Review

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Experimental evidence for sparse firing in the neocortex

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The advent of unbiased recording and imaging techniques to evaluate firing activity across neocortical neurons has revealed substantial heterogeneity in response properties *in vivo*, and that a minority of neurons are responsible for the majority of spikes. Despite the computational advantages to sparsely firing populations, experimental data defining the fraction of responsive neurons and the range of firing rates have not been synthesized. Here we review data about the distribution of activity across neuronal populations in primary sensory cortex. Overall, the firing output of granular and infragranular layers is highest. Although subthreshold activity across supragranular neurons is decidedly non-sparse, spikes are much less frequent and some cells are silent. Superficial layers of the cortex may employ specific cell and circuit mechanisms to increase sparseness.

Introduction

The number of neurons in the cerebral cortex has grown throughout phylogeny via an increase in the number of columns that are distributed across the cortical mantle [1]. Given the selective pressure that must have driven the expansion of the cerebral cortex, it is surprising that many neocortical neurons show very low firing rates. The sparse firing of neocortical neurons *in vivo* was not anticipated by decades of extracellular recordings (but see [2]), where detection of spiking neurons was not difficult. However, accumulating experimental evidence, using non-selective methods to assess the activity of identified, individual neurons, indicates that traditional extracellular recordings may have been strongly biased by selection of the most active cells.

What are the biological mechanisms that underlie the sparse firing of neocortical neurons? Why are there so many neurons if many do not transmit information to a subsequent stage of processing? Are sparse population responses an artifact of anesthesia, a reflection of a quiescent brain state, a consequence of impoverished laboratory animal experience, poor stimulus selection, or an essential feature of neural circuits? Because the spike is the primary mechanism by which information is transmitted in the central nervous system, understanding what factors determine which neurons will spike is of crucial importance.

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The computational and energetic advantages of sparsely firing neurons for efficient representation of sensory stimuli have been discussed in other excellent reviews on the subject [3–9]. Sparse sensory-evoked firing has perhaps been most thoroughly investigated in insects [10,11] and has also been observed in other vertebrates, such as songbirds [12]. Here we focus on experimental evidence in the mammalian neocortex indicating that firing neurons are rare, or that silent neurons are common, and evaluate possible mechanisms that produce or regulate sparse firing. The most convincing experimental data – with *post hoc* identification of recorded neurons for cell- and layer-assignment – has been obtained from analysis of neurons in rodent primary sensory cortex (but see [13–15]); thus, that material will be reviewed in greatest depth. In addition, we will address how the laminar location of a neuron influences its activity, with special attention to the superficial layers that receive dense innervation from input layers of the neocortex but fire at lower rates. Critical issues required to evaluate hypotheses about sparse firing across neocortical networks will be identified, and experiments to resolve these issues will be proposed.

Schemas for sparseness

The term 'sparse' has been widely used to describe the response properties of neocortical neurons. We have schematized different scenarios that give rise to the firing of only a few neurons in the cortical network to provide a framework in which to evaluate experimental data that support these models. There are at least 4 significantly different scenarios that can give the appearance of small ensemble activation, or sparseness (Figure 1). First, trial-to-trial variability in firing output, caused by either short-term or long-lasting changes in synaptic function or neural excitability, can lead to the appearance of sparse firing ('population sparseness'; Figure 1a). Trial-to-trial variability might arise from noise within the system. In both cases, neural responses appear to be sparsely distributed across the network at a given instant, but may average-out over longer periods of analysis (minutes to hours).

A second possibility is that the size of the responding ensemble is small and relatively fixed across trials ('lifetime sparseness'; Figure 1b). Generally, the computational advantages of having few firing neurons derive from this model. In contrast to Figure 1a, the identity of these ensembles is relatively constant, although they may shift

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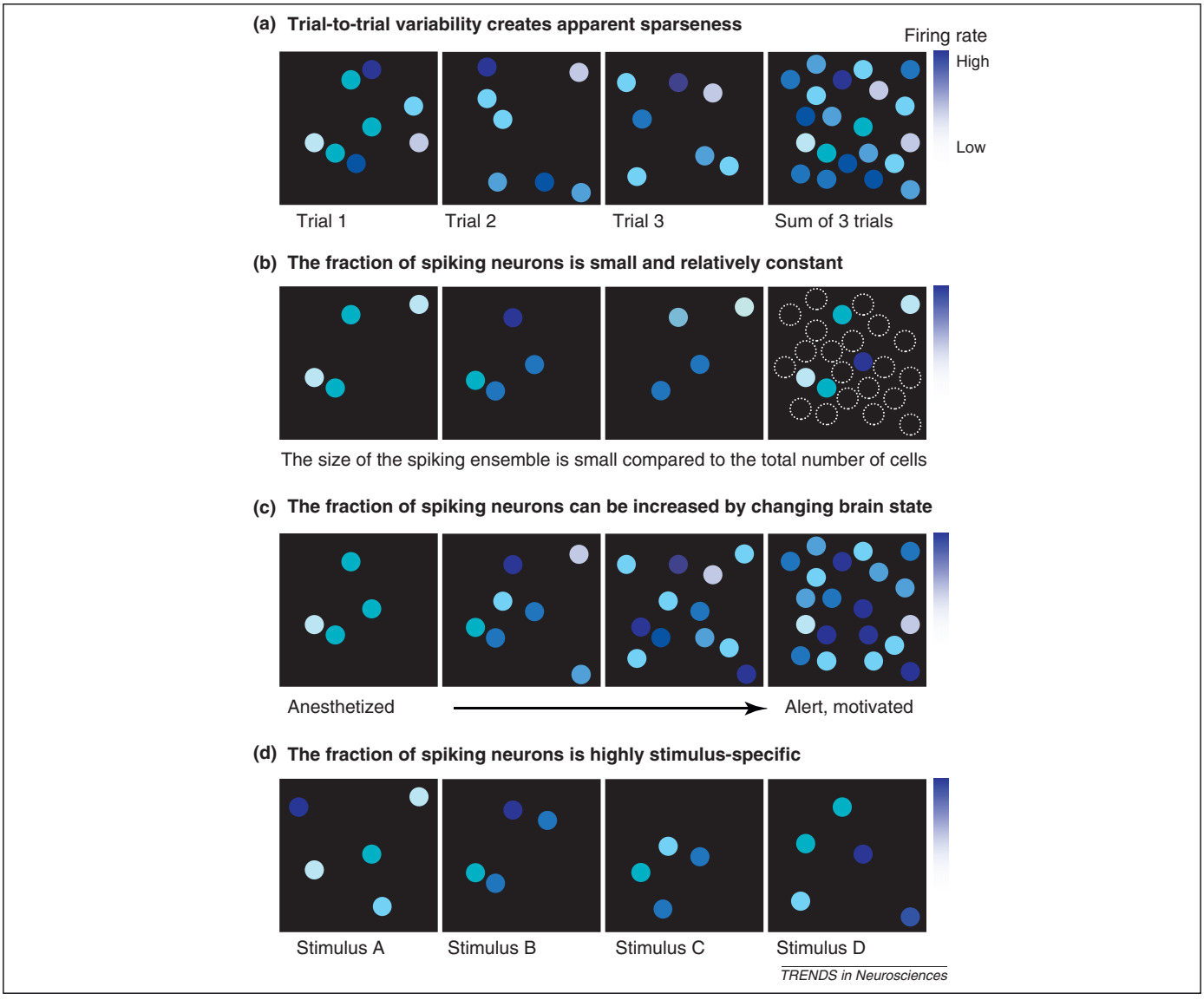

