Spatiotemporal characteristics of surround suppression in the primary visual cortex and the lateral geniculate nucleus of cat

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ABSTRACT
In the primary visual cortex (V1), a neuronal response to stimulation of the classical receptive field (CRF) is predominantly suppressed by stimulus presented outside the CRF (extra-classical receptive field, ECRF), a phenomenon referred to as ECRF suppression. To elucidate the neuronal mechanisms and origin of ECRF suppression in V1 of anesthetized cats, we examined the temporal properties of the spatial extent and orientation specificity of ECRF suppression in V1 and the lateral geniculate nucleus (LGN) using stationary-flashed sinusoidal grating. In V1, we found three components of ECRF suppression: 1) local and fast, 2) global and fast, and 3) global and late. The local and fast component, which resulted from within 2° of the boundary of the CRF, started no more than 10 ms after the onset of the CRF response and exhibited low specificity for the orientation of the ECRF stimulus. These spatiotemporal properties corresponded to those of geniculate ECRF suppression, suggesting that the local and fast component of V1 is inherited from the LGN. In contrast, the two global components showed rather large spatial extents about 5° from the CRF boundary and high specificity for orientation, suggesting that their possible origin is the cortex, not the LGN. Correspondingly, the local component was observed in all neurons of the thalamocortical recipient layer, while the global component was biased to other layers. Therefore, we conclude that both subcortical and cortical mechanisms with different spatiotemporal properties are involved in ECRF suppression.
KEYWORDS

contextual modulation; size tuning; extraclassical receptive field; surround suppression

INTRODUCTION

In the primary visual cortex (V1), neurons exhibit tuning properties for stimulus features such as orientation, direction, spatial and temporal frequencies, and stimulus size. The area evoking the maximal response is defined as the classical receptive field (CRF). The CRF response is generally reduced when a stimulus extends to the extraclassical receptive field (ECRF) outside the CRF, while ECRF stimulation alone fails to elicit a spike response. This suppressive response modulation is termed surround suppression, or ECRF suppression (Allman et al. 1985; Akasaki et al. 2002; DeAngelis et al. 1994; Ishikawa et al. 2010; Ozeki et al. 2004).

ECRF suppression is a good model for examining how both local signals within the CRF and global signals beyond the CRF are spatially and temporally integrated within the hierarchy of visual information processing. The strength of ECRF suppression depends on the stimulus context; that is, the relationship of figural features (stimulus-feature specificity) and spatial configurations (Akasaki et al. 2002; DeAngelis et al. 1994; Li & Li 1994; Mizobe et al. 2001) between the CRF and ECRF stimuli. For example, when the CRF and ECRF are stimulated with drifting sinusoidal gratings independently, the suppressive effects are stronger when the grating parameters (orientation, spatial frequency, direction, etc.) of the CRF and ECRF stimuli are similar.
to each other (Akasaki et al. 2002; DeAngelis et al. 1994; Knierim and van Essen 1992; Nothdurft et al. 1999). Stimulus contrast also influences the strength of ECRF suppression, where ECRF suppression becomes stronger as the stimulus contrast increases (contrast dependency) (Levitt and Lund 1997; Polat et al. 1998; Sadakane et al. 2006; Sceniak et al. 1999; Sengpiel et al. 1998; Wang et al. 2009). Because of this stimulus-feature specificity and contrast dependency, it has been proposed that ECRF suppression plays important roles in perceptual figure-ground segregation and saliency (Akasaki et al. 2002; Knierim and van Essen 1992; Li et al. 2000; Nothdurft et al. 1999, 2000), and the gain control of the neuronal response (Carandini M & Heeger 1994; Sengpiel et al. 1998).

