

Stimulus dependence of two-state fluctuations of membrane potential in cat visual cortex

Jeffrey Anderson¹, Ilan Lampl¹, Iva Reichova¹, Matteo Carandini² and David Ferster¹

¹ Department of Neurobiology and Physiology, Northwestern University, 2153 North Campus Drive, Evanston, Illinois 60208, USA

² Institute for Neuroinformatics, ETH/University of Zürich, CH-8057 Zürich, Switzerland

Correspondence should be addressed to D.F. (ferster@nwu.edu)

Membrane potentials of cortical neurons fluctuate between a hyperpolarized ('down') state and a depolarized ('up') state which may be separated by up to 30 mV, reflecting rapid but infrequent transitions between two patterns of synaptic input. Here we show that such fluctuations may contribute to representation of visual stimuli by cortical cells. In complex cells of anesthetized cats, where such fluctuations are most prominent, prolonged visual stimulation increased the probability of the up state. This probability increase was related to stimulus strength: its dependence on stimulus orientation and contrast matched each cell's averaged membrane potential. Thus large fluctuations in membrane potential are not simply noise on which visual responses are superimposed, but may provide a substrate for encoding sensory information.

When stimulated with an optimal drifting grating, a complex cell in visual cortex responds with a steady increase in average firing rate. Given these responses, one might expect membrane potential to respond in a similar manner, that is, with steady depolarization. This is indeed the case when responses of membrane potential are averaged over many stimulus trials¹. When monitoring the membrane potential on individual trials, however, we were struck by the presence of large, low-frequency, bistable fluctuations. Because the fluctuations are not time locked to the stimulus², however, when averaged over many stimulus trials, the individual events merged into a smaller but steady depolarization.

Large, two-state fluctuations occur in neurons throughout the brain^{3–10}, including the visual cortex (D.F. and M.C., *Soc. Neurosci. Abstr.* 22, 490, 1996), where they are remarkably synchronized in neighboring cells² and coincide with waves of activity traveling across the cortex¹¹ (A. Sterkin *et al.*, *Soc. Neurosci. Abstr.* 25, 784, 1999). It is probable that these fluctuations result from alternating patterns of synaptic activity^{6,8,12}, and thus represent bistability in the presynaptic cortical network. The functional impact of these fluctuations, however, is not known. In the context of sensory encoding, in particular, it is not clear if these fluctuations only reflect cortical spontaneous activity or if they contribute to the stimulus representation.

We investigated these issues by recording intracellularly from 95 neurons in primary visual cortex of anesthetized cats. We found that the amplitude of the fluctuations did not change with a stimulus. Instead, visual stimulation primarily affected the dynamics of the fluctuations, increasing the proportion of time the membrane potential spent in the depolarized state. This change in probability accounted for most of the depolarization observed in averaged membrane potential responses.

RESULTS

When averaged over several trials of an optimally oriented drifting grating, the responses of one of our complex cells conformed to previous reports of complex cell behavior^{1,13,14} (Fig. 1a and b). At the appearance of the stimulus (arrow), the mean firing rate rose by ten spikes per second (Fig. 1a), and the mean membrane potential rose by ten mV (Fig. 1b). The membrane potential recorded during individual trials, however, offered a radically different picture of the response (Fig. 1c). Large (>10 mV) fluctuations lasting from 50 ms to over a second (not shown) both preceded and followed the stimulus onset. Overall, the membrane potential tended to occupy 1 of 2 small ranges of potential, an up state and a down state, separated by 10–15 mV. The transitions between states were not locked to the stimulus: during visual stimulation, the rare down states occurred at different times in each trace. What was consistent from trace to trace, however, was a significant, visually evoked increase in the time the membrane potential spent in the up state. Visual stimulation also increased the mean potential of the up state by a few millivolts (see below).