Figure 1. Models of sparseness. **(a)** Variability across trials (from cell-intrinsic or network properties) creates the appearance of sparseness that is diminished with longer time-intervals for analysis. **(b)** A small but constant subset of cells fires more reliably across many trials than the rest of the population. **(c)** The fraction of firing neurons can be changed by brain state, for example, from sleep or anesthetized to waking. **(d)** Highly stimulus-specific neurons will create the appearance of sparseness. As stimulus space expands, more neurons will fire.

over very long time-scales (days to weeks). In both cases it is most likely that the most active cells receive stronger afferent drive for a given stimulus, or are intrinsically more excitable, and that these properties could not be modified instantly. In such a scenario, increasing stimulus strength might effectively recruit more cells into the responding population, but there would be a core set of cells that always show stronger firing output than their neighbors.

A third and often-discussed scenario is that sparseness is only apparent, a product of anesthesia or brain state (Figure 1c). Indeed, experimental evidence indicates that anesthesia suppresses response output [16–18] and the majority of studies have employed anesthesia to evaluate neural firing across large populations of cells for practical reasons. Brain state in unanesthetized animals might control the number of responsive neurons, such that the fraction of cells spiking in a quiet, resting animal could be very different from that in an awake, behaving animal performing a reward-driven task. This model does not specify whether the low abundance of firing cells stems from the depictions in Figures 1a or 1b, although it suggests that mean firing rates across the population may have been significantly underestimated. Direct experimental data to address this model have not been easy to obtain, but are likely to become available in the near future.

A final possibility is that the subset of responsive neurons is determined by the specific stimulus applied (Figure 1d). This possibility is related to the 'lifetime sparseness' model in Figure 1b, but differs in suggesting that the majority of neurons will reliably spike, given a diverse enough stimulus test-set. In such a case, the fraction of responsive cells may be underestimated because the experimental stimulus applied may not be appropriate to drive the neurons tested. Indeed, early studies of response properties in the primary visual cortex (V1) took off when it was discovered that the best stimulus to drive neural firing was oriented bars. Our collective understanding of the

optimal sensory stimulus to drive firing of neocortical neurons is still in its infancy. Especially in cases where the neuron requires convergent sensory or motor input (i.e. it is multimodal), a highly constrained experimental stimulus may not be optimized to drive a cell.

In the following sections each possibility will be discussed, with specific attention to different cortical layers and brain areas. We will focus on studies that have not used spike-sorting for cellular isolation because these methods can be biased by analysis of highly responsive cells.

Does sparseness exist? The distribution of firing across cortical layers

Compared to sensory-evoked responses at earlier stages of sensory processing, the fraction of stimulus-driven neurons in the neocortex is remarkably reduced. In the thalamus, firing is highly reliable. Individual thalamic neurons are innervated by only 1–2 ganglion cells from the retina or trigeminal nucleus [19,20], and the overall strength of inputs from a single fiber (~1 nA) is sufficient to drive a spike in the postsynaptic cell. Although this is a simplification of how inputs drive thalamic neurons, it is in stark contrast to how inputs are organized in cortical layer 4. The majority of thalamic inputs synapse within layer 4, the main input layer of the cortex. Terminals are highly distributed (one thalamic neuron projects to many layer 4 neurons) but weak (~50 pA) [21], a divergence that requires the firing of many thalamic neurons to drive a spike in a layer 4 neuron.

Mean evoked firing-rates across layer 4 neurons are typically higher than in upper layers. For example, in the somatosensory cortical area that represents the facial whiskers (i.e. barrel cortex), averaged evoked firing-rates from identified layer 4 neurons are 0.14–0.41 action potentials 100 ms after whisker deflection in anesthetized animals (Table 1) [22,23]. These averaged rates do not reveal what fraction of neurons were spiking, but analysis of published data indicates that the fraction of layer 4 neurons that ever spike in response to whisker touch is substantially less than the total population size in anesthetized animals [16,22,23]. Overall, in layer 4, over recording periods that range from ~5 to 60 min, approximately half the cells may be silent. Because these estimates are taken from many trials, the fraction of neurons spiking on a given trial is lower (e.g. Figure 1a).

