Heterogeneous firing rate response of mouse layer V pyramidal neurons in the fluctuation-driven regime

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**Key points**

- We recreated in vitro the fluctuation-driven regime observed at the soma during asynchronous network activity in vivo and we studied the firing rate response as a function of the properties of the membrane potential fluctuations.
- We provide a simple analytical template that captures the firing response of both pyramidal neurons and various theoretical models.
- We found a strong heterogeneity in the firing rate response of layer V pyramidal neurons: in particular, individual neurons differ not only in their mean excitability level, but also in their sensitivities to fluctuations.
- Theoretical modelling suggest that this observed heterogeneity might arise from various expression levels of the following biophysical properties: sodium inactivation, density of sodium channels and spike frequency adaptation.

**Abstract** Characterizing the input–output properties of neocortical neurons is of crucial importance for understanding the properties emerging at the network level. In the regime of low-rate irregular firing (such as in the awake state), determining those properties for neocortical cells remains, however, both experimentally and theoretically challenging. Here, we studied this problem using a combination of theoretical modelling and in vitro experiments. We first identified, theoretically, three somatic variables that describe the dynamical state at the soma in this fluctuation-driven regime: the mean, standard deviation and time constant of the membrane potential fluctuations. Next, we characterized the firing rate response of individual layer V pyramidal cells in this three-dimensional space by means of perforated-patch recordings and dynamic clamp in the visual cortex of juvenile mice in vitro. We found that individual neurons strongly differ not only in terms of their excitability, but also, and unexpectedly, in their sensitivities to fluctuations. Finally, using theoretical modelling, we attempted to reproduce these results. The model predicts that heterogeneous levels of biophysical properties such as sodium inactivation, sharpness of sodium activation and spike frequency adaptation account for the observed diversity of firing rate responses. Because the firing rate response will determine population rate dynamics during asynchronous neocortical activity, our results show that cortical populations are functionally strongly inhomogeneous in young mouse visual cortex, which should have important consequences on the strategies of cortical computation at early stages of sensory processing.

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**Abbreviations** EiF, exponential integrate-and-fire model; iLIF, inactivating leaky integrate-and-fire; LIF, leaky integrate and fire model; sfaLIF, LIF model with spike-frequency adaptation only.
Introduction

The neocortex of awake animals displays an activated state in which cortical activity manifests highly complex, seemingly noisy behaviour. At the level of single neurons the activity is characterized by strong subthreshold fluctuations and irregular firing at low rate: this constitutes the fluctuation-driven regime, which is believed to be central to cortical computations (Destexhe & Contreras, 2006). Sensory processing of natural stimuli also evokes a sparse response at low population rates; see for example Crochet et al. (2011) in mouse somato-sensory cortex or Baudot et al. (2013) in cat visual cortex. Understanding the dynamical and computational properties of this regime at the cellular and network level is a key challenge in systems neuroscience. Because the reliable computation performed during this regime happens on top of strong effects mediated by slow population dynamics (such as variable levels of ongoing activity at time scales \(T > 30-50 \text{ ms}\)), which, in turn, determine the integrative and computational properties at the cellular level (Destexhe & Paré, 1999; Chance et al. 2002; Rudolph & Destexhe, 2003; Rossant et al. 2011; Altwegg-Boussac et al. 2014), an accurate quantitative description of population dynamics (and its correlate in terms of membrane potential fluctuations) appears to be a necessary prerequisite to the comprehension of this regime.

In the present paper, we investigate the firing rate response as a response to membrane potential fluctuations: a form of neuronal transfer functions that lies at the core of theoretical models of population dynamics (see e.g. Amit & Brunel, 1997).

The sparse firing regime nonetheless constitutes a difficulty for experimentalists, as responses are of low amplitude and render experimental characterization challenging. In particular, characterizing the firing rate response of single neurons at low rates requires long recording times and stable properties. Here, we propose a characterization of the low rate response of single neurons that was made possible by the combination of the stability offered by the perforated-patch technique and a simple theoretically driven fitting procedure for the spiking response.

We identified three somatic variables to investigate single neuron responses: the mean, standard deviation and time constant of the membrane potential fluctuations at the soma. In comparison with previous work, reviewed in La Camera et al. (2008), our approach allows us (1) to investigate the response to fast membrane potential fluctuations characterizing the high conductance state of cortical networks (Destexhe et al. 2003) and (2) to perform a cell-by-cell comparison because of its formulation in terms of membrane potential variables. This characterization focuses on how these fluctuations are translated into output spikes on top of sub-threshold integration effects (Kuhn et al. 2004) and therefore highlights the contribution of active membrane properties. In addition, we also investigated the putative biophysical origin of the measured responses in established theoretical models of single neurons.

Methods

This Methods section is organized as follows: (1) we present the intracellular recording method used in this study: the perforated-patch technique, (2) we show how analytical calculus combined with the dynamic-clamp technique allowed us to control the membrane potential fluctuations on a cell-by-cell basis, (3) we explain the rationale behind our theoretical estimate of the firing rate response that led to the semi-analytical template used for fitting experimental responses.

Experimental preparation

Experiments were performed at the Unité de Neurosciences, Information et Complexité. Experimental procedures with animals were performed following the instructions of the European Council Directive 2010 86/609/EEC and its French transposition (Décret 2013/118). Swiss wild-type mice of either sex, 8–13 days old, were anaesthetized with inhaled isoflurane and decapitated, their brain was rapidly removed and immersed in cold ‘cutting’ solution (\(\sim 4 \degree\text{C}\)) containing the following (in mM): 110 choline chloride, 2.5 KCl, 1.25 \(\text{NaH}_2\text{PO}_4\), 26 NaHCO\(_3\), 8 MgCl\(_2\), 1 CaCl\(_2\), 10 glucose, pH equilibrated to 7.3 with \(\text{O}_2:\text{CO}_2 (95\%-5\%)\). Coronal slices (300 \(\mu\text{m}\) thick) were prepared with a vibratome (Leica VT1200 S, Leica Microsystems) and stored at room temperature in oxygenated aCSF containing the following (in mM): 126 NaCl, 2.5 KCl, 1.5 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 2 MgCl\(_2\), 2 CaCl\(_2\) and 10 glucose, pH 7.4. The slices were then transferred to the recording chamber (perfused with the same solution) where the temperature was maintained at \(34 \degree\text{C}\). Slices containing primary visual cortex were taken as the first four slices containing brain cortex starting from the most caudal one. The mouse visual cortex was chosen as this experimental model is the subject of intense investigation (see e.g. Okun et al. 2015) and would therefore provide a very interesting system to test the accuracy of the theoretical models of cortical dynamics constrained by the following results.

Electrophysiological recordings

We performed intracellular recordings of visually identified pyramidal cells located in the layer V of mouse cortex using the perforated-patch technique.
Patch electrodes (tip resistance: 1.5–2.5 MΩ) were pulled on a Sutter P-1000 apparatus (Sutter Instruments) and filled in a two step procedure. The pipettes were pre-filled with a solution containing the following (in mM): 130 potassium gluconate, KCl 7, NaCl 1, MgCl₂ 4, Hepes 10, pH adjusted to 7.3 with KOH (osmolarity 260 mOsm). The pipette was then back-filled with the same solution to which was added Amphotericin-B (Sigma Aldrich) previously dissolved in DMSO, the final concentration of Amphotericin-B was 60 μM. The reason for this two-step procedure is to allow a current flow out of the pipette (to preserve the tip from dirt) without pouring the perforant onto the target cell during the pipette approach. The perforation could therefore happen only after diffusion of Amphotericin-B through the 'clean' solution, this usually took 5–10 min after the pipette filling, thus allowing the cell-attached configuration to form in absence of the perforant molecule at the pipette’s tip.