This two-state behavior was present in most complex cells, though to varying degrees. Representative traces from 5 simple cells and 5 complex cells, accompanied by the membrane-potential histogram for each cell, illustrate the spectrum of 2-state behavior we recorded from 95 visual cortical cells (Fig. 2). About 60% (39 of 65) of the complex cells in our sample had clearly bimodal membrane-potential histograms, indicative of 2 states. By contrast, only 13% of simple cells (4 of 30) had bimodal histograms. It is possible that these proportions may slightly underestimate the prevalence of two-state behavior in our sample, as several cells (such as complex cell 4 in Fig. 2) showed evidence of two-state behavior in sample traces, but had merely skewed rather than bimodal distributions of membrane potential.

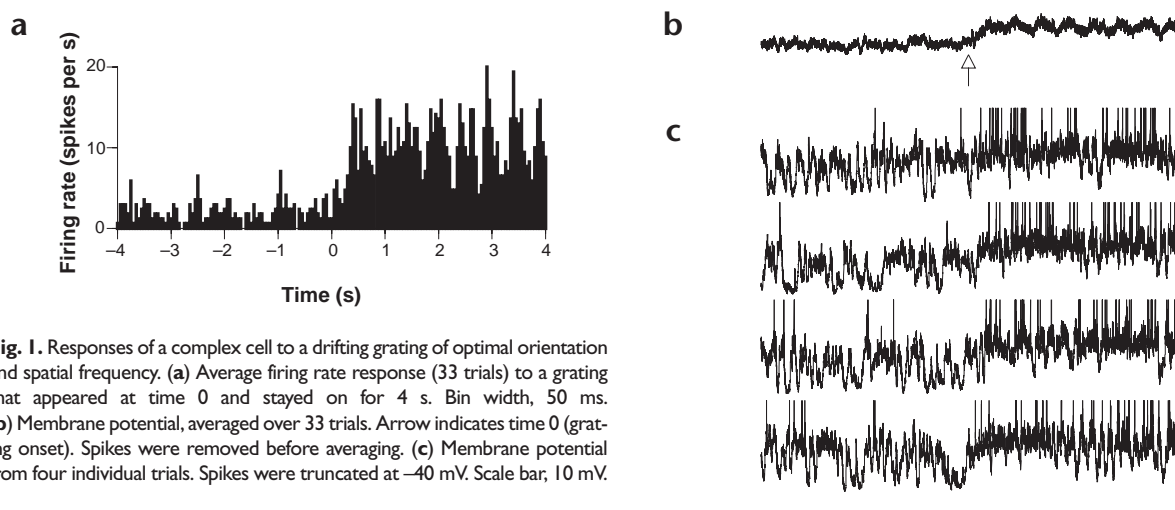


Fig. 1. Responses of a complex cell to a drifting grating of optimal orientation and spatial frequency. (a) Average firing rate response (33 trials) to a grating that appeared at time 0 and stayed on for 4 s. Bin width, 50 ms. (b) Membrane potential, averaged over 33 trials. Arrow indicates time 0 (grating onset). (c) Membrane potential from four individual trials. Spikes were truncated at -40 mV. Scale bar, 10 mV.

The effect of visual stimulation on membrane potentials of cells showing prominent fluctuations differed from the effect on those cells that lacked them. Figure 3a contains traces from five different complex cells with and without visual stimulation. We plotted histograms of membrane potential recorded with and without visual stimulation to quantify both the degree to which the responses comprised two distinct states and the visually evoked increase in time spent in the up state (Fig. 3b). Cell 1 gave responses similar to those predicted by averaged responses of complex cells: we observed no large fluctuations in membrane potential, the distribution of spontaneous membrane potential was clearly unimodal, and the distribution was shifted to the right and broadened by presentation of a visual stimulus. In cells 2–5, however, the membrane potential was distributed bimodally, with the two peaks representing the up and down states. Visual stimulation in these cases shifted much of the weight of the distribution from the down to the up state, as indicated by the relative area under each of the two peaks. In cells 3 and 4, visual stimulation additionally depolarized the mean potentials of the two states, shifting their peaks by five and eight mV.