A major challenge in assessing the sparseness of responding neurons is determining the size of the responding ensemble. Typically, this has been approached by recording individual neurons using whole-cell patch-clamp or sharp-electrode recording techniques. Ca^{2+}-imaging is perfectly suited to assess population size, assuming that cells take up a threshold level of Ca^{2+} indicator to report faithfully even a single spike. Unfortunately, use of Ca^{2+}-imaging to detect spiking activity across a large population is difficult at the depth required for analysis of layer 4 (but see [24]), so new techniques will be useful in determining the fraction of responsive cells. Such techniques might be the development of brighter Ca^{2+}-indicators that fluoresce in the red range, enabling better depth resolution for the examination of layer 4 firing.

Supragranular layers show many silent cells

Low evoked firing-rates in superficial layers have been recorded in a number of electrophysiological experiments (Table 1) [13,16,23,25–31]. For example, in primary auditory cortex of rat, acoustic stimuli failed to modulate firing in approximately half the neurons analyzed, and only 10% of neurons showed an increase in stimulus-evoked firing in awake animals [26].

The optical accessibility of superficial layers means that Ca^{2+}-imaging experiments are feasible and highly informative. Imaging studies in different sensory modalities, carried out in primary visual (V1) [17,32,33], somatosensory (S1) [34], olfactory [35,36], and gustatory cortex [37], confirm that only a small fraction of layer 2/3 neurons exhibit stimulus-evoked firing.

What is the precise fraction of the population that fires on a given stimulus trial? In S1 (barrel) cortex, the size of the responding ensemble can vary considerably from trial to trial, and is approximately 20% of the total layer 2/3 population in anesthetized animals [34]. This result is corroborated by electrophysiological measurements in both anesthetized and awake animals, which show that most of the spikes emanate from a small proportion (<20%) of the total recorded (e.g. [38–40]). Overall firing rates and the fraction of responsive cells appear to be higher in V1 (e.g. [41,42]). Although it is difficult to tease out firing rates for individual cells from many studies, one study reported that 63% of cells in layer 2/3 of V1 in anesthetized cat (61% in rat) show spiking activity in response to orientation bars [32] (Figure 2). In this case, it was suggested that the unresponsive cells might be due to poor indicator loading [32], and that the fraction of responsive cells has been substantially underestimated. Ca^{2+}-imaging studies in anesthetized mouse V1 show a similar fraction of responsive neurons, with 50–66% of imaged cells showing a Ca^{2+} transient in response to a natural movie [33,106].

The development of new techniques, such as genetically encoded Ca^{2+} indicators, to monitor neural activity in awake and behaving animals over long time-scales will be a crucial step towards understanding that parameters that regulate the size of the responding ensemble under more naturalistic conditions [38,39,43–45,107], as well as how it may change over time. In addition, the specific laminar mechanisms leading to especially sparse layer 2/3 responses, as well as how they can be modified by brain state or experience-dependent plasticity, are of particular interest [108,109].

Infragranular layers are less sparse

Despite the fact that the deeper cortical layers represent the major subcortical output layer, and appear to dominate the spiking of the column, there are surprisingly few anatomically identified recordings from deep layers. Layer 5 cells fire more spontaneously during behavior and in response to sensory stimulation than other layers (Table 1) [16,39,46]. Within layer 5, clear electrophysiological differences have been recorded with thick tufted (layer 5b) firing more than thin tufted (layer 5a) cells in the anesthetized and quiet waking state [16].

Even fewer recordings have been made from cortical layer 6. Those that have been made show a mix of silent

