Supplemental Text and Figures

Supplemental Methods

Fitting procedure

For each cell, simulated-annealing optimization techniques (Press *et al.*, 1992) were employed to fit equation 2 to the experimental data (Figure 5). The cost function minimized with respect to the model parameters $\{C, H, \tau, \tau_{arp}, \omega, \alpha\}$ was chosen to assess the goodness-of-fit by the χ^2-test (Press *et al.*, 1992):

$$\chi^2 = \sum_{h=1}^{M} \left[f_h - \Phi_{IF}(m_h, s_h) \right]^2 / \left[0.5 \cdot \left(\delta_h^+ + \delta_h^- \right) \right]^2 . \qquad \text{(S1)}$$

Each term of the sum above is weighted by the inverse of an accuracy interval for each experimental estimate f_h, derived by employing a phenomenological renewal model of spike emission, as discussed in La Camera *et al.*, (2006). Briefly, by specifying a desired confidence level q on the experimental estimates of the spike rates $\{f_h, h = 1,2,...,M\}$, f and its estimate $f_h = N_{sp\,h} / T$ are such that $Prob\{f_h - \delta_h^- \le f < f_h + \delta_h^+)\} \ge q$, with

$$\delta_h^{\pm} = | 0.5K^2 \pm K\sqrt{N_{sp\,h} + 0.25K^2} | /T \qquad \text{and} \qquad q = 1 - erfc(K/\sqrt{2}), \quad \text{(S2)}$$

where $N_{sp\,h}$ is the number of spikes observed in the time interval T. The last relationships imply that, by setting $K \ge 1$, the resulting accuracy interval $\delta_h^{\pm}$ approximately corresponds to a confidence q of at least 68% (see Figure 6).

<FIGURE S1 ABOUT HERE>

Supplemental Results

Calcium electrogenesis and sensitivity to fluctuations

Even when the apical dendrite was cut during the slicing procedure, the *f-I* curves displayed divergence and the *LIF* model did not fit the experimental data (n = 2; Figure S2). This suggests that somatic mechanisms can account for the sensitivity to fluctuations in the regime where input current mean m was above rheobase (i.e. *drift-dominated*). Nevertheless, we investigated whether a supralinear calcium influx in the dendrites (Larkum *et al.*, 1999b) could contribute to the observed sensitivity to the input fluctuations, even in the absence of dendritic inputs (Larkum *et al.*, 1999a).

<FIGURE S2 ABOUT HERE>

We looked for the somatic counterpart of dendritic calcium electrogenesis: an after-depolarization following a burst of action potentials. In the SSC this was shown to occur only when spikes fired at a sufficiently high frequency, above a threshold value defined as *critical frequency* and located typically between 80 and 150 Hz (Larkum *et al.*, 1999b).

Therefore, we systematically reanalyzed all of the recorded spike trains to look for bursts of at least five consecutive somatic action potentials that occurred with an instantaneous frequency higher than 80 Hz (i.e. *supra-critical*). Although five spikes at 80 Hz are not reported to fire a full (sixth) action potential in L5 pyramidal cells of the mPFC upon DC somatic stimulation, they do induce an after-depolarization (ADP) following the last spike (Thurley, K., Larkum, M.E. and Lüscher, H.-R., personal

communication). In correspondence to the same value of *m*, *supra-critical* bursts could have been induced by different levels of fast fluctuations in the input current (i.e. *s*) and the resulting ADPs might have facilitated somatic firing (Larkum *et al.*, 2004) increasing its rate. However, the conditions for *supra-critical* bursting defined above occurred very rarely in our experiments (i.e. nine traces out of a total of 3016 individual spike trains), and we indirectly conclude that ADPs were rare as well. In addition, for both *drift*- and *fluctuations-dominated* regimes, there was no correlation between *s* and the number of bursts (Kendall's tau test: $r_K = 0.07$ with $p_K < 10^{-7}$). Similarly, no correlation was found when looking for shorter bursts, composed of three or four supra-critical action potentials. Less than four cells out of 113 had a significant correlation between *s* and the number of bursts.