The neuronal mechanisms underlying ECRF suppression remain unclear and controversial. Currently, there are three network mechanisms being considered: 1) a reduction of excitatory thalamocortical inputs due to ECRF suppression in the LGN (Alitto & Usrey 2008; Bonin et al. 2005; Jones & Sillito 1991; Li & He 1987; Naito et al. 2007; Ozeki et al. 2004; Sadakane et al. 2006; Solomon et al. 2002; Sun et al. 2004; Webb et al. 2005) or retina (Alitto & Usrey 2008; Enroth-Cugell et al. 1983; Li et al. 1991; Passaglia et al. 2001; Solomon et al. 2006), 2) a decrease of excitatory inputs and/or increase of inhibitory inputs due to short-range and long-range lateral connections within V1 (Adesnik et al. 2012; Anderson et al. 2001; Angelucci et al. 2002; Cavanaugh et al. 2002b; Das & Gilbert 1999; DeAngelis et al. 1994; Hashemi-Nezhad & Lyon 2011; Haider et al. 2010; Levitt & Lund 2002; Ozeki et al. 2009; Sceniak et al. 2001; Walker et al. 1999, 2002), and 3) feedback connections from
higher-order areas (Angelucci et al. 2002, Angelucci & Bressloff 2006; Bair et al. 2003; Bullier et al. 2001; Levitt & Lund 2002; Ozeki et al. 2009; Schwabe et al. 2006). These three models are not mutually exclusive, because recent studies examining the spatiotemporal properties of ECRF suppression by drifting grating showed that a single network mechanism struggles to explain the widely varying aspects of ECRF suppression (Briggs and Usrey 2011; Liu et al. 2011; Webb et al. 2005). Those studies also suggested that the origin of ECRF suppression varies depending on the experimental conditions.

If multiple mechanisms are involved in a single suppressive phenomenon, we first need to distinguish the different components of ECRF suppression and then assess the neural circuits responsible for each component. We recently showed that the time course of the spatial property of ECRF suppression could help solve the first problem. The spatial-frequency tuning of ECRF suppression in V1 changes temporally from low-pass type to band-pass type, a transition that can be well explained by the spatial-frequency properties of subcortical and cortical circuits (Ishikawa et al. 2010). This change suggests that the origins of ECRF suppression in V1 change from subcortical to cortical networks over time. To confirm this hypothesis, we examined the temporal dynamics of tuning properties to stimulus size and orientation of ECRF suppression by using stationary flashed sinusoidal grating.

In the present study, we show that there are at least three components of ECRF suppression in V1: a local and fast component, a global and fast component, and a global and late component. Here we define the global components as suppression that
accumulates over space and therefore is strongest for the largest stimuli. The spatial extent of the local component in V1 matched well with that of LGN neurons, but that of the global components was fairly larger than those two. Moreover, the orientation selectivity of ECRF suppression in V1 was weaker in the local component than in the global ones, and corresponded to that of the LGN. Taking into account 1) the strong similarity of the local ECRF component in V1 to ECRF suppression in the LGN and 2) that the spatiotemporal properties of the global components of V1 are distinct from that of the LGN, we conclude that local and global components of ECRF suppression originate from subcortical and cortical mechanisms, respectively.
MATERIALS AND METHODS

The experimental protocol was approved by the Research Ethics Committee of Osaka University. All animal procedures were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (1996) and the Guidelines of the Animal Care Committee of the Osaka University Medical School. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Animal Preparation

Details of the experimental protocols for animal preparation and electrophysiological recording have been described previously (Ishikawa et al. 2010). Adult cats \(n = 15\) weighing 2–3.5 kg were used in this study. Dexamethasone (Decadron-A; Banyu Pharm., Tokyo, Japan) was injected (0.1 mg/kg, i.m.) 24–36 h before the start of the experiments. Atropine (0.02 mg/kg, i.m.) was injected 20 min before surgery. Animals were anesthetized using a mixture of isoflurane (Forane; Abbott Japan, Tokyo, Japan; 1–4%) and \(\text{N}_2\text{O} : \text{O}_2 \ (2 : 1)\). The electroencephalogram, electrocardiogram, and heart rate were continuously monitored throughout the experiment. The animals were mounted in a stereotaxic head holder, continuously paralyzed with pancuronium bromide (0.1 mg/kg/hr, i.v.) to minimize eye movements, and maintained under artificial ventilation. A craniotomy (3–4 mm wide) was made over area 17 (V1) and the LGN. The exposed cortical surface was covered with agar gel (2% in saline) after inserting an electrode into the brain. The nictitating membranes were
retracted using ophthalmic phenylephrine, and the pupils were dilated with ophthalmic atropine. The eyes were focused onto a CRF display 56 cm away using corrective, gas-permeable contact lenses. During the recording of neuronal activity, isoflurane was reduced to 0.2–0.4% in N₂O : O₂ (2 : 1), and then fentanyl citrate (Fentanest; Sankyo, Tokyo, Japan; 10 μg/kg/hr, i.v.) and droperidol (Droleptan; Sankyo; 125 μg/kg/hr, i.v.) were continuously infused through the femoral vein catheter to induce neuroleptanalgesia. Sodium pentobarbital (1–2 mg/kg/h, i.v.) was supplemented to the infusion solution when the animal's heart rate exceeded 240 beats/min or when it changed by more than 10% upon firmly pressing the ear skin of the animal. The rectal temperature was maintained at or near 38 °C by a thermostatically controlled heating pad. The end-tidal CO₂ was adjusted to 3.5–4.0%.