To measure the effects of visual stimulation on the dynamics of state transitions, we further studied 25 complex cells with high-amplitude (greater than 10 mV) fluctuations in membrane potential. To measure the duration of each state transition, two thresholds were set at one fourth and three fourths of the distance between the peaks of the membrane potential distribution. When the membrane potential rose above the upper threshold, a down-to-up transition was registered; when the membrane potential fell

below the lower threshold, an up-to-down transition was registered⁶. We plotted cumulative histograms of the durations of 4172 down and 4174 up events from these 25 cells (Fig. 3c). Our results for mean event durations in the spontaneous condition were similar to those previously observed⁶. During visual stimulation, the mean duration of the up events increased more than twofold, from 196 ± 9 ms (s.e.) to 410 ± 31 ms, whereas the duration of the down events decreased more than twofold from 135 ± 5 ms to 52 ± 2 ms. Because visual stimulation increased the average duration of up events more than it decreased the duration of down events, it also reduced the overall frequency of transitions. No serial dependence of events was observed in the spontaneous⁶ or stimulated conditions, and events were independent of the timing of the stimulus cycle. Visual stimulation substantially increased the total time spent in the up state—from $53 \pm 3\%$ to $84 \pm 3\%$. We observed such marked changes in the percentage of time spent in the up state in every cell showing prominent fluctuations. Time in the up state during a generally arousing stimulus (loud noise, tail pinch or visual stimulus outside the receptive field) was measured in three additional cells and found not to differ from baseline.

In addition to increasing the time spent in the up state, visual stimulation often evoked a small depolarization in the mean potential of each state (Fig. 3b, cells 3 and 4). To measure this effect, we identified the midpoint between the two peaks in each membrane-potential distribution. The potentials for the up and down states were defined as the mean potential on either side of the midpoint. The visually evoked changes in mean potential of the two states were then averaged together (Fig. 3d). The mean change in state potential was 2.6 ± 0.5 mV (range, -0.8 to 8.7 mV, $n = 25$). The overall response (the visually evoked change in mean membrane potential) was 5.7 ± 0.7 mV. By subtracting the

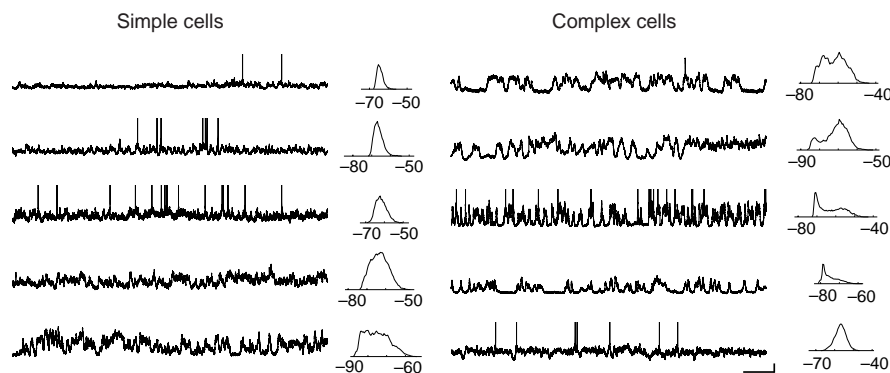


Fig. 2. Prevalence of two-state fluctuations in simple and complex cells. Each trace represents ten seconds of recording in the absence of visual stimulation. Each membrane-potential histogram (to right of each trace) was calculated from 20–90 s of recording without visual stimulation. Scale bars, 10 mV; 1 s.