Finally, for all of the cells whose response could be fit by the sLIF with high accuracy, no correlation was found between the number of bursts and the best-fit value of ω, which was employed as a measure of the cell sensitivity to *s*. Therefore, calcium electrogenesis in apical dendrites is unlikely to contribute to the divergent *f-I* relationships for different levels of input fluctuations *s*.

Sensitivity of the minimal interspike intervals to fluctuations

The increased sensitivity to *s* has been discussed so far in terms of mean firing rates for both the transient and steady state response. We demonstrated that such a phenomenon could be quantitatively described only when an IF model included an absolute refractory period that decreases with increasing *s* (eq. 3). One might then wonder, does the minimum interspike interval (ISI), which is an upper bound on the neuron′s absolute

refractory period, decrease as s increases? Further, details of the ISI distribution other than its mean, such as especially its minimum value, may be critical for obtaining quick information about stimulus variance (Lundstrom and Fairhall, 2006). Thus, we examined quantitatively the relationship between minimum ISIs and s.

We considered the smallest three ISIs ($ISI_1<ISI_2<ISI_3<...$) observed for different levels of input fluctuations s and mean m for all spike trains of 113 cells. For each cell, we estimated statistical correlations between s and the inverse of the length of the smallest three ISIs (see also Figure S3). We restricted our analysis only to the drift-dominated spiking regime where m is well above the rheobase current (e.g. m > 500pA as in Figures 1, 5). In this way, the more obvious correlations due to the fluctuation-dominated spiking regime did not bias the estimate.

Correlations were positive and strongly significant both for the steady state response as well as when the analysis was restricted to the initial transient (i.e. considering only the first two seconds of each recording). Spearman linear correlation coefficients, referred to the three smallest ISIs, averaged across all the cells and accompanied by a standard error, were r_1=0.51±0.003, r_2=0.74±0.002 and r_3=0.81±0.002.

<FIGURE S3 ABOUT HERE>

These results were extremely similar for both steady state and transient, and they were strongly confirmed in both conditions by the Kendall's Tau test: summarizing these results, the percentage of cells for which $r_K > 0.25$ with $p_K < 0.1$ was 52.8% for ISI_1, and 83.3% for both ISI_2 and ISI_3. Such results indicate that not only the mean of the ISI

distribution is affected by *s* but also its lower boundary (Figure S3). This result is not unexpected since the minimum ISI decreases with increasing *s* for both the standard HH model neuron and an HH model with slow sodium inactivation as in Figure 7 (data not shown).

Relation to previous work

It might be argued that, as opposed to previous experimental attempts, we employed much stronger mean currents *m* relatively to the cell input resistance, and thus characterized the discharge response over a greater range of input currents. Of course, if we had neglected enough data points collected in the *plateau* region of our *f-I* responses, as well as around the rheobase current where the curves start displaying divergence, the conventional LIF model could have better described our experiments. However, our criteria to choose the range for *m* closely followed those of Rauch *et al.* (2003) in the somatosensory cortex (SCC) experiments, where most of their recordings did not show divergence of *f-I* relationships, and where the conventional LIF model was a good description of their data. Instead, it is likely that mPFC pyramidal cells were able to sustain stronger mean input currents *m*, within the same frequency output range. As a consequence, we could explore the drift-dominated spiking regime in more detail, and examine a previously underestimated region. It is interesting to note that the input resistance of the cells included in the present study were larger than the values reported for the SSC. This is counterintuitive, as a lower input resistance would point towards a much wider input amplitude regime, to produce the same depolarization in the membrane voltage.