We recorded from n = 30 cells. After perforation, the access resistance R_S was 14.7 ± 6.9 MΩ. For each cell, the R_S value was plugged in into the amplifier-build bridge compensation system during the current-clamp recordings. At −75 mV, the recordings exhibited a leak current of −31.9 ± 26.8 pA (minimum observed resting potential: −76 mV), this current value was then set for each neuron as the holding current during the recording. Recorded pyramidal cells had an input resistance of 355.9 ± 184.1 MΩ and a membrane time constant at rest of 31.4 ± 12.0 ms. Recordings lasted 36.7 ± 20.9 min. In the absence of current injection, cells presented a quiescent activity.

The liquid junction potential was measured to be 6 mV and membrane potential recordings were corrected accordingly. Note that there might anyway be an unknown constant shift in the voltage value because the Amphotericin-B pores are selective channels (so that a non-zero reversal potential could appear if the cellular medium and our pipette medium are different). The absolute values of the membrane potential presented here should therefore be interpreted carefully (but this would only affect the \( \langle V_{\text{thre}}^{\text{eff}} \rangle_D \) quantity reported in this study).

### Measuring firing rate

We measured the firing rate simply by counting spikes over a fixed time window. Spikes were detected as upward crossings of −20 mV. The first 100 ms after fluctuating current onset were removed to avoid transient effects associated to the membrane potential rise. The duration of the stimulation was usually 5 s, and therefore the minimum (non-zero) rate was 0.2 Hz. Also, an online analysis was counting spikes and the stimulation was stopped when 20 spikes were reached (for example, in Fig. 4E, the middle episode is shorter than the other two ones); this was to avoid spending too much recording time in the high firing rate range.

### Dynamic clamp

Our dynamic-clamp system consists of an Intel Quad-Core computer equipped with an acquisition card (NI PCI-6251 ADC/DAC, Mseries, National Instruments) connected to the amplifier operating in current-clamp mode. The dynamic-clamp software is based on a custom ADC/DAC (analog-to-digital/digital-to-analog) program used for data acquisition and analysis (Elphy2, developed at Unité de Neurosciences, Information et Complexité (UNIC) by Gérard Sadoc) and interfaced with the NEURON simulator version 6.0 (Hines & Carnevale, 1997). NEURON was modified and recompiled to run under the INtime (TenAsys), a Real Time Operating System running alongside Microsoft Windows. The recordings were performed using a Multiclamp 700B amplifier (Molecular Devices). Stimulation protocols were run in real time with the acquisition card at 10 kHz. Acquisition and filter frequencies were set at 10 and 4 kHz, respectively. An unfiltered copy of the membrane potential signal was feeding the dynamic-clamp system.

### Single compartment approximation

Both for the experimentally recorded neurons and for the theoretical models, we use the single compartment membrane equation. The passive properties of a neuron are therefore described by a leak conductance \( g_L \), a capacitance \( C_m \) and a resting potential \( E_L \). With an additional current \( I(V, t) \), the membrane potential thus follows:

\[
C_m \frac{dV}{dt} = I(V, t) + g_L(E_L - V) \quad (1)
\]

Passive properties were fitted from the response to a hyperpolarizing current step for the recorded neocortical neurons in the subthreshold domain (around −75 ± 5 mV).

Though this approximation was found to be satisfactory (Fig. 4C), monitoring possible deviations is important in this study, as the approximation is used to shape the fluctuations of the membrane potential. We therefore performed a cell-by-cell quantification of the accuracy of the approximation as follows. We took the protocols that were used to determine the membrane properties; prior to each protocol, we recorded and averaged the response to 10 current pulses of 500 ms duration and \( \Delta I \sim 15pA \) amplitude, not the (noisy) continuous monitoring presented in Fig. 4. We averaged over trials the membrane potential response and fitted an exponential curve to this mean response, with a membrane time constant \( \tau_m^{\text{II}} \) and a
membrane resistance $R_m$. For all cells, we calculated the integral of the residual trace with respect to the RC circuit approximation. This allowed us to investigate whether the quality of the approximation had an impact on the excitability and sensitivities presented in the Results, we found no significant correlations between those quantities and the quality of the approximation ($c < 0.2$ and $P > 0.2$ for all characteristics, Pearson correlations), thus suggesting that the results of our study were not impacted by deviations from the single compartment approximation.

**Global autocorrelation time**

We present here a theoretical estimate for the speed of the membrane potential fluctuations.

In the case of a fluctuating synaptic input with temporal dynamics (e.g. resulting from a shotnoise of exponential synapses considered in this study, unlike the delta synapses considered in other studies; see Amit & Brunel (1997) for an example), the autocorrelation function is not an exponential function. Consequently, the resulting membrane fluctuations cannot be characterized by a single time constant $\tau_V$ (see the inset in Fig. 1). Nevertheless, the time constant taken from an exponential approximation of the normalized autocorrelation function corresponds to a first order description of the autocorrelation and will be the main contributor to the temporal dynamics of the fluctuations.

As a theoretical prediction for this global autocorrelation time, we take the half-integral of the normalized autocorrelation function:

$$\tau_V = \frac{1}{2} \int A(\tau) d\tau,$$

where $A(\tau)$ is the autocorrelation function of the $V$ fluctuations (see an example of $A(\tau)/A(0)$ in Fig. 1). From shotnoise theory (Daley & Vere-Jones, 2007), we will obtain the power spectral density of the $V_m$ fluctuations, so we re-express the global autocorrelation time as:

$$\tau_V = \frac{1}{2} \left( \int P_V(f) df \right)^{-1}.$$

In this study, this formula reduces to a very simple form (see next section). Note that the relations presented in this paper rely on the following convention for the Fourier transform: $\hat{F}(f) = \int F(t) e^{-2\pi i ft} dt$.

**A stimulation to investigate the dependency on the variables of somatic fluctuations**

We aimed to reproduce the dynamical state at the soma in the fluctuation-driven regime by reproducing membrane potential fluctuations with the control of the mean $\mu_V$, the standard deviation $\sigma_V$ of the subthreshold fluctuations and a global autocorrelation time $\tau_V$.

Our ‘input space’ is already a response of the neuron, thus we need a stimulation that would reliably produce this response. There exist multiple types of input that would lead to a given set of the ($\mu_V$, $\sigma_V$, $\tau_V$) variables. In the present study, to best characterize the dependency on those precise variables, we wanted a stimulation that would minimize the higher order terms appearing for realistic synaptic inputs, e.g. when injecting excitatory and inhibitory Ornstein–Uhlenbeck conductances (Destexhe et al. 2001; Fernandez et al. 2011). We chose the following stimulation.