**Physiological recordings**

Extracellular single-unit recordings were performed in V1 and the LGN with a tungsten-in-glass microelectrode (Levick 1972). The retinal eccentricity of the recorded CRFs was within 14° of the area centralis representation. Signals were amplified and filtered for spike activity using an AC amplifier (0.3–3 kHz; Model 1800; AM systems, USA). The single-unit activity was isolated by using a template-matching spike sorter (Multi Spike Detector, Alpha-Omega, Israel). The shape of the action potentials was continuously inspected to ensure that the same neurons were recorded throughout the recording period. Digital pulses by the template matching were acquired using a time-stamping board (Lisberger Tech., San Francisco, USA) at a sampling rate of 1
Visual stimulation and Characterization of receptive field properties

When a V1 neuron was isolated, the basic CRF properties were determined using a flashing or moving slit, drifting grating, and stationary grating. First, the minimum response field and its center were initially mapped onto a tangent screen placed 57 cm in front of the eyes of the cats by using a hand-held projector (Barlow et al. 1967). Each neuron was stimulated monocularly through the dominant eye. The preferences to slit light stimulus, such as bar orientation, moving direction and velocity, length, width, and the segregation of ON- and OFF- subregions, were manually assessed (Hubel and Wiesel 1962).

Next, quantitative analysis of the CRF was performed using drifting and stationary sinusoidal grating patches. The grating stimuli were generated by a VSG2/3F graphic board (Cambridge Research Systems, Rochester, UK) and displayed on a Sony 21-inch CRT monitor (CPD-G500J; Sony, Japan; mean luminance, 30 cd/m²; screen size, 40 × 30 cm²; non-interlaced refresh, 100 Hz; resolution, 1024 × 768 pixels). Nonlinearities in phosphor output were corrected by lookup tables. Parameters of grating including orientation, spatial frequency (SF), spatial phase, temporal frequency (TF), contrast, and size were controlled independently.

The drifting gratings were presented for 2 sec in a pseudo-random sequence for each parameter dimension, and each stimulus presentation was interleaved for 2–4 sec with a blank screen with the same mean luminance (30 cd/m²) as the gratings. Temporal
profiles of the responses to grating stimuli were displayed on-line as a raster plot and a peristimulus time histogram (PSTH). Concurrently, the total number of spikes evoked for the CRF stimulation period (mean response) was plotted as a parameter-tuning curve along a given dimension using custom-made software to examine the optimal values for each stimulus parameter.

Subsequently, CRF characterization was performed using stationary gratings. Stationary gratings were presented for 500 ms in a pseudo-random sequence for each stimulus parameter, and each stimulus presentation was done at various inter-trial intervals ranging between 2 and 4 s. Optimal parameters were determined according to the response tuning curves constructed for individual parameters. The contrast of the grating stimulus was determined as the luminance contrast eliciting sub-saturating responses (50–80% of maximal response) within the linear range of the contrast-response relationship. The same procedures were applied to LGN neurons except for additional CRF characterization specific to LGN neurons.

