Layer 4 in Primary Visual Cortex: Response Properties and Effects of Awake Brain States

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Layer 4 in Primary Visual Cortex: 
Response Properties and Effects of Awake Brain States

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University of Connecticut, 2013

Abstract:

In mammalian primary visual cortex (V1), layer 4 is the major input layer. It receives direct thalamocortical inputs, performs some of the earliest cortical computations and provides the main entrance of sensory information to the cortex. The first part of the current project investigated the response properties of two major cells classes in layer 4 of the awake rabbit V1: putative fast-spike inhibitory interneurons (suspected inhibitory interneurons, SINs) and putative excitatory cells with simple receptive fields. The results show that the properties of these layer 4 populations are markedly distinct. SINs are far less linear, more broadly tuned to orientation, direction, spatial/temporal frequency, are more sensitive to contrast, have much higher spontaneous and stimulus-driven activity than simple cells, and they always had spatially overlapping ON/OFF receptive subfields. Furthermore, cross correlation between lateral geniculate nucleus (LGN) and SIN spike trains confirmed a fast and precisely timed monosynaptic connectivity. The second part of the project investigated the effects of alert/nonalert awake brain states on the response properties of layer 4 SINs and simple cells. It was previously shown that different brain states profoundly affect the receptive field properties, spontaneous firing rates, and spike-train statistics of neurons in the LGN. The results of the current project show that, in layer 4, alertness increases the strength and reliability of visual responses in layer 4 simple cells; it broadens their temporal frequency tuning but preserves their contrast sensitivity and selectivity for orientation, direction of motion and spatial frequency. Alertness also increased the amplitude and reliability of visual
responses in layer 4 SINs but reduced their high spontaneous rate. Therefore, the net effect on
SINs could be either enhancement or reduction of mean firing rate. Finally, alertness selectively
suppressed the responses of layer 4 simple cells to stimuli moving orthogonal to the preferred
direction and high contrast stimuli, effectively enhancing their responses to mid-contrast moving
borders. Simulations of a direction/orientation detection model further demonstrate that enhanced
response to preferred stimulation, increased reliability and stimulus-dependent suppression seen
in layer 4 simple cells during the alertness can each contribute to increased cortical computational
speed.
Layer 4 in Primary Visual Cortex: 
Response Properties and Effects of Awake Brain States

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Layer 4 in Primary Visual Cortex: Response Properties and Effects of Awake Brain States

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2.5.1 Identification of layer 4 SINs and simple cells ................................................ 64
2.5.2 Response properties of layer 4 simple cells and SINs ......................................... 65
2.5.3 The sustained/transient distinction in layer 4 simple cells and SINs ..................... 66
2.5.4 SINs as conveyers of broadly tuned inhibition ................................................... 69
2.5.5 Thalamocortical specificity and mechanisms underlying diverse response properties of layer 4 populations ................................................................. 69

CHAPTER 3: Brain state effects on layer 4 of the awake visual cortex .......... 71
3.1 Abstract .................................................................................................................. 71
3.2 Introduction ......................................................................................................... 72
3.3 Materials and Methods ....................................................................................... 74
  3.3.1 Animal preparation and electrophysiological recording ................................ 74
  3.3.2 Brain state identification ............................................................................... 75
  3.3.3 Cortical layer 4 identification ....................................................................... 76
  3.3.4 Cell classification ....................................................................................... 76
  3.3.6 Offline data analysis .................................................................................... 78
3.4 Results ................................................................................................................... 81
  3.4.1 Alert/nonalert state effects on responsiveness .............................................. 81
  3.4.2 Brain state effects on spatial summation linearity ...................................... 87
  3.4.3 The alert state made visual responses not only stronger and more linear but also more reliable ............................................................ 87
  3.4.4 Contribution of spontaneous activity to state effects ................................. 90
  3.4.5 Brain state effects on orientation/direction tuning properties for simple cells 90
  3.4.6 Alert/nonalert state effects on the contrast response functions of simple cells 93
  3.4.7 Brain state effects on spatial/temporal tuning properties for simple cells .... 95
  3.4.8 Brain state effects on stimulus tuning properties for SINs ....................... 97
  3.4.9 Brain state effects on sustained response to flashing stationary stimuli .... 100
  3.4.10 Alertness enhances computational speed of feature detection ............... 102
3.5 Discussion .......................................................................................................... 106
  3.5.1 Alert/nonalert state effects in LGN and V1 ................................................. 106
  3.5.2 Alert/nonalert state effects beyond the multiplicative gain modulation .... 107
  3.5.3 Comparisons to previous studies in awake subjects .............................. 108
  3.5.4 The significance of getting drowsy ......................................................... 109

Abbreviations ...................................................................................................... 110
References ........................................................................................................... 111
List of Figures

Figure 2.1  pp19
Figure 2.2  pp34
Figure 2.3  pp36
Figure 2.4  pp39
Figure 2.5  pp41
Figure 2.6  pp43
Figure 2.7  pp46
Figure 2.8  pp48
Figure 2.9  pp51
Figure 2.10  pp53
Figure 2.11  pp57
Figure 2.12  pp60
Figure 3.1  pp82
Figure 3.2  pp83
Figure 3.3  pp85
Figure 3.4  pp88
Figure 3.5  pp91
Figure 3.6  pp93
Figure 3.7  pp95
Figure 3.8  pp98
Figure 3.9  pp100
Figure 3.10  pp103
List of Tables

Table 2.1  pp30
Table 2.2  pp31
Table 2.3  pp67
CHAPTER 1: Introduction

1.1 Layer 4 as the major input layer of primary visual cortex

The mammalian sensory cortices share common organizing principals. They can be divided into 6 anatomically and functionally distinctive layers, with layer 4 receiving the bulk of thalamic inputs from the lateral geniculate nucleus (LGN). As the major input layer of sensory cortices, layer 4 performs the earliest stage of cortical information processing and provides the main entrance of sensory information to the cortex.

1.1.1 Cell classes in layer 4

1.1.1.1 Anatomy

In general, neurons in layer 4 could be divided into two major groups: glutamatergic excitatory neurons and GABAergic inhibitory neurons, with the population of excitatory neurons (more than 80%) > 4 times higher than that of inhibitory cells (less than 20%, Gabbott and Somogyi, 1986; Fitzpatrick et al., 1987; Hendry et al., 1987; Meinecke and Peters, 1987; Demeulemeester et al., 1988). Morphologically most layer 4 excitatory cells can be classified as spiny stellate cells with multi-polar cell body and spinous dendrites, and a small number of layer 4 excitatory cells are pyramidal cells with triangular cell body, ascending apical dendrites and horizontal basal dendrites (Peters and Jones, 1984, vol 1; but see Staiger et al., 2004). The layer 4 inhibitory cells are made up by various morphological types: 1) basket cells with axons forming “basket” around cell bodies of excitatory neurons; 2) neurogliaform cells with small axonal plexus overlapping with their dendritic tree; 3) bipolar cells with narrow and vertical dendritic tree extending from each end of the cell body and 4) chandelier cells with distinctive short vertical axon
terminals innervating axon initial segments of pyramidal neurons. All of these inhibitory cells have smooth or sparsely spinous dendrites. However, other morphological types of inhibitory neurons (i.e. double bouquet cells with vertically axons extending from superficial layers to deep layers and Martinotti cells with distinctive ascending axons up to layer 1) are rather rare in layer 4 (but see Markram et al., 2004). Almost all cortical inhibitory cells have axons collaterals confined within cortex (either remaining near the parent cell body, or running horizontally parallel to the cortical surface or vertically across different cortical layers), so they are considered altogether as cortical inhibitory interneurons (Peters and Jones, 1984, vol 1). Please note these short descriptions of cortical interneuron morphologies here only capture general features of these cells and more detailed and comprehensive classifications of cortical interneurons are still developing (Ascoli et al., 2008; DeFelipe et al., 2013).

1.1.1.2 Biochemistry

The heterogeneity of cortical inhibitory interneurons is also reflected in their various biochemical markers. Several different types of calcium-binding protein such as parvalbumin (PV), calbindin (CB) and calretinin (CR); different types of neuropeptide such as somatostatin (SOM), cholecystokinin (CCK), vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY) are commonly used as biochemical markers to distinguish different type of cortical inhibitory neurons (Markram et al., 2004). The most comprehensive data of these biomarker expression in cortical inhibitory interneurons are from rodents. In rat visual cortex, all of PV+, SOM+ and CR+ cortical cells were found to be GABAergic and the expression of PV, SOM and CR were mutually exclusive. These three type of cells (PV+, SOM+ and CR+) together account for more than 80 percent of cortical GABAergic neurons (Gonchar and Burkhalter, 1997). However, in mouse visual cortex, some coexpression of SOM and CR were found (Gonchar et al., 2007) and further study show PV+, SOM+ and VIP+ inhibitory neurons form three mutually exclusive group which in total account for about 60% of total cortical GABAergic neurons (Xu
et al., 2010) while other studies showed PV+, SOM+ and ionotropic serotonin receptor 5HT3a positive neurons account for nearly 100% of cortical GABAergic neurons (see review Rudy et al., 2011). Despite these controversies, it is clear that PV+ and SOM+ neurons form the first and second major groups of cortical GABAergic neurons, respectively. In layer 4 of mouse primary visual cortex (V1) PV+ and SOM+ neurons account for more than 50% and 20% of total GABAergic neurons respectively (Xu et al., 2010). Finally, as they all use GABA as their neurotransmitter, cortical GABAergic neurons can be distinguished from excitatory cells by expressing GABA synthesizing enzymes (glutamate decarboxylase, GAD).

There are certain relationships between morphology and biochemical marker of cortical inhibitory interneurons. For example, basket cells typically express many neuronpeptides and PV seldom express SOM and VIP while Martiontti cells always express SOM but never express PV and VIP; chandelier cells usually express PV, bipolar cells usually express VIP and double bouquet cells express various biochemical markers (Markram et al., 2004). However, comprehensive and detailed reviews of the relationship between morphology and biochemical marker of cortical inhibitory neurons are beyond the scope of this introduction.

1.1.1.3 Electrophysiology

The layer 4 cortical neurons also show different electrophysiology properties. Based on their responses to current injection in vivo, the neurons in cat V1 can be divided into four types: regular spike (RS), fast spike (FS), intrinsic bursting (IB) and chattering (CH) (Nowak et al., 2003). RS cells respond adaptively to current injection with increasing inter-spike intervals (ISIs), while FS cells respond non-adaptively with constant and short ISIs. IB cells respond to the onset of current injection with a low frequency burst and to the rest of injection with a tonic train of action potentials while CH cells responds with repetitive
high-frequency bursts during the whole injection. Furthermore, RS and IB cells have wider action potential waveforms than FS and CH cells (Nowak et al., 2003). There are other classification systems of cortical neurons, but the RS/FS classification holds across various studies (Connors and Gutnick, 1990; Kawaguchi, 1993; Kawaguchi and Kondo, 2002; Markram et al., 2004; DeFelipe et al., 2013). Morphologically, in layer 4, RS and CH cells are found to be spiny stellate cells (putative excitatory) and FS cells are found to be sparsely spiny or aspiny nonpyramidal cells (putative inhibitory). IB cells are abundant in layer 5 but rare in layer 4 (Nowak et al., 2003). Biochemically, FS cells are commonly found to be PV positive (Kawaguchi, 1993; Kawaguchi and Kondo, 2002; Ma et al., 2010), while SOM+ cells are non-fast-spiking with broad action potential waveforms (Kawaguchi and Kondo, 2002; Ma et al., 2010).

In summary, layer 4 of primary sensory cortex consists of RS or CH glutamatergic excitatory neurons (>80% of total population) and GABAergic inhibitory neurons (<20%), within which about 50% are PV positive and fast-spiking (mostly likely basket cells), about 20% are SOM positive and non-fast-spiking; the rests show heterogeneous properties in morphology, biochemistry and electrophysiology. The rest of this introduction will be focusing on the three major cell types in layer 4: excitatory cells, PV+ inhibitory interneurons and SOM+ inhibitory interneurons.

1.1.1.4 Classification criteria based on extracellular recordings
Since FS cells show narrower action potential width than RS cells, their spike waveform recorded extracellularly are also narrower than those of RS cells, so the spike waveform width from extracellular recordings are commonly used as criterion to distinguish RS putative excitatory cortical cells and FS putative inhibitory cortical cells (Bruno and Simons, 2002; Andermann et al., 2004; Bartho et al., 2004; Hasenstaub et al., 2005; Lee et al., 2007; Mitchell et al., 2007; Atencio and Schreiner, 2008). However,
some spiny neurons including some identified projection neurons of visual (Swadlow, 1988), somatosensory (Dykes et al., 1988; Swadlow, 1989, 1990) and motor (Swadlow, 1994) cortices have short-duration spikes. Furthermore CH cells (they can be spiny stellate cells in layer 4 or pyramidal cells in other layers, Gray and McCormick, 1996; Nowak et al., 2003), and some pyramidal tract neurons (Takahashi, 1965) also have very brief spikes. As a result, at least some narrow spike FS cells identified by extracellularly recorded spike duration will be excitatory. Therefore, in the current project (Chapter 2 & Chapter 3) we used responses to thalamic electric stimulation to identify cortical fast-spike PV+ inhibitory interneurons (see Chapter2 & Chapter 3), which is proposed to be more accurate than the criterion of spike duration (Swadlow, 2003).

1.1.2 Response properties of different cell types in layer 4

In primary sensory cortices, different classes of neurons are associated with different response properties to the peripheral stimuli. In vivo studies in cat V1 have shown that both FS cells and RS cells can possess either simple or complex receptive field (Cardin et al., 2007). Furthermore, there are at least a subgroup of cortical FS cells lacking orientation/direction selectivity, and cortical FS cells as a group have broader orientation tuning width than RS, IB and CH cells (Nowak et al., 2008). The same phenomenon also holds just in layer 4 (Cardin et al., 2007). Another study found that the aspiny cells in layer 4 of cat V1 can be either simple (segregated ON/OFF receptive subfields) with orientation selectivity or complex (overlapping ON/OFF receptive subfields) without orientation selectivity (Hirsch et al., 2003). Therefore, in cat V1, the inhibitory interneurons show various response properties in terms of their receptive field structure and orientation selectivity. However, little information about relationship between receptive field properties and biochemical markers in cat visual cortex has been reported, probably due to the difficulties in combining in vivo electrophysiology and in vitro labeling. On the other hand, similar studies are a lot easier to be performed on rodents (especially mice) due to the availability of advanced
genetic tools. Recent studies have shown that although rodent visual cortex does not have well organized orientation columns (Ohki and Reid, 2007), the excitatory cells in mouse V1 are still sharply tuned in orientation (Niell and Stryker, 2008; Liu et al., 2009; Kerlin et al., 2010), while almost all PV+ cells show broad orientation tuning (Sohya et al., 2007; Kerlin et al., 2010). Furthermore, most excitatory cells in layer 2/3 and layer 4 of mouse V1 possess segregated ON/OFF receptive subfields or solitary receptive subfield (simple), while all PV+ inhibitory cells have overlapping ON/OFF subfields (complex) (Liu et al., 2009; Ma et al., 2010). Interestingly, SOM+ neurons in mouse visual cortex, with broad spike waveform, show various properties in terms of receptive field structure and orientation selectivity (Ma et al., 2010). Studies done on V1 of awake rabbits had the similar results, with a special type of cortical inhibitory cells (putative PV+ fast-spiking GABAergic interneurons, suspected inhibitory interneurons, SINs) identified extracellularly having diffusive overlapping receptive subfields and broad orientation tuning (Swadlow and Weyand, 1987; Swadlow, 1988).

Given the finding that different classes of cortical neurons have distinctive receptive field properties, it is reasonable to speculate that neurons with different receptive field properties play different roles in cortical computation. Recently, this question is under active investigations on mouse visual cortex by electrophysiology, genetic, optical approaches. One study showed that activation of cortical PV+ neurons markedly sharpens the feature selectivity of nearby neurons (Lee et al., 2012). This results are supported by an intracellular study showing broadly tuned inhibition could sharpen the orientation selectivity of layer 2/3 excitatory cells (Li et al., 2012). However, other studies reported that it is SOM+ neurons that affect the response selectivity (Wilson et al., 2012) while PV+ neurons affect response gain (Atallah et al., 2012; Wilson et al., 2012). The inconsistency among these studies is probably due to the difficulties in controlling the location (layers) of optical stimulation in vivo studies. Furthermore, SOM+ neurons were reported to provide surround inhibition in superficial layers (Adesnik et al., 2012) while, in layer 4,
SOM+ neurons were found to inhibit layer 4 PV+ neurons (Xu et al., 2013). Despite the controversies, these studies start to plot a functional cortical circuitry with a lot more details than the general laminar structure mentioned in the beginning of this introduction.
1.2 Effects of awake brain states on sensory responses

The “waking state” is not unitary. It contains different brain states which can be classified by behavior, physiological activity or neurotransmitter release (Harris and Thiele, 2011; Lee and Dan, 2012). Furthermore, psychological studies have shown different brain states can strongly affect perception (Dorokhov et al., 1998; Roge et al., 2002). Thus it is interesting and important to see how different brain states affect basic response properties at the early stage of cortical information processing. However, due to the difficulties of recording or imaging single cell activity when animal is awake but not attentive, most studies on awake animal were focusing on alert and attentive state (Kastner and Ungerleider, 2000; Reynolds and Chelazzi, 2004; Maunsell and Treue, 2006; Anton-Erxleben and Carrasco, 2013). Only a handful of studies have been done to investigate the effects of different awake brain states (from alert, vigilant state to nonalert drowsy state) on basic sensory response properties in sensory system. Most of them were done on rodents and rabbits.

1.2.1 Effects of awake brain states in rodent sensory cortex

Since only 3 studies of this kind were done in visual system (Niell and Stryker, 2010; Pinto et al., 2012; Polack et al., 2013) similar studies in other sensory systems are also included here. Studies of awake brain states on rodents use various definitions of different awake brain states. For example, an “active, vigilant state” can be defined by either cortical desynchronization (Harris and Thiele, 2011) or motivated behaviors, such as directed locomotion (Niell and Stryker, 2010; Polack et al., 2013), exploratory whisking (Poulet and Petersen, 2008) or engaging of behavioral tasks (Fanselow and Nicolelis, 1999; Otazu et al., 2009). Accordingly, on the other hand, an “inactive, quite state” can be defined by either cortical synchronization, or lack of locomotion, whisking or task engagement. Other studies mimic the active brain state by activating cholinergic projecting nucleus (Goard and Dan, 2009; Pinto et al., 2012).
But in general, cortical desynchronization is a significant feature of active awake brain state (see review Harris and Thiele, 2011).

Because of the variety of awake brain state definitions, it is difficult to compare the results across these states. One study using presence of locomotion as criterion found both RS cells and FS cells in mouse V1 significant increase their responsiveness during the “moving” state, but locomotion does not affect the responsiveness in LGN (Niell and Stryker, 2010). A recent study using the same criterion further reported, during the “moving” state, both excitatory cells and PV+ inhibitory cells in mouse V1 not only increase responsiveness but also decrease response variability (Polack et al., 2013). This is similar to another series of studies, which found, when stimulating nucleus basalis (cholinergic projecting center), neurons in both LGN and V1 significantly increase response reliability (Goard and Dan, 2009; Pinto et al., 2012). On the other hand, there are other studies showed contradictory results: during active exploring or behavior task performance, the responsiveness of cortical cells in primary somatosensory cortex (Fanselow and Nicolelis, 1999) and auditory cortex (Otazu et al., 2009) of awake rats were decreased. Thus, although different awake brain states have been shown to strongly affect cortical response properties in multiple sensory areas, it is difficult to make comparisons across these studies which use various species and various ways to define brain states. To better uncover state effects on cortical information processing, a generally accepted taxonomy of global brain states that applies across diverse species and cortical areas is needed.