The mean membrane potential is achieved through a constant current input:

$$I_{\mu_V} = g_L (\mu_V - E_L).$$

Varying the speed of the fluctuations $\tau_V$ is achieved by changing the total input conductance at soma $\mu_G$ (an increasing conductance reduces the effective membrane time constant of the membrane, events are integrated faster and this renders fluctuations faster). The total conductance $\mu_G$ is changed by introducing a current $I_{\mu_G}$ of static conductance $g_S = \mu_G - g_L$ and of reversal potential $E_{L1}$:

$$I_{\mu_G} (V) = g_S (\mu_V - V).$$

We introduce here the effective membrane time constant $\tau_{mem}^eff = \frac{C_m}{g_s + g_L}$.

An additional noisy current of zero mean creates the fluctuations around $\mu_V$ to control the standard deviation $\sigma_V$. This current is generated from two independent Poisson processes convolved with an exponential kernel: one excitatory, one inhibitory. They have the same presynaptic rate $\nu_{in}$ the same time constant for the exponential decay $\tau_b$ and opposite current increments $Q_I$ and $-Q_I$. This corresponds to the current $I_{\text{fluct}}(t)$:

$$\tau_b \frac{dI_{\text{fluct}}}{dt} = - I_{\text{fluct}} + Q_I \left( \sum_k \delta(t_{\text{eff}} - t) - \sum_k \delta(t_{i} - t) \right),$$

where $\{t_{\text{eff}}\}_{k \in \mathbb{N}}$ and $\{t_{i}\}_{k \in \mathbb{N}}$ are two sets of uncorrelated presynaptic events generated by the frequency $\nu_{in}$.

A single excitatory or inhibitory postsynaptic potential event arriving at $t = 0$ will have the following time course:

$$\text{PSP} (t) = \pm \frac{Q_I \tau_b}{g_L \left( \tau_{mem}^eff - \tau_b \right)} \mathcal{H}(t),$$

where $\mathcal{H}$ is the Heaviside function.
From shotnoise theory (Daley & Vere-Jones, 2007; see also El Boustani et al. (2009) for an application similar to ours), we can obtain the power spectral density of the \( V_m \) fluctuations \( P_V(f) \) as a response to the stimulation eqn (6):

\[
P_V(f) = \sum_{\text{syn}} v_{\text{syn}} \text{PSP}(f)^2 = \frac{2 v_{\text{in}} (Q I \tau_s)^2}{\mu_G (1 + 4 \pi^2 f^2 \tau_s^2)(1 + 4 \pi^2 f^2 (\tau_{\text{m}})^2)}.
\]

The variance of the membrane potential fluctuations is the integral of the power density spectrum:

\[
(\sigma_V)^2 = \int_{-\infty}^{\infty} P_V(f) \, df = \frac{v_{\text{in}} (Q I \tau_s)^2}{(\mu_G)^2 (\tau_s + \tau_{\text{m}})^2}.
\]

And the global autocorrelation time takes the very simple form (see eqn (3)):

\[
\tau_V = \frac{1}{2} \left( \frac{\int_{-\infty}^{\infty} P_V(f) \, df}{P_V(0)} \right)^{-1} = \tau_s + \tau_{\text{m}}^0.
\]

We rescale this relation with respect to the resting membrane time constant \( \tau_{\text{m}}^0 \):

\[
\frac{\tau_V}{\tau_{\text{m}}} = \frac{\tau_{\text{m}}^0}{\tau_{\text{m}}} + \frac{\tau_s}{\tau_{\text{m}}} = \frac{g_L}{\mu_G} + \frac{\tau_s}{\tau_{\text{m}}}.
\]

Because the mean synaptic conductance \( \mu_G \) should scale with the size of the membrane (because of the constant surfacic density of synapses), as does \( g_L \) (because of the constant surfacic density of leak channels), when the presynaptic bombardment increases, it is the rescaled quantity \( \mu_G \) that increases. Therefore, we investigated a fixed domain of the \( \tau_N = \tau_V/\tau_{\text{m}}^0 \) quantity.

Finally, the time- and voltage-dependent current \( I(V, t) \) inserted into the membrane eqn (1) or injected via the dynamic-clamp technique takes the form:

\[
\begin{align*}
I(V, t) &= I_{\mu_V} + g_S (\mu_V - V) + I_{\text{fluct}}(t) \\
\tau_S \frac{dI_{\text{fluct}}}{dt} &= -I_{\text{fluct}} + Q_I \left( \sum_k \delta(t_k - t) - \sum_k \delta(t_k - t) \right).
\end{align*}
\]

The three variables \( (\mu_V, \sigma_V, \tau_N^V) \) are achieved through the five variables of the input \( (I_{\mu_V}, g_S, v_{\text{in}}, \tau_s, Q_I) \). We have two additional degrees of freedom, so (1) to force the input to remain in the fluctuation-driven regime (many events of low amplitude) we arbitrarily set the presynaptic frequency to \( v_{\text{in}} = 2 \text{kHz} \) and (2) we fixed the current time constant to: \( \tau_s = 15\% \) (i.e. \( \tau_s = 4.5 \text{ ms} \) for \( \tau_{\text{m}}^0 = 30 \text{ ms} \)).

Thus, when we want to study the firing rate response as a function of \( (\mu_V, \sigma_V, \tau_N^V) \), with the membrane parameters
Following Platkiewicz & Brette (2011), the steady-state inactivating Adaptative Exponential and Fire model. It is constructed by combining the theoretical models proposed in Brette & Gerstner (2005) and Platkiewicz & Brette (2011).

\[
\begin{align*}
C_m \frac{dV}{dt} &= g_L (E_L - V) + I_{syn} (V, t) + k_a e^{\frac{-V}{\tau_w}} - I_w \\
\tau_w \frac{dI_w}{dt} &= -I_w + \sum_{t, \in \{t_{\text{spike}}\}} b \delta (t - t_i) \\
\tau_t \frac{d\theta}{dt} &= V_{\text{thres}} - \theta + a_i (V - V_i) H (V - V_i)
\end{align*}
\]

where \(I(V, t)\) is the current emulating synaptic activity that will create the fluctuations, \(I_w\) reproduces the \(I_n\) current (McCormick et al. 1985) and \(\theta(t)\) is a variable threshold whose temporal dynamics and voltage dependence accounts for the fast decrease in sodium channel availability at depolarized levels (Hille, 2001). The spiking mechanism is the following: when \(V(t)\) reaches \(\theta(t) + 5 k_a\), this triggers a spike at time \(t_i \in \{t_{\text{spike}}\}\), this increases the adaptation variable \(I_w\) by \(b\), the membrane potential is then clamped at \(E_L\) for a duration \(\tau_{\text{refrac}} = 5\) ms. Following Platkiewicz & Brette (2011), the steady-state threshold is described by a piecewise linear function (\(H\) is the Heaviside function).

The temporal dynamics of sodium inactivation and spike frequency adaptation were fixed to \(\tau_i = 5\) ms and \(\tau_w = 500\) ms, respectively. Also the threshold of the inactivation curve was fixed relative to the sodium activation threshold as \(V_i = V_{\text{thres}} - 8\) mV.

The leak potential of theoretical models was set to \(E_L = -70\) mV. All other parameters are varied along the study (see figure legends).