**Examination of ECRF suppression**

Following the preliminary CRF measurements, we conducted two types of main tests: (1) a size tuning test and (2) an orientation-contrast test. In the size tuning test, the response of each neuron was measured by pseudo-randomly changing the stimulus size (11 sizes ranging 0–10° in radius) of the stationary grating patches with optimal orientation, SF, and spatial phase. The grating stimuli were presented for 500 ms.
In the orientation-contrast test, a neuron’s CRF was stimulated with a circular sinusoidal grating using optimal parameters, and the ECRF was stimulated with an iso- or cross-oriented annular sinusoidal grating (ECRF stimulus). The outer radius of the ECRF annulus stimulus was 10° or sufficiently larger than the outer edge of the neuron’s CRF, and the inner radius of the ECRF stimulus was the CRF radius. The CRF and ECRF stimuli were presented simultaneously and disappeared after 500 ms and 50 ms, respectively. We adopted this stimulus condition based on our recent finding (Ishikawa et al. 2010) that the suppressive effect of ECRF stimulus is summed temporally, and that shortening the presentation duration is advantageous for enhancing the difference of the feature-dependent persistency of suppression. The stimulus condition does not affect our conclusion, because the characteristic features of the orientation-selectivity of ECRF suppression and its temporal dynamics in individual cells were already observed before the offset of the ECRF stimulus.

In both size tuning and orientation-contrast tests, each stimulus condition was tested by at least more than 10 repetitions.

Data analysis

Cell classification

V1 cells were classified as either simple or complex according to the spatial segregation of ON/OFF subregions (Hubel and Wiesel 1962) and the ratio of the first harmonic (F1) and the DC (F0) of the peristimulus time histogram (PSTH) of the response to a drifting grating patch with optimal temporal frequency, SF, and orientation
LGN cells were classified as X or Y type cells on the basis of commonly used criteria, that is, the pattern of the response to a standing contrast (Cleland et al. 1971) and the linear summation of the excitation (Enroth-Cugell and Robson 1966; Hochstein and Shapley 1976). We analyzed only X type cells because the sustained response, which is characteristic to the responses of X cells to stationary stimuli, was ideal for examining the temporal dynamics of ECRF suppression.

**Strength of ECRF suppression**

To quantify the strength of the ECRF suppression, we calculated a suppression index (SI) according to the following equation:

\[ SI = 1 - \frac{R(CRF + ECRF)}{R(CRF)}, \]

where \( R(CRF) \) and \( R(CRF + ECRF) \) are the responses to CRF stimulation alone and to combined stimulation of the CRF and ECRF, respectively. An SI value of 1 means complete suppression; 0, no suppression.

**Size tuning curve analysis**

To compare the spatial properties between V1 and LGN neurons quantitatively, we estimated the CRF radius and ECRF radius by fitting the size tuning curves.
The CRF radius was estimated by a ratio of Gaussians (RoG) model (Cavanaugh et al. 2002a; Zhang et al. 2005).

\[ R(x) = R_0 + K_c L_c(x) / [1 + K_i L_i(x)], \]

where, \( L_c(x) = [\sigma_c * \text{erf}(x/\sigma_c)]^2 \), \( L_i(x) = [\sigma_i * \text{erf}(x/\sigma_i)]^2 \).

Here, \( R_0 \) is the spontaneous rate, \( x \) is the stimulus diameter, and \( K_c \) and \( K_i \) respectively represent the amplitude of the center and surround Gaussians. The spatial extents of the center and surround Gaussians are represented by \( \sigma_c \) and \( \sigma_i \). During curve-fitting, we always constrained the functions so that \( \sigma_c < \sigma_i \). Values of \( K_c \), \( K_i \), \( \sigma_c \), and \( \sigma_i \) were optimized to provide the least squared error fit to the data. Fitting procedures of the stimulus-size tuning curve were done with the MATLAB optimization toolbox using the FMINCON nonlinear least-squares function. The CRF radius was determined as the stimulus radius at which the response of the fit was maximal.

The outer boundary of the ECRF (ECRF radius) was estimated by fitting the descending limb of the size tuning curve with a Gaussian function (Ozeki et al. 2004):

\[ R(x) = Ke^{-(x-a)^2/b^2} + d, \quad x \geq a, \]

where \( R \), \( x \), and \( a \) are the firing rate, stimulus radius, and CRF radius, respectively, \( b \) is the space constant of the Gaussian function fitted to the data outside the CRF radius \( (x \geq a) \), and \( K \) and \( d \) represent the amplitude of the Gaussian function and offset of the asymptotic response from spontaneous activity, respectively. The
stimulus radius that evoked 95% maximal ECRF suppression was defined as the ECRF radius. We also calculated the ECRF width by subtracting the CRF radius from the ECRF radius.