Table 1. Laminar analysis of spiking activity in sensory cortex

Behavioral state	Spontaneous rate (Hz)[a,b]	Evoked rate (Hz)[c,d]	Stimulus	Recording method[e]	Area	Refs[f]
Layer 2/3						
Anesthetized (urethane)	0.32 +/− 0.49	Mean: 0.11 +/−0.14 (APs/100 ms post-stim)	Ramp + hold	Juxta	S1[g]	[23]
Anesthetized (ketamine/ xylazine)	0.32 +/− 0.03*	–	–	Ca^{2+}[,h]	V1	[17]
Anesthetized (urethane)	0.28 +/− 0.01* 0.24 +/− 0.04*	–	–	Ca^{2+} Juxta	S1	[34]
Anesthetized (urethane)	0.068 +/− 0.21	Mean: 0.031 +/− 0.056 (APs/100 ms post-stim)	Ramp + hold	WC	S1	[48]
Awake	0.61 +/− 0.99 median 0.13	–	–	WC	S1	[27]
Awake resting	0.31 +/− 0.21	–	–	Juxta	S1	[16]
Awake resting	0.87 +/− 1.38	–	–	WC	S1	[25]
Awake resting	1.1 +/− 0.3 0.9 +/− 0.5	–	–	WC Juxta	S1	[43]
Awake resting	0.44 +/− 0.02*	–	–	Ca^{2+}	V1	[17]
Awake resting	0.2 +/− 0.2 0.1 median 0.0–0.5 range	–	–	WC	S1	[38]
Awake behaving	0.3 +/− 0.9 0.04 median 0.0–3.9 range	1.7 +/− 5.0 0.2 median 0.0–20.8 range (across touch sequence) 0.12 +/− 0.23 0.02 median 0.0–0.87 range (probability of AP firing within 50 ms of touch)	Active touch	WC Juxta	S1	[38]
Awake behaving	1.31 +/− 2.02	–	–	WC	S1	[25]
Awake behaving	1.00 +/− 0.4	–	–	WC	S1	[43]
Awake behaving	0.18 +/− 0.16	–	–	Juxta	S1	[16]
Awake behaving	0.49 +/− 0.03*	–	–	Ca^{2+}	V1	[17]
Awake trained behavior	–	3.04 +/− 7.36 0.18 +/− 1.58 median +/− IQR (across touch sequence)	Active touch	Juxta	S1	[39]
Layer 4						
Anesthetized (Urethane)	0.58 +/− 0.36	0.41 +/− 0.41 (AP/100 ms post-stim)	Ramp + hold	Juxta	S1	[23]
Anesthetized (Urethane)	0.053 +/− 0.12	0.14 +/− 0.29 (AP/100 ms post-stim)	Ramp + hold	WC	S1	[22]
Awake resting	1.93 +/− 2.02	–	–	Juxta	S1	[16]
Awake behaving	1.77 +/− 2.29	–	–	Juxta	S1	[16]
Awake trained behavior	–	11.96 +/− 16.50 3.48 +/− 11.57 median +/− IQR (across touch sequence)	Active touch	Juxta	S1	[39]
Layer 4/5						
Awake trained behavior	Air 4.6 +/− 0.9	Rough 9.4 +/− 0.8 Smooth 7.3 +/− 0.7	Active touch	Tetrode	S1	[91]
Layer 5						
Anesthetized (Urethane)	1.08 +/− 0.38 slender-tufted 3.65 +/− 1.32 thick-tufted	0.15 +/− 0.35 slender-tufted 0.64 +/− 0.47 thick-tufted (APs/100 ms stim)	Ramp + hold	Juxta	S1	[23]
Anesthetized (Urethane)	Layer 5A: 0.39 +/− 0.14 Layer 5B: 0.77+/− 0.28	Layer 5A: 0.12 +/− 0.03 Layer 5B: 0.13 +/− 0.05 (APs/100 ms post-stim)	Ramp + hold	WC	S1	[46]
Awake resting	1.62 +/− 1.81 slender-tufted 4.12 +/− 3.22 thick-tufted	–	–	Juxta	S1	[16]
Awake behaving	4.94 +/− 7.22 slender tuft 4.53 +/− 4.84 thick-tufted	–	–	Juxta	S1	[16]

Table 1 (*Continued*)

Behavioral state	Spontaneous rate (Hz)[a,b]	Evoked rate (Hz)[c,d]	Stimulus	Recording method[e]	Area	Refs[f]
Awake trained behavior	–	11.87 +/− 14.34 9.13 +/− 13.47 median+/− IQR (across touch sequence)	Active touch	Juxta	S1	[39]
Layer 6						
Anesthetized (Urethane)	0.47 +/− 0.46	0.31 +/− 0.35 (APs/100 ms post-stim)		Juxta	S1	[23]
Awake resting	0.52 +/− 0.47	–	–	Juxta	S1	[16]
Awake behaving	0.32 +/− 0.38	–	–	Juxta	S1	[16]
Awake trained behavior	–	2.30 +/− 4.09 0.48 +/− 8.11 median +/− IQR (across touch sequence)	Active touch	Juxta	S1	[39]
No defined layer						
Anesthetized (isoflurane)	Mean 0.15 (median 0.06)			Tetrode	S1	[86]
Slow-wave sleep	Mean 3.8 (median 2.9)					
Awake behaving	Mean 6.1 (median 4.6)					
Awake running in textured environment	Mean 7.8 (median 4.0)					

Abbreviations: –, not determined; AP, action potential; IQR, interquartile range; Juxta, juxtacellular recordings; stim, stimulation; WC, whole-cell current clamp records.

[a]All values are from regular-spiking excitatory neurons cells within confirmation of laminar location, determined from published reports with *post hoc* analysis.

[b]All values are means +/− standard deviations unless indicated by * where standard error is presented.

[c]Values from published work that presented evoked responses as normalized to spontaneous firing rates or were expressed as overall response probabilities were not included in the table.

[d]Because of variations in the selection post-stimulus time-window, the specific details for individual studies are noted. Values are presented as Hz where appropriate.

[e]Summarized electrophysiological studies employed either whole-cell current clamp, juxtacellular recordings, or tetrode recordings.

[f]With the exception of [39] and [86], all data presented are from studies where individual neurons have been labeled and identified *post hoc* for laminar location. Studies without published firing-rate values presented in the text (e.g. [21,26,31]) have been omitted from the table, although results from these studies are consistent with the laminar differences described above. The evoked firing-rate in [23] is presented as the increase in firing (i.e. AP rate 100 ms post-stimulus subtracted from the pre-stimulus spontaneous AP rate).

[g]S1 was somatosensory (barrel) cortex in all cases.

[h]For Ca^{2+}-imaging studies, resolution of single spikes to allow calculation of spike rates and silent cells was reported.

and active cells, for example in somatosensory and auditory cortex [16,31,47]. This lack of data is probably due to the relative technical difficulty associated with recording and imaging in deeper layers. However, technical advances in deep-layer Ca^{2+}-imaging [24] alongside sustained effort in combining electrophysiological recordings with single-cell reconstructions [16,31] seem certain to provide important data on deeper layers in the near future.

Cell-to-cell differences regulate the size of the responding ensemble: differential wiring of pyramidal neurons

What are the potential circuit and cellular mechanisms that might lead to the consistent activation of particular pyramidal cells? A common property of cortical sensory representations is the broad distribution of thalamic input across many neocortical neurons, where individual synaptic connections are weak [21]. This schema can generate sparse responses in the postsynaptic population because the convergent activation of many presynaptic neurons is required to generate a spike. Indeed, intracellular recordings from all primary sensory cortical areas show broad subthreshold tuning [22,23,38,43,48–50], but much sharper suprathreshold tuning [35,51–53]. Thus, broadly distributed but individually weak synapses can lead to increased sparseness across layers. However, this explanation does not address why some neurons might consistently show higher firing-responses than others.