1.2.2 Alert/nonalert state effects in rabbit visual system

The effects of different awake brain states on response properties of rabbit visual system has also been systematically investigated (Swadlow and Weyand, 1985, 1987; Swadlow and Gusev, 2001; Bezduchnaya
et al., 2006; Cano et al., 2006; Stoelzel et al., 2009; Bereshpolova et al., 2011). These studies applied uniform criterion to define awake brain state. That is defining “alert state” by showing prominent “theta” activity (5-7 Hz) in hippocampal EEG with cortical desynchronization, and defining “nonalert state” by showing large amplitude irregular activity in hippocampal EEG with cortical synchronization. The transition between these states is often very rapid (<1 sec) and can happen hundreds of times a day (Bezdudnaya et al., 2006). During both states, animal are sitting still without locomotion.

The alert/nonalert brain state profoundly affects spiking statistics and receptive field properties in LGN. During the alert state, LGN cells have significantly higher spontaneous firing rate, higher response gain, higher temporal frequency peak, broader temporal frequency tuning and generate a lower number of bursts than during the nonalert state (Swadlow and Gusev, 2001; Bezdudnaya et al., 2006; Bereshpolova et al., 2011). LGN cells also have higher maintained response to stationary stimulation during the alert state than during the nonalert state (Swadlow and Weyand, 1985). On the other hand, thalamocortical synaptic transmission is remarkably stable across the states (Stoelzel et al., 2009), suggesting that layer 4 neurons, the major recipients of LGN input, may inherit the brain state effects from LGN. Surprisingly, unlike LGN cells, suspected inhibitory interneurons (SINs) in layer 4 decreased their spontaneous firing rate during the alert state while layer 4 simple cells kept their spontaneous firing relatively constant (Bereshpolova et al., 2011). These surprising results suggested that brain state can have a different effect on visual cortex than thalamus. Notably, the two major cell classes in layer 4, the SINs and the putative excitatory simple cells, have markedly different response properties in awake rabbits (Chapter 2), suggesting very different roles in cortical computation. The study described in Chapter 3 follows this series of studies and further investigated the alert/nonalert brain state effects on the response properties of different cell classes in layer 4 of awake rabbits.
CHAPTER 2: Layer 4 in primary visual cortex of the awake rabbit: contrasting properties of simple cells and putative feed-forward inhibitory interneurons

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2.1 Abstract

Extracellular recordings were obtained from two cell classes in layer 4 of the awake rabbit V1: putative inhibitory interneurons (suspected inhibitory interneurons, SINs) and putative excitatory cells with simple receptive fields. SINs were identified solely by their characteristic response to electrical stimulation of the LGN (3+ spikes at > 600 Hz), and simple cells were identified solely by receptive field structure, requiring spatially separate ON and/or OFF subfields. Notably, no cells met both criteria, and we studied 62 simple cells and 33 SINs. Fourteen cells met neither criterion. These layer four populations were markedly distinct. Thus, SINs were far less linear (F1/F0 < 1), more broadly tuned to stimulus orientation, direction, spatial and temporal frequency, more sensitive to contrast, had much higher spontaneous and stimulus-driven activity, and always had spatially overlapping ON/OFF receptive subfields. SINs responded to drifting gratings with increased firing rates (F0) for all orientations and directions. However, some SINs showed a weaker modulated (F1) response sharply tuned to orientation and/or direction. SINs responded at shorter latencies than simple cells to stationary stimuli, and the responses of both populations could be sustained or transient. Transient simple cells were more sensitive to contrast than
sustained simple cells and their visual responses were more frequently suppressed by high contrasts.

Finally, cross correlation between LGN and SIN spike trains confirmed a fast and precisely timed monosynaptic connectivity, supporting the notion that SINs are well-suited to provide a fast feed-forward inhibition onto targeted cortical populations.
2.2 Introduction

The cerebral cortex is organized in six layers that can be distinguished based on cell morphology, functional connections and response properties. Among all layers, layer 4 has been studied more intensively because it is the layer that receives the bulk of the thalamic inputs and provides the main entrance of sensory information to the cortex. In V1, layer 4 circuits are involved in generating neuronal response properties such as orientation and direction selectivity that are essential for visual processing. While these properties are thought to emerge from the interplay between excitation and inhibition, we still do not have a complete understanding of how inhibitory and excitatory neurons of this layer differ in their responses to visual stimuli. Moreover, a full understanding of neuronal mechanisms involved in visual perception requires the study of subjects that are awake and capable of perception. To these ends, we have investigated the response properties of layer 4 putative excitatory and inhibitory neurons (suspected inhibitory interneurons, SINs) in awake rabbits. Both rabbits and rodents are closely related to primates (superorder of Euarchontoglires, also known as supraprimates, Murphy et al., 2001; Asher et al., 2009) but, unlike primates, the rabbit eye remains nearly motionless for considerable periods of time, making quantitative receptive field (RF) analyses feasible in awake subjects (Bezdudnaya et al., 2006; Cano et al., 2006).

We have previously shown that the great majority of neurons in LGN of awake rabbits have concentric RFs that are bimodally distributed into sustained and transient classes (Swadlow and Weyand, 1985; Bezdudnaya et al., 2006; Cano et al., 2006). We also demonstrated that transient and sustained concentric LGN neurons differ in their contrast sensitivity (Cano et al., 2006), temporal tuning properties (Bezdudnaya et al., 2006), and project to different depths within cortical layer 4 (Stoelzel et al., 2008). Here, we move this investigation to V1 and compare the response properties of layer 4 simple cells and SINs. We show that these populations differ dramatically along multiple RF property dimensions. Thus,
when compared with simple cells, SINs are very broadly tuned to stimulus orientation, direction, temporal frequency and spatial frequency, and they respond in a much more non-linear manner (low F1/F0 ratios), and at higher firing rates to drifting grating stimulation. We also show that, like LGN cells, simple cells and SINs can be subdivided into sustained and transient subclasses that differ significantly in their contrast sensitivity. We finally show, using cross-correlation methods, that many layer 4 SINs do, indeed, receive direct input from retinotopically aligned LGN neurons (i.e., that these putative inhibitory internuerons receive a potent feed-forward thalamic input, Swadlow, 2003; Gabernet et al., 2005; Cruikshank et al., 2007) and, as such, are optimally positioned to shape the response properties of layer 4 simple cells (Hirsch et al., 1998; Troyer et al., 1998; Miller et al., 2001; Liu et al., 2011).
2.3 Materials and Methods

Recordings were obtained from monocular V1 of 3 awake adult female Dutch-Belted rabbits. All experiments were conducted with the approval of the University of Connecticut Animal Care and Use Committee in accordance with National Institutes of Health guidelines.

2.3.1 Animal preparation and electrophysiological recording

The general surgical procedures for chronic recordings have been described previously (Bezdudnaya et al., 2006; Stoelzel et al., 2008; Bereshpolova et al., 2011) and are reported only briefly here. Initial surgery was performed under ketamine-acepromazine anesthesia using aseptic procedures. After removal of the skin and fascia, stainless steel screws were installed on the dorsal surface of the skull and fused together with acrylic cement. A stainless steel rod, oriented in a rostrocaudal direction, was then cemented to the acrylic mass. The rabbit was held rigidly by this rod during the electrode implantation and recording sessions. Silicone rubber was used to buffer the wound margins from the acrylic cement on the skull. Following at least 10 days of recovery, recordings of neuronal activity were obtained in the awake state through a small hole in the skull.

Extracellular single-unit recordings and cortical local field potentials (LFP) were obtained from the monocular region of V1 of awake rabbits. Single unit activity for most cells was studied using fine-diameter (40 μm) quartz-insulated platinum/tungsten electrodes tapered and sharpened to a fine tip (impedance, 1.5-3 Mohm). A group of seven such electrodes was chronically implanted in a concentric array (200 μm separation), with tips initially located just above the dura. Each of these electrodes was independently controlled by a miniature microdrive (Swadlow et al., 2005). Multi-unit activity from superficial layers of the superior colliculus (SC) was simultaneously recorded via 1-3 electrodes of same type but lower impedance (<1.5 Mohm) controlled by a similar 3-channel microdrive system. A small
number of cells were studied using 16-channel silicone probes, with recording site diameters of 33 μm (NeuroNexus Technologies). Two stimulating electrodes (parylene-c insulated platinum/iridium micro wire) were implanted in LGN for identification of cortical neurons (below). Hippocampal EEG was recorded using two electrodes implanted above and below the CA1 layer and used, along with cortical EEG, for monitoring brain states. All electrophysiological activity was acquired using a Plexon data acquisition system.

In another set of experiments, SINs in V1 layer 4 were recorded simultaneously with LGN cells from topographically aligned regions of dorsal LGN as described in our earlier studies (Swadlow and Gusev, 2002; Stoelzel et al., 2009; Bereshpolova et al., 2011). In this set of experiments, only spontaneous activity and response to sparse noise (see below) of both LGN cells and cortical SINs were recorded.

2.3.2 Brain state identification

During recordings, no anesthetic agents were applied, the eyes were open and subjects responded to external stimuli (novel sound and gentle touch, etc). Our goal was to record when subjects were awake, either alert or nonalert, in roughly equal proportions. In rabbits, wakefulness can be divided into alert and nonalert states. The alert state is indicated by hippocampal “theta” activity (5-7 Hz) and cortical desynchrony, and the nonalert state indicated by hippocampal high-voltage irregular activity, and more slow wave activity in the neocortex (Swadlow and Gusev, 2001; Bezdudnaya et al., 2006; Cano et al., 2006; Stoelzel et al., 2008; Stoelzel et al., 2009; see recent discussion in Bereshpolova et al., 2011). The hippocampal EEG was segmented by visual inspection into alert versus nonalert states based on the presence of theta activity (5–7 Hz) or high-voltage, irregular activity, respectively (Swadlow and Gusev, 2001; Bezdudnaya et al., 2006; Cano et al., 2006; Stoelzel et al., 2009). This segmentation was aided and verified by fast Fourier transformation (FFT) analysis. Some non-periodic sensory stimulation (random sounds, tactile stimulation, movements in room) is often required to keep rabbits from transitioning from
the awake, nonalert state to sleep (early signs of which are indicated by cortical spindle activity), and such stimulation was applied when needed. During recording sessions, the average percentages of time the animals spent in the alert state and nonalert state were 32.94±1.36% and 36.28±2.20%, respectively. In some cases, when rabbits became drowsy during extended recordings, we provided novel sounds to generate alertness. If they were difficult to arouse, we let them sleep for a while, and did not collect data during these periods.

### 2.3.3 Cortical layer 4 identification

Depth range of cortical layers was determined by electrode depth and reversal of stimulus evoked field potentials that occur at known depths. Brief full field flash evoked LFPs were recorded at different depth. As previously verified histologically (Stoelzel et al., 2008), the top of layer 4 was identified as 100 μm below a prominent current sink/source reversal point to the flash stimuli and the bottom of layer 4 was identified as 400 μm below the top of this layer. Only neurons within this narrow (400 μm) depth zone were included in this study.

### 2.3.4 Identification of SINs and simple cells

For each cell, the response to electrical stimulation of the thalamus (rectangular voltage pulses, 0.2 ms duration, range: < 1 V – 45 V) was recorded (latency of first spike, spike number after stimulation and minimal inter-spike interval of these spikes, Figure 2.1A). SINs were identified by a high-frequency discharge of 3 or more spikes to this stimulus, with peak frequencies of > 600 Hz (Swadlow, 1988, 1989, 1991, 1995, 2003). Cells identified in this manner have spikes of short duration (see below). However, many cortical projection neurons also have short duration spikes (Swadlow, 1988, 1990, 1991, 1994) and we have, therefore, argued that spike duration is not a sufficient criterion for identifying SINs (Swadlow, 2003). Cortical simple cells were identified by possessing a RF with one, two, or three spatially separated...
ON and/or OFF subfields. Cells that could not be classified as simple cells or SINs were grouped as “other cells” and were not subject to further analysis. Two cells that were antidromically activated by thalamic stimulation, were thought to be dendritic recordings (Bereshpolova et al., 2007) and were excluded from this study.
Figure 2.1. Responses to thalamic electrical stimulation and spike waveform durations of different cell types. A, Conceptual diagram of a cell’s response to a thalamic electrical stimulation. B-D, Distribution of spike waveform durations (B), latencies to the onset of stimulation (C) and minimal inter-spike intervals (ISIs) of responding spikes (D) of different cell types. ‘NA’ in panel D represents cells that responded to thalamic stimulation with < 2 spikes.
2.3.5 Receptive field and visual response property measurements

All visual stimuli for cortical cells were presented by a CRT monitor (primary monitor, Nec MultiSync, 40x30cm, mean luminance, 48cd/m², refresh rate: 160Hz). The cells’ RFs were mapped by sparse noise stimulation. High-contrast light and dark squares (1 x 1 - 5 x 5 degrees, mostly 2 x 2 degrees) were presented pseudorandomly, in a grid of 30 x 22 degrees on the primary monitor. Each grid space was 1 degree and each square was presented for either 18.75 or 31.25 ms. For some layer 4 simple cells which responded poorly to flashing squares, small flashing bars in their preferred orientation were used instead to map their RFs. The cell’s raw ON and OFF RF matrices were generated by reverse correlation method (Jones and Palmer, 1987; Stoelzel et al., 2008, below). After mapping, the cell’s RF center was constantly tracked by dynamic SC multi-unit RF position, and all the visual stimuli thereafter were presented to the cell’s RF center (see below).

In the rabbit LGN, the response of concentric neurons to stationary stimuli can be classified as either ‘sustained’ or ‘transient’, and this distinction is robust and bimodal (Bezdudnaya et al., 2006). However, the sustained response is severely attenuated when animals are not alert in both LGN (Swadlow and Weyand, 1985) and V1 (Swadlow and Weyand, 1987). Therefore, we classified cortical neurons as sustained or transient based on tests that were done in the alert state. The cell’s sustained/transient property was measured with flashing stationary stimuli, which were optimized to elicit the strongest response possible. The stimulus was either a circle or rectangle optimized for size, orientation, and contrast polarity (dark or light). The stimulus was presented either 1 sec on and 1 sec off or 2 sec on 2 sec off (see below).

After assessing sustained/transient responses, circular drifting gratings were used to measure the tuning properties of the cell. The drifting grating was optimized by orientation, spatial frequency, temporal frequency, size and contrast. After optimizing the grating, the cell’s tuning properties were measured by
pseudorandomly varying one of the four (orientation, spatial frequency, temporal frequency or contrast) grating parameters while keeping the other four at the optimal values. Each presentation of a particular parameter combination lasted for 3 to 8 sec with 2 sec gap in between and all tested parameter combinations were presented for at least 100 times. For some cells, the spontaneous activity was also recorded for at least 10 minutes with the monitor screen set at a mid-luminance level.

2.3.6 Monitoring eye position

The eye position of the awake rabbit is generally very stable (Collewijn, 1971; Fuller, 1981; Swadlow and Weyand, 1985, 1987; Bezdundnaya et al., 2006). During recording sessions, the eye position was continuously monitored by mapping the SC multi-unit RF center position with sparse noise on a second LCD monitor (Acer AL1516, 30x23 cm, mean luminance, 36cd/m², refresh rate, 75Hz). For most cells, the pupil position and size were also simultaneously monitored by a high-frequency (220 Hz) infrared camera system (ViewPoint EyeTracker® system, Arrington Research, Inc.) placed about 40 cm from the eye. If an eye movement occurred during testing, the relation between the RF centers of the cortical cell and SC multiunit RF center was used to dynamically place the stimuli on the cortical RF center. During the offline analysis, we discarded data recorded within ±15 sec around the eye movement by sliding a time window of 25-30 sec with a step of 5 sec over time and detecting the steps in which the standard deviation of SC RF centers was > 1 degree. Visual responses to drifting gratings were measured by generating peristimulus time histograms (PSTHs) during stable eye periods and the PSTH peaks at the fundamental frequency were aligned across these periods. Only the cells with significant responses at the fundamental frequency were processed with this alignment method.

2.3.7 Offline data analysis
Spikes from cortical single units were isolated during the experiment and verified off-line by using Plexon cluster analysis software. All data analysis was then performed with NeuroExplorer (Nex Technologies, Inc.) and MATLAB (The MathWorks, Inc.).

2.3.7.1 Spike waveform
Spike waveform durations were calculated as the durations from the beginning of negative component to the end of positive component of the spike (Bereshpolova et al., 2011). The negative or positive components were identified by lower or higher than 20% of negative or positive peak amplitudes, respectively. If the end part of the spike waveform did not reach the 20% threshold, the whole waveform was estimated by elongating the raw waveform through a linear fitting of the end part (from the peak of positive component to the end). Then the spike durations were calculated from the elongated waveforms.

2.3.7.2 Spatial properties of ON-OFF RF subfields
For each cell, the raw ON and OFF RF matrices were calculated by spike-trigger averaging the stimulus within a 20ms time window around the peak response. A Gaussian filter was applied to smooth these matrices. Finally, the processed ON and OFF RF maps were generated by applying a threshold as 30% of maximum pixel value.

To measure the spatial segregation of subfields, the local similarity index (LSI) was calculated as normalized dot product of the processed ON and OFF RF maps (DeAngelis et al., 1999; Usrey et al., 1999; Alonso et al., 2001).

\[
\text{LSI} = \frac{(\text{RF}_{\text{ON}} \cdot \text{RF}_{\text{OFF}})}{\sqrt{(\text{RF}_{\text{ON}} \cdot \text{RF}_{\text{ON}})(\text{RF}_{\text{OFF}} \cdot \text{RF}_{\text{OFF}})}}
\]
The values of LSI range from 0 to 1. LSI equals 1 if the ON and OFF RF maps are identical and perfectly superimposed and equals 0 if the ON and OFF RF maps are completely separated.

Dominant RF subfield area and width were used to estimate the size of RF subfields. To get these measurements, processed ON and OFF RF maps went through cubical interpolation at resolution of 0.1 degree. Then RF subfield was defined by a region of contiguous pixels with values > 30% of maximum pixel (the higher value of ON peak and OFF peak). Finally the dominant RF subfield was defined as the stronger subfield, ON or OFF. For each dominant subfield, the area and the width (on the orientation orthogonal to the cell’s optimal orientation) were measured.

2.3.7.3 Sustained index and sustained/transient classification

In the alert state, a sustained index (SI) was calculated as the ratio between the cell’s maintained response and the baseline activity. The maintained response was measured as the mean firing rate within 0.5 to 1.0 sec after the onset of optimal stationary stimuli, and the baseline activity was measured as the mean firing rate within the time window of either -1 to 0 or -0.5 to 0 sec relative to the onset of stimuli. Sustained cells were defined by having SIs larger than 2 and having an absolute maintained response of more than 1 spk/sec. Cells with SIs lower than 2 were classified as transient. Cells that failed to reach these criteria (SI larger than 2 but the absolute maintained activity lower than 1 spk/sec) remained as ‘sustained/transient unclassified’.