Finally, because of the variability of membrane time constants in the experimental data (indeed, the data show variations not only in input resistance, also in \(\tau_{\text{in}}\)), the comparison for the firing rate response between data and theoretical models requires careful treatment. Because when the synaptic bombardment raises, the ratio of input conductance with respect to the leak conductance \(\frac{g_i}{g_l}\) raises, we scanned a fixed range in terms of \(\tau_{\text{in}}^N = \tau_{\text{in}}^0\) (see previous section). But this means that for a given range of \(\tau_{\text{in}}^0\), there will be different range of \(\tau_{\text{in}}\) when there is a change in membrane time constant \(\tau_{\text{in}}^0\), and consequently a different range of output firing frequency. The solution that we adopted for a relevant comparison between data and theoretical models is to simulate the models with different membrane time constants \(\tau_{\text{in}}^0\) reproducing the variability in experimental data. In Fig. 2 only, a single model was numerically simulated with the parameters by the randomization of the scanned input points and the use of multiple seeds.

**Theoretical models of neurons**

The general model considered in this study is the inactivating Adaptative Exponential and Fire model.
(\(g_L = 2.5\) nS, \(C_m = 80\) pF to get \(\tau_m^0 = 32\) ms as the average of the intracellular data). For all other figures (Figs 3, 6 and 7), where the sensitivities are presented, we simulated three models, all with the same leak conductance \(g_L = 2.5\) nS, but with three membrane capacitances \(C_m\) to reach \(\tau_m^0 \in (20, 32, 44)\) ms to reproduce the standard deviation of the data (see previous section). The presented sensitivities were then the average of the sensitivities of the three models. Note that, even if the scaling of the firing response with the resting membrane time constant is clear in theoretical model \((\nu_out \propto 1/\tau_m\) for a given \(\tau_m\) space), this effect was not significantly visible in the data (Pearson correlations between mean excitability and membrane time constants, \(P > 0.1\) presumably masked by the heterogeneity discussed in this paper).

### Starting from a simple approximation for the firing rate

The starting approximation for the firing rate given some fluctuation properties is similar to the one introduced in Amit & Brunel (1997). Because our situation is different (our input has a temporal dynamics and includes

\[\text{Figure 2. The analytical template (eqns (18) and (9)) can capture the firing rate response of various theoretical models.} \]

Shown for the leaky integrate-and-fire model (LIF) with \(V_{thre} = -47\) mV (kept for all following models), the exponential integrate-and-fire model (EIF) with \(k_a = 2\) mV, the LIF model with spike-frequency adaptation only (sfa-LIF) with \(b = 20\) pA, the inactivating leaky integrate-and-fire model (iLIF) with \(a_i = 0.6\) and the iAdExp model that combines all the previously mentioned mechanisms with \(k_a = 2\) mV, \(b = 6\) pA and \(a_i = 0.6\). A, response to depolarizing current step, dashed line: continuous line: response to depolarizing current step, dashed line: continuous line: response to hyperpolarizing current step. For the LIF and iAdExp models, we show in red the dynamics of the threshold \(\theta(t)\). B, firing rate response in the \((\mu_V, \sigma_V)\) space. Various colours indicate various levels of the global autocorrelation \(r_{\mu_V}^V = r_V / \tau_m^0\). C, projections along the standard deviation \(\sigma_V\) axis for different mean polarization levels \(\mu_V\). Data (points) and fitted analytical template (thick transparent lines) are shown. Note the shifts in the scanned \((\mu_V, \sigma_V, \tau_m^0)\) domain to reach a comparable firing range despite a reduced excitability (see main text). D, phenomenological threshold \(V_{thre}^{eff}\) that leads to the fitted firing rate response; the coefficients of the linear functions can be found in Table 1.
additional conductance), we justify here the refinement that led to the estimate used in our study.

If a neuron has membrane potential fluctuations described by a mean \( \mu_V \), a standard deviation \( \sigma_V \) and a typical autocorrelation time \( \tau_V \), then we can divide a time axis of length \( T \) (with \( T \gg \tau_V \)) into \( N \) bins of length \( \tau_V \). Within each of this bin, we consider that a reorganization of the membrane potential values occurs, then the bins can be considered independently, and in each, we sample randomly from the Gaussian distribution defined by \( \mu_V \) and \( \sigma_V \). We remain in the low firing regime (\( \nu_{\text{out}} \leq 30 \text{ Hz} \)), so that we can neglect the repolarization dynamics and saturation effects. Then, if a spike occurs when the membrane potential crosses a threshold \( V_{\text{eff}} \), the probability of having a spike within a bin is the probability of being above this threshold \( \Pr(V \geq V_{\text{eff}}) \). The number of spikes during the time \( T = N \tau_V \) is \( k = N \Pr(V \geq V_{\text{thre}}) \). The definition of the stationary firing rate is \( \nu_{\text{out}} = k/N \), so that we get:

\[
\nu_{\text{out}} = \frac{\Pr(V \geq V_{\text{thre}})}{\tau_V},
\]

i.e. in the case of a Gaussian distribution for the membrane potential:

\[
\nu_{\text{out}} = \frac{1}{2 \tau_V} \text{erfc} \left( \frac{V_{\text{eff}} - \mu_V}{\sqrt{2} \sigma_V} \right),
\]

where the subthreshold variables \( (\mu_V, \sigma_V, \tau_V) \) can be calculated as a response to the synaptic input as detailed in the previous section.

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**Table 1.** Fitted coefficients of the linear phenomenological threshold for the theoretical models shown in Fig. 2

<table>
<thead>
<tr>
<th>Model</th>
<th>( P_0 ) (mV)</th>
<th>( P_\mu ) (mV)</th>
<th>( P_\sigma ) (mV)</th>
<th>( P_T ) (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIF</td>
<td>-49.74</td>
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<td>0.31</td>
<td>-0.51</td>
</tr>
<tr>
<td>EIF</td>
<td>-46.9</td>
<td>1.69</td>
<td>1.47</td>
<td>-3.6</td>
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<td>sfaLIF</td>
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<td>4.29</td>
<td>3.91</td>
<td>0.56</td>
</tr>
<tr>
<td>iLIF</td>
<td>-46.11</td>
<td>2.33</td>
<td>-1.06</td>
<td>3.62</td>
</tr>
<tr>
<td>iAdExp</td>
<td>-48.78</td>
<td>4.72</td>
<td>5.25</td>
<td>-1.35</td>
</tr>
</tbody>
</table>

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Figure 3. Extracting the mean properties of a single neuron response: excitability and sensitivities to the variables of the fluctuation-driven regime

A, illustration for the LIF model. From the fitting procedure we obtain an analytical description of the firing rate response (centre plot). We focus the analysis on the domain \( D \) of the low-rate fluctuation-driven regime (see main text); its extent is delimited by the white square in the bottom and top-right insets. The mean phenomenological threshold in the \( D \) domain quantifies the excitability (top left: large dashed line). Then for each variable (top right: \( \tau_V \), bottom left: \( \sigma_V \), bottom right: \( \mu_V \)), we show the projections of the firing response along this dimension for different combinations of the two other variables within the \( D \) domain (dotted lines). The mean derivative (represented by arc angle and large dashed line) with respect to the variable in \( x \)-axis over different combinations of the remaining variables quantifies the mean sensitivity to this variable. B, excitabilities for the LIF, EIF, sfaLIF and iLIF models (parameters as in Fig. 2). C, mean sensitivities to \( \mu_V \), \( D \), mean sensitivities to \( \sigma_V \). E, mean sensitivities to \( \tau_V \) for the four models.
Fitting

To render the fitting of the phenomenological threshold easier, we insured that the linear coefficients of eqn (19) take similar values by normalizing the \((\mu_V, \sigma_V, \tau_V)\) space. The normalization factors \(\mu_V^0 = -60 \text{ mV}, \delta \sigma_V = 10 \text{ mV}, \sigma_V^0 = 4 \text{ mV}, \delta \sigma_V^0 = 6 \text{ mV}, \tau_V^0 = 0.5, \delta \tau_V^0 = 1\) arbitrarily delimits the fluctuation-driven regime (a mean value \(x^0\), and an extent \(\delta x^0, \forall x \in [\mu_V, \sigma_V, \tau_V]\)). It is kept constant all along the study.