A subset of cells might respond with higher firing-activity because they are wired more strongly into the circuit, receiving greater excitatory drive (or, conversely, less inhibition; Box 1) than other cells [40] (Figure 1b). Differences in wiring of neocortical neurons confined to a molecularly defined subset are likely to occur via stochastic processes [54]. Identification of neurons that fire more spikes, either through the use of fluorescence-coupled activity-dependent genes [55,56], or *in vivo* Ca^{2+}-imaging for targeted recordings or anatomical analysis [57,58], will facilitate a mechanistic understanding of how sparseness is generated. Determining whether the population of neurons with a higher firing-probability to a specific stimulus remains constant over long time-intervals is an important experimental goal.

It is possible, however, that subcircuits tuned to a specific stimulus parameter arise during cortical development [59]. Indeed, preferential connectivity between subsets of layer 4 and layer 2/3 neurons has been observed [60], and between layer 2/3 neurons with similar orientation preference in V1 [58]; this feature might result from some peripheral input that drives activity in one pathway and not in the other, thus establishing subcircuits in an activity-dependent manner. In auditory cortex, evoked responses can differ greatly across layer 2 and layer 3 neurons, which show different patterns of connectivity [61]. Consistent with this hypothesis, layer 2/3 neurons in visual cortex which share orientation preference have a