The reason for using the different fitting functions to determine the CRF and ECRF was the quality of the fits. Since the spatial extent of the ECRF is estimated to be the visual field within which the suppressive effect is spatially accumulated, the accuracy of the measurements strongly depends on precisely fitting the descending limb (downslope) of the size tuning curves. The Gaussian function is well suited to fit the downslope. The fitting process is completely independent of the upslope, and therefore provides a much better fit.

To quantify how well the above functions fit the data, we estimated the error between the empirical model and actual data. The error was calculated as the mean fractional error (Sceniak et al. 2002):

\[
E = \frac{1}{N} \sum_{j=1}^{N} \frac{(\text{theory}_j - \text{data}_j)^2}{\left(\frac{1}{N} \sum_{j=1}^{N} \text{theory}_j\right)^2}
\]

To compare fits for models with different numbers of degrees of freedom, we used the normalized E value, \( E_N \) (Hoel et al. 1971, Cavanaugh et al. 2002a):

\[
E_N = \frac{E}{df},
\]

where \( df \) was the number of degrees of freedom in the model.
Local suppression

To assess the relative strength of near ECRF suppression derived from less than 1° outside the CRF in V1 neurons, we calculated “% near ECRF effect” according to the following formula:

\[
\% \text{ near ECRF effect} = \frac{\text{SI}_{\text{near}}}{\text{SI}_{\text{mean}}} \times 100, 
\]

where \(\text{SI}_{\text{near}}\) is the SI calculated from the response to a stimulus 0.7–0.8° larger than the CRF in radius, and \(\text{SI}_{\text{mean}}\) indicates the mean value of SIs obtained from responses to all stimuli larger than the CRF. The mean value but not maximal value of the SI was used to minimize the influence of the data variance of a single data point on % near ECRF effect. This analysis was applied only for early responses within 80 ms after the stimulus onset in which a certain population of V1 neurons showed strong suppressive effects of near ECRF stimulation.

Temporal profile of suppression

Onset latency of ECRF suppression (suppression latency) was estimated according to the method by Müller et al. (2003). Cumulative spike counts histograms were constructed as the time course of responses to CRF and CRF + ECRF stimulations, and the difference was taken. The onset latency was defined as the time at which the differential histograms started to deviate continually for more than 40 ms downward from 0.

Orientation selectivity of ECRF suppression
To quantify the orientation selectivity of the ECRF suppression and its temporal dynamics, we compared the first 150 ms responses to combined stimulations of the CRF and ECRF with cross-oriented and iso-oriented gratings, and determined less and more effective ECRF orientations for each cell. We then calculated the orientation selectivity index (OSI) according to the following equation:

$$\text{OSI} = \frac{[(\text{less suppressed responses}) - (\text{more suppressed responses})]}{\text{CRF response}},$$

where less and more suppressed responses are responses to more and less effective ECRF orientations, respectively.

**Statistical analyses**

The total response evoked during stimulus presentation in the size tuning test were first analyzed by the Kruskal-Wallis test to examine the statistical significance of the suppressive effect of ECRF stimulation on the CRF response. Neurons showing significant ECRF suppression were analyzed further for the spatiotemporal properties of ECRF suppression by the Kruskal-Wallis test, Steel-Dwass test (Steel 1960; Dwass 1960), and Friedman test. A Tukey’s test for the equality of proportions was used for statistical analysis of the laminar distribution of each type of ECRF suppression.

**Electrolytic lesions and histology**

At the end of each penetration, three to four electrolytic lesions were produced by applying tip-negative currents (3–4 µA for 10 s) along the length of each electrode penetration. After the recording experiments, the animals were deeply anaesthetized.
with sodium pentobarbital (50 mg/kg, i.v.) and perfused transcardially with buffered saline (pH 7.4) followed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Blocks of the occipital cortex were cut out and immersed in 30% sucrose in PBS for 48 h. Sixty-micrometer-thick frozen coronal sections were sliced on a microtome and kept in PBS. Sections were stained with cresyl violet or cytochrome-oxidase staining (Wong-Riley 1979). The laminar location of the recording sites was then identified under a light microscope. Figure 1 A–C exemplify micrograms of three serial sections stained with cytochrome oxidase and three electolitic lesions (white arrows). Shrinkage of the cortical tissues was corrected by multiplying the ratio of the measured dye mark distance with the value expected from the micrometer reading. Cortical layers were classified into layers II/III (supragranular layer), layer IV (granular layer), and layers V/VI (infragranular layer) based on Otsuka and Hassler (1962).
RESULTS