The fitting consisted first in a linear regression in the phenomenological threshold space of eqn (19), followed by a non-linear optimization of eqn (18) on the firing rate response. Both fittings were performed with the leastsq method in the optimize package of SciPy.

Numerical simulations

All numerical simulations of single cell dynamics have been performed with custom code written in the numerical library of python: numpy and optimized with the numba library. For the neuronal model, each point (a mean output frequency and its standard deviation across trials) corresponds to numerical simulations running with a time step \(dt = 0.01 \text{ ms}\), for a duration of 10 s and repeated 4 times with different seeds (one simulation duration: \(\approx 2 \text{ s}\) of real time on a Dell Optiplex 9020 desktop computer).

Results

The paper is organized as follows: we start by defining the fluctuation-driven regime at the soma and designing a method to reproduce this somatic dynamical state under dynamic-clamp experiments. We also derive a flexible template for the firing rate response, whose accuracy is demonstrated on various theoretical models. Then, we investigate the firing rate response of layer V pyramidal cells in mouse juvenile cortex and we analyse the individual features of single neuron responses. Finally, we explore the putative biophysical origin of the observed response in theoretical models of neocortical neurons.

A three-dimensional description of the dynamical state at the soma in the fluctuation-driven regime

Determining the cellular input–output functions is complex because input of neocortical neurons is mostly in dendrites and output spikes are generated in initial segments of an axon as reviewed in Stuart & Spruston (2015) and Debanne et al. (2011). Input will therefore crucially shape the cell’s input-output relationship. Various parameters of presynaptic activity can arbitrarily control the properties of the membrane potential fluctuations at the soma. Those properties can be quantified by identifying three somatic variables that provide a reduced description of the dynamical state at the soma in the fluctuation-driven regime: the mean \(\mu_V\), the standard deviation \(\sigma_V\) of the membrane potential fluctuations and their typical autocorrelation time \(\tau_V\) (see Methods). For example, the excitatory–inhibitory balance controls the mean depolarization at the soma, the mean synaptic bombardment impacts the standard deviation and the speed of the membrane potential fluctuations. Other effects such as synchrony in the presynaptic spike trains or the ratio between distally and proximally targeting synaptic activity also affect the statistical properties of the fluctuations. The effects of synaptic input and its dendritic integration on somatic variables can be investigated theoretically using cable theory (Tuckwell et al. 2002) and will be the focus of a future communication.

Because the spike initiation site lies electrotonically close to the soma (Debanne et al. 2011), we assume that those three purely somatic variables will define the firing rate uniquely. In this study we investigate the firing response in terms of those somatic variables (illustrated in Fig. 1).

We therefore designed a stimulation protocol to reproduce an awake-like dynamical state at the soma and investigate the firing rate response in this three-dimensional space. We evaluate the parameters of stochastic current and static conductance that would result in a particular configuration of the \(V_m\) fluctuations \((\mu_V, \sigma_V, \tau_V)\) for passive membrane using a single-compartment approximation (Kuhn et al. 2004). This procedure allows us to focus on how active currents convert fluctuations into spikes. Another advantage of this approach is that it naturally rescales the input with respect to the individual cellular properties \((R_m, C_m, E_i)\) and therefore allows a cell-by-cell comparison. In addition, we investigated domains of the dimensionless variable \(\tau_V^N = \frac{\tau_V^C}{R_m}\) instead of absolute values of autocorrelation time \(\tau_V^C\) to account for the scaling of the synaptic inputs with membrane area (eqn (11) in Methods).

Template for the firing rate response of single neurons

A key challenge for the in vitro characterization of input–output relationship is to extract a reliable quantitative estimation of its functional form from a limited number of experimentally sampled points. One approach consists in fitting the response to the formula derived from a specific theoretical model, such as a leaky integrate-and-fire neuron (Rauch et al. 2003; Lundstrom et al. 2009). This strategy has three drawbacks: (1) the complexity of the analytical formula requires a careful numerical determination and thus renders fitting procedures non-trivial, (2) it does not generalize easily to biophysically realistic synaptic input.
(e.g. reproducing synaptic dynamics; see Brunel & Sergi, 1998) and (3) the low number of parameters of simple theoretical models (e.g. a single spike threshold for the leaky integrate-and-fire model) imposes that the membrane parameters (e.g. leak conductance and membrane capacitance) are free parameters to have enough degrees of freedom.

We propose here a different strategy: we introduce a flexible analytical template fully determined by membrane parameters, which are experimentally measured, and some free parameters, which can be fitted using a simple two-step minimization procedure.

The basis for the template relies on a simple estimate, analogous to Amit & Brunel (1997), for the firing rate response of the LIF model:

\[
v_{\text{out}} (\mu_V, \sigma_V, \tau^N_v) = \frac{\Pr(V \geq V_{\text{thre}})}{\tau_v} = \frac{1}{2} \left[ 1 - E\text{rfc} \left( \frac{V_{\text{thre}} - \mu_V}{\sqrt{2} \sigma_V} \right) \right].
\]  

(18)

It is obtained heuristically by splitting the time axis into bins of length \(\tau_v\); the spiking probability is then the probability that the membrane potential is above the threshold \(V_{\text{thre}}\) (see Methods). In comparison with earlier approaches (Amit & Brunel, 1997; Kuhn et al. 2004), we take here the global autocorrelation time \(\tau_v\) instead of the membrane time constant \(\tau^0_m\). We used this approximation as a baseline trend for the firing rate response and the properties of an individual cell will be described by deviations from this baseline behaviour.

We found that those deviations could be accurately accounted for by replacing the hard threshold of the approximation \(V_{\text{thre}}\) by a linear phenomenological threshold:

\[
V^\text{eff}_{\text{thre}} (\mu_V, \sigma_V, \tau^N_v) = P_0 + P_\rho \frac{\mu_V - \mu^0_V}{\delta \mu_V} + P_\sigma \frac{\sigma^0_V - \sigma_V}{\delta \sigma_V} + P_\tau \frac{\tau^N_v - \tau^0_v}{\delta \tau^N_v}.
\]  

(19)

The quantities \((\mu^0_V, \delta \mu^0_V, \sigma^0_V, \delta \sigma^0_V, \tau^N_v, \delta \tau^N_v)\) are constant rescaling factors of the \((\mu_V, \sigma_V, \tau^N_v)\) space; see Methods.

A practical advantage of the template (eqn (18)) is that, given some firing rate data \(v_{\text{out}}(\mu_V, \sigma_V, \tau^N_v)\), we can invert the equation to get the phenomenological threshold as a function of the output firing rate:

\[
V^\text{eff}_{\text{thre}} (v_{\text{out}}, \mu_V, \sigma_V, \tau^N_v) = \sqrt{2} \sigma_V E\text{rfc}^{-1} \left( 2 \tau^N_v \tau^0_m v_{\text{out}} \right) + \mu_V, \forall v_{\text{out}} > 0,
\]  

(20)

where \(E\text{rfc}^{-1}\) is the inverse of the complementary error function.

