



FIRING PATTERN MODULATION BY OSCILLATORY INPUT IN SUPRAGRANULAR PYRAMIDAL NEURONS

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Abstract—Neocortical neurons *in vivo* receive periodic stimuli due to feedforward input from the periphery as well as local cellular and circuit properties. In order to understand how neurons process such information, the responses of neurons to periodic sine wave current stimuli of varying frequencies and amplitudes were investigated. Sine wave stimuli were injected into pyramidal cells of young adult ferret visual cortical slices *in vitro* using sharp microelectrodes. To simulate higher resting membrane potentials observed *in vivo* a slight depolarizing current was injected to bring the neuron just to threshold. Initially, neurons discharged at least one action potential per sine wave cycle, but as the frequency was increased, a point was reached where this one-to-one responsiveness was lost. This critical frequency was dependent upon the injected sine wave amplitude and the magnitude of the underlying steady-state depolarization, and was correlated with spike width. Larger steady-state depolarizations and thinner action potentials corresponded to higher critical frequencies. Thus, when a neuron was very active it could respond in a one-to-one fashion over a greater range of frequencies than with the smallest DC offset.

The results suggest that the frequency-following characteristics of individual cortical neurons can be modulated by the activity state of the neuron itself. © 2002 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: frequency tuning, devil's staircase, input/output transformation, sine wave.

Neurons *in vivo* are confronted with both periodic and aperiodic inputs. Classically, experimenters have used periodic inputs such as sine wave gratings moved across a neuron's receptive field at different frequencies in order to study visual responses *in vivo* (e.g. Maffei et al., 1973; Ferster and Jagadeesh, 1991). Previous experiments using a wide range of such stimuli have helped to define the input/output transformations of single neurons within the context of an intact network. However, *in vitro* methodologies have been employed to study the response of individual neurons to periodic inputs. Sinusoidal currents have been injected into a wide variety of cells (Guttman et al., 1980; Carandini et al., 1996; Nowak et al., 1997; Hunter et al., 1998; Leung and Yu, 1998; Fellous et al., 2001). Recordings from squid giant axons in response to sinusoidal inputs revealed that axons could respond with at least one action potential per cycle at low input frequencies and once every two or three cycles at higher input frequencies. Frequencies between whole number firing states caused axons to respond in a chaotic or random manner (Guttman et al., 1980). Investigations of cortical neuron responses to sine wave inputs have suggested that cortical neurons behave as band-pass filters and once a cutoff frequency is reached the neurons

cease to respond (Carandini et al., 1996; Fellous et al., 2001).

In this study, we conducted a detailed investigation of cortical neuron responses to injected sinusoidal currents of different amplitudes, frequencies and baselines. It was determined that the ability of a neuron to respond with at least one action potential per sine wave cycle depended upon both the neuron's intrinsic properties (spike width) as well as its level of activation.

EXPERIMENTAL PROCEDURES

Preparation of slices and solutions

Slices were obtained from male or female ferrets, 4–7 months old (Marshall Farms). The ferrets were deeply anesthetized with sodium pentobarbital (30 mg/kg) and decapitated. The brain was quickly removed and the hemispheres separated with a mid-line incision. Coronal slices of visual cortex were cut using a DSK microslicer (Ted Pella). In order to maintain tissue viability a modification of the technique developed by Aghajanian and Rasmussen (1989) was employed. During the preparation of the cortical slices the tissue was kept in a solution where NaCl had been replaced with sucrose while the osmolarity was maintained at 307 mOsm. After preparation the slices were maintained in an interface style chamber (Fine Scientific Tools) and allowed to recover for at least 2 h at 34–36°C. The bathing medium contained (in mM): 124 NaCl, 2.5 KCl, 2 MgSO₄, 1.25 NaH₂PO₄, 1.2 CaCl₂, 26 NaHCO₃, 10 dextrose, and was aerated with 95% O₂, 5% CO₂ to a final pH of 7.4. For the first 10 min that the slices were in the recording chamber the bathing medium contained an equal mixture of the bathing and slicing solutions with 2.0 mM CaCl₂ which was subsequently reduced to 1.2 mM in the bathing solution, an extracellular

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Ca^{2+} concentration similar to what has been observed in cats *in vivo* (Hansen, 1985).

Sharp microelectrodes were pulled from medium-walled glass (1BF100; WPI) on a Sutter Instruments P-80 micropipette puller and beveled on a Sutter Instruments beveler to a final resistance of 80–120 M Ω . Electrodes were filled with 2 M potassium acetate with 1.5–2% (w/v) biocytin for subsequent histological identification of recorded cells. Recordings were made at 34–36°C.