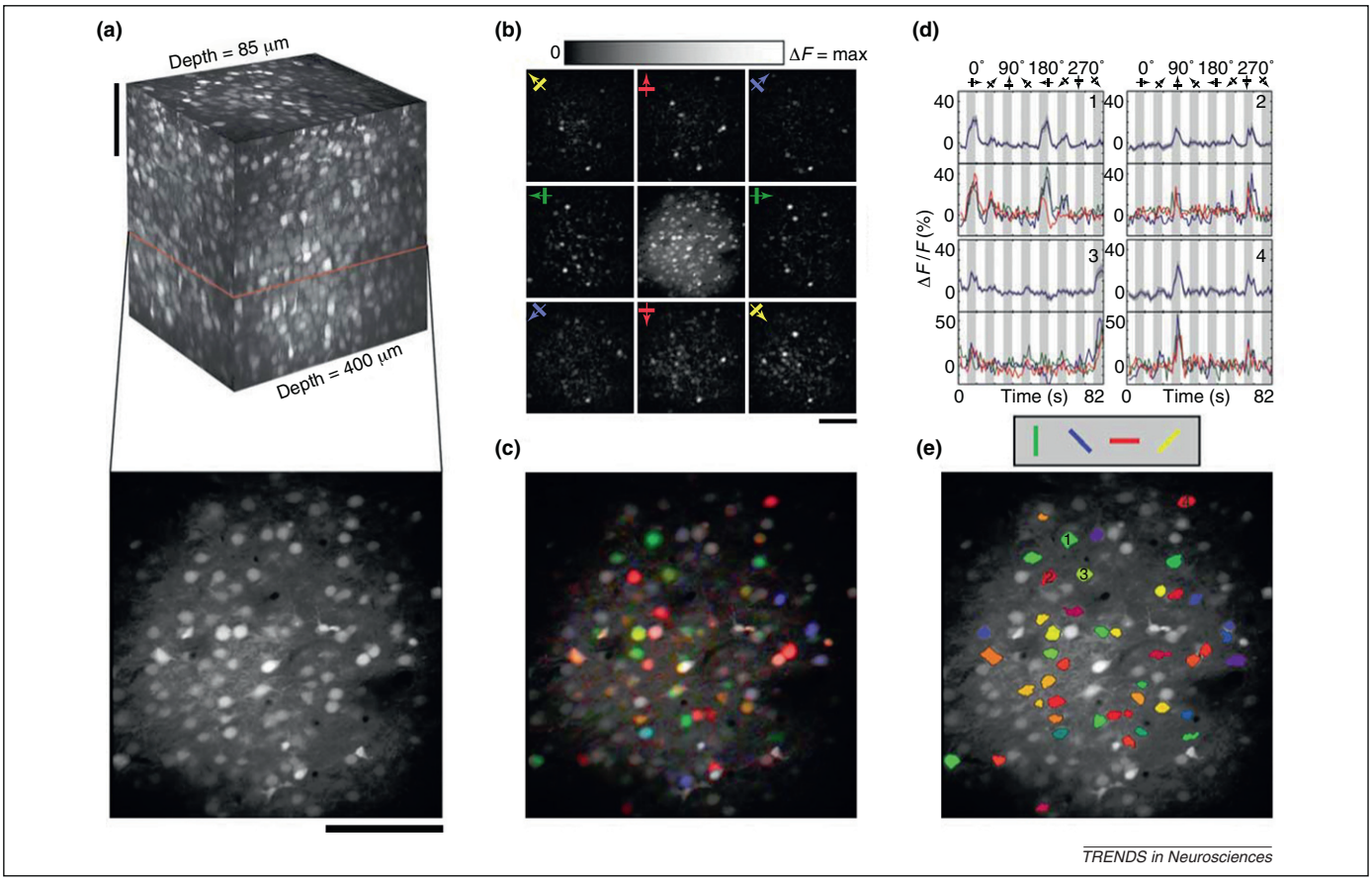

Figure 2. Functional maps of selective responses in rat visual cortex with single-cell resolution. **(a)** *In vivo* images of cortical cells stained with a calcium indicator, OGB-1 AM. The top panel shows a volume of stained cells (depth of 85–400 mm including 3000 cells) reconstructed from images obtained with 1 mm spacing in depth; the bottom panel is an anatomical image at 290 mm below the pia, averaged over all frames during the visual stimulation protocol. **(b)** Single-condition maps (DF) for eight directions of visual stimuli (outer panels; the arrows indicate stimulus direction). Each map is the average of eight repeats. In this and subsequent figures, the scale bar (DF) only applies to the outer panels. The central panel is redrawn from **(a)**. **(c)** Pixel-based orientation map, in which hue is determined by the best orientation, overlaid with the anatomical image in **(a)**. **(d)** Time-courses of four orientation-selective cells (1–4) in **(e)**. The upper traces show the average response to eight repeats (SEM in gray); lower traces show three individual repeats. Visual stimulation periods for eight directions are indicated by grey bars. **(e)** Cell-based orientation map. Responsive cells ($P = 0.01$, ANOVA; 45/115) are colored according to preferred orientation. Scale bars, 100 μm. Adapted, with permission, from [32].

significantly higher probability of being connected to each other than those cells with an orthogonal orientation [33]. In layer 5, it has been observed that subgroups of neurons may share very strong connections, a feature that might result in correlated firing of these cells [62,63]. Experiments that address differential wiring and its impact on sparse firing in neocortical neurons are an important priority.

Cell-to-cell differences regulate the size of the responding ensemble: intrinsic firing properties

Assuming that the population of neurons within a given class or lamina is equivalent, differential firing of neocortical neurons might arise from intrinsic conductances that facilitate firing in some neurons and not others. This could be dynamic, regulated for example by prior activity (e.g. [64–66]), or might be entirely stochastic. Support for this general hypothesis has come from a long history of analysis of the intrinsic firing properties of neocortical neurons [67–69]. Differences in the intrinsic firing properties of otherwise equivalent pyramidal neurons could lead some cells to spike consistently and others to be relatively silent. For example, brain-slice experiments comparing cell properties across cortical layers have shown a more hyperpolarized resting membrane potential in layer 2/3 as compared to layer 5 pyramidal neurons that could result in lower *in vivo* firing rates [70,71]. However, in a recent study from our labs, the increased spike-output of a class of layer 2/3 pyramidal neurons was not associated with greater intrinsic excitability [40] It is not yet clear whether sparseness could be generated by an identified subclass of neocortical pyramidal neurons.

Behavioral state regulates the size of the responding ensemble

Performing an experiment under the appropriate behavioral conditions can have dramatic impact on the sensory response and the size of the responding ensemble (Figure 1c). Animals process sensory information while they are awake and behaving, but the vast majority of *in vivo* mammalian experiments have been performed on anesthetized or sedated animals. Furthermore, it is often not possible to determine the size of the ensemble because in many cases mean firing-rates (not the fraction of responding cells) are reported.

Anesthesia alters both correlations between neurons and reduces firing rates by approximately 30% in visual cortex [17]; this results in cell type-specific reductions in

Box 1. Inhibitory neurons and sparseness

In many studies, a small subset of neurons that respond with multiple spikes and reliably across trials has been identified. Fewer studies have attempted to classify these cells as excitatory or inhibitory. In studies that distinguish between these two classes (using genetically targeted fluorescent protein expression), it has become clear that many of the high-firing neurons may in fact be interneurons [38,43,58,92–95], a subpopulation that may constitute 20% of all neocortical neurons. Indeed, without genetic markers to label specific cell types, or anatomical recovery and reconstruction of the specific neuron recorded, it is difficult to interpret much of the data that suggest sparse responses. Because many regular-spiking interneuron subtypes are less stimulus-selective than excitatory neurons (but have similar spike waveforms to pyramidal neurons [43]) and show higher evoked firing rates, extracellular recordings may have selectively focused on these cells. This bias would lead to an overestimate of firing rates, and indicates that excitatory neuron responses are even more sparse.

Inhibitory neurons will themselves generate sparse responses across pyramidal cells. Sensory input strongly drives inhibitory neuron firing and rapidly restricts the spread of excitation in the cortex [96]. Several subtypes of inhibitory neurons show extremely broad connectivity, where a single inhibitory neuron can be synaptically connected to virtually all nearby pyramidal cells [97–99]. Strong recurrent inhibition reduces overall firing output from pyramidal cells, increasing the sparseness of response probabilities. For example, a recent study found that increasing the size of the visual stimulus generated more spikes in inhibitory interneurons that reduce the spiking response of nearby pyramidal neurons [41]. Changing interneuron output by neuromodulation [100,101] could alter the number of responding pyramidal cells, offering a point of control to modulate sparseness.

spontaneous firing rates in somatosensory cortex [16]. Despite the relative increase in firing rates in awake animals, population analysis of firing activity in awake animals also suggests that sparseness may be maintained [16,39,44], especially in layer 2/3. A recent study has confirmed that sparse firing is present during active whisker-exploration [38], suggesting that it may result from strong inhibitory input (Box 1).

Some of the effects of anesthesia on firing rate may depend upon the type of anesthesia administered, and on the extent of thalamic inactivation [72]. Unexpected presentation of stimuli to awake, resting animals triggers large-amplitude spiking responses – perhaps reflecting a sensory 'wake-up call' – whereas stimulation during attentive or active behaviors evokes a smaller amplitude response [25,73] (but see [74]).

The gold standard for examining sensory coding is to record and manipulate neuronal activity while the animal is performing a task. Inspired by decades of primate work, rodents are now being trained to perform sophisticated head-restrained and freely moving behaviors while recording or manipulating neuronal activity with powerful battery of novel techniques [13,39,75–77]. Interestingly, a recent study using juxtacellular recordings in all cortical layers in mice during a whisker-dependent tactile discrimination task showed firing rates with a higher than expected mean population firing-rate (and a higher fraction of responsive cells), especially in layer 5 [39] (Figure 3). Single-cell stimulation in infragranular layers may also be sufficient to drive behavior in some cases (Box 2). However the data still support a sparse model for cortical layers 2/3 and 6, where a minority (approximately 10%) of neurons fired the majority of spikes (Figure 3). Studies in awake, motivated animals with careful cell identification will significantly add to our understanding of sparseness in cortical responses.