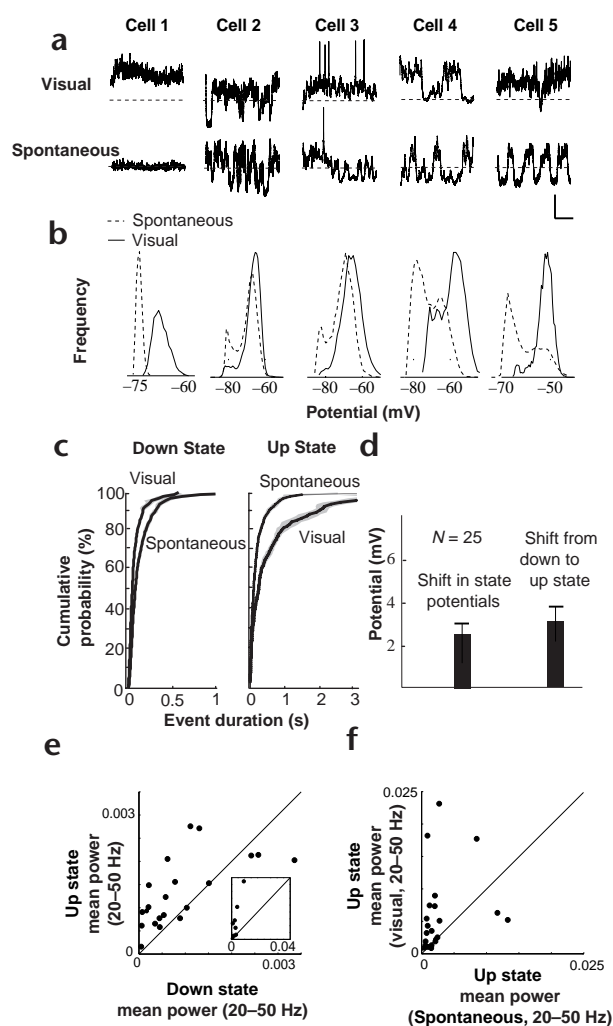


Fig. 3. Visual responses of complex cells. (a) Traces from five complex cells during spontaneous activity (lower) and during stimulation with an optimal drifting grating (upper). Horizontal reference lines show mean potential of spontaneous activity for each cell. Scale bars, 10 mV, 1 s. (b) Membrane potential histograms calculated from 4–45 stimulus trials during spontaneous activity (dotted lines) and visually evoked activity (solid lines). (c) Cumulative distributions of up- and down-event durations pooled from 25 complex cells with prominent 2-state fluctuations in membrane potential. Solid lines show probability that event duration would be less than or equal to a given value. Shaded regions show 95% confidence intervals for event duration. Confidence intervals were calculated using a bootstrap method by taking 1000 subsets of the complete data set. (d) Two components of the membrane potential response for 25 cells, the shift in the mean potential of the up and down states and the shift in mean potential attributable to the increase in time spent in the up state. These two components sum to the visually evoked shift in mean potential. (e) Difference in the mean power in the 20–50 Hz range of the membrane potential during up and down events. (f) Difference in the mean power (20–50 Hz) of up states before and during visual stimulation.

change in state potential from the overall response for each cell, we concluded that the remaining 3.1 ± 0.4 mV of the visual response must have arisen from the increased probability of the up state.

Yet a third effect of visual stimulation in complex cells is an increase in the amplitude of the high-frequency components of the membrane potential^{2,15}. Visual stimulation substantially

increased the variance of the potential, especially during the up state (Figs. 1b and 3a, for example). To quantify this effect, we calculated the mean power of the membrane potential within the 20–50 Hz range. In the eight cells of Fig. 3c, the mean high-frequency power in the absence of visual stimulation in the up state was more than triple that in the down state (Fig. 3e). Visual stimulation evoked a further twofold increase in high-frequency fluctuations in the up state (Fig. 3f).

For cells with prominent fluctuations, changes in the up- and down-state probabilities, changes in the potentials of the states themselves and changes in high-frequency components of the potential all contributed to a cell's visual selectivity. Orientation-tuning curves constructed from all three measures and from mean membrane potential and spike rates (Fig. 4) paralleled one another closely. On average, the preferred orientation for mean membrane potential (as determined from a Gaussian fit to the tuning curves) differed from that of time in the up state by $16 \pm 7^\circ$, from that of change in mean state potential by $5 \pm 2^\circ$ and from that of high-frequency (20–50 Hz) power by $23 \pm 14^\circ$ ($n = 5$). The tuning width (full width at half height) for spikes was $45 \pm 12^\circ$ ($n = 4$); for mean membrane potential it was $78 \pm 10^\circ$ ($n = 5$), for time in the up state it was $78 \pm 12^\circ$ ($n = 5$), for change in state potential it was $82 \pm 20^\circ$ ($n = 5$), and for high-frequency power it was $50 \pm 10^\circ$ ($n = 5$). Contrast-response curves constructed from the three measures of membrane potential response also paralleled one another closely (Fig. 4).