On the significance of the response sensitivity to fluctuations

The dependence of the neuronal discharge rate on the input fluctuations in the *drift-dominated* regime (see Figure S1) could be demonstrated quantitatively only through the significance of the fit of distinct IF models (Figure 5, but see Figure 2). Direct inspection of isolated data points is insufficient to quantify such a sensitivity; data points of all curves should be considered simultaneously. In fact, with reference to the fit of the sLIF model parameter ω, *a priori* it is difficult to assess by eye whether the experimental *f-I* curves collected for increasing values of s (i.e. $s_1, s_2, \ldots, s_n$) are significantly apart one from the other within a given confidence level on the spiking frequency estimates. In other words, the values of the maximal theoretical discharge rate (i.e. the horizontal asymptotes in Figures 3, 5),

$$\{s_1 / (s_1 \tau_{arp} + \omega), s_2 / (s_2 \tau_{arp} + \omega), s_3 / (s_3 \tau_{arp} + \omega), \ldots, s_n / (s_n \tau_{arp} + \omega)\}$$

might be closer than the error bars computed by any procedure and by any statistical hypothesis underlying the estimate of f, even when $\omega>0$. This is especially true for larger values of s. Therefore, the best-fit of the parameters of simple IF models represents an effective way to assess the diversity of single-cell *f-I* curves, as it considers globally the data points.

Finally, the addition of the extra-parameter ω might suggest intuitively that the new model had a better chance to improve data-fit performances. Although this is generally

true in minimizing a mean square error, possibly resulting in an overfitting, it is not the case for the statistical test employed here (i.e. the χ^2-test). Such a test explicitly takes into account the number of free model parameters: increasing the number of parameters makes the test on the goodness of the fit harder to pass, as the number of *degrees of freedom* of the χ^2 distribution decrease accordingly (Press *et al.*, 1992).

Although, by no means can we deduce the *true* model for a pyramidal cell under noisy current injection, we suggested here a more adequate way to assess the experimental data: the test performance of the sLIF model versus the *LIF* model is a strong indication that significant differences have been found compared to previous work.

Supplemental References

Larkum, ME, Kaiser, KM, Sakmann, B (1999a) Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. Proc Natl Acad Sci U S A 96(25), 14600-4.

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Supplemental Figure Captions

Figure S1: The divergence of *f-I* curves corresponding to distinct levels of input fluctuations is highly reproducible and significant. As in Figure 1A, panels **A-D** report the response functions in four distinct cells. Panels **C-D** focus particularly on the plateau region of the *f-I* curves. Different markers and gray levels represent amplitude

fluctuations s of [50 150 300] pA in the input current. The comparison of the relative distances among experimental points, collected by repeating the same combination (*m,s*) and obtained across distinct values of *s*, demonstrates the good degree of reproducibility of the mean firing rates and the significance of the displacement of individual *f-I* curves.

Figure S2: Active currents in the apical compartments are not necessary to account for the extra sensitivity to input fluctuations. Depending on the depth within the brain tissue, sometimes slicing procedure completely ablated portions of a neuron. *A posteriori* in a neuron whose reconstructed morphology indicated that the apical dendrite had been cut, discharge responses were compared to a control case, chosen for having similar passive membrane properties and *f-I* curves (standard deviation s = 50 pA, 150 pA, and 300 pA; see inset of upper right panel). The *f-I* curve divergence was quantified by the value of the model parameter ω for both statistically significant best-fits. ω was even larger in (**B**) with respect to (**A**), suggesting that dendritic calcium electrogenesis is unlikely to account for the extra-sensitivity to input fluctuations. *V-I* and action potential trajectories (**A-C**), investigated for the same range of DC current *i*, were very similar in the two cells.

Figure S3: Inter-Spike Interval (ISI) distributions reflect a sensitivity to input fluctuations. For the same two cells as in Figure 4, panels report the inter-spike interval distributions of the spike trains evoked for three distinct values of *m* (i.e. 800 pA, 1000 pA, and 1200 pA) and across distinct input fluctuations regimes (**A-C** and **D-F**).