Cortical states and the size of the responding ensemble

Neocortical neurons are spontaneously active, displaying sub- and supra-threshold oscillations at a broad range of frequencies during different behavioral states [78]. At first glance this activity appears to be large in amplitude (subthreshold oscillations of 20 mV are common during periods of quiet wakefulness in mouse [25,27,38,43,79] and rat [80,81] somatosensory cortex), highly variable, and strongly influences the spiking behavior of cortical neurons – not ideal conditions for decoding small-amplitude sensory stimuli. However, when mice enter active or attentive behavioral states, and slow, large-amplitude oscillations are

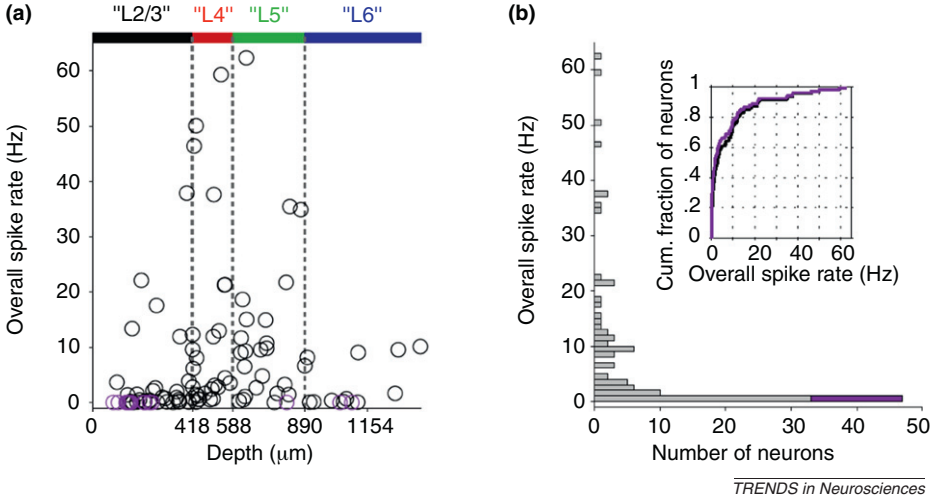

Figure 3. Most spikes are fired by a small number of neurons. **(a)** Firing rate for each neuron during sensory-based task performance in mice (averaged across all behavioral epochs), as a function of cortical depth. Each circle corresponds to a single neuron (purple, 'silent neurons'). Laminar boundaries are indicated by colored bars at top and by vertical dashed lines. Note that some cells (black circles) have very low but non-zero firing rates. **(b)** Histogram of the firing rate data in **(a)**. The purple bar indicates the number of 'silent neurons.' Inset, cumulative histogram of the same data, both omitting (black) and including (purple) the silent neurons. Adapted, with permission, from [39].

Box 2. Sparse cortical stimulation for perception and behavior

Are a small number of firing neurons in the cortex sufficient for sensory perception or motor behavior? Recent technical advances have allowed stimulation of spikes in small numbers of cortical neurons while simultaneously monitoring their effect on behavior. Electrical stimulation of a small number of cortical neurons activates a sparse and variable population of cortical neurons [102]. For example, a 200 ms burst of 10 spikes elicited in only one neuron in rat motor cortex can elicit small-amplitude whisker movements [103].

It is even possible to condition rats to report a small number of spikes from very few neurons in sensory cortex [75,104]. Stimulating single neurons with trains of single spikes can have a small but measurable impact on network activity [90] (Figure I). It is surprising that effects from stimulating such a small population or even single neurons are measurable at all at the network or behavioral level, however in general these effects are relatively small (but see [105]).