A total of 120 V1 cells and 32 X type LGN cells were extracellularly recorded from adult cats. Of these, 90 V1 cells and 19 LGN cells were recorded stably for more than two hours. We first analyzed the size tuning of these cells for the statistical significance of ECRF suppression using the Kruskal-Wallis test, in which CRF responses and responses to stimuli larger than the CRF (CRF + ECRF) were compared for the total number of spikes evoked during a 500 ms presentation of stationary grating patches. Significant ECRF suppression was observed in 62 V1 cells (simple cell, n = 32; complex cell, n = 30) and 17 LGN cells. Temporal dynamics of the spatial extent and orientation selectivity of ECRF suppression were analyzed further for cells showing significant suppression.

Three types of ECRF suppression in V1

In the size tuning test, individual V1 neurons exhibited varying magnitudes and time courses in their visual responses to grating stimuli of varying sizes. To extract common spatiotemporal features of the ECRF suppression, we first observed the time courses of the ECRF suppression by subtracting the PSTH of the CRF response from the PSTHs of individual CRF + ECRF responses bin-by-bin over time (differential PSTHs). By surveying the temporal dynamics of ECRF suppression in relation to stimulus size, we found that ECRF suppression can be classified into three types based on the characteristics of the suppression of the early CRF response, which is defined as within approximately 80 ms of the stimulus onset. These characteristics include the
presence or absence of early ECRF suppression and the degree and onset latency of the suppressive effect of local ECRF stimulation when the ECRF stimulus is slightly larger than the CRF. Below, we first demonstrate two distinct types with early ECRF suppression (Fig. 1 D–G), and then describe a third type without early ECRF suppression.

Figure 1 exemplifies two simple cells showing ECRF suppression with distinct spatiotemporal properties. The time courses of the visual responses and ECRF suppression are depicted as line graphs of the PSTHs (D and E) and differential PSTHs (F and G), respectively. Each neuron was stimulated with stationary sinusoidal grating patches using optimal parameters (orientation, SF, and phase) and varying sizes. The CRF radii of Cell-1 and Cell-2 were 1.7° and 2.5°, respectively, and the CRF responses are indicated in red and magenta thick lines in D and E. The other thin lines indicate responses to stimuli of varying sizes (CRF + ECRF), all larger than the CRF. Both cells exhibited a similar temporal pattern of the CRF response in which a phasic response peaked at 100 ms, followed by a sustained response with gradual decay. Both CRF responses were reduced when the stimulus was presented beyond the CRF, but the time course of ECRF suppression and the relationship between stimulus size and strength of suppression (size tuning property) were largely different between the two cells. The characteristic features were clearly observed in early suppression within 80 ms after stimulus onset.

In the size tuning test, the ECRF is defined as the visual field within which the suppressive effect is spatially accumulated, and the outer boundary of the ECRF is
determined as the stimulus size beyond which no additional accumulation of the suppressive effect occurs. Therefore, the temporal dynamics of the spatial extent of the ECRF are reflected in a stimulus-size-dependency of response reduction (suppressive effect) in the PSTHs and differential PSTHs. No or weak size-dependency of response suppression means localization of the ECRF. On the other hand, size-dependency over a wide range means largeness of the ECRF.

ECRF suppression in Cell-1 was characterized by early and spatially localized suppression (< 80 ms, pale yellow zones in Fig. 1D and F) followed by late and spatially summating global suppression (> 80 ms, uncolored zones). In the pale yellow zone, all CRF + ECRF stimuli including even the 2.5° radius, which was only 0.8° larger than the CRF radius, caused transient and strong suppression followed by sustained suppression of various degrees. The suppression bottomed out at about 60 ms to almost the same extent (81.8 ± 4.1% decrement for all CRF + ECRF stimuli, 83.3% for the 2.5° radius, Fig. 1 D and F), and showed no size-dependency. This suggests that in Cell-1 the stimulation of the local region near the boundary of the CRF was enough to evoke almost maximal suppression. In this study, the suppression that originated from the local region near the CRF is referred to as a local component of the ECRF (local ECRF suppression).