Electrophysiology

All recordings were obtained from the supragranular layers of ferret visual cortex. Once a stable intracellular recording had been obtained (resting V_m of -60 mV or more negative, overshooting action potentials, ability to generate repetitive spikes to a depolarizing current pulse), the cell was classified according to its discharge pattern in response to an injected current pulse (120 ms, $+0.5$ nA) as intrinsically bursting, regular-spiking, fast-spiking or chattering (McCormick et al., 1985; Brumberg et al., 2000). The focus of the present study was to characterize the frequency-following properties of regular-spiking cells. In general, there is less synaptically driven spontaneous activity *in vitro* than *in vivo* and the resting membrane potential is more hyperpolarized (see Pare et al., 1998; Volgushev et al., 1998). To approximate the more depolarized membrane potentials observed *in vivo* a small depolarizing steady-state current was injected (0.2–0.7 nA) to bring the neuron to threshold. The enhanced conductances that are observed *in vivo* are not mimicked by this depolarization (Pare et al., 1998; Destexhe and

Pare, 1999). Superimposed on this steady-state depolarization was a sine wave stimulus (Grass Instruments waveform generator) systematically varied in both frequency (0.2–200 Hz) and amplitude (peak-to-peak, 0.1–0.5 nA). Additionally, the magnitude of the steady-state depolarization was systematically varied to study how the level of activation of an individual neuron affects its frequency-following capabilities (see Fig. 1A). The steady-state depolarization was noted instead of membrane voltage, since in each case the smallest steady-state depolarization was sufficient to exceed threshold, thus larger magnitude depolarizations did not further depolarize the neuron, but just increased its firing rate prior to the injection of the sine wave stimulus.

Data collection and analysis

For intracellular recording, data were collected via an Axoclamp 2B intracellular amplifier (Axon Instruments) and digitized at 44 kHz (Neuro-Corder DR-886, Neuro Data Instruments) and recorded to VCR tapes for subsequent off-line analysis. Analysis was done off-line using the Spike2 data collection system (Cambridge Electric Design) or Axoscope 8.0 (Axon Instruments). Several metrics were used to study the frequency-following capabilities of neocortical pyramidal cells. Spike half-width was determined by injecting a depolarizing current pulse from rest to elicit a single action potential. Action potential amplitude was determined from threshold to peak, the width was measured at 50% of its peak amplitude. The number of action potentials per cycle was determined by counting the number of action potentials that occurred during one full cycle

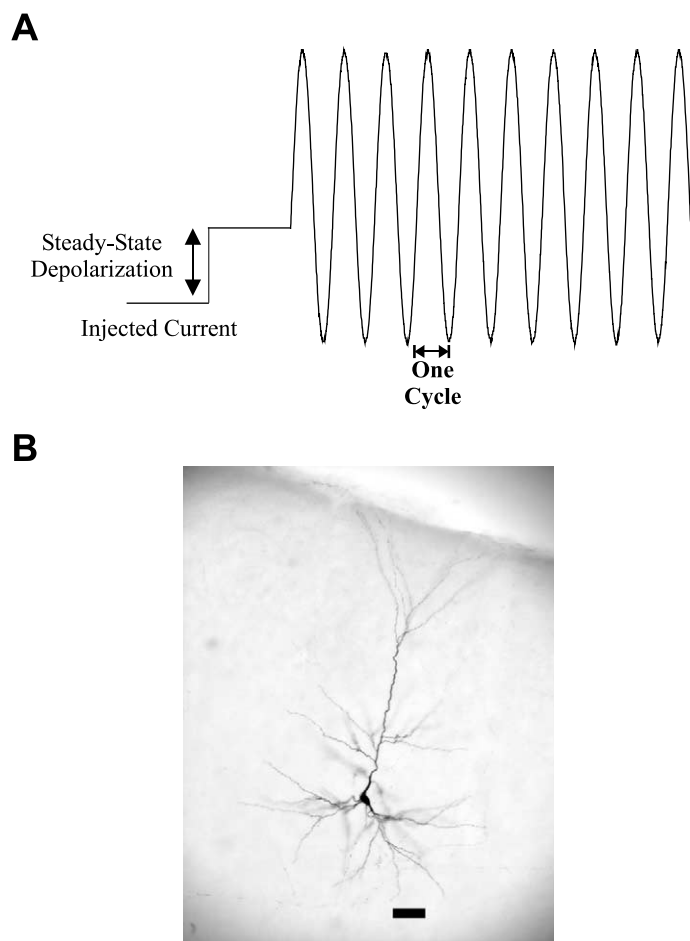


Fig. 1. (A) Representation of the steady-state depolarization and subsequent superimposition of a sine wave stimulus which was injected into the neurons under study. (B) A representative layer II pyramidal cell filled with intracellular injection of biocytin. The pial surface is towards the top right. Scale bar = 50 μm .