We used this property to design the final fitting procedure: given some data \(v_{\text{out}}(\mu_V, \sigma_V, \tau^N_v)\), we calculated the phenomenological threshold data using eqn (20) and fitted the \((P_0, P_\rho, P_\sigma, P_\tau)\) coefficients by linear regression. Then starting from those coefficients we performed a non-linear least-square fitting. Those two steps guarantee that the non-linear optimization starts from a good initial guess and ensures that the gradient–descent method converges close to the global minimum.

### Firing rate response of theoretical models

We start by demonstrating the accuracy and flexibility of this phenomenological description on the firing rate response for various theoretical models (Fig. 2).

The model considered in this study is the inactivating adaptative exponential-and-fire model (Methods), which extends the model of Brette & Gerstner (2005) by adding an inactivation mechanism (Platkiewicz & Brette, 2011). Several widespread theoretical models are special cases of this model: the leaky integrate-and-fire (LIF), the exponential integrate-and-fire (EIF; Fourcaud et al. 2003), the inactivating leaky integrate-and-fire (iLIF; Platkiewicz & Brette, 2011). We also define a LIF model with spike-frequency adaptation only (sfaLIF).

We show on Fig. 2 that the template is able to describe the firing rate response of those various theoretical models. The impact on firing of those different biophysical properties of those models could all be accurately captured by differences in the linear phenomenological threshold (Fig. 2D).

We compared the four-parameter description to simpler and more complex models in which the phenomenological threshold is: constant (1 parameter), linear function (4 parameters) or a second-order polynomial of \((\mu_V, \sigma_V, \tau^N_v)\) (10 parameters). The goodness of fit of the single-parameter description was 84.6 ± 8.9%; it increased to 99.0 ± 0.5% for the four-parameter description, and then to 99.6 ± 0.2% for the quadratic phenomenological threshold with 10 parameters. We conclude that the four-parameter fit is a good compromise between goodness of fit and number of parameters.

In the absence of active mechanisms, membrane potential fluctuations are statistically identical in all theoretical models (by design, they are the same leaky RC circuit). The active mechanisms may nonetheless have an impact on the membrane potential fluctuations themselves and will, by this means, impact the firing response. In our description, those effects are captured in the dependency of phenomenological...
threshold on input variables. For example, the stationary spike-frequency adaptation level induces a net hyperpolarizing current, which, in our description, leads to an increased phenomenological threshold (sfaLIF vs. LIF in Fig. 2D).

**Link between the biophysical properties and the characteristics of the firing rate response in theoretical models**

To capture the particular features determining the properties of neuronal computation in the fluctuation-driven regime, we now turn to analysing firing rate responses of the models. We define four simple quantities that provide a reduced description of the response of a single neuron: a mean excitability (mean phenomenological threshold) and average sensitivities to variations of mean \( \mu_V \), standard deviation \( \sigma_V \) and speed of the fluctuations \( \tau_V \). These quantities were averaged for all combination of the three input variables consistent with awake-like conditions (low-rate, 1–15 Hz; fluctuation-driven regime, \( \mathcal{D} \) domain in Fig. 3).

The LIF model provides a basic picture for the firing rate response (see LIF in Figs 2 and 3A). Spiking in the LIF model increases with mean depolarization and the standard deviation (bottom panels in Fig. 3A), while it decreases with the global auto-correlation time (top right panel in Fig. 3A). More sophisticated biophysical mechanisms implemented in the considered theoretical models (exponential activation, adaptation, etc.) affect those baseline characteristics. First of all, such mechanisms suppress spiking and therefore reduce the mean excitability of all the models (Fig. 3B). The effect on average sensitivities is more complex.

The substitution of the hard threshold of LIF with an exponential function in EIF imitates the gradual opening of sodium channels in time. This property has a very strong impact on the dependency on the speed of the fluctuations (Fig. 3E). In contrast to LIF, fast fluctuations do not lead to an increase of spiking. This effect, which occurs due to the inability of the smooth sodium activation curve to extract fast varying fluctuations (Fourcaud et al. 2003), is well captured by our analysis: the sensitivity to \( \tau^v_V \) is much reduced for the EIF with respect to the LIF model.

The spike frequency adaptation of sfaLIF reproduces the effect of a calcium-dependent potassium current (\( I_m \) current) that tends to hyperpolarize neocortical pyramidal neurons at each spike occurrence (McCormick et al. 1985). This is an effect that attenuates firing and because it is proportional to firing itself we expected it would reduce the dependencies to all variables. Indeed the sensitivities to \( \mu_V \) and \( \sigma_V \) are strongly attenuated with respect to LIF (Fig. 3C and D). In contrast, the sensitivity to \( \tau^v_V \) is only mildly affected. The temporal dynamics of the hyperpolarizing current (\( \tau_w = 500 \text{ ms} \)) impedes short inter-spike intervals in the output spike train, consequently slow fluctuations are more strongly dampened than fast fluctuations, which restores the sensitivity to \( \tau^v_V \) (Fig. 3E).

The iLIF model reproduces the fast inactivation properties of sodium channels (see Hille (2001) for a review). Close to threshold, sodium channels tend to rapidly inactivate (\( \tau_i = 5 \text{ ms} \)). This mechanism clearly favours fast and high amplitude fluctuations, which allows a spike to be triggered before the channels become unavailable. Indeed, the sensitivity to \( \sigma_V \) and \( \tau^N_V \) is strongly enhanced (see Fig. 3D and E).

**Response of juvenile mouse layer V pyramidal neurons in vitro with the perforated-patch technique**

We now use the above analytical tools to determine experimentally firing rate responses in vitro. Scanning the response of neocortical neurons in the fluctuation-driven regime is experimentally challenging because it is characterized by an irregular firing at low rates (\( \sim 0.1–20 \text{ Hz} \)). To obtain a meaningful estimation of the firing rate response we need long and stable recordings (Rauch et al. 2003; Köndgen et al. 2008). Both to obtain this stability and to ensure the integrity of the intracellular medium (in particular to maintain a physiological \( \text{Ca}^{2+} \) dynamics), we chose the perforated-patch technique (Rae et al. 1991; Lippiat, 2009), in which electrical access is obtained by inserting a conducting pore (Amphotericin B protein permeant only to monovalent ions) in a patch of membrane (Wendt et al. 1992; Kyrozis & Reichling, 1995).

Although the technique may sometimes limit the quality of the electrical access to the cell, we achieved very low ratios between the access resistance and the membrane resistance (4.7 ± 2.6%; see also Rae et al. 1991), thus allowing for reliable use of the dynamic-clamp technique (Destexhe & Bal, 2009).

We monitored the stability of recordings by means of three quantities: (1) the resting membrane potential, (2) the membrane resistance and (3) the variations of the firing rate probability, and we formulated strict criteria for the stability of the recordings (see Fig. 4E).

The resulting dataset contains \( n = 30 \) cells, and totals to 65455 spikes fired at an average frequency of 3.62 Hz, i.e. within the low-rate, fluctuation-driven regime defined above (0.2–15 Hz). This relatively large amount of data was necessary to extract the biophysical relations between the fluctuations properties and the stationary firing rate.