2.3.7.4 Latency to flash stimulation

The latency to flash stimulation was measured from the cell’s response to optimal flashing stimuli. The PSTH (with bin size equal to 1 ms) around the onset of the stimuli was generated, and smoothed by
applying a sliding boxcar filter with width of 30 ms. Each stimulus frame was time-stamped and, because stimuli presented higher in the field occur slightly sooner than those presented lower in the field, stimulus onset time was corrected for the position of the stimulus on the screen. Latency was defined as the time at which the smoothed function first pass 40% of its maximum value (Jin et al., 2011).

2.3.7.5 Drifting grating parameter tuning properties

The mean firing rate (F0) and first harmonic component (F1) of the PSTH responding to drifting grating stimuli were calculated by Fourier analysis. The maximum F1 and maximum F0 responses (with or without the spontaneous activity) were measured for each cell. Spatial summation linearity was measured as the mean of two F1/F0 ratios, one ratio measured from the PSTH with strongest F1 response and the other from the PSTH with strongest F0 response. Reliability was measured with an optimized grating stimulus as the Fano factor (variance/mean) with a bin size equal to the period of the stimulation. Almost all simple cells (>95%; defined by segregated ON and OFF RF subfields) had stronger F1 than F0 responses and all SINs had stronger F0 than F1 responses (see results). Therefore, the F1 response was used to measure tuning properties for simple cells and the F0 response to measure tuning properties for SINs (if not otherwise stated).

Contrast tuning responses were tested on 8 different contrasts ranging from 1% to 95% (Cano et al., 2006). Because some cells showed decreased responses at high contrast (high contrast suppression, see results), F1 and F0 responses were fitted by a hyperbolic model with (Peirce, 2007) or without (Naka and Rushton, 1966; Albrecht and Hamilton, 1982) high contrast suppression modification.

\[
\text{F0 response without high contrast suppression: } y = R_0 + A \cdot x^n/(C^n + x^n) \\
\text{F0 response with high contrast suppression: } y = R_0 + A \cdot x^n/(C^{sn} + x^{sn})
\]
F1 response without high contrast suppression: \( y = A \times x^n / (C^n + x^n) \)

F1 response with high contrast suppression: \( y = A \times x^n / (C^{kn} + x^{kn}) \)

Where \( A \) is the response amplitude, \( R_0 \) is baseline activity, \( x \) is contrast and \( C \) is the contrast at half maximum response. The parameters \( A \) and \( C \) from the equations above are generally used to describe the response amplitude and the contrast generating the half maximum response (C50), respectively (Albrecht and Hamilton, 1982; Cano et al., 2006). However, the parameters extracted from the fit do not faithfully represent the values of the contrast response function in the model with high contrast suppression, which can span to contrast values larger than 100%. Therefore, the response amplitude \( (R_{con}) \) and the C50 were calculated by limiting the fitting function within a contrast ranging from 0% to 100%. All contrast response functions were fitted with and without high contrast suppression and the \( R^2 \) of fitness normalized by degree of freedom \( (df) \) was measured in both fits (Peirce, 2007):

\[
R_{nor}^2 = 1 - (1 - R^2) / df
\]

The model with higher \( R_{nor}^2 \) was chosen to extract the parameters \( R_{con} \) and C50.

Orientation tuning was measured with drifting gratings differing in orientation/direction by 15 - 30 degrees. Tuning curves were fitted by von Mises distribution functions (Nowak et al., 2008).

\[
y = R_0 + A_1 \exp[k \cos(x - OR_{peak} - 1)] + A_2 \exp[k \cos(x - OR_{peak} + \pi) - 1)]
\]

where \( A_1 \geq A_2 \)
Here the independent variable ‘x’ (the moving direction of the tested drifting grating) is presented in radians. From this model, \( R_0 \) represents the baseline of the curve, \( A_1 \) and \( A_2 \) represent the response amplitude to the preferred direction (\( \text{OR}_{\text{peak}} \)) and to the opposite of preferred direction, respectively. ‘k’ is a width factor. To measure the orientation selectivity and direction selectivity, the circular variance (CirVar, Ringach et al., 2002), orientation selectivity index (OSI) and direction selectivity index (DSI) were calculated as follows:

\[
\text{CirVar} = 1 - \frac{\sum R_j e^{i \theta_j}}{\sum R_i};
\]

\[
\text{OSI} = \frac{\left( R_{\text{pref}} - R_{\text{orth}} \right)}{\left( R_{\text{pref}} + R_{\text{orth}} \right)};
\]

where \( R_{\text{orth}} = \frac{R_{\text{pref}+} + R_{\text{pref}-}}{2} \); 

\[
\text{DSI} = \frac{\left( R_{\text{pref}} - R_{\text{oppo}} \right)}{\left( R_{\text{pref}} + R_{\text{oppo}} \right)};
\]

where \( j \) represents all the directions in the orientation tuning curve; \( R_j \) and \( \theta_j \) represent the cells response (spk/sec) and the angle (in radians) of \( j \)th direction, respectively; \( R_{\text{pref}} \) represents the cell’s response in the preferred direction; \( R_{\text{orth}} \) represents the cell’s mean responses of the two directions orthogonal to the preferred direction; and \( R_{\text{oppo}} \) represents the cell’s response to the opposite of the preferred direction. All three measurements (CirVar, OSI and DSI) have values ranging from 0 to 1, with higher CirVar meaning weaker orientation selectivity; higher OSI meaning stronger orientation selectivity and higher DSI meaning stronger direction selectivity.

Spatial frequency responses were tested from 0.00825 to 1.32 cpd (for some cells from 0.05 to 1.32 cpd). The response tuning data were fitted by a Gaussian model.
\[ y = R_0 + R_{SF} \cdot \exp\left[-\frac{(x - SF_{\text{peak}})^2}{2\sigma^2_{SF}}\right] \]

From the model, the amplitude (\(R_{SF}\)) and the spatial frequency with peak response (\(SF_{\text{peak}}\)) were extracted. Cells with response at lowest tested spatial frequency higher than 50% of peak response were defined as spatially low pass cells, otherwise as spatially band-pass cells. Spatial frequency tuning width for low-pass cells were defined by the range from lowest tested spatial frequency to the spatial frequency with half maximum response on logarithmic scale (with base 10). While tuning width for band pass cells was measured as the range between two spatial frequencies with half maximum response on logarithmic scale (\(SF_{\text{width}}\)) as follows:

\[ SF_{\text{width}} = \log_{10}\left(\frac{(SF_{\text{peak}} + 1.17741 \cdot \sigma_{SF})}{(SF_{\text{peak}} - 1.17741 \cdot \sigma_{SF})}\right) \]

Temporal frequency responses were tested on 6 different temporal frequencies (0.5, 1, 2, 4, 8 and 16 Hz). The temporal frequency tuning curve was fitted by Gaussian model on logarithmic scale (with base 2, modified from Bezudnaya et al., 2006).

\[ y = R_0 + R_{TF} \cdot \exp\left[-\left(\log_2(x) - \log_2(TF_{\text{peak}})\right)^2/2\sigma^2_{TF}\right] \]

From the model, the amplitude (\(R_{TF}\)) and the temporal frequency with peak response (\(TF_{\text{peak}}\)) were extracted. Temporal frequency tuning width was measured as the width at half height on logarithmic scale (\(TF_{\text{width}}\), with base 2).

\[ TF_{\text{width}} = 2.35482 \cdot \sigma_{TF} \]
Goodness of fitting showed the models used here faithfully described the tuning properties of the cells’ responses ($R^2$, contrast tuning: $0.972\pm0.007$, n=65; temporal frequency tuning: $0.925\pm0.010$, n=78; spatial frequency tuning: $0.919\pm0.010$, n=72; orientation tuning: $0.855\pm0.016$, n=82; overall: $0.915\pm0.006$, n=297). Only the fittings with $R^2$ values larger than 0.5 were included in further analysis.

2.3.7.6 Testing LGN input to SINs

For some LGN cells and cortical SINs, cross-correlograms were generated from simultaneously recorded spontaneous spike trains. Functional connectivity between these pairs was accessed by searching for a peak in the correlogram at intervals of 1.1–2.5 ms following the LGN spike. This peak was defined by the presence, within this 1.5 ms window, of two bins out of three successive bins, each showing significantly higher firing rate than baseline activity (measured by activity between –4 ms and +1 ms of the LGN spike time). When a significant peak was detected, onset latency and peak latency were measured as the relative timing of the first and highest bin in the peak, respectively, and an ‘efficacy’ value was calculated based on a brief window (peak ± 0.6 ms) by counting the number of SIN spikes that occurred during this window, subtracting the baseline, and dividing this value by the number of total LGN spikes (Swadlow and Gusev, 2002).

All the p-values provided in result section represent the results of independent sample t-test, if not specified. Data are provided as ‘mean ± standard error’.
2.4 Results

We recorded 109 cells in layer 4 of V1 from 3 female rabbits. 62 of these were simple cells (56.9%), 33 were SINs (30.3%) and 14 could not be placed into either category, and were defined as “other” cells (12.8%, Table 1). These “other” cells were not studied in detail. Receptive field properties are summarized in Table 2.
Table 2.1. Cell numbers of each group

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Number of cells</th>
<th>% of whole population</th>
</tr>
</thead>
<tbody>
<tr>
<td>simple cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sustained</td>
<td>27</td>
<td>24.77%</td>
</tr>
<tr>
<td>transient</td>
<td>19</td>
<td>17.43%</td>
</tr>
<tr>
<td>Sus/Tran unclassified</td>
<td>16</td>
<td>14.68%</td>
</tr>
<tr>
<td>SINs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sustained</td>
<td>8</td>
<td>7.34%</td>
</tr>
<tr>
<td>transient</td>
<td>25</td>
<td>22.94%</td>
</tr>
<tr>
<td>Other</td>
<td>14</td>
<td>12.84%</td>
</tr>
<tr>
<td>All</td>
<td>109</td>
<td>100%</td>
</tr>
</tbody>
</table>
### Table 2.2. Comparisons between Layer 4 simple cells and SINs

<table>
<thead>
<tr>
<th></th>
<th>Layer 4 simple cells</th>
<th>Layer 4 SINs</th>
<th>Significance</th>
<th>Overlap distribution</th>
<th>Bimodality (Hartigan) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike duration (ms)</td>
<td>1.54±0.12</td>
<td>0.59±0.02</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Spont activity (spk/sec)</td>
<td>0.55±0.08</td>
<td>21.96±2.18</td>
<td>***</td>
<td>NO</td>
<td>***</td>
</tr>
<tr>
<td>Latency to flash stimulation (ms)</td>
<td>34.22</td>
<td>28.16</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Evoked F0 (spk/sec)</td>
<td>9.61±0.93</td>
<td>59.76±4.20</td>
<td>***</td>
<td>NO</td>
<td>**</td>
</tr>
<tr>
<td>Evoked F0 w/o spont (spk/sec)</td>
<td>7.51±1.01</td>
<td>31.94±3.76</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Evoked F1 (spk/sec)</td>
<td>14.42±1.51</td>
<td>32.97±3.73</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>RF subfield structure (local similarity index)</td>
<td>segregated/unipolar (0.028±0.007)</td>
<td>overlapped (0.666±0.023)</td>
<td>***</td>
<td>NO</td>
<td>***</td>
</tr>
<tr>
<td>RF subfield width (deg)</td>
<td>3.17±0.13</td>
<td>8.74±0.57</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Spatial summation (F1/F0 ratio)</td>
<td>linear (1.50±0.04)</td>
<td>nonlinear (0.53±0.04)</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Fano factor</td>
<td>1.15±0.10</td>
<td>1.33±0.08</td>
<td>N.S.</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Orientation selectivity index</td>
<td>0.81±0.02</td>
<td>0.17±0.02</td>
<td>***</td>
<td>YES</td>
<td>**</td>
</tr>
<tr>
<td>Circular variance</td>
<td>0.53±0.03</td>
<td>0.94±0.01</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Direction selectivity index</td>
<td>0.69±0.03</td>
<td>0.11±0.02</td>
<td>***</td>
<td>YES</td>
<td>***</td>
</tr>
<tr>
<td>C50 (%)</td>
<td>27.17±2.65</td>
<td>10.83±1.94</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Peak SF (cpd)</td>
<td>0.20±0.02</td>
<td>0.22±0.05</td>
<td>N.S.</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>SF bandwidth (oct)</td>
<td>0.71±0.07</td>
<td>1.20±0.10</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>Peak TF (Hz)</td>
<td>5.58±0.57</td>
<td>6.26±0.98</td>
<td>N.S.</td>
<td>YES</td>
<td>N.S.</td>
</tr>
<tr>
<td>TF bandwidth (oct)</td>
<td>2.87±0.17</td>
<td>4.70±0.43</td>
<td>***</td>
<td>YES</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

***: p<0.001. **: p<0.01. N.S.: p>0.05. 
§: median. ¶: independent t-test. ¢: K-S test. #: C50s are different between sustained and transient cells. Refer to table 3 for results of simple cells and SINs breaking down in to sustained and transient groups.
2.4.1 Cell classification, spike duration and responses to thalamic stimulation

Many extracellular studies rely on spike duration for cell classification. Here, SINs were identified by a discharge of 3 or more spikes to thalamic stimulation at frequencies exceeding 600 Hz (Figure 2.1A). SINs had significantly shorter spike durations (0.590±0.017 ms) than simple cells (1.539±0.126 ms, p<0.001), and only one SIN (3%) had a spike duration longer than 0.8 ms. By contrast, 52 simple cells (84%) had spike durations longer than 0.8 ms. A small number of simple cells did, however, have very short spike durations. Spike waveforms of 3 simple cells and one SIN are shown in Figure 2.2 (A1-D1, inset). The distribution of spike durations of these populations showed some overlap (Figure 2.1B). Moreover, the overall distribution was not significantly bimodal (Hartigan’s test, p=0.116).

SINs also had significant shorter latencies (Figure 2.1C) to synaptic activation via thalamic stimulation (1.794±0.087 ms) than simple cells (3.174±0.124 ms, p<0.001). Whereas all but three SINs (90.9%) had latencies of < 2 ms, 47/54 simple cells (87%) that responded to thalamic stimulation had latencies in excess of 2 ms. Simple cells not only had longer latencies, but also responded to thalamic stimulation with fewer spikes (usually 1 spike) than SINs (by definition, at least three spikes, median value = 4 spikes). Simple cells also showed higher thresholds to thalamic stimulation than SINs (53 simple cells vs. 33 SINs, 6.1±0.09 V vs. 1.8±0.02 V, p<0.001).

By virtue of our criteria for identification, SINs were required to respond to thalamic stimulation with three or more spikes, at minimal inter-spike intervals (ISIs) of < 1.67 ms (600 Hz). Figure 2.1D shows these minimal ISIs for SINs and for cells that did not meet this criterion. The great majority of simple cells responded with only one spike (47 cells) or no spikes (8 cells) to thalamic stimulation (right column.
in Figure 2.1D), and only two simple cells had minimal ISIs of < 1.6 ms. These latter two cells were not classified as SINs because they responded with only two (not three) spikes to thalamic stimulation.

2.4.2 Spatial structure of the RF

RF maps of each sign (ON or OFF) were obtained from all simple cells and SINs by reverse correlation method. Simple cells were of the “S1” variety, with a single ON (n=28), or OFF (n=25) subfield or the “S2” variety, with parallel ON and OFF subregions (n=9). Please note, by applying a threshold as 30% of peak response to remove noise (methods), we may underestimate the population of S2 simple cells due to the possibility of cutting off very weak subfields. For the identified S2 simple cells, the alignment of ON and OFF subfields was in agreement with their preferred orientation (i.e., the preferred orientation was predictable from the position of the subfield maxima, mean deviation was 10.6 degrees, maximum deviation was 30 degrees). By contrast, all SINs possessed overlapped ON and OFF subfields. Figure 2.2 shows examples of the RF maps of one ON S1 simple cell (A1), one OFF S1 simple cell (B1), one S2 simple cell (C1) and one SIN (D1).
Figure 2.2. Receptive field maps, spike waveforms, perievent rasters and PSTHs of three simple cells and a SIN. A1-A3, an example of S1 OFF simple cells. A1, receptive field maps, grid scale: 1 degree, each contour: 12% of peak value. LSIs (local similarity indices) were shown on top of the maps. Inset: spike waveforms, horizontal scale bar: 0.4 ms; vertical scale bar: 0.1 mV. Shadowed areas represent “mean ± standard deviation”. A2, Perievent rasters for two periods. For reasons of clarity, only first 40 iterations were shown. A3, PSTHs. F0 responses, F1 responses, Fano factors and spontaneous activity were listed on the upper right corner. Unit for F0, F1 and spontaneous activity: spk/sec. B1-B3, an example of S1 ON simple cell. C1-C3, an example of S2 simple cell. D1-D3, an example of SIN. Gray dashed line in panel D3 shows the spontaneous activity level for the SIN. The spontaneous activity levels for simple cells are too low to indicate with a dashed line.
In visual cortex, simple/complex classification is often based on the spatial separation of the cell’s ON/OFF subfields, with simple cells having separated ON/OFF subfields and complex cells having overlapped ON/OFF subfields (Hubel and Wiesel, 1962; Hirsch and Martinez, 2006). Here we used the local similarity index (LSI) to measure the overlap of the cell’s ON/OFF subfields. LSI takes value from 0 to 1, with lower value meaning less overlap and more ON-OFF separation and higher value meaning more overlap and less ON-OFF separation (see methods). The LSI distribution of all cells was significantly bimodal (Hartigan’s test, p<0.001, Figure 2.3A,B), with all simple cells having LSI less than 0.2 (0.028±0.007) and all SINs have LSI larger than 0.3 (0.666±0.023). This means all simple cells had single unipolar subfield or separate ON/OFF subfields and all SINs had overlapping ON/OFF subfields. It is not surprising that simple cells had low LSI, as this was our classification criterion. What is notable is the finding that all SINs had overlapping ON/OFF subfields (i.e. complex), considering that our SINs were defined solely on their responses to thalamic electrical stimulation. Simple cells also showed significantly smaller dominant subfield width (3.169±0.131 deg) and dominant subfield area (9.713±0.692 deg^2) than SINs (width: 8.739±0.568 deg, p<0.001; area: 61.738±7.677 deg^2, p<0.001, Figure 2.3C,D). Although, the distributions of dominant subfield width and area were not significantly bimodal (Hartigan’s test, for dominant subfield width p=0.816, for dominant subfield area p=0.776), these measures do enhance the segregation between simple cells and SINs by RF properties (Figure 2.3A).
Figure 2.3. Receptive field subfield structures of simple cells and SINs. A, Scatter plot of local similarity index (LSI) against dominant subfield width from all simple cells and SINs. B, Distribution of LSI. C, Distribution of dominant subfield width. D, Distribution of dominant subfield area.
2.4.3 Spontaneous activity

The spontaneous firing rates of simple cells and SINs were dramatically different, with all simple cells having a spontaneous rate lower than 2spk/sec (mean: 0.551±0.084 spk/sec, n=28) and all SINs having a spontaneous rate higher than 5spk/sec (mean: 21.955±2.182 spk/sec, n=19, p<0.001). Our data further reveal that the spontaneous firing rate distribution of these cells was significantly bimodal on logarithmic scale (Hartigan’s test, p<0.001, Figure 2.4A), with two peaks (each formed by single type of cells) having no overlap at all.