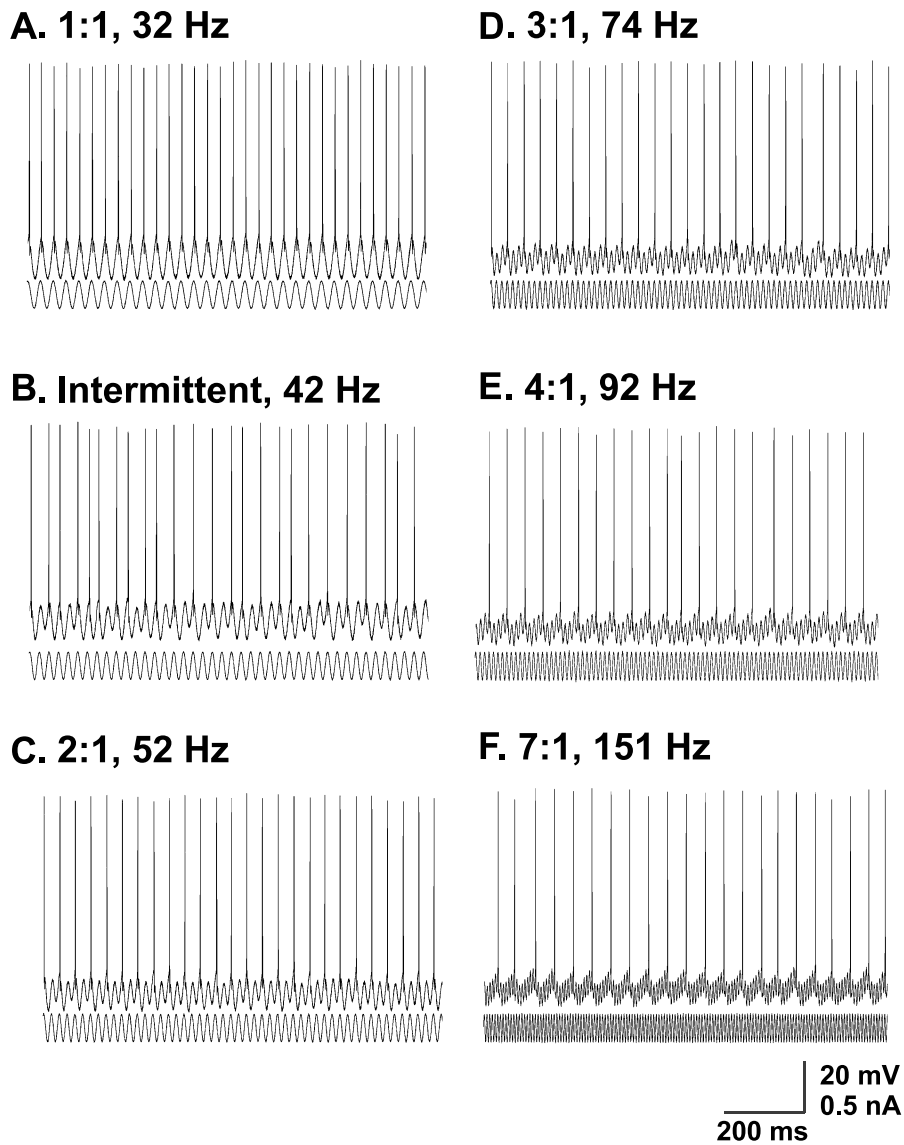


Fig. 2. Response of a supragranular pyramidal cell (pictured in Fig. 1B) to sinusoidal input. Top traces represent membrane voltage and the bottom trace is the current input which was superimposed on a +0.2 nA steady-state depolarization. At low frequencies the neuron responded with one action potential per stimulus cycle (A). As input frequency increased the neuron began to skip cycles (B). Whole number firing ratios (C–F) were separated by intermittent firing regimes (not pictured, e.g. B).

(360°) of the sine wave and then averaging over all presentations of a particular frequency. The phase of the first spike was computed by measuring when the first action potential of each cycle occurred in relation to the first upward zero crossing of the injected sine wave (in degrees) and then averaging over all cycles of a particular frequency. Statistics were computed using either Microcal Origin (Microcal Software) or Statview (Abacus Systems) on a PC.