We investigated whether the combination of the analytical template (and its fitting procedure) with our experimental recording protocols was able to produce a
reliable characterization of the firing rate response of layer V neocortical neurons in juvenile mouse visual cortex.

For each of the $n = 30$ cells, we obtained a given scan of the $(\mu_V, \sigma_V, \tau_V)$ space and applied our fitted procedure. We show the data and the fit for four examples in Fig. 5. The goodness-of-fit of our template was high (goodness-of-fit of $88.6 \pm 9.4\%$, compared to only $38.4 \pm 35.3\%$ for the constant threshold and $90.6 \pm 9.2\%$ for the quadratic phenomenological threshold); the small divergence is due to intrinsic irregularity of the low-rate spike process (sampled over $5\,s$ per episode; Fig. 4E). In spite of this variability, the linear threshold averages the intrinsic firing irregularity and produces a reliable characterization of the firing rate response.

We quantified the robustness of the experimental characterization with cross-validation: we split the measurements into two sets and investigated whether the first half of data would give the same phenomenological
 threshold as the second half. We found a good agreement if the number of scanned configuration of input space \((\mu_V, \sigma_V, \tau_V^\text{thre})\) was \(n_{\text{points}} \geq 70\) (Pearson correlations, \(c>0.8\) and \(P < 1e-5\) for the correlations between first and second half of data, Fig. 5B). The high Pearson correlations between the response characteristics in the two subsets indicates that the characterization is robust for \(n_{\text{points}} \geq 35\) scanned combinations of input parameters. Among the 30 cells used for further analysis, the cell with the minimum number of points had \(n_{\text{points}} = 42\) scanned combinations, i.e. meeting the above criterion for the robustness.

**Single neurons show strongly heterogeneous firing rate responses**

A striking feature in the response of the recorded cells is the differences in their response.

We illustrate this property on the four examples shown in Fig. 5A. Cells 1 and 4 show a very strong dependency on speed of the fluctuations, whereas cells 2 and 3 are almost insensitive to the \(\tau_V^\text{thre}\) variable (different colours in Fig. 5A). The dependency on the standard deviation of fluctuations \(\sigma_V\) is steeper for cell 2 than for cells 3 and 4. Also the sensitivity to \(\mu_V\) seems to be variable, a 10 mV depolarization has stronger effect on responses of cell 4 than 3. Finally, the cell excitabilities are also highly variable, so that they reach the 1–15 Hz firing range at various depolarization levels (e.g. compare cells 2 and 3). Similar differences are present in all recorded pyramidal cells (Fig. 6A).

This strong heterogeneity raises the question of whether there is an underlying structure in the variations of the characteristics of the firing rate response and how could it be explained by diverse biophysical mechanisms.

First, when plotted in the four-dimensional space of the firing rate response characteristics the data did not seem to distribute into distinct clusters (Fig. 6B). Presented on two-dimensional projections, the excitability and sensitivities to input variables of different cells co-vary

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**Figure 5. Characterization of the firing rate response of the recorded neocortical pyramidal neurons**

A, four examples of the firing rate response of single neurons, showing data (diamonds, error bars indicate variability estimated as the standard deviation from responses to multiple trials where available) and fitted template function (continuous line); the cells are indexed from 1 to 4 to identify them in the heterogeneity analysis (Fig. 6).

B, for 21 neurons scanned with at least of 70 different combinations of input statistics, we split the dataset into two and investigated the similarity of the coefficients between the two subsets. The relatively high and significant \((P < 0.05, \text{Pearson correlation})\) correlation coefficients between characterizations in the first and second datasets indicate a robust characterization of the firing rate response.
(Pearson correlation, c, Fig. 6B). We then looked for the four-dimensional structure of those co-variations in the response characteristics by means of a principal component analysis (Fig. 6C). No single co-variation of the sensitivities could explain a strong percentage of the observed heterogeneity, suggesting that the correlation structure is weak.

Nonetheless, two vectors explained 75% of the variations in the data. The first vector corresponds to a co-variation of the sensitivities to mean and amplitude of the fluctuations. This co-variation can be achieved in the sfaLIF model, varying the weight of spike frequency adaptation concomitantly varies the sensitivities to $\mu_V$ and $\sigma_V$ (Fig. 7C). The second vector corresponds to a co-variation of a decrease in the excitability and an increase in the sensitivity to the speed of the fluctuations. The variability in excitabilities is quite remarkable, to reproduce it in the LIF model one needs variations of the threshold $V_{\text{thre}}$ that spans nearly 15 mV (see Fig. 7). The variability in the sensitivity to $\tau_V^N$ covers a wide

![Figure 6. Heterogeneity and underlying structure of the firing response of neocortical cells](image-url)

A, histogram of data from recorded cells showing the mean excitabilities and sensitivities to the variables of the fluctuations. The dashed coloured lines show the values of theoretical models for comparison. B, scatter plot of the mean excitability and sensitivities to the variables of the fluctuation-driven regime; the cells shown in Fig. 5 are highlighted with larger markers. C, principal component analysis; the inset show the vector coordinates of the two first components.
Discussion

In this paper, we have provided a study of the spiking responses of mouse cortical neurons, in the fluctuation-driven regime, using injection of synthetic synaptic bombardment using dynamic clamp in vitro. In our view, the principal contributions of the present paper are the following: (1) to have identified, theoretically, a set of three somatic variables that characterize the response to fluctuating input; (2) having determined, theoretically, an analytic template for the spike response using these variables, which renders the experiment feasible; (3) to have designed an experimental protocol where these variables could be fully implemented by current and conductance injection to characterize the spiking response; (4) to have performed a full characterization of the spiking response of pyramidal cells in juvenile mouse cortex in vitro; (5) to have identified a possible biophysical origin for the observed diversity in the firing responses, using computational models. We discuss below the implications of these findings, and how they relate to previous work.

Compared to previous studies (La Camera et al. 2008), we focused on the low-rate regime and we extended the domain of somatic in vivo-like conditions to cover a broad range of synaptically induced activity. In particular, we investigated the dependency on the firing rate for high somatic conductance and low autocorrelation time of the membrane potential fluctuations. Scanning the response to low autocorrelation time allowed us to highlight the impact of sodium inactivation because this is the regime

![Figure 7. Variations in the expression of biophysical mechanisms explain the observed cellular heterogeneity in their firing rate response](image)

A, increasing the threshold \( V_{\text{thre}} \) of the LIF model. Note that this only affects the excitability and negligibly the sensitivities to \( \mu_v \), \( \sigma_v \) and \( \tau_N^D \). B, decreasing the sharpness of the sodium activation curve in the EIF model, \( k_a = 0 \text{ mV} \) corresponds to the LIF model, \( k_a = 3.7 \text{ mV} \) corresponds to a very smooth activation. Note the strong impact on the sensitivity to \( \tau_N^D \). C, increasing spike frequency adaptation in the sfaLIF model, \( b = 0 \text{ pA} \) corresponds to the LIF model, \( b = 35 \text{ pA} \) corresponds to a strongly adapting model. Note the concomitant variations of the sensitivities to \( \mu_v \) and \( \sigma_v \). D, increasing sodium inactivation in the iLIF model, \( a_i = 0 \text{ corresponds to the LIF model, } a_i = 0.7 \text{ corresponds to a strongly inactivating model. Note the strong impact on the increase in sensitivity to } \sigma_v \text{ and } \tau_N^D. E, histogram of the data from the } n = 30 \text{ neurons for the four characteristics of the firing rate responses.}
where the temporal dynamics of this feature is likely to play a role ($\tau_{\text{inact}} \sim 5$ ms).