2.4.4 F0 and F1 responses, spatial summation linearity and reliability

After mapping the RF, we measured the cell’s response to its optimal drifting grating and calculated the maximum F0 response (with or without spontaneous firing rate subtracted), maximum F1 response, F1/F0 ratio and Fano factor. Examples of perievent rasters (Figure 2.2, A2-C2 for simple cells and D2 for SIN) and PSTHs (Figure 2.2, A3-C3 for simple cells and D3 for SIN) to optimal drifting gratings of simple cells and a SIN are shown in Figure 2.2. Among these examples, simple cells were strongly modulated at the frequency of the drifting grating while SINs responded with pronounced increase in mean rate superimposed to a weak sinusoidal modulation.

Both maximum F0 responses (Figure 2.4B) and maximum F1 responses (Figure 2.4C) were higher in SINs than in simple cells and these differences were highly significant (F0: 57 simple cells vs. 30 SINs, 9.612±0.926 spk/sec vs. 59.764±4.204 spk/sec, p<0.001; F1: 14.422±1.506 spk/sec vs. 32.965±3.727 spk/sec, p<0.001). Bimodality was seen in maximum F0 distribution (Hartigan’s test: p=0.007, Figure 2.4B) but not in maximum F1 distribution (Hartigan’s test: p=0.701, Figure 2.4C).
The F1/F0 ratio is a common method to test the linearity of spatial summation in the RF and has been frequently used as a criterion to define simple and complex cells (Skottun et al., 1991). Due to the differences in linearity of spatial summation, the F1/F0 ratio should be larger than 1 for simple cells and lower than 1 for complex cells. In this study, we measured the linearity of spatial summation (F1/F0 ratio) in 57 simple cells and 30 SINs. All but a few simple cells (94.74%) had F1/F0 ratios higher than 1 (mean for all simple cells: 1.497±0.036), and all SINs had F1/F0 ratios lower than 1 (mean for all SINs: 0.532±0.039, simple vs. SIN: p<0.001, Figure 2.4D). Thus, SINs showed features of complex cells in terms of spatial summation linearity, as they did in terms of spatial RF structure. However, although two peaks are evident in the overall histogram shown in Figure 2.4D, Hartigan’s test for bimodality did not reach statistical significance (p=0.247). Moreover, the distribution of F1/F0 lost any appearance of bimodality when we subtracted the spontaneous activity (Hartigan’s test, p=0.975, see below, Figure 2.4D, inset, below)

The reliability of visual responses is an important factor in information transmission and is usually quantified as a variance/mean ratio called Fano factor (e.g. Kara et al., 2000; Alitto et al., 2011). Cells with highly reliable visual responses and limited variability from trial to trial (low variance) should have a low Fano factor while cells that are more variable should have higher Fano factors. We measured the Fano factors of 30 simple cells and 18 SINs under drifting grating stimulation, using a full grating cycle as the time bin. In these cells, we collected a large number of spikes (simple: 6140±788 spikes; SINs: 66322±6499 spikes) to make the measurements of Fano factor as accurate as possible. We found no significant difference between simple cells and SINs in the average Fano Factor (simple cells vs. SINs, 1.153±0.096 vs. 1.330±0.077, p=0.206) and the two groups combined showed no hint of bimodality (Hartigan’s test, p=0.790, Figure 2.4E).
Figure 2.4. Spontaneous firing rates and responses to optimal drifting gratings of simple cells and SINs. A, Distribution of spontaneous firing rates. B, Distribution of F0 responses to optimal drifting gratings. Inset: SINs had significantly higher F0 responses than simple cells with or without spontaneous activity removed. ***, p<0.001. C, Distribution of F0 responses to optimal drifting gratings. D, Distribution of F1/F0 ratios. Inset: distribution of F1/F0 ratios after removing spontaneous activity. E. Distribution of Fano factors.
2.4.5 Orientation and direction selectivity

Measurements of orientation/direction tuning curves in 55 simple cells and 27 SINs demonstrate that simple cells are much better tuned to orientation/direction than SINs. Figure 2.5 shows orientation tuning curves from 3 simple cells (left panels) and 3 SINs (right panels). The simple cells were sharply tuned and two of the three were responding to only one direction (Figure 2.5A-B) while the other one responded to both directions (Figure 2.5C). All three SINs were unselective to orientation/direction (Figure 2.5D-F). The distribution of OSI was significantly bimodal (Figure 2.6A, Hartigan’s test, p=0.002) with simple cells forming a peak around high OSI (0.803±0.024) and SINs forming another peak around low OSI (0.175±0.015). The distribution of DSI was also significantly bimodal (Hartigan’s test, p<0.001, Figure 2.6B). However, although simple cells had significantly higher DSI than SINs (0.683±0.034 vs. 0.107±0.016, p<0.001), some simple cells were orientation selective but not direction selective (Figure 2.6C). In summary, all simple cells were orientation selective and most were direction selective, while no SINs showed either direction selectivity or orientation selectivity. Since OSI and DSI are calculated from only 2-3 data points from tuning curves, they may not describe completely the orientation/direction tuning of layer 4 cells. To document more completely these properties we also measured circular variance (Levick and Thibos, 1982; Ringach et al., 1997; Ringach et al., 2002), which uses all data points in the entire orientation/direction cycle. Circular variance takes values from 0 to 1, with lower values meaning tighter orientation tuning and higher values meaning broader orientation tuning. Consistent with OSIs, simple cells had significantly lower circular variance than SINs (simple vs. SIN, CirVar, 0.526±0.030 vs. 0.937±0.010, p<0.001, Figure 2.6D). Notably, no SIN had a circular variance lower than 0.8, emphasizing a pronounced lack of orientation selectivity. As expected, the circular variance was highly correlated with the orientation selectivity in both SINs and simple cells (Figure 2.6D inset).
Figure 2.5. Receptive field structures and orientation tunings of three simple cells (A, B and C) and three SINs (D, E and F). For each cell, left part shows the PSTHs to drifting gratings in four example directions (designated by the arrow heads located at upper left corner of the box). Each PSTH plots 2 periods of the stimulation. Vertical scale bar: 10 spk/sec. Horizontal scale bar: 0.25 sec. Right part shows the orientation tuning curves (fitted by von Mises distribution function, F1 for simple cells and F0 for SINs). OSI: orientation selectivity index. DSI: direction selectivity index. CirVar: circular variance. Upper right part shows the receptive subfields of the cell, each contour: 12% of peak value. Note that, panel A represents the same cell shown in Figure. 2.2C and panel B represents the same cell shown in Figure. 2.2B. The dashed lines in PSTHs and tuning curves of SINs indicate their spontaneous activity levels. The spontaneous activity levels for simple cells are too low to indicate with a dashed line.
The preferred direction and orientation distribution of simple cells (Figure 2.6E-F) show that most our simple cells prefer approximately horizontal orientations and up/down directions with a bias < 30 degrees which is consistent with previous studies (Bousfield, 1977; Murphy and Berman, 1979; Swadlow and Weyand, 1987). S2 simple cells showed significantly larger OSIs than S1 simple cells (S2 vs. S1: 0.9±0.026 n=8 vs. 0.797±0.026 n=46, p=0.009), suggesting that S2 simple cells respond less to the orthogonal orientation than S1 simple cells. The circular variances of S2 cells also suggested more selectivity than those of S1 cells, but this difference did not reach statistical significance (S2 vs. S1: 0.410±0.067 vs. 0.547±0.033, respectively, p=0.108). There were no significant differences in the DSIs between S2 and S1 simple cells (S2 vs. S1, DSI, 0.685±0.085 vs. 0.694±0.036, p=0.923).
Figure 2.6. Orientation and direction tuning properties of simple cells and SINs. A, Distribution of OSIs (orientation selectivity indices). B, Distribution of DSIs (direction selectivity indices). C, Scatter plot of OSIs against DSIs. D, Distribution of circulation variances (CirVar). Inset: OSI and CirVar are significantly correlated. E, Distribution of preferred directions of simple cells. Length of the each arrow represents frequency. F, Distribution of preferred orientations of simple cells. Orientation of each bar represents the orientation of stimulating drifting grating. Length of the each bar represents frequency. SUP: superior. POS: posterior.
As noted, the above results concerning orientation/direction tuning were derived from F1 responses of simple cells and F0 responses from SINs (see methods). This is because F1 and F0 responses represent the dominant responses for linear simple cells and nonlinear SINs, respectively (see results of F1/F0 ratio), and it is a common way to analyze simple and complex cells in visual cortex (e.g. Hawken et al., 1996; O'Keefe et al., 1998; Contreras and Palmer, 2003; Cardin et al., 2007). Interestingly, although the F0 responses of all SINs are unselective to orientation and direction as described above, the F1 responses of some SINs show considerable orientation and/or direction selectivity (Figure 2.7), despite the fact that they are weaker than F0 responses in all directions. Figure 2.7A shows orientation tuning curves from both F0 and F1 responses of three SINs. The F0 responses of these three SINs did not present strong orientation/direction selectivity. However, their F1 responses were strikingly diverse and showed no orientation selectivity (Figure 2.7A left), strong orientation selectivity (Figure 2.7A middle) or strong direction selectivity (Figure 2.7A right). So we compared the orientation tuning properties between F0 and F1 response for both simple cells and SINs (Figure 2.7B-E). For simple cells, all three measurements (OSI, DSI and CirVar) calculated from F1 responses strongly correlated with those from F0 responses, and all data points distributed tightly around the diagonal line (correlation coefficient, CirVar: 0.970 Figure 2.7B, OSI:0.926 Figure 2.7C, DSI:0.944 Figure 2.7D), which is consistent with previous measurements in macaque V1 (Ringach et al., 2002). By contrast, the results from SINs deviated strongly from the diagonal line because their F1 responses can be more orientation or direction selective than F0 responses (Figure 2.7B-D). Population statistics also indicated that the F1 responses of SINs were more orientation/direction selective than the F0 responses (paired t-test, F1 vs. F0, OSI: 0.428±0.040 vs. 0.181±0.017, p<0.001; DSI: 0.287±0.047 vs. 0.112±0.018, p=0.002; CirVar: 0.833±0.026 vs. 0.935±0.011, p=0.001, Figure 2.7E). Note, however, that although the F1 responses of some SINs showed orientation/direction selectivity to some extent, the selectivity was not as pronounced as in simple cells (simple F1 vs. SIN F1, OSI: 0.817±0.023 vs. 0.428±0.040, p=0.001; DSI: 0.695±0.034 vs. 0.287±0.047,
p<0.001; CirVar: 0.513±0.031 vs. 0.833±0.026, p<0.001, Figure 2.7E). Moreover, the F1 responses of
SINs were clearly smaller than the F0 responses even at their preferred orientations (Figure 2.7A).
Figure 2.7. F1 responses of SINs are more orientation/direction selective than their F0 responses. A, orientation tuning curves of F0 and F1 responses from three SINs. Gray dashed lines indicate spontaneous activity level. Note that all three SINs show F0 responses with poor orientation/direction selectivity, however, their F1 response could be unselective (left), orientation selective (middle) or direction selective (right). B-D, scatter plot of orientation tuning parameters of F1 responses against those from F0 responses for both simple cells and SINs. (B: CirVar; C: OSI; D: DSI). E, Statistical comparisons for these parameters between F0 and F1 responses and between simple cells and SINs. OSI: orientation selectivity index. DSI: direction selectivity index. F1 vs. F0: paired t-test. Simple vs. SIN: independent t-test. ***: p<0.001. **: p<0.01. N.S.: not significant, p>0.05.
2.4.6 Sustained/transient classification and contrast tuning

Simple cells and SINs can generate either sustained or transient responses when presented with optimal stationary stimulus. We studied this response property by quantifying a “sustained index (SI)”, which was defined as the ratio between the cell’s maintained firing rate (measured 0.5 – 1.0 sec after stimulus presentation) and the baseline firing rate (measured during the one sec or half sec prior to stimulus onset). Cells with SIs lower than 2 were classified as transient cells. Cells with SIs greater than 2 and an absolute maintained firing rate higher than 1 spk/sec were classified as sustained cells. The remaining cells (with SIs higher than 2 but absolute maintained firing rate lower than 1 spk/sec) remained unclassified with respect to the sustained/transient distinction. Based on these criteria, we classified 27 simple cells as ‘sustained’ (SI: 14.100±3.689), 19 as ‘transient’ (SI: 0.651±0.126) and 16 as ‘sustained/transient unclassified’. Of the 33 SINs studied, 8 were ‘sustained’ (SI: 2.541±0.363) and 25 were ‘transient’ (SI: 1.040±0.082). Figure 2.8 shows PSTHs to optimal stationary stimuli from one sustained simple cell (Figure 2.8A), one transient simple cell (Figure 2.8B), one sustained SIN (Figure 2.8C), one transient SIN (Figure 2.8D), as well as the population averages of these four groups (Figure 2.8E,F). Figure 2.8G,H show the distribution of SIs for simple cells and SINs, respectively, on logarithmic scale. Note that simple cells are significantly more likely to be sustained than SINs ($\chi^2$ test, p=0.006).
Figure 2.8. Sustained/transient responses of simple cells and SINs. A-D, response PSTHs to stationary flashing visual stimuli of a sustained simple cell (A), a transient simple cell (B), a sustained SIN (C) and a transient SIN (D). SIs (sustained indices, ratio between sustained component to baseline, see Methods) are labeled for each cell. E, population average for sustained and transient simple cells. F, population average for sustained and transient SINs. Shaded area represents mean ± SEM for each bin. G, sustained index distribution of simple cells. H, sustained index distribution of SINs. Dashed line marks the cutoff criterion for classifying sustained and transient cells. Vertical scale bar in A, B and E: 10 spk/sec; in C, D and F: 50 spk/sec.
Sustained and transient cells had similar average latencies to electrical stimulation (sustained vs. transient, simple cells: 3.139±0.198 ms vs. 3.189±0.193 ms, p=0.861; SINs: 1.638±0.075 ms vs. 1.844±0.112 ms, p=0.318), spike durations (simple cells: 1.632±0.213 ms vs. 1.302±0.141 ms, p=0.253; SINs: 0.603±0.036 ms vs. 0.586±0.019 ms, p=0.660), LSI (simple cells: 0.017±0.008 vs. 0.044±0.015, p=0.130; SINs: 0.691±0.061 vs. 0.658±0.024, p=0.545), spontaneous activity (simple cells: 0.444±0.055 spk/sec vs. 0.851±0.288 spk/sec, p=0.211; SINs: 17.799±4.876 spk/sec vs. 23.064±2.445 spk/sec, p=0.339), F1/F0 ratio (simple cells: 1.542±0.041 vs. 1.441±0.077, p=0.213; SINs: 0.499±0.107 vs. 0.543±0.041, p=0.641), Fano Factor (simple cells: 1.155±0.129 vs. 1.254±0.219, p=0.679; SINs: 1.161±0.139 vs. 1.414±0.086, p=0.125), OSI (simple cells: 0.805±0.037 vs. 0.845±0.042, p=0.481; SINs: 0.197±0.029 vs. 0.165±0.018, p=0.356), DSI (simple cells: 0.709±0.046 vs. 0.742±0.071, p=0.684; SINs: 0.117±0.034 vs. 0.104±0.019, p=0.720) and circular variance (simple cells: 0.524±0.053 vs. 0.486±0.051, p=0.624; SINs: 0.929±0.022 vs. 0.941±0.011, p=0.611). However, they showed striking differences in their contrast response functions (especially for simple cells), as illustrated in Figure 2.9A-D for the four example cells from Figure 2.8A-D, respectively, and in Figure 2.9 E-F for the normalized population average (sustained and transient simple cells, Figure 2.9E; sustained and transient SINs, Figure 2.9F). The sustained simple cells were less sensitive to low contrasts (had significantly higher C50s) than the transient simple cells (C50, sustained simple cells: 33.038±3.942%, n=21; transient simple cells: 11.930±2.170%, n=10, p<0.001, Figure 2.9G). sustained SINs also had higher C50s (16.971±5.219%, n=7) than transient SINs (8.444±1.556%, n=18), however, this difference was not significant (p=0.161, Figure 2.9G). The inset in Figure 2.9E shows the population average response for sustained and transient LGN neurons in alert rabbits (from Cano et al., 2006). Note that the differences in the contrast response functions of layer 4 sustained and transient simple cells are very similar to those of the sustained and transient LGN neurons, respectively. However, unlike LGN transient cells, transient simple cells showed a certain level of high contrast suppression: they responded to intermediate contrasts better than to high contrasts (Figure 2.9E). This phenomenon was very prominent in some simple cells. For the example cell illustrated in Figure
2.9B, the response at intermediate contrasts was almost twice as strong as at the highest contrast. We calculated the percentage of the cells which showed high contrast suppression in each of the four groups (sustained simple cells, transient simple cells, sustained SINs and transient SINs). Transient cells were more likely to show high contrast suppression than sustained cells, especially if they were simple cells. High contrast suppression could be demonstrated in 50% (5 out of 10) of transient simple cells, 33.33% (6 out of 18) of transient SINs, 28.57% (6 out of 21) of sustained simple cells and 28.57% (2 out of 7) of sustained SINs (Figure 2.9H). Whether the cell showed high contrast suppression or not was decided by the best fit of the cell’s contrast tuning curve (fit with or without high contrast suppression). We used $R_{nor}^2$ as the criterion for goodness of fitting to control for the additional parameter used in the fit with high contrast suppression (see methods). By using this justification, we controlled the effect of noisy fluctuation of contrast response functions and limited the possibility of false positive identification of high contrast suppression. In summary, our results demonstrate that transient cells are very sensitive to low contrasts and are more likely to show high contrast suppression. By contrary, sustained cells are less sensitive to low contrasts and more likely to increase their responses monotonically with contrast. The differences in the contrast response function between sustained and transient cells are more prominent in simple cells than in SINs.
Figure 2.9. Contrast response properties of sustained and transient cells for both simple cells and SINs. A-D, contrast response functions of the same cells plotted in Figure 2.8A-D, respectively. Raw data were fitted by hyperbolic function (see methods). C50s are labeled for each cell. E, normalized population average contrast response function of sustained and transient simple cells. Inset: population average contrast response functions of sustained and transient LGN cells (modified from Cano et al., 2006 with permission). F, normalized population average contrast response functions of sustained and transient SINs. G, comparison of C50s from these four cell groups (sustained simple cells, transient simple cell, sustained SIN and transient SIN). Sustained vs. transient: independent t-test. ***: p<0.001. N.S.: no significance, p>0.05. H, percentage of cells showing high contrast suppression within each of these four cell groups.
2.4.7 Latency to flash stimulation

Simple cells showed significantly longer latencies to flash visual stimuli than SINs (median for 57 simple cells: 34.22 ms; median for 33 SINs: 28.16 ms, p<0.001, K-S test) and sustained cells (simple cells and SINs, combined) showed significantly longer latencies than transient cells (median for sustained cells: 34.32 ms, n=35; median for transient cells: 28.68 ms, n=44, p=0.012, K-S test, Figure 2.10). Within each cell type group, sustained simple cells showed significantly longer latencies than transient simple cells (median for 27 sustained simple cells: 37.49 ms; median for 19 transient simple cells: 29.67 ms, p=0.017, K-S test, Figure 2.10 inset, left), while this difference was not significant in SINs (median for 8 sustained SINs: 28.58 ms; median for 25 transient SINs: 27.10 ms, p=0.191, K-S test, Figure 2.10 inset, right).
Figure 2.10. Distribution of latencies to flash visual stimulation of simple cells and SINs. Inset: distribution broken down into sustained/transient groups for simple cells and SINs.
2.4.8 Spatial and temporal frequency tuning