Histology

After a recording was complete the slices were subsequently fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for subsequent biocytin immunoreactivity (Horikawa and Armstrong, 1988). Slices were subsequently placed in 20% sucrose in 0.1 M phosphate buffer, sectioned on a freezing stage sliding microtome at 60 μ m thickness and reacted using an ABC kit (Vector Labs). Sections were mounted, dehydrated, cleared and coverslipped using Permount mounting medium, were examined using a Zeiss Axioplan2 microscope and images

captured using a ProgRes camera and Adobe Photoshop (Adobe) on a Macintosh. Neurons were classified as pyramidal or non-pyramidal according to their morphologies and their laminar location was confirmed.

RESULTS

Frequency-dependent firing patterns

The data reported are from 13 morphologically identified regular-spiking supragranular pyramidal neurons (Fig. 1B). Figure 2 is typical of the responses observed in response to sine wave current injections. A slight depolarizing current (+0.2 nA) was injected to bring the neuron under study to threshold and a sine wave stimulus was superimposed. Neuronal threshold was opera-

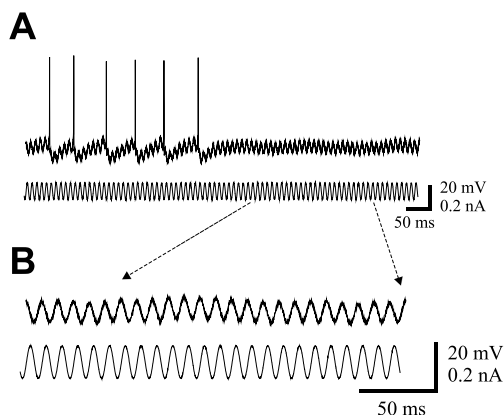


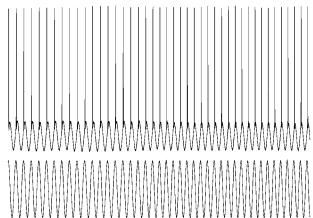
Fig. 3. At high frequencies of stimulation, neurons would respond initially with several action potentials (A) and then exhibit sub-threshold oscillations at the frequency of the injected sine wave (B). The frequency of injected sine wave was 100 Hz.

tionally defined as the minimum magnitude of the steady-state depolarization necessary to evoke a continuous train of action potentials. In Fig. 2, the top trace in each panel shows membrane voltage in response to the sine wave current injection presented in the lower trace.

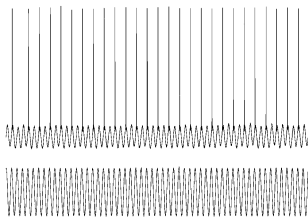
Neurons responded in stereotyped fashion to injected sine wave currents, at low frequencies neurons responded with at least one action potential per cycle of the sine wave (Fig. 2A). By convention, activity evoked by sinusoidal inputs is quantified as a ratio: number of cycles of the stimulus: number of action potentials (see Glass et al., 1980). For instance, in Fig. 2A, with one action potential for every sine wave cycle, the ratio is 1:1. In Fig. 2C there are two cycles for each action potential and thus the ratio is 2:1. In the example represented in Fig. 2 stable firing regimes were identified for every integer up to 7:1 (Fig. 2F). Between each whole number ratio, neurons exhibited irregular or intermittent firing patterns. Over a large range of sine wave frequencies the stable firing regimes (e.g., 1, 2, 3:1) always alternated