There is little doubt that the amount of time spent in the up state influenced a cell's spike activity; the cell in Fig. 1c never fired when the membrane potential was in the down state. Once a cell was in the up state, however, an increase in the mean potential of the up state should have increased the firing rate even further. An increase in the amplitude of high-frequency components of the membrane potential during the up state should have done the same, as threshold would be crossed more frequently and would be effectively lowered during a rapid rise in membrane potential^{16–18}. We measured the contributions of these two components to the visually evoked responses of three cells by plotting firing rate against each component over multiple stimulus trials (Fig. 5). Strong positive correlations in both cases suggested that mean state potential and high-frequency component amplitude, together with time spent in the up state, acted synergistically to increase firing rate. Additionally, because each aspect of the membrane potential responses was tuned for stimulus orientation, each must have contributed to the tuning of the firing rate responses.

DISCUSSION

Here we investigated the visual responses of complex cells with prominent low-frequency membrane potential fluctuations. We found that the visual response of a cell as reflected in the averaged membrane potential could be attributed to a combination of two factors, a change in the dynamics of the membrane potential fluctuations, in which the neuron spent more time in the depolarized state, and a tonic depolarization, in which the states themselves were depolarized. The relative contributions of these two modes of response varied among complex cells, with some cells showing only tonic depolarization and other cells showing only a change in the dynamics of membrane potential fluctuations. Most cells fell between these two extremes, showing a combination of both modes of visual response. We observed two-state fluctuations in nearly two of three complex cells, and in cells with large fluctuations, an increase in the time spent in the depolarized state was usually the dominant mode of visual response. Both modes of visual response contributed to the observed increase