We did not find any significant differences in spatial and temporal tuning properties between sustained and transient simple cells (sustained vs. transient, peak spatial frequency: $0.228\pm0.039$ cpd vs. $0.176\pm0.024$ cpd, $p=0.394$; spatial bandwidth: $0.670\pm0.080$ oct vs. $0.598\pm0.107$ oct, $p=0.607$; peak temporal frequency: $5.979\pm0.875$ Hz vs. $6.644\pm1.156$ Hz, $p=0.644$; temporal bandwidth: $2.836\pm0.215$ oct vs. $3.091\pm0.392$ oct, $p=0.540$), or between sustained and transient SINs (sustained vs. transient, peak spatial frequency: $0.128\pm0.036$ cpd vs. $0.241\pm0.061$ cpd, $p=0.126$; spatial bandwidth: $1.180\pm0.232$ oct vs. $1.201\pm0.119$ oct, $p=0.935$; peak temporal frequency: $5.171\pm2.006$ Hz vs. $6.618\pm1.145$ Hz, $p=0.534$; temporal bandwidth: $5.503\pm0.764$ oct vs. $4.430\pm0.513$ oct, $p=0.290$). Therefore, in the following analyses we grouped sustained and transient cells together and only looked for simple/SIN differences. The spatial frequency tuning was measured in 44 simple cells and 28 SINs. Each cell class showed considerably diversity in both the peak spatial frequency and the bandwidth, as illustrated in Figure 2.11A left panels for three simple cells, one of which was low-pass (upper panel) and the other two were band-pass (middle and lower panels). Figure 2.11A right panels show spatial frequency tuning curves from three SINs, within which one was band-pass (upper panel) and the other two were low-pass (middle and lower panels). The peak spatial frequency did not differ between simple cells ($0.198\pm0.024$ cpd) and SINs ($0.217\pm0.049$ cpd, $p=0.730$, Figure 2.11B). However, SINs showed a significantly wider spatial frequency tuning (SF_width: $1.197\pm0.104$ oct) than simple cells (SF_width: $0.711\pm0.066$ oct, $p=0.001$, Figure 2.11C). Within the 44 simple cells studied, 11 (25%) were classified as low-pass and 33 (75%) as band-pass. Within the 28 SINs, 19 (67.86%) were classified as low-pass and 9 (32.14%) as band-pass. Therefore, the SINs were statistically more likely to be low-pass than simple cells ($\chi^2$ test, $\chi^2=12.93$, $p<0.001$). Interestingly, S2 simple cells showed significantly narrower spatial frequency tuning (S2 vs. S1: $0.433\pm0.057$ oct n=4 vs. $0.739\pm0.071$ oct n=40, $p=0.004$) than S1 simple cells and all S2 simple cells tested for spatial frequency tuning (n=4) were band-pass. S2 simple cells also showed higher spatial
frequency peaks than S1 simple cells, but this difference was not significant (S2 vs. S1: 0.323±0.066 cpd vs. 0.185±0.025 cpd, p=0.108).

The temporal frequency tuning was measured in 50 simple cells and 28 SINs, as illustrated in Figure 2.11D for three simple cells (left panels) and three SINs (right panels). There was no significant difference in peak temporal frequency between simple cells (5.579±0.566 Hz) and SINs (6.257±0.983 Hz, p=0.626, Figure 2.11E). However, SINs showed significantly wider temporal frequency tuning width (4.698±0.432 oct) than simple cells (2.873±0.171 oct, p=0.004, Figure 2.11F). No significant differences in temporal frequency tuning were found between S1 and S2 simple cells.

Thus, although simple cells and SINs did not differ on preferred spatial and temporal frequencies, SINs responded to a broader range of spatial and temporal frequencies than simple cells.

Drifting gratings for SINs were optimized based on their F0 responses because the F1 responses were usually small and noisy. However, some SINs had robust F1 responses that were well fitted to our temporal and/or spatial frequency models (goodness of fitting, $R^2>0.8$). In these SINs, we measured the tuning parameters for both F1 and F0 responses and, as we found for orientation selectivity (Figure 2.7), the widths of their spatial and temporal frequency tuning were significantly smaller when measured with F1 responses than with F0 responses (paired t-test, F1 vs. F0, SF width: 1.160±0.078 oct vs. 1.420±0.098 oct, p=0.029, n=19; TF width: 2.868±0.336 oct vs. 3.711±0.288 oct, p=0.049, n=21). No significant differences were seen in spatial and temporal frequency peaks between F1 and F0 responses of SINs (paired t-test, F1 vs. F0, SF peak: 0.055±0.011 cpd vs. 0.143±0.042 cpd, p=0.071, n=19; TF peak: 6.776±1.112 Hz vs. 6.967±1.109 Hz, n=21, p=0.866). These results are consistent with the F1 and F0
differences that we report for orientation tuning and demonstrate that the F0 responses of SINs are less selective than their F1 responses.
Figure 2.11. Spatial and temporal frequency tuning properties of simple cells and SINs. A, spatial frequency tunings of three simple cells and three SINs. Raw data were fitted by Gaussian function. Peak spatial frequency, tuning bandwidth and pass mode (low-pass/band-pass) are labeled for each cell. B-C, comparison of peak spatial frequency and spatial frequency tuning bandwidth between simple cells and SINs, respectively. D, temporal frequency tunings of the same three simple cells and three SINs. Raw data were fitted by Gaussian function on logarithmic scale. Peak temporal frequency and temporal frequency tuning bandwidth are labeled for each cell. E-F, comparison of peak temporal frequency and temporal frequency tuning bandwidth between simple cells and SINs, respectively. Dashed lines indicate spontaneous activity levels for SINs. Simple vs. SIN: independent t-test. N.S.: no significance, p>0.05. ***: p<0.001.
2.4.9 Simple cells with brief spikes have properties like other simple cells, not like SINs

As mentioned above, some of simple cells in this study had short duration spikes (Figure 2.1B) that were indistinguishable from those of most SINs. It is interesting to ask if such cells behaved, in other respects, like simple cells, or like the SINs of our study. To answer this, we examined the response properties of all simple cells with spike durations of $< 0.7\text{ms}$ ($n=8$). We found that these 8 simple cells responded to electrical stimulation of the thalamus in a manner characteristic of other simple cells, not of SINs. Thus, none had latencies (Figure 2.1B) of $< 2\text{ ms}$. Three of these cells did not respond to thalamic stimulation, and the remaining 5 had latencies of $2.0-4.0\text{ ms}$ (mean $= 3.1\text{ ms}$). In addition, only one of these 8 cells responded to thalamic stimulation with more than a single spike (this cell responded with two spikes, with a minimal inter-spike interval of $2.0\text{ ms}$). Thus, these 8 simple cells are not at all similar to SINs in their responses to thalamic stimulation. Moreover we found that the eight simple cells with short-duration spikes were also very different from SINs (and very similar to the overall population of simple cells) in their spontaneous firing rates (ranges: $0.15-0.57\text{ spk/sec}$), in their LSIs ( $0-0.21$), in their F1/F0 ratios ($1.28-1.83$) and in their OSIs (0.52-0.97) and DSIs (0.46 to 1.0).

2.4.10 SINs receive monosynaptic input from LGN neurons

By definition, all SINs in the above experiments responded to thalamic electrical stimulation with at least three spikes, and most did so at short latencies (Figure 2.1C). Although this is highly suggestive of monosynaptic input to SINs from LGN axons, this is not necessarily the case, as such synaptic activation could be mediated via the recurrent collateral of antidromically activated corticothalamic axons. To investigate whether SINs do, indeed, receive monosynaptic input from LGN axon terminals we performed cross-correlation analysis for five additional SINs that each were recorded simultaneously with at least one LGN neuron that was in precise retinotopic alignment (e.g. Reid and Alonso, 1995; Alonso et al., 2001; Swadlow and Gusev, 2001, 2002). We found that each of the five SINs did, indeed, show clear
evidence of monosynaptic input from at least one LGN cell, showing a sharp, brief increase in SIN spike probability at a short latency (onset latency: 1.3-1.8 ms; peak latency: 1.5-2.1 ms) following the thalamic spike (Figure 2.12). Four additional LGN neurons were retinotopically aligned with at least one of these SINs but did not make a functional connection, and one LGN cell made a functional impact on each of the two SINs that it was aligned with (notably, these two SINs had dominant ON responses, but the LGN neuron was OFF center, Figure 2.12C,D). However, this dataset is too small to draw conclusions about the “rules” for connectivity (Alonso and Martinez, 1998; Alonso et al., 2001; Swadlow and Gusev, 2002) between LGN cells and SINs. What is clear from these data, is that the SINs of layer 4 do, indeed, receive monosynaptic input from the LGN.
Figure 2.12. Layer 4 SINs receive monosynaptic thalamocortical inputs. A-E, Crosscorrelograms of five simultaneously recorded cell pairs between retinotopically aligned LGN cells and layer 4 SINs. The x-axis represents the relative timing to LGN spikes. Efficacy of each connection is shown for each crosscorrelogram. Insets: the RF maps of LGN cells and SINs. Red/blue: ON/OFF subfield of SINs, respectively. ON or OFF dominancy was labeled for each SIN. White/black patches: RF of ON/OFF centered LGN cells, respectively. Note that in C-D, a single LGN OFF-center cell contracted two layer 4 SINs, each with an ON-dominated receptive fields.
2.4.11 The effect of spontaneous activity on SIN response properties

In the current study, we found that SINs maintained very high spontaneous activity (Fig. 2.4A) in the absence of visual stimulation. This could imply that the simple/SIN differences in response properties may be solely due to their very different spontaneous rates. Therefore, it is important to compare the RF properties between simple cells and SINs after removing their spontaneous activity. These comparisons were done on the subset of our cell populations for which we had good measures of spontaneous activity and the main conclusions of this analysis are summarized below.

1. The maximum F0 responses from SINs were still significantly higher than those from simple cells after subtraction of spontaneous activity (maxF0-spont: 26 simple cells vs. 15 SINs, 7.513±1.013 spk/sec vs. 31.941±3.760 spk/sec, p<0.001, Figure 2.4B inset).

2. The F1/F0 ratios of simple cells and SINs measured without spontaneous activity (calculated through rectifying PSTHs by spontaneous firing rate, Chen et al., 2009) were significantly different (F1/F0 rectified, 36 simple cells vs. 15 SINs, 1.632±0.046 vs. 0.863±0.140, p<0.001), but the distribution did not appear bimodal (Hartigan’s test, p=0.975, Figure 2.4D inset). After removing spontaneous activity, 96% (25 out of 26) of simple cells had F1/F0 ratios larger than 1 while only 46% (7 out of 15) of SINs had F1/F0 ratios higher than 1. This difference between simple cells and SINs, while highly significant, is not as prominent as that seen when spontaneous activity is included, indicating that the spontaneous activity does affect the F1/F0 calculation for SINs.

3. Subtracting spontaneous activity from F0 did not affect simple/SIN difference on orientation tuning (F0-spontaneous activity, 28 simple cells vs. 15 SINs, OSI: 0.872±0.037 vs. 0.406±0.070, p<0.001; DSI:
0.768±0.052 vs. 0.159±0.032, p<0.001; CirVar: 0.413±0.053 vs. 0.786±0.062, p<0.001), and bimodal distributions were still found in DSI and circular variance (Hartigan’s test, OSI: p=0.311; DSI: p=0.003; CirVar: p=0.043). However, the orientation selectivity differences between F1 and F0 responses of SINs (Figure 2.7) were reduced by subtracting spontaneous activity from F0 responses (paired t-test, 14 SINs, F1 vs. F0-spont, OSI: 0.470±0.055 vs. 0.388 vs. 0.072, p=0.270; DSI: 0.307±0.059 vs. 0.167±0.034, p<0.001; CirVar: 0.809±0.039 vs. 0.794±0.066, p=0.836).

Finally, the measurements of C50 from the contrast response function, temporal/spatial frequency peak and temporal/spatial frequency tuning width were independent from baseline measurements (Methods). Therefore, the subtraction of spontaneous activity does not affect the values of these parameters. In summary, these results indicate that removing the spontaneous activity from F0 responses affect the calculation of some RF properties for SINs, however, the main differences between SINs and simple cells that we report can still be demonstrated.
2.5 Discussion

2.5.1 Identification of layer 4 SINs and simple cells

SINs were identified by high-frequency (>600Hz) discharge of 3+ spikes following thalamic stimulation, and simple cells were identified solely by their RF properties, requiring spatially discrete ON and/or OFF subregions. Notably, no neuron met both criteria. All SINs had overlapping ON/ OFF subfields, and most simple cells responded with only one spike to thalamic stimulation. Moreover, SINs had much shorter latencies and minimal inter-spike intervals to thalamic stimulation than simple cells (Figure 2.1CD), and threshold intensities were much lower (Results). Such responsiveness of SINs to thalamic (and visual, Figure 2.4) stimulation, probably reflect a combination of factors, including membrane properties (Azouz et al., 1997; Cardin et al., 2007) large unitary thalamocortical EPSPs (Gibson et al., 1999; Gabernet et al., 2005; Cruikshank et al., 2007; Hull et al., 2009; Cruikshank et al., 2010; Bagnall et al., 2011), and extensive thalamocortical convergence onto SINs (Swadlow and Gusev, 2002; below).

Although not used as an identification criterion, SINs had shorter-duration spikes than simple cells. However, there was overlap in these distributions (Figure 2.1B). We have emphasized the need for criteria other than spike duration for extracellular identification of fast spike GABAergic interneurons (e.g. Swadlow, 2003). Notably, some spiny neurons, including identified projection neurons of sensory (Dykes et al., 1988; Swadlow, 1989, 1990) and motor (Takahashi, 1965) cortices, and “chattering” cells (Gray and McCormick, 1996) have short-duration spikes. Because of this and because the few simple cells with short-duration spikes were, in all other ways, similar to other simple cells, not SINs, we think it very unlikely that they were fast spike GABAergic interneurons. However, some simple cells could have been GABAergic interneurons of other classes (e.g., SOM+ interneurons), which have longer-duration
spikes and receptive fields and other properties distinct from those of fast spike parvalbumin-positive interneurons (e.g. Gibson et al., 1999; Ma et al., 2010; Adesnik et al., 2012).

2.5.2 Response properties of layer 4 simple cells and SINs

Although simple cells were required to have spatially separate ON and/or OFF subfields, there were no receptive field requirements for SINs. Nevertheless, ON/OFF subfield separation (LSI) of simple cells and SINs was strongly bimodal and subfield sizes (width, area) were much smaller in simple cells than SINs (Figure 2.3B-D).

SINs had much higher spontaneous firing rates than simple cells and the distribution was strongly bimodal (Figure 2.4A). The high firing rates of SINs is consistent with results in awake mouse (Niell and Stryker, 2010), and results in other layers of awake rabbit V1 (Swadlow, 1988), S1 (Swadlow, 1989, 1990), S2 (Swadlow, 1991), and motor cortex (Swadlow, 1994), where SINs have the highest spontaneous firing rate. SINs also had higher F0 and F1 responses than simple cells (Figure 2.4B-C), but responded with similar reliability (Figure 2.4E).

The F1/F0 ratio is often used to define simple vs. complex V1 neurons, with ratios of >1 used to classify “linear” simple cells, and those <1 to classify “non-linear” complex cells (Skottun et al., 1991). We found that F1/F0 ratios were <1 for all SINs and >1 for nearly all simple cells (54/57, Figure 2.4D). However, when spontaneous firing rates were subtracted from F0 responses (Figure 2.4D, inset), the distinction between these populations was less categorical, although still highly significant.
Simple cells displayed considerable orientation selectivity (and often directional selectivity) in their F0 and F1 responses (Figure 2.6, 2.7). By contrast, SINs were broadly tuned to orientation/direction in their F0 responses (Figure 2.6). Interestingly, the relatively small F1 responses of SINs were sometimes more orientation/direction selective than the much larger F0 responses (Figure 2.7). Nevertheless, across multiple comparisons, SINs were far less selective for orientation/direction than simple cells.

V1 simple cells similar to those reported here are found in rats (Girman et al., 1999), mice (Niell and Stryker, 2008; Liu et al., 2009), squirrels (Heimel et al., 2005; Van Hooser et al., 2005) and in other layers of rabbit V1 (Swadlow and Weyand, 1987; Swadlow, 1988). Notably, orientation selectivity of V1 simple cells can be very sharp, despite the lack of orientation columns (Ohki and Reid, 2007). Similarly, high spontaneous firing rates, low (nonlinear) F1/F0 ratios, spatially overlapping ON/OFF RF subfields and broad orientation/direction tuning occur in putative fast spike interneurons in layer 4 of anesthetized mice (Niell and Stryker, 2008; Liu et al., 2009), and broad orientation tuning is seen in parvalbumin-positive neurons in layer 2-3 of mouse (Kerlin et al., 2010; but see Runyan et al., 2010) and in SINs of superficial and deep layers of awake rabbit (Swadlow and Weyand, 1987; Swadlow, 1988). In cat V1, however, some fast-spiking GABAergic interneurons have sharp orientation tuning, display spatially separate ON/OFF subfields or F1/F0 ratios indicative of simple cells (Azouz et al., 1997; Hirsch et al., 2003; Cardin et al., 2007; Nowak et al., 2008). Thus, differences in the complexity of visual processing seen in different mammalian lines may be reflected by corresponding differences in the diversity of interneuron response properties.

2.5.3 The sustained/transient distinction in layer 4 simple cells and SINs

66
LGN concentric neurons can be classified as sustained or transient, based on response to maintained stimulation of the RF center (Cleland et al., 1971). In LGN of awake rabbits, the distribution of response duration to sustained stimulation is bimodal (Swadlow and Weyand, 1985; Bezdudnaya et al., 2006; Stoelzel et al., 2008). Notably, transient cells are much more sensitive to low contrast than sustained cells (Cano et al., 2006), similar to differences between transient magnocellular and sustained parvocellular LGN cells in new world primates (Usrey and Reid, 2000). Here, although many simple cells and SINs could be clearly classified as sustained or transient, some were ambiguous and the distinction was not as clear as in LGN. Nevertheless, transient simple cells were much more sensitive to low contrasts than sustained simple cells, and suppressed their responses more strongly at high contrasts (Table 3). A similar pattern was seen in rabbit LGN transient cells, but high contrast suppression seems to originate in V1 (Figure 2.9E, inset).
Table 2.3. Comparison of contrast responses between sustained and transient groups for both layer 4 simple cells and SINs

<table>
<thead>
<tr>
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<th>Layer 4 simple cells</th>
<th>Layer 4 SINs</th>
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<tr>
<td></td>
<td>sustained</td>
<td>transient</td>
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<tr>
<td>C50 (%)</td>
<td>33.04±3.94</td>
<td>11.93±2.17***</td>
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<tr>
<td>% of cells with high contrast suppression</td>
<td>28.6%</td>
<td>50%</td>
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</tbody>
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***: p<0.001; *: p<0.5; N.S.: p>0.05 (independent t-test, comparing to sustained group within same cell type).
2.5.4 SINs as conveyers of broadly tuned inhibition

Theoretical studies suggest that a broadly tuned suppression, generated within the local microcircuit, may sharpen the tuning properties of recipient spiny neurons, and control gain (Heeger, 1992; Troyer et al., 1998; McLaughlin et al., 2000), and these ideas have received experimental support (Carandini et al., 1997; Xing et al., 2011; Shapley and Xing, 2013). The receptive fields of most layer 4 excitatory cells in mouse V1 consist of only one subfield that is sharply tuned for orientation (Niell and Stryker, 2008; Liu et al., 2009, as we reported here in rabbit V1). Recent work on mice has shown that broadly tuned inhibition is important in sharpening orientation selectivity of such cells (Liu et al., 2011). Furthermore, activation of cortical parvalbumin-positive GABAergic cells sharpens the orientation selectivity of excitatory cells (Lee et al., 2012; but see Wilson et al., 2012). Consistent with these findings, the fast and strong feedforward response of SINs, and their broad orientation, spatial and temporal tuning, make them well-suited to convey a fast, broadly tuned inhibition onto local spiny neurons.