Vary Sine Wave Amplitude

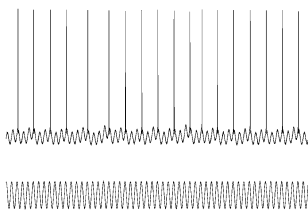
A. 1:1, 0.975 nA



B. 2:1, 0.671 nA



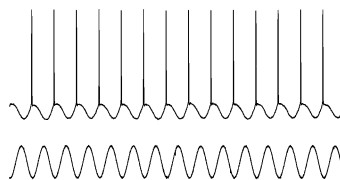
C. 3:1, 0.515 nA



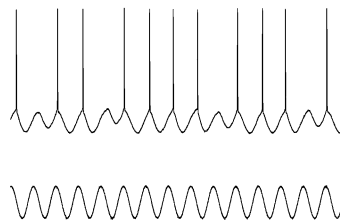
25 mV
0.5 nA
500 ms

Vary Steady-State Depolarization

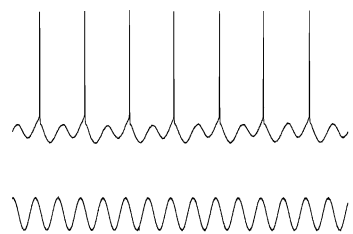
D. 1:1, +0.4 nA



E. Intermittent, +0.3 nA



F. 2:1, +0.2 nA



75 mV
0.5 nA
200 ms

Fig. 4. Varying the sine wave amplitude while holding the steady-state depolarization (+0.2 nA) and sine wave frequency (28 Hz) constant moved the neuron from a 1:1 (A) to a 2:1 (B) and finally a 3:1 (C) firing regime. Not pictured are the intermittent regimes that separated the three different regimes. Varying the steady-state depolarization while fixing both the sine wave amplitude (peak-to-peak +0.25 nA) and frequency (15 Hz) moved a different neuron from a 1:1 (D) to a 2:1 (F) regime with an intermittent regime separating the two (E).

with regimes of intermittent firing. Even in these intermittent regimes the neuron's response was driven by the stimulus, but what cycle of the sine wave would evoke an action potential was unpredictable.

All regular-spiking neurons tested ($n=13$ of 13) displayed similar behavior. The frequency at which the transition between regular and intermittent firing began differed from neuron to neuron (see below). At high frequencies (≥ 100 Hz) neurons would respond initially with a burst of action potentials and then demonstrate subthreshold oscillations at the input frequency of the sine wave input ($n=4$ of 4). In Fig. 3A the neuron responded with several action potentials following a high frequency sine wave input (100 Hz) before it stopped generating action potentials, presumably due to the buildup of Na^+ channel inactivation (Hille, 1992, Henze and Buzsaki, 2001). The subthreshold oscillations that result are driven by the sine wave input (see Fig. 3B).

Amplitude-dependent firing patterns

Varying the amplitude of the sine wave also resulted in a progression from regular to intermittent firing ($n=5$ of 5). In Fig. 4A–C the frequency of the injected sine wave was fixed at 28 Hz and steady-state depolarization was kept at +0.2 nA while the amplitude was systematically modulated. The response shown in Fig. 4 is typical of those neurons where amplitude variations were examined. At large amplitudes the neuron responded in a 1:1 fashion (Fig. 4A). As the amplitude of the sine wave was decreased the neuron showed 2:1 (Fig. 4B) and 3:1 (Fig. 4C) firing regimes. Similar to the case where the frequency was modulated there were intermittent firing regimes between each integer step (data not shown).

Steady-state depolarization-dependent firing patterns

As explained in Experimental procedures, the sine wave injection current was superimposed upon a steady-state depolarizing offset. In six neurons the magnitude of this depolarization was systematically varied resulting in a response progression similar to those described above (see Fig. 4D–F). In Fig. 4D–F the amplitude and frequency of the sine wave were held constant while the steady-state depolarization was varied (+0.2 to +0.4 nA). In response the neuron moved from a 1:1 (Fig. 4D) to an intermittent (Fig. 4E) to a 2:1 (Fig. 4F) firing regime. The magnitude of the steady-state depolarization is noted instead of the membrane potential, since even the smallest depolarization was a suprathreshold stimulus and increasing the magnitude of the depolarization increased the basal firing rate of the neuron without much effect on the membrane potential.

Importantly, larger depolarizations allowed the neurons to respond to a wider range of frequencies in a 1:1 fashion ($n=6$ of 6). As will be discussed below, this ability suggests that more depolarized neurons will be able to respond with at least one action potential per cycle over a larger frequency range.