We formulated a two-step procedure to circumvent the issue of spatially distributed inputs in neocortical neurons where the intermediate quantities are the properties of the $V_m$ fluctuations at the soma. Other investigators studied the response to the noisy current input properties at the soma and then addressed the problem of how dendritic integration shapes the properties of this current (Giugliano et al. 2008; La Camera et al. 2008). Our approach can be seen as a way to include the mean conductance effect due to changes in background synaptic activity. The reason we presented the data as a function of the $V_m$ fluctuations properties and not the quantity that the experimentalist actually controls (the current and the conductance) is that it allows us to compare individual cells and that the spiking response is dominated by sub-threshold integration effects, e.g. for the same variance of input current, an increasing conductance would decrease the $V_m$ fluctuation amplitude by shunting effects (Kuhn et al. 2004). The effects of the spike-related mechanisms are then difficult to distinguish from those subthreshold effects. Here, we used calculus to design a stimulation protocol that allows us to control the fluctuations and therefore to focus on understanding the single neuron computation on top of the fluctuation properties.

Starting from a simple approximation, we showed that bringing the problem into a phenomenological threshold space was a simple way to describe the firing rate response of neocortical neurons. Other investigators have already reported that a shift in the threshold was a convenient way to account for increasing biophysical complexity (Brunel & Sergi, 1998; Platkiewicz & Brette, 2010). Unlike these two studies, the form of our phenomenological threshold was not derived mathematically (it was arbitrarily taken as linear) but we believe that the descriptive power of this very simple form further confirms the idea that the threshold space is a convenient space to work in.

We showed that this template is able to describe the response of neuronal models of varying complexity. We then used this simple description to design a robust characterization of the firing rate response of single neurons experimentally. This approach, combined with the long and stable recordings provided by the perforated-patch technique, was our way of circumventing the experimental and theoretical difficulties of assessing a relevant firing rate response in a low-rate irregular firing regime.

We now discuss the biophysical mechanisms relevant for the firing rate response of layer V pyramidal cells in juvenile mouse visual cortex.

First, spike frequency adaptation was shown to be an important mechanism of the firing rate response. Notably, all models lacking spike frequency adaptation (LIF, EIF, iLIF) had a sensitivity to $\mu_V$ higher than all recorded cells. This mechanism is therefore crucial to reproduce the attenuated sensitivity to depolarizations of layer V pyramidal cells in mouse visual cortex.

Many cells showed a weaker sensitivity to the speed of the fluctuations than the LIF model ($n = 20$ out of 30). This could be reproduced in theoretical models by implementing a smoother activation curve for the sodium channels (EIF models of varying sharpness). This observation contrasts with reports from studies in more mature pyramidal neurons in rat neocortex (Köndgen et al. 2008; Ilin et al. 2013), where it was found that pyramidal cells could have a very sharp activation curve that would enable them to extract very fast input. Nevertheless, even at the soma, the neurons of our recordings show a rather smooth activation curve: $k_v \sim 1.5$ mV (not shown) from the dynamic $I$–$V$ curve analysis (Badel et al. 2008), rendering this possibility unlikely.

Surprisingly some cells showed a stronger sensitivity to the speed and amplitude of the fluctuations than the LIF model ($n = 10$ out of 30). By penalizing slow and low amplitude fluctuations, sodium inactivation seems to be able to explain this phenomena. Our observation is thus analogous to the phenomena described in Fernandez et al. (2011) for pyramidal cells in rat CA1, where the authors found that a high conductance state (corresponding to fast fluctuations in our study) could evoke more spikes than a low conductance state (slow fluctuations here). Their study provides evidence for the role of fast sodium inactivation in the sensitivity to the speed of the fluctuations and is therefore compatible with our modelling results. Because of its role in promoting large amplitude and/or fast fluctuations, sodium inactivation seems to be a key property in shaping the input–output properties of layer V pyramidal cells in the fluctuation-driven regime.

Finally, we did not discuss the impact of other sub-threshold non-linearities usually present in pyramidal cells such as the $I_h$ current. This mechanism is weakly expressed in our recordings from pyramidal cells (see response to current steps in Fig. 4C). Nevertheless, we investigated its effect on the firing response (not shown). Because of its high pass filtering behaviour, it would have an effect very similar to that of sodium inactivation: penalizing slow fluctuations and therefore increasing the sensitivity to $\tau_V$.

It must be noted that the present analysis was performed on data acquired on immature neurons (P8–P13) during the most rapid phase of electrophysiological maturation (McCormick & Prince, 1987). We investigated whether the firing response properties correlated with the post-natal day of the recording (Pearson correlation). We found no significant correlation for the three sensitivities to fluctuations ($P > 0.1$) and a weak correlation ($c = 0.4$, $P = 0.02$) for the excitability. Various developmental stages therefore poorly explain the observed variability,
suggesting that the firing response heterogeneity is an intrinsic property of the pyramidal cell population throughout the P8–P13 period. There is still a possibility that this is a phenomenon specific to this post-natal period that disappears in adult phenotypes. Nonetheless, variability in cellular excitability (as evaluated from action potential threshold) is routinely found in cortical cells in adult mice (Crochet et al. 2011; Okun et al. 2015; Yang et al. 2015), thus suggesting that electrophysiological heterogeneity is, at least partially, preserved in adult cortex and constitutes an important property of cortical assemblies. Its precise extent remains to be evaluated and should be the focus of future studies.

The main perspective for future work is to further explore the variable sensitivities of neurons to fluctuations and their putative functional consequences. We introduced new quantitative measures to quantify the sensitivity of cells to various properties of the fluctuations, which suggest several applications to the present work. First, the present analysis should be combined with a model of dendritic integration to understand how different sensitivities may have an impact on the cellular input–output function. Second, at the network level, previous theoretical work (Mejias & Longtin, 2012) investigated how variability in the excitability level had an impact on network computation, and this should be generalized to the variability in the additional quantities introduced in this paper. Our results and measurements should thus be incorporated into mean-field descriptions of cortical dynamics to understand the properties emerging at the population level. Last, our study provided a simple and tractable response function of high empirical accuracy that could be useful for biologically inspired algorithmic computation.

In conclusion, the present work shows that the spiking response of cortical neurons is highly inhomogeneous in juvenile mouse visual cortex, not only at the level of neuronal excitabilities but also in relation to their sensitivity to fluctuations. This provides quantitative insight into how neuronal diversity may impact population dynamics in the low-rate fluctuation-driven regime.

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Additional information

Competing interests

The authors declare no competing financial interests.

Author contributions

G.O. and A.D. co-supervised the experimental and theoretical part, respectively, of this work. A.D. and T.B. initially designed the project, C.D. and Y.Z. performed preliminary whole cell experiments, G.O. designed the perforated-patch protocols. Y.Z. designed the dynamic-clamp protocols, performed experiments, numerical simulations and analysed data. Y.Z., B.T. and A.D. discussed the results and wrote the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the present work.

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