2.5.5 Thalamocortical specificity and mechanisms underlying diverse response properties of layer 4 populations

Separate ON/OFF subregions of V1 simple cells (and orientation preferences) are thought to originate from selective thalamocortical inputs with ON or OFF RF centers precisely aligned with the cortical RF subfields (Hubel and Wiesel, 1962; Tanaka, 1983; Reid and Alonso, 1995; Alonso et al., 2001; but see Mata and Ringach, 2005). By contrast, a promiscuous, unselective thalamocortical input would be expected to generate RFs with ON/OFF spatial overlap and poor orientation selectivity (Alonso and Swadlow, 2005). A promiscuous, unselective thalamocortical functional connectivity has been demonstrated in somatosensory cortex of rats and rabbits, where layer 4 SINs (Swadlow, 1995; Swadlow and Gusev, 2002) or fast spike cells (Bruno and Simons, 2002) receive potent inputs from a large proportion of topographically aligned thalamocortical neurons (also see Inoue and Imoto, 2006; Sun et al.,...
In these systems, most somatosensory thalamocortical neurons are sharply tuned for whisker deflection direction, and many such neurons (with a diversity of directional preferences) converge upon individual SINs/fast spike cells, which show minimal directional tuning. Notably, SINs of rabbit somatosensory cortex, like those of V1, respond at very short latency to peripheral stimulation (Swadlow, 1995). In the visual thalamocortical system, a similar unselective input from ON-center and OFF-center LGN neurons could generate the overlapping ON/Off RFs seen in V1 SINs, and our data provide examples of an OFF-center LGN neuron providing input to SINs with ON-dominated RFs (Figure 2.12C-D). We suggest that a promiscuous thalamocortical input onto fast spike interneurons of layer 4 may be a general feature of sensory neocortex. This, together with a highly convergent input from other cortical neurons (Bock et al., 2011) and the electrical coupling observed among neighboring fast spike interneurons (Gibson et al., 1999, 2005; Galarreta and Hestrin, 2002) may account for the high responsiveness and broad tuning of these cells to many aspects of sensory stimulation.
CHAPTER 3: Brain state effects on layer 4 of the awake visual cortex

3.1 Abstract

Awake mammals switch between alert and nonalert brain states hundreds of times per day. Here we show that alertness increases the strength and reliability of visual responses at the input layers of visual cortex (layer 4 simple cells). It broadens their temporal frequency tuning but preserves their contrast sensitivity and selectivity for orientation, direction and spatial frequency. Alertness also increases the response amplitude and reliability of layer 4 suspected inhibitory neurons (SINs) but reduces their high spontaneous rate. Therefore, the net effect on SINs can be either enhancement or reduction of mean rate. Finally, alertness selectively suppresses the responses of layer 4 simple cells to high contrast stimuli and stimuli moving orthogonal to the preferred direction, effectively enhancing mid-contrast moving borders. Using computer modeling, we demonstrate that enhanced reliability, higher gain, and increased orientation flank inhibition of simple cells during alertness could each significantly increase the speed of cortical feature detection.
3.2 Introduction

The “waking state” is not unitary. It contains different brain states which can be classified by behavior, physiological activity or neurotransmitter release (Harris and Thiele, 2011; Lee and Dan, 2012). It has been shown that these different awake brain states strongly affect information processing in sensory thalamus and cortices in cats (Worgotter et al., 1998), mouse (Poulet and Petersen, 2008; Gentet et al., 2010; Niell and Stryker, 2010; Pinto et al., 2012; Polack et al., 2013), rats (Fanselow and Nicolelis, 1999; Castro-Alamancos, 2004; Goard and Dan, 2009; Otazu et al., 2009) and rabbits (Swadlow and Weyand, 1985, 1987; Swadlow, 1988; Bezdudnaya et al., 2006; Cano et al., 2006; Bereshpolova et al., 2011). Our previous studies have shown that awake rabbits readily shift between alert and nonalert brain states, measured by electroencephalogram (EEG). The transition between these states is often very rapid (<1 sec) and profoundly affects spiking statistics and RF properties in LGN. During the alert state, LGN cells have significantly higher spontaneous firing rate, higher response gain, higher temporal frequency peak, broader temporal frequency tuning and generate fewer bursts than during the nonalert state (Swadlow and Gusev, 2001; Bezdudnaya et al., 2006; Bereshpolova et al., 2011). LGN cells also have higher maintained response to stationary stimulation during the alert state than during the nonalert state (Swadlow and Weyand, 1985). On the other hand, thalamocortical synaptic transmission is remarkably stable across the states (Stoelzel et al., 2009), suggesting that layer 4 neurons, the major recipients of LGN input, may inherit the brain state effects from LGN. Surprisingly, unlike LGN cells, SINs in layer 4 decreased their spontaneous firing rate during the alert state while layer 4 simple cells kept their spontaneous firing relatively constant (Bereshpolova et al., 2011). These surprising results suggested that brain state can have a different effect on visual cortex than thalamus. Notably, the two major cell classes in layer 4, the SINs and the putative excitatory simple cells, have markedly different response properties in awake rabbits (Zhuang et al., 2013), suggesting very different roles in cortical computation. Here, we examine the visual response properties of identified layer 4 simple cells and SINs in V1 of awake rabbits during both alert and nonalert states. We found that the alert state increased the strength and reliability of visual
responses in both layer 4 cell classes while preserving their contrast sensitivity and spatial selectivity. At the same time, alertness selectively suppressed the visual responses of layer 4 simple cells to high contrast stimuli and stimuli moving orthogonal to the preferred direction. When fed into a simple computational model, these results demonstrate that the enhanced reliability, enhanced response strength and selective response suppression of layer 4 simple cells during alertness could significantly increase the speed of cortical computations that may underlie feature detection.
3.3 Materials and Methods

Recordings were obtained from monocular V1 of 4 awake adult female Dutch-Belted rabbits. All experiments were conducted with the approval of the University of Connecticut Animal Care and Use Committee in accordance with National Institutes of Health guidelines.

3.3.1 Animal preparation and electrophysiological recording

The general surgical procedures for chronic recordings have been described previously (Bezdudnaya et al., 2006; Stoelzel et al., 2008; Bereshpolova et al., 2011; Zhuang et al., 2013) and are reported only briefly here. Under ketamine-acepromazine anesthesia, 8 stainless steel screws were installed on the exposed surface of the skull and a stainless steel rod, oriented in a rostrocaudal direction, was tightly attached to the screws and skull by acrylic cement. The rod was then used to rigidly hold the rabbit during the electrode implantation and recording sessions. The space between the wound margin and the acrylic cement was filled with silicone rubber. Following at least 10 days of recovery, recordings of neuronal activity were obtained in the awake state through a small hole in the skull.

Extracellular single-unit recordings and local field potentials (LFP) were obtained from the monocular region of V1 of awake rabbits. Single unit activity for most cells was studied using fine-diameter (40 µm) quartz-insulated platinum/tungsten electrodes tapered and sharpened to a fine tip (impedance, 1.5-3 Mohm). A group of seven such electrodes was chronically implanted in a concentric array (200 µm separation), with tips initially located just above the dura. Each of these electrodes was independently controlled by a miniature microdrive (Swadlow et al., 2005). Multi-unit activity from superficial layers of the SC was simultaneously recorded by a similar 3-channel microdrive system. A small number of cells were studied using 16-channel silicone probes, with recording site diameters of 33 µm (NeuroNexus
Technologies). Two stimulating electrodes (parylene-c insulated platinum/iridium micro wire) were implanted in LGN for identification of cortical neurons. Hippocampal EEG was recorded using two electrodes implanted above and below the CA1 layer and used, along with cortical EEG, for monitoring brain states. All electrophysiological activity was acquired using a Plexon data acquisition system.

### 3.3.2 Brain state identification

During recordings, no anesthetic agents were applied, the eyes were open and subjects responded to external stimuli (novel sound and gentle touch, etc). In rabbits, wakefulness can be divided into alert and nonalert states. The alert state is indicated by hippocampal “theta” activity (5-7 Hz) and cortical desynchrony, and the nonalert state indicated by hippocampal high-voltage irregular activity, and more slow wave activity in the neocortex (Swadlow and Gusev, 2001; Bezdudnaya et al., 2006; Cano et al., 2006; Stoelzel et al., 2008; Stoelzel et al., 2009; Bereshpolova et al., 2011). Some non-periodic sensory stimulation (random sounds, tactile stimulation, movements in room) is often required to keep rabbits from transitioning from the awake, nonalert state to sleep (early signs of which are indicated by cortical spindle activity), and such stimulation was applied when needed. In some cases, when rabbits became drowsy during extended recordings, we provided novel sounds to generate alertness. If they were difficult to arouse, we let them sleep for a while, and did not collect data during these periods. During recording sessions, subjects spent roughly equal amount of time in each state. For each of the cells that we studied, the ratio of time spent in the alert state to time spent in the nonalert was close to 1 (1.06±0.06). For all recorded files, the hippocampal EEG was segmented by visual inspection into alert versus nonalert states based on the presence of theta activity (5–7 Hz) or high-voltage, irregular activity, respectively (Swadlow and Gusev, 2001; Bezdudnaya et al., 2006; Cano et al., 2006; Stoelzel et al., 2009; Bereshpolova et al., 2011). This segmentation was aided and verified by fast Fourier transform analysis. For some recorded
files, comparisons between states were also performed using data around the alert/nonalert state transition points (5 sec before and 5 sec after).

3.3.3 Cortical layer 4 identification

Depth range of cortical layers was determined by electrode depth and reversal of stimulus evoked field potentials that occur at known depths. Brief full field flash evoked LFPs were recorded at different depth. As previously verified histologically (Stoelzel et al., 2008), the top of layer 4 was identified as 100 μm below a prominent current sink/source reversal point to the flash stimuli and the bottom of layer 4 was identified as 400 μm below the top of this layer. Only neurons within this narrow (400 μm) depth zone were included in this study.

3.3.4 Cell classification

Classification of cortical layer 4 cells follows the criteria published before (Zhuang et al., 2013). Layer 4 SINs were defined by responding to thalamic electrical stimulation with a high-frequency discharge of 3 or more spikes with peak frequencies of > 600 Hz (Swadlow, 1988, 1989, 1991, 1995, 2003), while layer 4 simple cells were defined by possessing a receptive field with one, two or three spatially separated ON and/or OFF subfields. Layer 4 cells which did not meet either criteria were classified as ‘other’ and were excluded from further analysis.

Cortical layer 4 simple cells and SINs were further classified into sustained/transient categories (Zhuang et al., 2013). The cell’s response to optimal flashing stationary stimuli (1 sec on 1 sec off or 2 sec on 2 sec off) in the alert state was recorded. Then a sustained index (SI) was calculated as the ratio between the cell’s maintained response (firing rate within 0.5 to 1.0 sec after the onset of stimuli) and the baseline
activity (firing rate within either -1 to 0 or -0.5 to 0 sec relative to the onset of stimuli). Cells with SIs larger than 2 and with an absolute maintained response of more than 1 spk/sec were defined as sustained cells while cells with SIs lower than 2 were defined as transient cells. Cells that failed to reach these criteria remained as ‘sustained/transient unclassified’. For some cells, responses to flashing stimuli during the nonalert state were also recorded to measure state effects, but only the responses recorded during alert state were used to classify the sustained/transient property.

3.3.5 Visual stimulation and eye movement control

Visual stimulation protocol were similar to what have been published before (Zhuang et al., 2013). All visual stimuli were presented by a CRT monitor (Nec MultiSync, 40x30cm, mean luminance, 48cd/m2, refresh rate: 160Hz). First the cell’s ON-OFF subfields were mapped with sparse noise stimulation by reverse correlation. After mapping, the receptive field center was constantly tracked by dynamic calculation of receptive field position from multi-unit recordings in the superior colliculus. All the visual stimuli thereafter were presented to the cell’s receptive field center. Then the cell’s sustained/transient property was measured with flashing stationary stimuli, which were optimized to elicit the strongest response possible. After assessing sustained/transient responses, circular drifting gratings were used to further measure the cell’s response properties. The drifting grating was optimized by orientation, spatial frequency, temporal frequency, size and contrast. The measurements were restricted to periods of time with stable eye position (smaller than ±1 degree), verified by both the receptive field position of multi-unit recordings in the superior colliculus and movies of eye position using a high-frequency infrared camera. After recording the cell’s response to optimal drifting grating, the cell’s tuning properties were measured by pseudorandomly varying one of the four (orientation, spatial frequency, temporal frequency or contrast) grating parameters while keeping the other four at the optimal values. Each presentation of a particular parameter combination lasted for 3 to 8 sec with 2 sec gap in between and all tested parameter
combinations were presented for at least 100 periods. During tuning property testing, the rabbit’s eye position was constantly monitored and data around eye major movements was discarded in offline analysis (Zhuang et al., 2013). For some cells, the spontaneous activity was also recorded for at least 10 minutes with the monitor screen set at a mid-luminance level.

3.3.6 Offline data analysis

Spikes from cortical single units were isolated during the experiment and verified off-line by using Plexon cluster analysis software. All data analysis was then performed with NeuroExplorer (Nex Technologies, Inc.) and MATLAB (The MathWorks, Inc.). The offline data analysis methods have been reported earlier (Zhuang et al., 2013), and are described only briefly here.

Latency to flash stimulation

The latency to flash stimulation was measured from the cell’s response to optimal flashing stimuli. The PSTH (with bin size equal to 1 ms) around the onset of the stimuli was smoothed by a sliding boxcar filter with width of 30 ms. Then latency was defined as the time at which the smoothed function first pass 40% of its maximum value (Jin et al., 2011).

F0, F1, F1/F0 ratio and Fano factor

The mean firing rate (F0) and first harmonic component (F1) of the PSTHs, F1/F0 ratio and Fano factor were calculated from the cell’s response to the optimal drifting grating. For the cells with spontaneous activity recordings, the ‘F0-spontaneous firing rate’ and ‘F1F0 ratio without spontaneous activity’ (calculated from PSTHs rectified by spontaneous firing rate, Chen et al., 2009) were also calculated.
Drifting grating parameter tuning properties

All the tuning properties are analyzed in the same way described before (Zhuang et al., 2013). Because almost all simple cells (> 95%) had stronger F1 than F0 responses and all SINs had stronger F0 than F1 responses (see results), the F1 response was used to measure tuning properties for simple cells and the F0 response to measure tuning properties for SINs. The response amplitude for all tuning curves (R_con, R_or, R_SF, R_TF), were calculated as the range between baseline and the maximum response. Contrast tuning response functions were fitted by a hyperbolic model with (Peirce, 2007) or without (Albrecht and Hamilton, 1982) modification account for high-contrast suppression, justified by the R² of fitness normalized by degree of freedom (Cavanaugh et al., 2002; Peirce, 2007). C₅₀ was defined as the contrast at which the cell’s response first reach the middle point of that range. Orientation tunings were fitted by von Mises distribution functions (Nowak et al., 2008). The orientation/direction selectivity was measured by circular variance (CirVar), orientation selectivity index (OSI) and direction selectivity index (DSI) calculated as 1 − |(∑ R_i e^{i2θ_j})/∑ R_i|, (R_{pref} − R_{orth})/(R_{pref} + R_{orth}), and (R_{pref} − R_{oppo})/(R_{pref} + R_{oppo}), respectively, where R_i and θ_j represent the cells response (spk/sec) and the angle (in radians) of jth direction, respectively; R_{pref} represents the cell’s response in the preferred direction; R_{orth} represents the cell’s mean responses of the two directions orthogonal to the preferred direction; and R_{oppo} represents the cell’s response to the opposite of the preferred direction. Orientation tuning width was measured by the half width at half height (OR_HWHH) after removal of baseline. Spatial frequency tuning curves were fitted by Gaussian model. Peak spatial frequency (SF_peak) was measured as the spatial frequency at which maximum response occurred and spatial frequency tuning width (SF_width) as the width at half of the maximum response without baseline on logarithmic scale (with base 10). Temporal frequency tuning curves were fitted by Gaussian model on logarithmic scale (with base 2). Peak temporal frequency (TF_peak) was measured as the temporal frequency at which maximum response occurred and temporal
frequency tuning width (TF_width) as the width at half of the maximum response without baseline. All these measurement were done for both alert and nonalert state. Goodness of fitting showed the models used here faithfully described the tuning properties of the cells’ responses ($R^2$, alert: 93.0±0.8%; nonalert: 93.7±0.7%). Only the fittings with $R^2$ values larger than 0.5 were included in further analysis.

**Model simulation**

The orientation tuning curves of 8 simulated cells took the shape of population average of all our layer 4 simple cells in each state (Figure 3.5B) but preferred on every 45 degrees, and Gamma process was used to generate spike trains with given firing rate and Fano factor (Inter spike interval distribution ~ $\Gamma(1/Fano, Fano/Rate)$)(Nawrot et al., 2008). The detector calculates the vector sum of the 8 simulated firing rates each in the direction of the 8 cells’ preferred direction, and then matches this vector sum (simulated vector sum) to the vector sums calculated in the same way but from the original tuning curves (tuning curve vector sum). The direction gives the smallest difference between simulated vector sum and tuning curve vector sum will be the output of the detector as detected direction. For each stimulating direction, simulation ran for 1500 trials and integration time took step of 20 ms.
3.4 Results

We measured the response properties of 50 simple cells and 30 SINs in layer 4 of V1 during alert/nonalert brain states (n = 3 adult, female awake rabbits). The animals sit calmly on a bench for several hours and no anesthesia was needed during recording sessions. All statistical comparisons reported below were quantified as mean ± standard error with p-values obtained by paired sample t-tests.