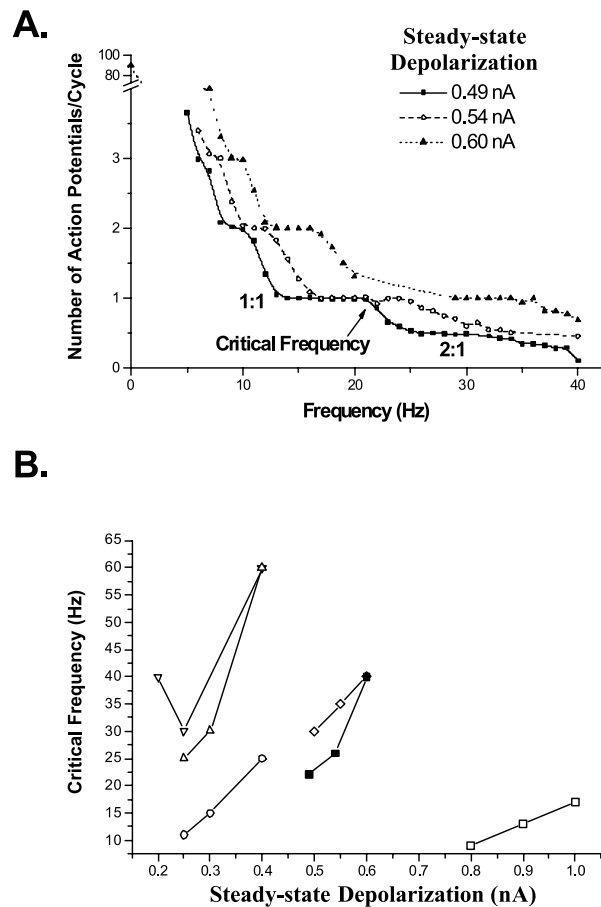


Fig. 5. Increasing the steady-state depolarization shifts the frequency–response curve to the right (A). Plotting the frequency where the 1:1 regime is lost (critical frequency) versus the magnitude of the steady-state depolarization in two representative examples (B) demonstrates that the critical frequency is a function of how activated the neuron is. The filled symbols in B represent the neuron in A.

Stable firing regimes

Next the range of frequencies that display regular, non-intermittent, firing regimes and how these ranges depend on the neuron's level of activation (depolarization) was examined. Figure 5A shows a representative sample from the present population of neurons. Plotted on the x -axis is the input frequency of the injected sine wave and on the y -axis the average number of action potentials per cycle of the sine wave for three different magnitudes of depolarization. The frequency at which the transition was made between regular, 1:1 firing and intermittent firing was termed the *critical frequency* (arrow in Fig. 5A). Increasing the sine wave frequency further revealed the existence of a 2:1 regime. At low sine wave frequencies it was possible to elicit more than one action potential per cycle as can be seen in the low frequency ranges in Fig. 5A.

In all neurons ($n=6$ of 6) in which the steady-state depolarization was systematically varied the frequency–response curve shifted to the right with larger depolarizing offsets. To quantify this observation the critical frequency was identified for each neuron in response to

three different steady-state depolarizations. Figure 5B shows that the sine wave frequency at the end of the 1:1 regime (critical frequency) increased as a function of the steady-state depolarization for six different neurons (represented by different symbols). Comparing the critical frequency in response to the smallest and largest depolarization for each cell revealed that these differences were significant (paired *t*-tests, P 's < 0.05, $n = 6$).

Spike half-width is correlated with the critical frequency

Figure 6 demonstrates that critical frequency is also dependent on a neuron's intrinsic properties. To assess this the width of the action potential without the application of a steady-state depolarization was determined (see [Experimental procedures](#)) and related to that neuron's critical frequency. In Fig. 6A the critical frequency (*y*-axis) is plotted as a function of the action potential width measured at half amplitude (*x*-axis). A linear regression showed that there is a significant interaction, the narrower the spike the higher the critical frequency ($n = 13$, $r = -0.76$, $P < 0.003$). A similar analysis comparing critical frequency with input resistance (Fig. 6B) revealed no correlation ($n = 13$, $r = 0.12$, $P > 0.5$). Thus neurons with thin action potentials are able to follow inputs to higher frequencies than neurons with broader action potentials. Along with a neuron's state, therefore,

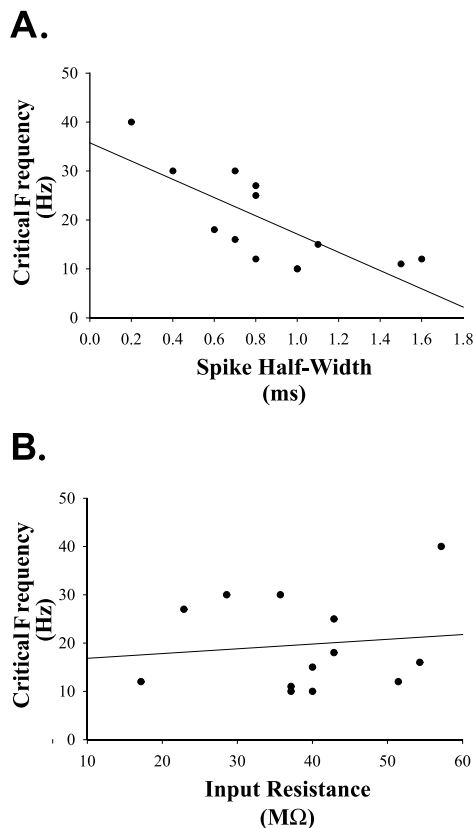


Fig. 6. The critical frequency is related to spike width (A), but not input resistance (B). Panel A plots the critical frequency (*y*-axis) versus spike width at half amplitude (*x*-axis) which reveals a significant correlation (plotted line of linear regression). A similar correlation is not seen when critical frequency is plotted against input resistance (B).