Eighty milliseconds after stimulus onset, the suppressive effects weakened for all CRF + ECRF stimuli, and the neuronal responses started to recover toward the level of the CRF response, as seen in the white zones of Fig. 1D and F. It should be noted that the response recovery and the subsequent sustained responses strongly depended on
stimulus size. The size-dependency of ECRF suppression suggests that the ECRF suppression was generated from a wide visual field and the suppressive effects were spatially summated. Taken together with the early and local ECRF suppression, our results suggest that the spatial extent of ECRF suppression was not temporarily constant, rather it enlarged progressively with the time course of the response. The suppression originating from a wide-range ECRF is referred to as the global component of ECRF suppression (global ECRF suppression). We call the cells showing early local suppression and late global suppression type I cells.

On the other hand, Cell-2 was characterized by global suppression from the first 80 ms (pale yellow zones in Fig. 1E and G) in which not only the strength of suppression but also the onset latency depended strongly on stimulus size. When stimulus size was increased by only 0.9º from the CRF radius (2.5º) to 3.4º, the stimulation caused a weak and delayed ECRF suppression with an onset latency of about 90 ms. As the stimulus size was enlarged further, the magnitude of suppression increased progressively and the onset latency of suppression became shorter to nearly the onset latency of the CRF response (Fig. 1G). Thus, ECRF suppression exhibited a clear size-dependency from the early time window (pale yellow zones in Fig. 1E and G). The size-dependent decrease of the response was observed in a wide area of more than 5º in radius, and it continued until at least 200 ms from the stimulus onset. We call the cells showing global suppression from this early time window type II cells.

The ECRF suppression of the type I cell (Cell-1) was composed of early local suppression and late global suppression. In contrast, the type II cell (Cell-2) showed
only global suppression with size-dependent onset latency. Thus, the distinctive
difference between the two types of ECRF suppression was the spatiotemporal
properties of early suppression, especially the suppressive effect of ECRF stimulation
near the CRF.

There was a third type (type III) of ECRF suppression, which had distinct
spatiotemporal properties from types I and II cells, although it was not frequently
observed. This type was characterized by a late global suppression without early
suppression. Thus, type III can be discriminated by the absence of early suppression. We
describe an example response of a type III cell in a later section (see Fig.4).

Type III cells may represent the possibility that non-suppressed cells categorized
on the basis of the total responses during the 500 ms visual stimulation show significant
ECRF suppression in the early time window. To confirm this point, we analyzed the
statistical significance of ECRF suppression during an early time period (0–80 ms) for
non-suppressed cells, but found no significant suppression.

Spatiotemporal properties of ECRF suppression in V1

We found that three types of ECRF suppression can be distinguished qualitatively
by the presence or absence of early ECRF suppression and its spatiotemporal properties.
Therefore, we performed quantitative cell classification according to the nature of early
ECRF suppression. First, we tested the statistical significance of early suppression by
comparing CRF only and CRF + ECRF responses in the first 80 ms following stimulus
onset (Steel-Dwass test). Most cells (51/62 cells, 82%) showed significant suppression.
The cells without significant early (< 80 ms) suppression were classified as type III. Next, we quantified the spatial and temporal properties of the early suppression to distinguish between types I and II cells. Type I cells are characterized by fast and localized suppression adjacent to the boundary of the CRF, whereas type II cells show global suppression with size-dependent onset latency. Therefore, we evaluated the relative strength of the suppressive effect with local ECRF stimulation to the mean strength of suppression with all ECRF stimulations as a “% near ECRF effect” (see Material and Methods). We also calculated the difference of the onset time between the CRF response and ECRF suppression evoked by stimulation of the ECRF near the CRF (near ECRF suppression) for each cell as an “Onset delay of near ECRF suppression”, in which the onset time of near ECRF suppression was calculated by averaging the onset times for the first and second smallest stimulus sizes among stimulus sizes larger than the CRF size. The scatter plot of “Onset delay of near ECRF suppression” versus “% near ECRF effect” (Figure 2) demonstrates that two cell populations are clearly distinguishable by these parameters. Therefore, cells with % near ECRF effect values larger than or equal to 55% and with Onset delay of near ECRF suppression smaller than 20 ms were classified as type I, and those less than 55% and larger than 20 ms as type II. Although there appears to be a continuum of their response properties, for the remainder of the paper we distinguish the two cell types using the above definitions so as to reveal important aspects of the subsequent analyses. As a result, of the 62 V1 neurons studied, 28 were classified as type I (simple, 17; complex, 11), 23 as type II (simple, 12; complex, 11), and 11 as type III (simple, 3; complex, 8). Since there was no
classification bias for simple and complex cells, both types of cells were pooled for the following analyses.