3.4.1 Alert/nonalert state effects on responsiveness

Our previous studies demonstrated that LGN cells are more responsive to visual stimuli during the alert state than the nonalert state (Bezdudnaya et al., 2006; Cano et al., 2006). Here, we studied the effect of these changes in brain state at the main input layers of the V1. V1 layer 4 cells were stimulated with optimal drifting gratings when awake animals switched between alert and nonalert states. The visual stimulation was optimized on contrast, orientation, spatial/temporal frequency and size for each cell. The effect of alertness was measured in both layer 4 simple cells (Figure 3.1 A) and layer 4 SINs (Figure 3.1 B). During the transitions in brain state, we studied the visual responses to a drifting grating over periods of time with highly stable eye position (± 1 deg, Figure 3.1). During the alert state, there was prominent hippocampal theta activity (HippEEG) and cortical desynchrony (CtxEEG), which changed to hippocampal high voltage irregular activity and cortical synchrony during the nonalert state. The transitions between alert and nonalert states occurred very quickly, within 1-2 seconds (Figure 3.1). We measured both transition directions from nonalert to alert states (Figure 3.1 A, B, left) and from alert to nonalert states (Figure 3.1 A, B, right). However, since we did not find any differences in neuronal response modulations between the two transition directions, most comparisons are made between alert and nonalert states, independently of the transition direction.
When rabbits became alert, both simple cells and SINs responded stronger to optimal drifting gratings. We studied visual responses by calculating the mean response to the drifting grating (F0) and the response modulation at the frequency of the stimulus, which is the amplitude of the first Fourier harmonic (F1). In simple cells, both F0 responses (Figure 3.2 A, B) and F1 responses (Figure 3.2 C, D) were consistently and significantly higher during the alert state than the nonalert state (alert vs. nonalert, n=43, F0s: 9.8±1.0 vs. 6.7±0.7 spk/sec, p<0.001; F1s: 14.6±1.6 vs. 10.1±1.2 spk/sec, p<0.001, Figure 3.2), a finding that could be replicated if we compared data only around the time of a state transition (alert vs. nonalert, 5 sec -5 sec transitions, n=30, F0s: 9.4±1.1 vs. 7.8±1.0 spk/sec, n=30, p<0.001; F1s: 14.6±1.6 vs. 10.1±1.2 spk/sec, n=30, p<0.001). In contrast, the effect of alertness on SINs was much more diverse. In some SINs, the F0 responses increased during the alert state and in others it decreased (Figure 3.2 A). As a consequence of this diversity, the mean F0 response of the SIN population showed no significant difference between two states (alert vs. nonalert, F0s of SINs: F0: 48.6±4.1 vs. 47.1±2.9 spk/sec, n=26, p=0.68, Figure 3.2 A, B), a finding that was also replicated if we compared data only around state transitions (5 sec before, 5 sec after, alert vs. nonalert, n=18, F0s: 51.0±4.0 vs. 47.8±2.7 spk/sec, p=0.18; F1s: 24.4±4.1 vs. 17.9 vs. 2.7 spk/sec, p=0.008). Unlike the average F0 response, the average F1 response of SINs was higher during the alert than nonalert states (alert vs. nonalert, F1s of SINs: 32.7±4.6 vs. 24.6±3.6 spk/sec, n=26, p<0.001, Figure 3.2 C, D).
Figure 3.1. Responses to optimal drifting gratings of a layer 4 simple cell and a layer 4 SIN during alert/nonalert transitions. A: simple cell. B: SIN. For each cell, one transition from the nonalert state to the alert state (upper left), one transition from the alert state to the nonalert state (upper right), spike wave form (lower left, inset: receptive subfield map), peri-event rasters (lower middle) and PSTHs (lower right) around transition points (from about 5 sec before to about 5 sec after) are shown. For each state transition example, from top to bottom, visual stimulation time course, the cells’ spike response, snap shots of eye movie (every second), hippocampal EEG (HippEEG) and cortical EEG (CtxEEG) are shown. For spike waveforms, green and orange traces: cell spikes; black traces: background hash activity; shaded areas: standard deviation. For receptive subfield maps, each contour: 10% of maximum activity. For rasters and PSTHs, 4 stimulation cycles are shown. Dashed gray line: spontaneous activity level (spontaneous level of the simple cell is too low to indicate with a dashed line).
Figure 3.2. The state effects on F0 and F1 responses of layer 4 simple cells and layer 4 SINs. A: scatter plot of F0 responses of each cell in the two states. Inset: scatter plot with spontaneous activity removed. B: population statistics of state effect on F0 responses. Inset: population statistics with spontaneous activity removed. Vertical calibration: 10 spk/sec C, D: scatter plot and population statistics for F1 responses, respectively. ***: p < 0.001; NS: p > 0.05, paired t-test.
The lack of significant state effect for the F0 responses of SINs could be due to two possibilities: 1) changes in brain state have variable effects on the F0 responses of each SIN; or 2) changes in brain state have reliable effects on the F0 responses of each SIN but the effects are different across SINs. To examine this, we calculated F0s in both states for every state transition point during which the animal switched from alert to nonalert states and from nonalert to alert states (≤ 5 sec before and ≤ 5 sec after, Bereshpolova et al., 2011). Figure 3.3 shows three example SINs, with each panel representing a particular SIN and each dot representing a single state transition point. The left panel shows a SIN with significantly higher F0s during the alert state in almost all state transitions (Figure 3.3 A); the middle panel shows a SIN with roughly equal F0s in both states (Figure 3.3 B) and the right panel shows a SIN with significantly higher F0s during the nonalert state in almost all state transitions (Figure 3.3 C). As shown in this figure, the state effects on SINs were very consistent during recording sessions and independent of the direction of the state transition (alert to nonalert, or nonalert to alert). These results demonstrate that, although changes in brain state do not affect the mean F0 response of the entire layer 4 SIN population, they affect differently individual SINs. The F0 response of a SIN can increase, decrease or remain the same, as rabbits transition from nonalert to alert states.
Figure 3.3. The state effects on F0 responses are diverse for different SINs, but consistent for individual SINs. For three example SINs (A, B and C), the F0 responses in both states around state transitions (5 sec before 5 sec after) are shown. For each panel, every filled square represents an individual state transition from the nonalert state to the alert state and every open square represents a individual state transition from the alert state to the nonalert state. A: a SIN has constantly higher F0s during the alert state than during the nonalert state; B: a SIN has about the same F0s during both states; C: a SIN has constantly lower F0s during the alert state than during the nonalert state. Insets: mean F0s in each state of each type of transition points for the particular cell. Grey lines: spontaneous activity level. Arrow at the bottom shows the direction of state transition (left to right = alert to nonalert, right to left = nonalert to alert). Vertical calibration: 20 spk/sec. ***: p < 0.001; NS: p > 0.05, paired t-test.
3.4.2 Brain state effects on spatial summation linearity

F1/F0 ratios are a common measurement of spatial summation linearity for visual cortical cells. We previously showed that layer 4 simple cells (F1/F0 > 1) are much more linear than SINs (F1/F0 <1, Zhuang et al., 2013). Here we measured the F1/F0 ratios of layer 4 cortical cells in both states, alert and nonalert. While the F1/F0 ratio of layer 4 simple cells did not change with brain state (alert vs. nonalert, 1.50±0.04 vs. 1.48±0.04, n=43, p=0.25), SINs showed significantly higher F1/F0 ratios in the alert state than in the nonalert state (alert vs. nonalert, 0.50±0.05 vs. 0.40±0.04, n=26, p<0.001, Figure 3.4 A, B) and similar results could be demonstrated if we restricted the comparative analysis to temporal periods around state transitions (5 sec before 5 sec after, alert vs. nonalert, F1/F0 for simple cells: 1.59±0.05 vs. 1.56±0.04, n=30, p=0.068; F1/F0 for SINs: 0.49±0.07 vs. 0.40±0.05, n=18, p=0.001). Thus, SINs showed a small but significant increase in their F1/F0 ratios during alertness indicating that the alert state makes visual responses more linear within cortical layer 4, even in cells that show a pronounced spatial non-linearity. It should be noted that all SINs had F1/F0 ratios of < 1, regardless of brain state, and all but two simple cells had F1/F0 ratios of > 1 in both brain states.

3.4.3 The alert state made visual responses not only stronger and more linear but also more reliable

The response reliability was measured as the Fano factor, which is the cycle by cycle spike count variance divided by the mean spike count in a single cycle. Both layer 4 simple cells and SINs increased their response reliability during the alert state (Fano factor, alert vs. nonalert, simple cells: 0.88±0.07 vs. 1.31±0.11, n=30, p<0.001; SINs: 0.87±0.08 vs. 1.71±0.09, n=18, p<0.001, Figure 3.4 C, D), a finding that could be replicated in temporal periods around state transitions (Fano factor, 5 sec before 5 sec after, alert vs. nonalert, simple cells: 0.90±0.08 vs. 1.18±0.09, n=30, p<0.001; SINs: 1.18±0.29 vs. 1.60±0.18, n=18, p<0.001). Interestingly, the increase in response reliability was more than 2 times higher in cortical layer
4 neurons than in the LGN inputs (percentage of Fano factor increase in the nonalert state, simple cells: 
33.71±2.31%, n=30; SINs: 49.37±3.06%, n=18; LGN cells with concentric receptive fields:
15.74±5.73%, n=11; simple vs. LGN: p=0.0012; SIN vs. LGN: p<0.001, independent t-test, Figure 3.4 C, inset). This finding indicates that the effect of alertness on response reliability is not simply transmitted from LGN but it is amplified in visual cortex.
Figure 3.4. The state effects on spatial summation linearity (F1/F0 ratios) and reliability (Fano factor) of layer 4 simple cells and layer 4 SINs. A: scatter plot of F1/F0 ratios during both states for all cells. Inset: scatter plot after removing spontaneous activity. B: population statistics of state effect on F1/F0 ratio. Inset: population statistics after removing spontaneous activity. Vertical calibration: 1. C: scatter plot of Fano factors during both states of all cells. Inset: scatter plot and population statistics of Fano Factors for LGN concentric cells. D: population statistics of state effect on Fano factor. ***: p < 0.001; *: p < 0.05; NS: p > 0.05, paired t-test.
3.4.4 Contribution of spontaneous activity to state effects

Layer 4 SINs have very high spontaneous activity (Zhuang et al., 2013), which decreases by 44% during the alert state (Bereshpolova et al., 2011). Therefore, the change in the F1/F0 ratios of SINs with alertness could be due to changes in spontaneous rate. To investigate this possibility, we measured the state effects on F0 responses and F1/F0 ratios after removing spontaneous activity for both layer 4 simple cells and SINs. The removal of spontaneous activity did not affect the state effects on simple cells; their F0 responses were still higher during the alert state (F0-spontaneous: alert vs. nonalert, 8.9±1.1 vs. 6.1±0.9 spk/sec, n=27, p<0.001, Figure 3.2 A, B, insets) and the F1/F0 ratios did not change (F1/F0 without spontaneous activity: 1.61±0.05 vs. 1.65±0.03, n=27, p=0.065, Figure 3.4 A, B, insets). However, the removal of spontaneous activity did affect the state effects in SINs. When the spontaneous rate was subtracted, the F0 responses of almost all SINs became significantly higher during the alert than nonalert states (F0-spont for SINs, alert vs. nonalert, 33.0±5.0 vs. 22.2±3.2 spk/sec, n=17, p=0.008, Figure 3.2 A, B, insets). Moreover, the F1/F0 ratios of SINs were no longer higher during the alert state (F1/F0 w/o spont for SINs, 0.83±0.12 vs. 0.84±0.12, n=17, p=0.71, Figure 3.4 A, B, insets). These results indicate that, because the alert state markedly reduces the high spontaneous rate of SINs, their visual responses become more linear and the F0 responses can either increase (in SINs with the lowest spontaneous rates) or decrease (in SINs with the highest spontaneous rates). In contrast, because layer 4 simple cells have much lower spontaneous rates than SINs (and their spontaneous rates do not change with state), their F0 responses are consistently increased during alertness.

3.4.5 Brain state effects on orientation/direction tuning properties for simple cells

Unlike SINs, layer 4 simple cells show strong selectivity for both the orientation and direction of motion (Figure 3.5 A). Because this orientation/direction selectivity plays an important role in visual cortical processing, it is important to investigate in what extent it is affected by changes in brain state. Careful
inspection of example single cells revealed a pronounced increase in response amplitude during the alert state (alert vs. nonalert, $R_{am}: 19.2\pm4.3 \text{ vs. } 17.5\pm4.2 \text{ spk/sec, } n=17, p=0.03$, Figure 3.5 C), which barely affected orientation and direction selectivity. To quantify this observation, we calculated orientation/direction tuning for the population average of all tested simple cells (with normalization and after aligning the preferred direction of each cell to 0 degree, Figure 3.5 B). The alert state did not affect the population orientation tuning width (alert vs. nonalert, HWHH: 29.7±4.1 \text{ vs. } 30.9±4.9 \text{ deg, } n=17, p=0.42$, Figure 3.5 C) and direction selectivity (DSI: 0.75±0.07 \text{ vs. } 0.74±0.06, n=17, p=0.47, Figure 3.5 C). However, it caused a slight but significant improvement in orientation selectivity and circular variance (OSI: 0.86±0.04 \text{ vs. } 0.82±0.04, n=17, p=0.015; CirVar: 0.42±0.07 \text{ vs. } 0.50±0.06, n=17, p=0.008, Figure 3.5 C). The improvement in orientation selectivity was caused both by a response increase in the preferred direction (ratio between alert vs. nonalert: 1.21±0.09 : 1, n=16, p=0.03, one sample t-test, Figure 3.5 D) and a response suppression in the orthogonal direction (ratio between alert vs. nonalert: 0.64±0.11 : 1, n=16, p=0.004, one sample t-test, Figure 3.5 D). These results demonstrate that the alert state increases the strength of visual responses in layer 4 simple cells without broadening their orientation/direction tuning. Importantly, the alert state improves orientation selectivity by enhancing visual responses in preferred direction and suppressing them in the orthogonal direction.
Figure 3.5. The state effects on orientation tuning of layer 4 simple cells. A: orientation tuning curves of two example layer 4 simple cells. B: population average of F1 orientation tuning curves of layer 4 simple cells. Tuning curves for each cells were normalized to the peak response (the bigger one of alert peak and nonalert peak) before averaging. Preferred direction (marked by arrow) for each cell are aligned as 0 degree. Two directions orthogonal to the preferred direction are marked by arrow head. C: population statistics of orientation tuning parameters during the alert and nonalert state. **: p < 0.01; *: p < 0.05, NS: p > 0.05, paired sample t-test. D: Average F1 responses (normalized by the F1 responses during the nonalert state) of layer 4 simple cells in preferred direction and orthogonal directions. Dashed line: 100%. **: p < 0.01; *: p < 0.05, one sample t-test.
3.4.6 Alert/nonalert state effects on the contrast response functions of simple cells

The contrast response functions of layer 4 simple cells increased their response amplitude in the alert state (alert vs. nonalert, \( R_{\text{con}} \): 18.8±2.8 vs. 15.9±2.5 spk/sec, \( n=25 \), \( p<0.001 \)) without changing the contrast that generated half-maximum response (\( C_{50} \), 26.6±3.4% vs. 28.6±3.2%, \( n=25 \), \( p=0.25 \)). As we showed previously (Zhuang et al., 2013), layer 4 simple cells can respond weaker to stimuli with high contrast than intermediate contrast. Importantly, here we demonstrate that this high-contrast suppression is strongly affected by brain state (Figure 3.6 A, B). The high-contrast suppression was calculated as the percentage of response suppression at the highest contrast relative to the maximum response. Interestingly, in layer 4 simple cells that showed high-contrast suppression (\( n=15 \)), the suppression was nearly 2 times higher during the alert state than the nonalert state (alert vs. nonalert, 29.0±5.8% vs. 15.4±4.8%, \( n=15 \), \( p=0.023 \), Figure 3.6 B). These results indicate that, in a subset of layer 4 simple cells, the alert state selectively amplifies visual responses to intermediate luminance contrasts. It should be noted that the proportion of layer 4 simple cells showing high-contrast suppression (57% in our sample, 15/26) may be underestimated due to limitations of CRT monitors in reaching the luminance levels and luminance contrasts that are found in natural scenes.
Figure 3.6. The state effects on contrast response function of layer 4 simple cells. A: contrast response function of an example layer 4 simple cell showing strong reduction of response at high contrast during the alert state but no reduction during the nonalert state. B, population average of contrast response function of layer 4 simple cells which showed high contrast suppression in either state (n=15). For each cell, the contrast response function were normalized to their maximum response (the larger one of alert peak and nonalert peak). Inset: average percentage of reduction in both state for these simple cells. C: contrast response function of an example layer 4 simple cell showing no high-contrast suppression during either state. D: population average of contrast response function of layer 4 simple cells which showed no high-contrast suppression in either state (n=11). *: p < 0.05; **: p < 0.01; ***: p < 0.001, paired t-test.
3.4.7 Brain state effects on spatial/temporal tuning properties for simple cells

The amplitude of the spatial frequency tuning also increased during the alert state (Figure 3.7 A, alert vs. nonalert, R_{sf}: 19.9±4.7 vs. 15.9±4.6, n=13, p<0.001), however, the increase in amplitude did not affect the peak and width of the tuning curves (SF_peak: 0.21±0.06 vs. 0.21±0.06 cpd, n=13, p=0.48; SF_width: 0.24±0.05 vs. 0.23±0.04 oct, n=13, p=0.85, Figure 3.7 B). Therefore, the alert state increased the strength of visual responses in layer 4 simple cells without affecting their selectivity for spatial patterns.

While the alert state did not affect the selectivity for stimulus orientation, direction of motion and spatial frequency, it had a pronounced effect on the temporal frequency tuning. The alert state increased the amplitude of the temporal frequency tuning (alert vs. nonalert, R_{tf}: 13.3±3.1 vs. 10.5±2.5 spk/sec, n=22, p=0.002, Figure 3.7 C) and, as shown previously for LGN (Bezdudnaya et al., 2006), it made the temporal frequency tuning broader (TF_width: 3.1±0.3 vs. 2.6±0.3 oct, n=22, p=0.03, Figure 3.7 D). There was also a slight increase in the temporal frequency peak during the alert state but, unlike in LGN (Bezdudnaya et al., 2006), it did not reach significance (TF_peak: 6.4±1.0 vs. 5.6±0.9 Hz, n=22, p=0.057, Figure 3.7 D). Notice that all the tuning curves of layer 4 simple cells were measured from F1 responses to drifting gratings and, therefore, the analysis was independent of the spontaneous activity. It is possible that the lack of significant changes in temporal frequency peak may reflect a limitation of CRT monitors, which cannot faithfully drift gratings beyond 16 Hz.
Figure 3.7. The state effects on spatial and temporal tuning of layer 4 simple cells. A: spatial frequency tuning curves of two example layer 4 simple cells. B: population statistics of peak spatial frequency and spatial tuning width in both state. C: temporal frequency tuning curves of two example layer 4 simple cells. D: population statistics of peak temporal frequency and temporal tuning width. *: p < 0.05; NS: p > 0.05, paired t-test.
3.4.8 Brain state effects on stimulus tuning properties for SINs