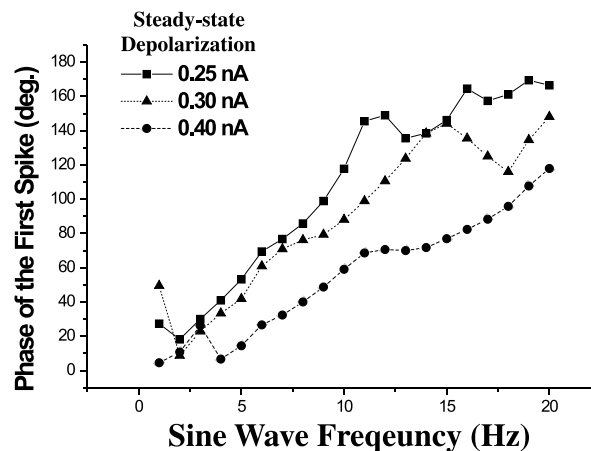


Fig. 7. Increasing the steady-state depolarization phase advances the first action potential per cycle. At low sine wave input frequencies (*x*-axis), the first action potential per cycle occurs relatively early in the sine wave cycle (*y*-axis), action potentials occur later in the cycle for higher input frequencies.

the ability of a neuron to respond 1:1 may also be modulated via changing currents that effect action potential generation.

Increasing the magnitude of the steady-state depolarization offsets advances the spike

The generation of an action potential relative to a neuron's input might be important in encoding certain temporal characteristics of the input. One benefit of using sine wave stimuli is that the resultant output, in this case action potentials, can be related not only to the frequency or amplitude of the stimulus, but also to its phase. The input/output phase relationship was examined for each neuron with one example represented in Fig. 7. As the frequency of the sine wave increased the action potential occurred at a later phase in the sine wave cycle ($n = 13$ of 13). Similar results have been seen in neurons coupled by gap junctions, increasing the frequency of the sine wave injected into one neuron increased the phase lag in the coupled cell increased (Galarreta and Hestrin, 1999). This phenomenon appears to be a general feature of placing a RC circuit in series with a sine wave current injection. In both cases the neurons appear to be acting as low pass filters. Additionally, as the DC offset was increased the action potentials occurred earlier in the cycle ($n = 4$ of 4). Thus increasing the steady-state depolarization caused the action potentials to become phase-advanced (a downward shift in the curve in Fig. 7). One possible explanation for this observation could be related to the threshold for action potential generation. If the neuron has a fixed threshold, it would be predicted that as the steady-state depolarization was increased the threshold would be reached sooner (the action potential occurring earlier in relation to the sine wave cycle). Consistent with the idea of a fixed threshold is the finding that when the time between the zero crossing of the sine wave and the initiation of the first action potential is computed, the neuron fires sooner when it is depolarized to a greater extent ($P < 0.05$).

DISCUSSION

The ability of pyramidal neurons to respond to injected sine wave currents is a function of the frequency, the amplitude of the injected sine wave, as well as the magnitude of the steady-state depolarization. Neurons responded with at least one action potential per sine wave cycle when the injected sine wave was at relatively low frequency or large amplitude. As either the frequency was increased or the amplitude decreased a critical frequency was reached such that the neuron moved from a 1:1 firing regime, to an intermittent regime, to a 2:1 regime and so forth. The critical frequency was also a function of spike width, neurons possessing action potentials with thinner half-widths could respond in a 1:1 manner over a larger frequency range. Finally, the activation level of the neuron was crucial in determining its response. With a larger steady-state depolarization, a neuron remained in the 1:1 regime over an almost two-fold greater range of frequencies as compared to small steady-state depolarizations.

Why skip cycles?

It is evident that even in response to the largest steady-state depolarization the regular-spiking neurons can be moved out of the 1:1 regime. The exact physiological mechanism underlying the different firing regimes and the transition between them is not clear. However, there are several possibilities: synaptic inputs, intrinsic membrane properties, channel noise or the dynamics of the Na⁺ and K⁺ channels that underlie the action potentials. It is unlikely that synaptic inputs are the basis of either the intermittent or 2:1 regime, since there was relatively little spontaneous activity observed *in vitro* (see Pare et al., 1998). The different regimes could be due to the dynamics of the membrane itself. It is possible that neurons that are more electrotonically compact (possessing shorter time constants) charge their membranes quicker and thus can follow higher frequency sine wave inputs. Similarly, if the site of current injection is farther away from the action potential trigger zone it might not be able to follow high frequency sine wave inputs, due to filtering. Another possible candidate is the dynamics of the Na⁺ channel (see Hille, 1992). At low input frequencies enough channels could recover from inactivation and generate at least one action potential per sine wave cycle. At higher frequencies it is possible that a significant portion might not yet have recovered from inactivation and thus a spike can be missed and sometimes it can be generated (intermittent regime). The fact that there is no significant change in spike amplitude during the injection of the sine wave argues against a significant role that Na⁺ channel inactivation might play in this phenomenon. The exact mechanism underlying the different firing regimes remains to be determined.