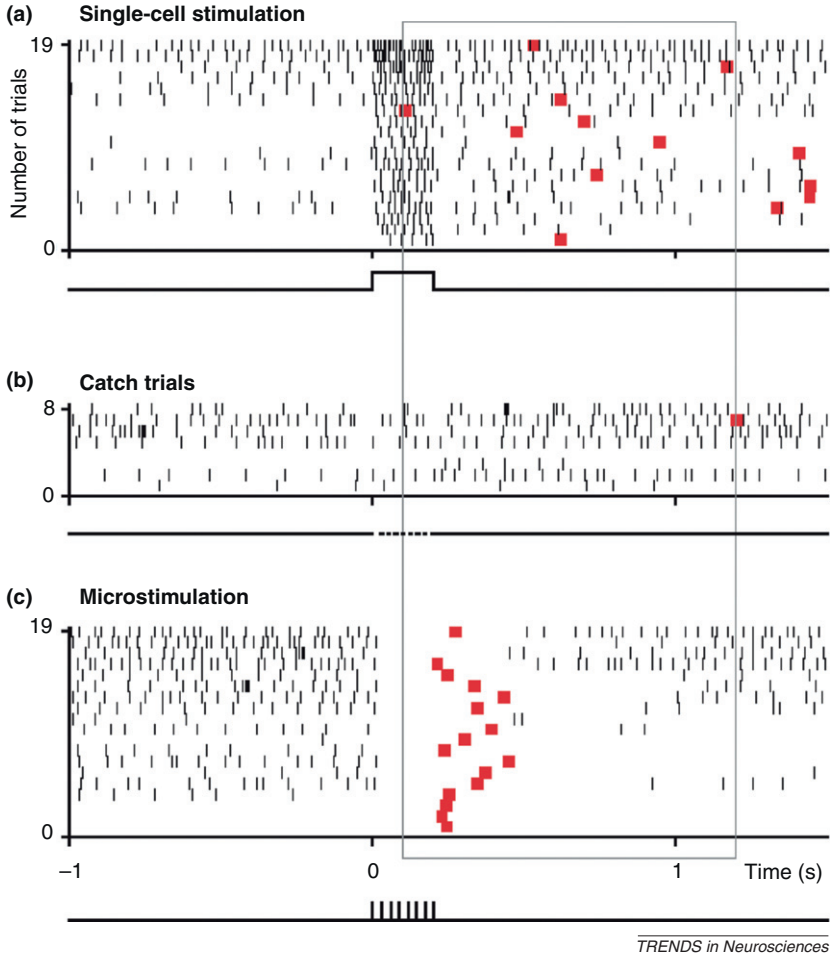

Figure I. Behavioral response to stimulation of a single cortical neuron. **(a)** Juxtacellular stimulation (at 0 s) of a single layer 5b cortical pyramidal neuron in an awake rat increases the firing rate of the cell (each spike is one tick on the raster plot) in each of the 19 trials. In 47% of trials, stimulation also triggers a lick (marked by red square) to obtain a water reward [90]. **(b)** Catch trials with no stimulation show few behavioral responses. **(c)** Microstimulation of many cells in the same cortical region drives behavioral responses (in 71% of trials in this example) [90]. Adapted, with permission, from [90].

replaced by smaller-amplitude, higher-frequency oscillations, evoked firing-rates are reduced. It will be exciting to investigate whether similar changes in membrane potential and firing rates are present in different cortical regions, and in different species, during trained behavioral tasks.

Such changes in cortical state can dramatically alter sparseness, creating small time-windows where cortical neurons are more likely to fire [25,73,74,81,82]. In auditory cortex, extracellular recordings show that spiking responses in awake, attending animals are suppressed compared to passive listening [73]. In somatosensory cortex, deflection of a single whisker in an awake animal can lead to spike suppression associated with decreased excitatory postsynaptic potential (EPSP) amplitude [25]. In V1, on the other hand, animal movement triggers an increase in evoked spiking responses of individual neurons [74]. These effects could be due to depression of the thalamocortical synapse [83] due to high thalamic firing-rates [79], or to subcortical neuromodulatory input from cholinergic or noradrenergic centers [84,85]. Furthermore, attentional processes – release of neuromodulatory factors – may also alter firing rates and sparseness [78].

Maybe responses are not sparse?

It is important to consider that the relatively small fraction of responsive cells, especially in superficial layers, might be merely an artifact of non-optimal stimulus presentation or impoverished behavioral conditions (Figure 1d). This is

more likely in whisker somatosensory cortex, where a typical stimulus consists of deflection of single whisker or of all whiskers by an airpuff – a far cry from how the whiskers might be using during behavior.

Consistent with this, extracellular recordings that use spike-sorting to record individual neurons in freely moving animals have reported higher firing-rates than have studies using head-fixed or immobilized animals [86]. However, cells that fire few spikes are still nearly impossible to detect in these studies. Although firing rates observed in S1 during awake behavior [39] are higher, these studies confirm that superficial layers contain a substantial number of silent cells. Visual stimuli typically engage a larger fraction of the network. In addition, in auditory cortex, primate recording studies using naturalistic vocalizations typically report higher firing-rates (and potentially more dense population responses) [87]. Stimulus optimization is a crucial parameter in assessing sparseness.

Why is it important to determine the size of the response ensemble? Across primary sensory cortex, synaptic connections are typically small, from 0.02 to 1 mV [70,88,89], such that spiking from a single cell is insufficient to drive a spike in a downstream neuron (but see [90]). With spike thresholds typically ~30 mV above the resting membrane potential, near-synchronous (i.e. within 10–50 ms) activation of tens to hundreds of cells might be required to drive downstream neurons. Too few neurons that spike in response to a stimulus effectively ends the transfer of information across the chain. There is probably a balance between the computational advantages of sparse neural activity (which can carry more information than more dense representations [7]) and the disadvantages of representations that are too sparse (which carry no information if they cannot drive downstream spikes). To evaluate computational models that rely on sparse firing of large populations of neocortical neurons, it will be important to perform experimental recordings with high temporal and spatial resolution from identified neurons, preferably in the context of an awake, attentive, and motivated animal.

Concluding remarks

In summary, it is crucial to confirm that sparse representations are used by the cerebral cortex, before determining the computational advantages of this phenomenon as a coding strategy (Box 3). Significant confusion between instantaneous or population sparseness (Figure 1a) and lifetime sparseness (Figure 1b) must be addressed by long-term recording or imaging studies, where individual neurons can be reliably monitored in the context of rich sensory experience. Few studies have directly addressed the long-term firing activity of neocortical neurons, and extracting data about silent or very low firing-rate neurons from published work is fraught with difficulty. Despite this, supragranular layers in particular exhibit a higher fraction of silent or nearly silent neurons. The broad range of firing rates of neurons, especially in supragranular layers of primary sensory cortex, may reflect the differential wiring of cortical circuits for distinct forms of sensation, perception, and learning.

Box 3. Outstanding questions

- Why does sparseness change across cortical layers, with many more silent neurons being observed in supragranular layers?
- How can high-throughput measures to address sparseness be developed for analysis of layer 4 and deep cortical layers?
- Are the cells that show higher firing rates a stable subset of the population?
- What kind of synaptic connectivity might underlie differential firing across populations of neurons within a layer?
- Can neuromodulation alter sparseness?
- Does motivated behavior with a naturalistic stimulus reduce the apparent sparseness of neocortical responses?

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