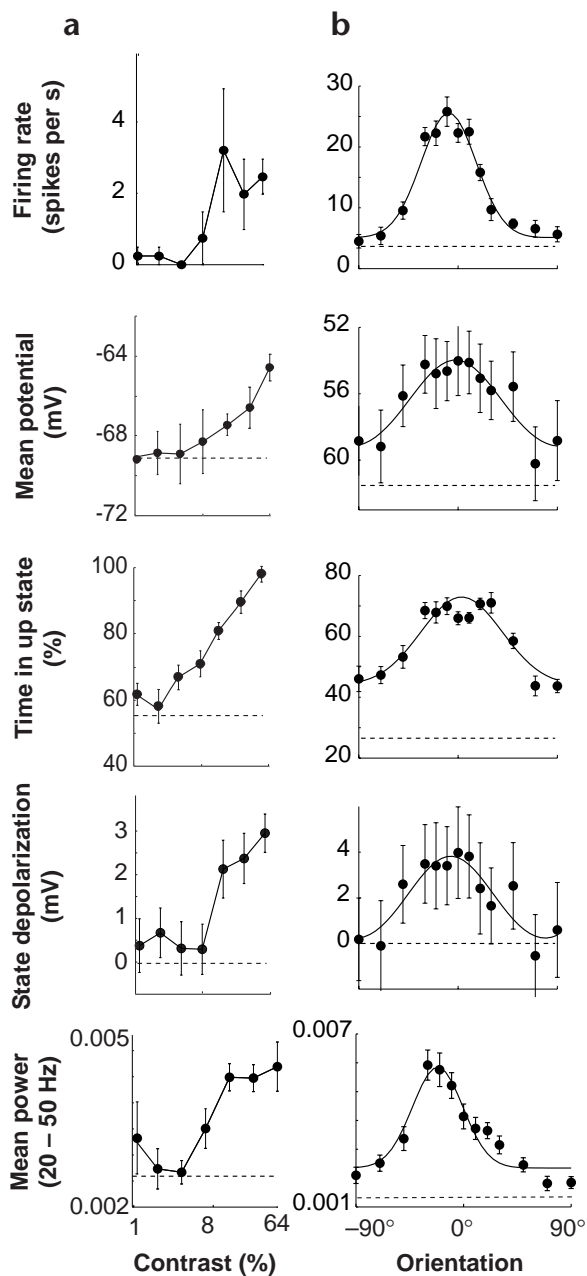


Fig. 4. Visual response properties derived from membrane potential fluctuations in complex cells. **(a)** Contrast–response curves for spikes, mean potential, time in the up state, shift in the state potentials and mean power at 20–50 Hz of the membrane potential. Data represent averages of five to ten responses to drifting gratings at each stimulus contrast, presented for four s in random order (error bars, s.e.). Horizontal dashed lines show the value of each parameter in the absence of visual stimulation. **(b)** Orientation tuning curves for the same five parameters in another cell. Solid lines show fits to a Gaussian curve.

in firing rate in response to visual stimuli. In addition, a third component contributed to the increase in firing rate; this involved increased amplitude of high-frequency fluctuations observed while the neuron occupied the depolarized state.

Average membrane potential, time in the up state and mean up-state potential were tuned for orientation with the same pre-

ferred orientation, on average. The high-frequency component also had the same preferred orientation as the other three components, but, similar to spike responses¹⁹, was more narrowly tuned. This difference opened the possibility that the different components were not all mediated by the same cortical circuitry, and that each might modulate a cell's response independently.

Previous paired intracellular recordings in cortical neurons demonstrate that membrane potential fluctuations occur nearly simultaneously in nearby neurons within the cortical circuit². Field-potential^{20,21} (D.F. and M.C., *Soc. Neurosci. Abstr.* 22, 490, 1996) and optical recordings (A. Sterkin *et al.*, *Soc. Neurosci. Abstr.* 25, 784, 1999), in conjunction with intracellular recording, show that activity can correlate over large segments of the cortical network. This necessarily means that the bistable fluctuations do not arise intrinsically from voltage-dependent mechanisms, but are largely synaptic in origin^{6,8,12}. The observation that intracellular current injection does not significantly change the distribution of time spent in up and down states supports this conclusion². Thus visual stimulation may not act independently on individual cortical cells, but may interact at the network level with the mechanism generating synchronized fluctuations within a neuronal population.

The difference in bimodality between simple and complex cells is striking, and suggests that network architecture in cortical layers containing simple cells (layers 4 and 6) differs from that found in layers containing primarily complex cells (layers 2, 3 and 5)²². It is proposed that intercolumnar interconnections in the cortex operate as a positive-feedback amplifier^{23–25}. Such an amplifier, when overdriven, could produce exactly the two-state behavior we observed. It may be, then, that feedback operates exclusively in those layers where complex cells are found, which are also the layers with the richest synaptic interconnections.

Fluctuations in membrane potential such as those described here are observed in neurons of the cortex^{10,26–28}, thalamus⁹ and striatum^{3–8}, both in awake³ and in anesthetized² animals. Because our results were all collected from anesthetized animals, further experiments will be required to determine the extent to which these mechanisms operate in the alert animal. The observation that these fluctuations were modulated in a predictable way by visual stimuli, however, presents the possibility that they may participate in the cortical representation of stimuli. This possibility is strengthened by the findings that spontaneous single-neuron activity in the visual cortex is highly correlated with local population activity (A. Sterkin *et al.*, *Soc. Neurosci. Abstr.* 25, 784, 1999), and that this population activity shows similar spatial organization to that evoked by visual stimuli²⁹. Bimodality in the cortical network could be an advantageous mechanism for representing a continuous variable such as stimulus orientation or stimulus contrast, as modulating the probability of the up state (in which spiking easily occurs) allows firing rate to be varied in a continuous manner. In a manner similar to stochastic resonance, this computational strategy may be less sensitive to threshold nonlinearities and synaptic noise than would be a graded response close to the spike threshold.