Expansion of frequency following

The results from the present study complement a related study by Carandini et al. (1996) which found

that pyramidal neurons in guinea-pig visual cortex could respond similarly to sine wave stimulation. They interpreted their results in terms of how changing the activation of a neuron (magnitude of the steady-state depolarization) modulated the threshold of spike initiation, but they did not focus on the firing pattern of the neurons. In the present study it was determined that as the magnitude of the steady-state depolarization was increased the neuron could remain in the 1:1 regime for a wider range of input frequencies. Consistent with these results, Hunter et al. (1998) demonstrated the existence of both stable (1:1) and intermittent firing regimes that could be transitioned between by modulating either the magnitude of the steady-state depolarization or the sine wave amplitude in *Aplysia*. Additionally, Fellous et al. (2001) showed the expansion of the 1:1 regime was dependent upon the amplitude of the injected sine wave in rat prefrontal neurons.

One possible interpretation of the present results is the possibility that a network can regulate the gain of its constituent neurons. At low levels of network activity, corresponding to a low steady-state depolarization, the neuron could respond in a 1:1 fashion to a limited number of input frequencies. As the neuron becomes more activated, corresponding to a larger steady-state depolarization or more network activity, this range is expanded. One consequence of increased network activity is increased ionic and synaptic conductances (Pare et al., 1998; Destexhe and Pare, 1999) which are not approximated by the steady-state depolarization and whose impact on the behavior of the neuron is undetermined.

The intermittent regimes present between the whole number firing regimes (e.g., 1:1, 2:1) have been observed in a variety of cell types and species. In similar experiments carried out in cardiac cells (Chiavlo and Jalife, 1987; Heschler and Speicher, 1989), mollusk neurons (Hayashi and Ishizuka, 1992) and squid axons (Guttman et al., 1980), the intermittent regimes were shown to be fractal in nature. The behavior exhibited in these previous reports and the pyramidal neurons in this study has been characterized as a 'devil's staircase' (e.g. 1:1, 2:1, 3:1... firing regimes interrupted by chaotic firing regimes) which results when an endogenous oscillator such as a neuron is driven at varying frequencies (see Glass et al., 1980; Wang, 1994; Coombes and Bressloff, 1999). In cardiac cells certain classes of arrhythmias (Wenckebach) are characterized by a devil's staircase phenomenon (Chiavlo et al., 1990). It is possible that certain electrogenic pathologies in the brain might be preceded by a devil's staircase-type phenomenon. *In vitro* studies within the CA3 region of the hippocampus have shown that epileptiform activity following mossy fiber tract stimulation can follow a devil's staircase pattern (Hayashi and Ishizuka, 1995). Furthermore, Hayashi and Ishizuka (1995) demonstrated that the CA3 population spike can be entrained in a 1:1, 2:1 or 3:1 regime with chaotic regimes separating these regimes as a result of mossy fiber stimulation.

Is there any advantage for a neuron to respond in a 1:1 fashion? There is a history both *in vivo* and *in vitro* of studying the input/output transformations of neurons (e.g. McCormick et al., 1985). It is often assumed that by

responding with at least one action potential per stimulus cycle there is high throughput and a maximization of information transfer from one stage of processing to the next. Interestingly, neurons that are closest to the periphery stimulus such as mechanoreceptors can respond in a 1:1 fashion over the widest range of frequencies (Gardner et al., 1992). In the visual system geniculate neurons can follow sine wave stimuli at higher frequencies than layer IV cortical neurons (Orban et al., 1985; Hawken et al., 1996). The conservation of the 1:1 regime may reflect a need for higher processing centers to receive as much information from the periphery as possible in order to perform their cortical calculations. For example, when the visual system is confronted with a complex scene and as a result is highly activated (similar

to a large steady-state depolarization), neurons within the visual cortex could respond to a wider range of inputs. Thus the cortex would have a mechanism where it could self-regulate its gain. Furthermore, it has been shown that increasing the amplitude of an injected sine wave (Fellous et al., 2001) increases spike timing reliability which may be important for the encoding temporal stimuli (for review see deCharms and Zador, 2000).

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