In general, the effect of brain state on SINs was similar to simple cells, however, there were some exceptions that deserve to be mentioned (Figure 3.8). Like with simple cells, the alert state did not change the contrast that generated half maximum response (alert vs. nonalert, \(C_{50}: 11.8\pm2.5\%\) vs. \(10.7\pm1.7\%, n=22, p=0.41\)), the spatial frequency tuning (\(SF_{\text{peak}}: 0.21\pm0.06\) vs. \(0.16\pm0.04\) cpd, \(n=20, p=0.24\); \(SF_{\text{width}}: 1.28\pm0.12\) vs. \(1.25\pm0.11\) oct, \(n=20, p=0.70\)) and the orientation tuning of SINs (HWHH: \(71.1\pm7.8\) vs. \(66.1\pm10.3\) deg, \(n=8, p=0.86\); OSI: \(0.17\pm0.03\) vs. \(0.18\pm0.03, n=8, p=0.37\); CirVar: \(0.95\pm0.02\) vs. \(0.94\pm0.02, n=8, p=0.12\); DSI: \(0.15\pm0.03\) vs. \(0.14\pm0.03, n=8, p=0.37\)). However, while the alert state broadened the temporal frequency tuning of simple cells and increased their high contrast suppression, these changes were not observed in SINs (TF_{\text{peak}}: \(6.25\pm1.43\) vs. \(5.51\pm1.15\) Hz, \(n=16, p=0.30\); \(TF_{\text{width}}: 4.55\pm0.56\) vs. \(3.98\pm0.45\) oct, \(n=16, p=0.47\); percentage of suppression at high contrast: \(11.4\pm3.4\%\) vs. \(8.5\pm3.6\%, n=12, p=0.268\)). Furthermore, the averaged SIN population did not show significant changes in the strength of visual responses with brain state (alert vs. nonalert, \(R_{\text{con}}: 48.1\pm5.2\) vs. \(44.2\pm3.6\) spk/sec, \(n=22, p=0.22\); \(R_{\text{oc}}: 66.1\pm10.3\) vs. \(62.5\pm8.2\) spk/sec, \(n=8, p=0.52\); \(R_{\text{SF}}: 45.4\pm6.2\) vs. \(47.2\pm5.5\) spk/sec, \(n=20, p=0.61\); \(R_{\text{TF}}: 37.8\pm7.0\) vs. \(30.2\pm4.3\) spk/sec, \(n=16, p=0.055\)). Finally, the effect of brain state on individual SINs was much more diverse than on simple cells. While the alert state consistently increased the visual responses of simple cells, the effect of alertness on SINs could be a response increase (Figure 3.8 A), a response decrease (Figure 3.8 C) or no change (Figure 3.8 B). This larger diversity of effects in SINs is mainly explained by their spontaneous firing rate, which is much higher than in simple cells and is reduced during the alert state. Please note that, although the response tuning properties of layer 4 SINs were measured from F0 responses, the calculations of response strength, \(C_{50}\), orientation tuning width, spatial/temporal peak and tuning width were all independent from the baseline measurements and, therefore, they are not be affected by removal of spontaneous activity. Only three parameters – orientation selectivity, direction selectivity and circular variance –, could be potentially affected by subtraction of spontaneous activity but they were not (after removing spontaneous
activity, n=5, alert vs. nonalert, OSI: 0.29±0.07 vs. 0.36±0.08, p=0.31; CirVar: 0.90±0.05 vs. 0.86±0.06, p=0.16; DSI: 0.27±0.08 vs. 0.24±0.06, p=0.57).
Figure 3.8. Example tuning curves of orientation, contrast, spatial and temporal frequency (left to right) in both states from 3 layer 4 SINs (A, B and C). A: a SIN shows constantly higher F0 responses during the alert than during the nonalert state; B: a SIN shows about the same F0 responses during both states; C: a SIN shows constantly lower F0 responses during the alert state than during the nonalert state. Tuning curves in black represent data from mixed state (not enough to be cut into the alert/nonalert state). Dashed lines: spontaneous activity level.
3.4.9 Brain state effects on sustained response to flashing stationary stimuli

Both layer 4 simple cells and SINs can be divided into sustained and transient categories, based on their responses to flashing stationary stimuli (Zhuang et al., 2013). Because sustained V1 responses are strongly affected by brain state (Swadlow and Weyand, 1987), it is important to investigate specifically these effects in layer 4 neurons. Sustained responses were measured with flashing stationary stimuli optimized for each neuron. Then, a sustained index (SI) was calculated as the ratio between the sustained response (within 0.5 to 1.0 sec after the stimulus onset) and the baseline activity. The alert state did not change the latency to flashing stimuli, as measured in sustained simple cells (alert vs. nonalert, 47.6±5.6 vs. 44.2±3.9 ms, n=20, p=0.43), transient simple cells (39.6±9.3 vs. 40.1±9.8 ms, n=2, p=0.50), sustained SINs (30.4±2.3 vs. 30.4±2.1 ms, n=6, p>0.9), transient SINs (28.2±1.9 vs. 28.2±2.0 ms, n=6, p>0.9), all simple cells (43.7±4.4 vs. 41.6±3.1 ms, n=27, p=0.511) and all SINs (29.3±1.5 vs. 29.3±1.4 ms, n=12, p>0.9). However, it caused a pronounced increase in the sustained responses of sustained simple cells (Figure 3.9 A-C, alert vs. nonalert, sustained firing rate: 3.0±0.5 vs. 1.3±0.2 spk/sec, n=20, p<0.001; Sustained Index: 39.4±15.2 vs. 4.9±0.4, n=20, p=0.034,) and sustained SINs (sustained firing rate: 21.3±4.9 vs. 7.0±5.2 spk/sec, n=6, p<0.001; Sustained Index: 3.3±0.8 vs. 1.5±0.4, n=6, p=0.008, Figure 3.9 D-F). Therefore, as for other neurons in area V1 (Swadlow and Weyand, 1987), the alert state strongly increases the sustained responses of layer 4 neurons, both in simple cells and SINs.
Figure 3.9. Both sustained simple cells and sustained SINs have higher sustained response during the alert than during the nonalert state. Each figure plots the cell’s response PSTH to optimal flashing stationary stimulus during either state (red: alert; blue: nonalert). A, B: responses from a sustained simple cell. C: population average responses of all sustained simple cells (N=19). D, E: responses from a sustained SIN. F: population average responses of all sustained SINs (N=6). Inset: comparison of “Sustained Index (SI)” between the alert and the nonalert state for each population. Note that the transient component of the response was little affected by state. **: p < 0.01; *: p < 0.05, paired t-test.
3.4.10 Alertness enhances computational speed of feature detection

The effects of alertness that we describe on layer 4 neurons could have an important impact on the speed of cortical processing, which is essential for survival. To investigate this functional implication, we simulated eight layer 4 simple cells with preferred directions separated by 45 degrees (Figure 3.10 A) and tuning curve shapes matching the average tuning measured in our data (Figure 3.10 B). The eight simple cells converged on a direction detector that read the firing rates of each cell with different integration times. The stimulus moving in a given direction (Figure 3.10 A, magenta arrow) generated a spike train in each cell through a Gamma process defined by a mean firing rate and Fano factor. The firing rate was given by the representation of the stimulus in the orientation tuning curve of each cell and the Fano factor by the average Fano factor measured during the alert (0.88) or nonalert states (1.31) in simple cells. After generating spike trains, the simulated firing rates of each cell were calculated in a certain time window (integration time) and fed to a direction detector. The direction detector performed a vectorial sum of the 8 cells, the vector angles being the preferred directions of the cells and the vector modules the mean rates of each spike train for a given integration time. The detection error was defined as the absolute value of the difference between the stimulus direction and the detected direction. We simulated the relationship between the integration time and the detection error for three stimulus directions (12.5, 11.25 and 0 degrees, Figure 3.10 C,D,E respectively).

The responses of simple cells during alert and nonalert states differed in 3 main parameters that are relevant to this model: Fano factor, response strength and response suppression to stimuli moving orthogonal to the preferred direction (Figure 3.10 E). To assess the contribution of each of these three parameters in direction detection, we simulated four conditions (Figure 3.10 C-E): 1) tuning curves with the shape and response reliability of the alert state (A); 2) tuning curves with the shape and response reliability of the nonalert state (NA); 3) tuning curves with the shape of the nonalert state but the high
reliability of the alert state (nonalert high reliability, NA HR); 4) tuning curves with the shape of the nonalert state but scaled up to match the maximum response of the alert state and the high response reliability of the alert state (nonalert scaled high reliability, NA HR S). We estimated the effect of high response reliability from the difference in detection time between condition NA and NA HR, the effect in response strength from the difference between NA HR and NA HR S and the effect of response suppression in the orthogonal direction from the difference between NA HR S and A (Figure 3.10 E square inset).

The results of the simulation demonstrate that the detection error decreases exponentially as the integration time becomes longer. Moreover, all three factors, response reliability, response strength and response suppression, are important in increasing the speed of cortical processing during the alert state. If we use a detection error of 20 degrees as a threshold, the detection time was 457±12.9 ms during the nonalert state (NA), 142 ms shorter if we added the high response reliability of the alert state (NA, HR: 315±8.3 ms), 47 ms shorter if we added the response strength of the alert state (NA HR S: 268±6.8 ms) and 42 ms shorter if we added the orthogonal-response suppression of the alert state (A: 226±5.8 ms). These results demonstrate that the alert state can increase the speed of cortical computation (i.e. compute stimulus direction) by more than 200 ms, a difference that could have an important behavioral impact (i.e. quickly escape in the opposite direction). Moreover, the results demonstrate that the most important factor in making cortical computation faster is the increase in response reliability followed by the increase in response strength and orthogonal suppression.
Figure 3.10. Model showing that low response variance, high response gain and orthogonal suppression all contribute to the increase of detection speed during the alert state. A: conceptual diagram of direction detection model. When stimulated in certain direction, each of 8 coding cells generates simulated spike train by mean firing rates and Fano factor. Firing rates of simulated spike trains in integration time window were fed to vector sum detector to predict stimulus direction. The differences between detected direction and stimulus direction (detection error) were measured to evaluate the performance of the system. B: Direction tuning curves of two example cells in A. Each of the 8 coding cells have identical tuning curve shape as the simple cell population average shown in Figure 3.5 B, but
with preferred directions every 45 deg apart. Grey arrows and dashed lines: three simulated stimulus directions. Red solid line: alert; blue solid line: nonalert. C,D,E: the relationship between integration time and detection error under each of three stimulating direction. “A”: simulation with tuning curves and reliability during the alert state; “NA”: simulation with tuning curves and reliability in the alert state; “NA HR”: simulation with tuning curves in the nonalert state and reliability in the alert state; “NA HR S”: simulation with tuning curves in the nonalert state scaled up to match the maximum in the alert state and reliability in the alert state. Horizontal line: threshold (20 degrees) for measuring detection time. Insets in C,D,E statistical results of detection time under each stimulus direction. Grey box in E was magnified to show the contributions of each component of state effects to the increase of computational speed in the alert state. Supp: orthogonal suppression.
3.5 Discussion

We demonstrated that changes in brain state have pronounced, cell-specific effect on the responses of neurons at the input layers of visual cortex. For layer 4 simple cells, the alert state increased the strength and reliability of visual responses, made the temporal frequency tuning broader but preserved the contrast sensitivity, linearity of spatial summation (F1/F0) and selectivity for spatial patterns. Moreover, it increased orientation selectivity by selectively enhancing visual responses at the preferred direction and suppressing them at the orthogonal direction. For layer 4 SINs, the alert state increased the response strength and reliability while reducing the spontaneous firing rate, making the SIN visual responses more linear. Finally, model simulation showed that three separable effects of alertness on layer 4 simple cells (increase of reliability, increase of response gain, increase of flank inhibition) could each significantly enhance the speed of cortical feature detection during the alert state.

3.5.1 Alert/nonalert state effects in LGN and V1

Because LGN neurons are strongly affected by brain state and layer 4 is the major recipient of LGN inputs in visual cortex, it is important to discuss how LGN and cortical layer 4 are differently affected by brain state. Some effects in LGN and cortical layer 4 were only quantitatively different. 1) Both LGN cells and layer 4 cells increased their F1 responses to drifting gratings during the alert state but the effects were much stronger in LGN. During the alert state, F1 responses increased by \( \sim 88\% \) in LGN cells (Cano et al., 2006), while the response increase was only \( \sim 44\% \) for layer 4 simple cells and \( \sim 33\% \) for layer 4 SINs (Figure 3.3 C, D). 2) Both LGN cells and layer 4 cells increased their response reliability during the alert state, but the increased in response reliability was more pronounced in cortical cells. During the alert state, the average Fano factor decreased by \( \sim 17\% \) for LGN cells (Figure 3.5 A inset), while the reduction was \( \sim 32\% \) for layer 4 simple cells and \( \sim 50\% \) for layer SINs (Figure 3.5 A, B). Some effects were almost identical in LGN and cortical layer 4. During the alert state, sustained cells in both LGN (Swadlow and
Weyand, 1987) and layer 4 (Figure 3.9) increased their sustained responses to stationary stimuli and broadened their temporal frequency tuning (LGN: (Bezdudnaya et al., 2006); layer 4 simple cells: Figure 3.7 D).

Some effects of brain state were specific for layer 4 simple cells. 1) The alert state increased the orientation selectivity of layer 4 simple cells by selectivity enhancing responses in the preferred direction and suppressing them in the orthogonal direction (Figure 3.5). These effects are not found in LGN cells with concentric receptive fields. Interestingly, in directionally selective LGN neurons, alertness also suppresses responses to movement in the null direction (Hei et al., 2012). 2) The alert state suppressed the responses of a large number of layer 4 simple cells to high-contrast stimuli (Figure 3.6). Such high-contrast suppression is much weaker and rare in LGN cells (Cano et al., 2006). Therefore, some of the effects of alertness at the inputs layers of the cortex are inherited from LGN, others are inherited and then amplified and others are purely intracortical.

3.5.2 Alert/nonalert state effects beyond the multiplicative gain modulation

Changes in response strength have been proposed to account for a variety of neurophysiological functions (Salinas and Sejnowski, 2001). Consistently, brain state changes in the contrast response function of LGN neurons are largely explained by a multiplicative gain change (Cano et al., 2006), and this is also the case for the contrast response function and spatial frequency tuning of layer 4 neurons. However, a multiplicative gain cannot explain several effects of brain state in cortical layer 4 such as the increase in orientation selectivity, high-contrast suppression and the pronounced increase in response reliability. The increase in response reliability during the alert state was profound, and was independent of the increase in mean firing rate. In fact, while all layer 4 cells increased their response reliability during the alert state
(Figure 3.4 C), some SINs decreased their mean firing rate (Figure 3.2 A). These results demonstrate that
the alert state does more than amplify visual responses; it selectively increases responses to preferred
directions of motion and suppresses responses to non-preferred directions and high contrasts. Our
direction detection model (Figure 3.10) demonstrates that the combined increase in response strength,
reliability and suppression has an important impact on the speed of cortical feature detection.

3.5.3 Comparisons to previous studies in awake subjects

It is important to emphasize that the EEG-defined alert/nonalert brain states studied here are “global”
brain states, not local states such as those generated by selective attention (Kastner and Ungerleider,
2000; Reynolds and Chelazzi, 2004; Maunsell and Treue, 2006; Anton-Erxleben and Carrasco, 2013).
Other studies on rodents have found some awake brain states were associated with cortical
desynchronization (Poulet and Petersen, 2008; Niell and Stryker, 2010; Pinto et al., 2012), similar to our
alert state. But it is important to note that the brain states previously studied by others may not be directly
comparable to the awake alert vs. awake-but-nonalert brain states examined here. For example, Niell and
Stryker (Niell and Stryker, 2010) compared the visual response properties of V1 simple cells and putative
fast-spike interneurons when mice were running, vs. balancing on a ball, and found the “running” state
increases responsiveness of layer 2/3 regular spike cells, but has diverse effects on firing rates of narrow
spike cells. However, whereas these authors concluded that the LGN does not contribute significantly to
the effects seen at the cortex (no changes in LGN response magnitude during locomotion). We have
previously shown that LGN cells are powerfully influenced by state (Swadlow and Weyand, 1985;
Bezdudnaya et al., 2006; Cano et al., 2006), suggesting that some of the effects seen in layer 4 (e.g., the
gain changes) are inherited from the LGN, while others (nonlinearities in the contrast response functions,
Figure 3.6 B) may not. While another study found enhanced reliability during stimulation of the N.
Basalis (Pinto et al., 2012), similar to our present result showing enhanced reliability of responses during
the alert state (lower Fano factor). Finally, based on the cortical EEG, the alert and nonalert global EEG states defined by the current study are highly relevant with “synchronized” and “desynchronized” state mostly defined in rodents (Harris and Thiele, 2011; Lee and Dan, 2012), respectively.

3.5.4 The significance of getting drowsy

The mechanisms of visual attention in the awake, alert brain have been intensely studied (Kastner and Ungerleider, 2000; Reynolds and Chelazzi, 2004; Maunsell and Treue, 2006; Anton-Erxleben and Carrasco, 2013). However, awake subjects are not always alert and attentive. In familiar environments, they readily switch between alert and nonalert states, yet they must be able to perceive salient features in their environment when nonalert. Here, we show that a non alert, “drowsy” state significantly reduces response gain, reliability, and suppression to non-preferred stimuli in V1 of awake subjects. Moreover, our simulations demonstrate that each of these factors can reduce computation speed during the alert state. Thus drowsiness strongly affects visual information processing at very early stage, and this raises questions concerning changes in perception (Dorokhov et al., 1998; Roge et al., 2002) and behavior that have been associated with drowsiness. For example, “drowsy driving” kills >1000 people and causes tens of thousands of injuries each year in the United States alone [National Highway Traffic Safety Administration (2011). National Highway traffic safety administration review on drowsy driving, http://www.nhtsa.gov/people/injury/drowsy_driving1/drowsy.html], and it is sometimes assumed that this is largely due to the increased likelihood of falling asleep when nonalert. By contrast, the present results suggest that state-related changes in perceptual mechanisms, including the computational speed underlying perceptual discriminations, may play an important role in such statistics.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>alert</td>
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<tr>
<td>CB</td>
<td>calbindin</td>
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<td>CCK</td>
<td>cholecystokinin</td>
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<td>CH</td>
<td>chattering</td>
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<td>CirVar</td>
<td>circular variance</td>
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<tr>
<td>CR</td>
<td>calretinin</td>
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<tr>
<td>CtxEEG</td>
<td>cortical EEG</td>
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<td>DSI</td>
<td>direction selectivity index</td>
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<tr>
<td>EEG</td>
<td>electroencephalogram</td>
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<tr>
<td>EPSP</td>
<td>excitatory post-synaptic potential</td>
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<td>FFT</td>
<td>fast Fourier transformation</td>
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<td>FS</td>
<td>fast spike</td>
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<td>GABA</td>
<td>γ-Aminobutyric acid</td>
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<td>GAD</td>
<td>glutamate decarboxylase</td>
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<td>HippEEG</td>
<td>hippocampal EEG</td>
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<td>HWHH</td>
<td>half width at half height for orientation tuning</td>
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<tr>
<td>IB</td>
<td>intrinsic bursting</td>
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<tr>
<td>ISI</td>
<td>inter-spike interval</td>
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<tr>
<td>LFP</td>
<td>local field potential</td>
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<tr>
<td>LGN</td>
<td>lateral geniculate nucleus</td>
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<tr>
<td>LSI</td>
<td>local similarity index</td>
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<tr>
<td>NA</td>
<td>nonalert</td>
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<tr>
<td>NA HR</td>
<td>nonalert high reliability</td>
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<td>NA HR S</td>
<td>nonalert scaled high reliability</td>
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<tr>
<td>NPY</td>
<td>neuropeptide Y</td>
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<tr>
<td>OSI</td>
<td>orientation selectivity index</td>
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<tr>
<td>PSTH</td>
<td>peristimulus time histogram</td>
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<tr>
<td>PV</td>
<td>parvalbumin</td>
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<td>RF</td>
<td>receptive field</td>
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<td>RS</td>
<td>regular spike</td>
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<td>SC</td>
<td>superior colliculus</td>
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<td>SF peak</td>
<td>spatial frequency tuning peak</td>
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<td>SF width</td>
<td>spatial frequency tuning width</td>
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<td>SI</td>
<td>sustained index</td>
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<td>suspected inhibitory interneurons</td>
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<tr>
<td>SOM</td>
<td>somatostatin</td>
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<tr>
<td>TF peak</td>
<td>temporal frequency tuning peak</td>
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<td>TF width</td>
<td>temporal frequency tuning width</td>
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<tr>
<td>V1</td>
<td>primary visual cortex</td>
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<td>VIP</td>
<td>vasoactive intestinal peptide</td>
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References


115