METHODS

Recordings were obtained from anesthetized cats as previously described^{2,30}. Visual stimuli consisted of drifting sinusoidal gratings optimized for spatial and temporal frequency under computer control. Current-clamp recordings were made in the whole-cell configuration (Figs. 2 and 3; $n = 50$ cells)³¹ or with sharp microelectrodes (Figs. 1–5; $n = 45$ cells)². Patch electrodes (4–14 M Ω) were filled with 135 mM potassium gluconate, 5 mM

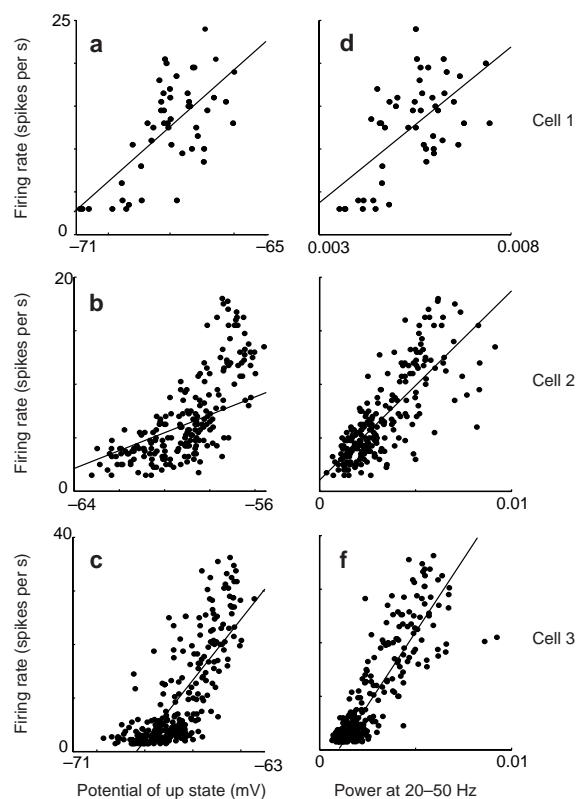


Fig. 5. Contribution of membrane potential fluctuations to changes in firing rate during the up state. (a–c) Relationship between mean potential of the up state and firing rate in three cells. (Cell 1 is the cell in Fig. 1.) Each point corresponds to one stimulus trial lasting two or four s. (d–f) Relationship between the mean power in the 20–50 Hz range and spike rate for the same 3 cells.

HEPES, 2 mM MgCl₂, 1.1 mM EGTA, 0.1 mM CaCl₂, 3 mM ATP, 2 mM GTP and 0.02 mM GMP (pH 7.4). A junction potential of 10 mV was subtracted from all patch recordings³². For recordings with patch electrodes ($n = 50$), mean membrane potential ranged from -91 mV to -46 mV (mean, -66 mV), access resistances ranged from 40 M Ω to 286 M Ω (mean, 141 M Ω), and input resistances ranged from 30 M Ω to 145 M Ω (mean, 75 M Ω). Sharp electrodes were filled with 2 M potassium acetate. For recordings with sharp electrodes ($n = 47$), mean membrane potential ranged from -85 mV to -40 mV (mean, -67 mV), access resistances ranged from 40 M Ω to 125 M Ω (mean, 90 M Ω), and input resistances ranged from 28 M Ω to 80 M Ω (mean, 48 M Ω). A cell was classified as complex if the component of the membrane potential response at the frequency of the drifting grating was less in amplitude than half of the evoked increase in mean potential¹⁹. In all recordings, action potentials were truncated at spike threshold by linear interpolation before subsequent analysis. Removal of spikes from records rather than truncating the spikes did not significantly affect the analysis. Experimental procedures were approved by the Northwestern University Committee on Animal Care and Use.

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