowski, 1995; Sejnowski, 1995; Stevens and Zador, 1995; deCharms and Merzenich, 1996; Rieke *et al.*, 1996; Victor and Purpura, 1996; Reich *et al.*, 1997). There are many possible ways in which timing could be used to encode and process information, such as the relative timing of spikes in a population of neurons or a code based on precise interspike intervals. One line of investigation has focused on observations of highly repeatable spike trains in the neocortex in response to repeated, structured inputs both *in vivo* and *in vitro* (Bair and Koch, 1996; Berry *et al.*, 1997; Beylin and Snodderly, 1997; Reich *et al.*, 1997; de Ruyter van Steveninck *et al.*, 1997), raising the possibility that precise spike timing could convey sensory information.

Modification of spike timing, implied by cholinergic reduction in spike frequency adaptation to a constant current pulse (Fig. 1), might disrupt information encoded by spike times. However, in the presence of carbachol, the timing of the spikes, but not interspike intervals, was preserved with fluctuating inputs, despite an increase in excitability due to the insertion of additional spikes (Fig. 2). Thus, information carried by cortical spike timing is likely to be stable during changes in the local concentrations of acetylcholine. The responses of cortical neurons in the awake squirrel monkey auditory cortex elicited by species-specific vocalizations were studied by focal application of acetylcholine and norepinephrine (Foote *et al.*, 1975). Although spike time displacement was not directly measured in this study, visual inspection of the published recordings reveals that the cholinergic and noradrenergic modulation had effects on spike timing that were similar to the cholinergic effects *in vitro* reported here.

Visual features, such as form and color, correlate with the temporal structure of the spike trains recorded from neurons in monkey inferior temporal cortex (McClurkin *et al.*, 1996). Neurons in primate auditory cortex respond with changes in the correlation between pairs of neurons even when there is no significant change in their firing rates to steady tones (deCharms and Merzenich, 1996). Despite this evidence for the coding of sensory information in cortical spike timing, there is no direct *in vivo* evidence that spike timing information is actually used in the cortex (but see Roelfsema *et al.*, 1994). For example, a direct link between sensory perception and spike timing might be made if, spike trains of the same firing rate but different timing can be elicited and, at the same time, different percepts can be induced.

The effects of cholinergic modulation observed with fluctuating stimuli have implications for the possibility of temporal coding in the neocortex. The preservation of spike timing suggests that although acetylcholine enhances neuronal excitability, a stable code based on spike timing could be maintained under varying concentrations. Thus, neuromodulators may actually enhance spike timing information by recruiting additional spikes to code excitatory fluctuations that previously were subthreshold. Spike timing could complement information conveyed by the firing rate and take advantage of the high precision with which synaptic plasticity is apparently regulated by the relative timing of presynaptic and postsynaptic events. By probing neurons with stimuli resembling *in vivo* conditions, it may be possible to uncover other emergent properties and create new links between cellular mechanisms and system level functions.

Notes

We thank Zachary Mainen, David McCormick, Barak Pearlmutter, Raphael Ritz, Kechen Zhang, Anthony Zador, Brian Christie, David Horn, and Bartlett Mel for their critical comments and helpful discussions. Research supported by the Howard Hughes Medical Institute, the National Institutes of Health, and Schweizerische Studienstiftung.

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