In our sample, a few type II cells showed moderate % near ECRF effect with small Onset delay of near ECRF suppression (Fig. 2). This result may suggest a contribution of local components in early ECRF suppression. Although this possibility cannot be excluded, the subsequent population analyses demonstrate the influence is marginal in the population data.

The characteristic features of the spatiotemporal profiles of the ECRF suppression observed in the example cells in Fig. 1 were also observed at the cell population level. We constructed a population average of PSTHs and differential PSTHs for each cell type and each cell’s CRF size. Figure 3 shows examples of the PSTHs constructed from cells with a CRF of 1.7º and 2.5º, common sizes in our sample. Type I cells (Fig. 3A, C, E, and G) showed early local suppression insensitive to stimulus size and later size-dependent global suppression, and their spatiotemporal features of ECRF suppression was not different between simple and complex cells (data not shown).

In contrast, type II cells (Fig. 3B, D, F, and H) exhibited a clear global suppression throughout the response period. Thus, the spatiotemporal properties of ECRF suppression on the population average in each cell type resembled those of single cells, suggesting that cells can be successfully classified according to the characteristic features of the ECRF suppression. Population averages of the PSTHs and differential PSTHs were not constructed for type III cells because of the small number of cells.

Figure 4 illustrates examples of type I (Fig. 4A; Cell-3 and Cell-4), type II (Fig.
499 4B; Cell-5 and Cell-6), and type III (Fig. 4C; Cell-7 and Cell-8) cells. The responses for
500 the three stimulus conditions, CRF only, CRF + ECRF with smallest ECRF (local ECRF
501 stimulus), and CRF + ECRF with largest ECRF (global ECRF stimulus), as determined
502 from a set of stimulus sizes tested, are respectively illustrated as PSTHs (top) and
differential PSTHs (bottom). Each cell shows characteristic features of each type of
504 ECRF suppression. Type I cells (Fig. 4A, Cell-3 and Cell-4) displayed a local
505 suppression in the early time window (0–80 ms, gray zone in each histogram), in which
506 the ECRF stimulation of radius 2.5°, which is only 0.8° larger than the CRF size, caused
507 a suppressive effect equivalent to the largest ECRF stimulation tested (radius 10°) in
508 magnitude and time course. Thereafter, the suppressive effect by local and global ECRF
509 stimulations began to dissociate from each other with time, indicating the addition of
global suppression. Type II cells (Cell-5 and Cell-6) showed global suppression
510 throughout the response. Although local ECRF stimulation evoked suppression, the
512 strength was substantially weaker than the global ECRF stimulation over the response
513 period. Different from types I and II cells, type III cells (Cell-7 and Cell-8) exhibited a
514 delayed onset of global ECRF suppression only. ECRF suppression began no earlier
515 than 50 ms after stimulus onset and the strength of the late suppression was strongly
516 dependent on the stimulus size.

The spatiotemporal properties of ECRF suppression should reflect the nature of
the neuronal circuits engaged. To better understand the circuit mechanisms responsible
for ECRF suppression, we quantitatively examined the temporal and spatial profiles of
ECRF suppression for each cell type.

26
We analyzed the onset latencies of ECRF suppression (suppression latency) and CRF response (CRF latency). Figure 5 shows the relationship between suppression latency and stimulus radius. This figure also contains results for LGN neurons, which we describe in the next section, “Spatiotemporal properties of ECRF suppression in the LGN”. There was no significant difference in the CRF latency among type I, II, and III cells (type I, 36.3 ± 1.8 ms, N = 28; type II, 35.3 ± 1.7 ms, N = 23; type III, 38.3 ± 4.3 ms, N = 11; $P = 0.67$, Kruskal-Wallis test). On the other hand, suppression latency and its dependency for stimulus-size showed marked differences among the three types.

Type I cells are characterized by ECRF suppression of early local suppression followed by late global suppression. Therefore, the suppression latency of type I cells reflects the nature of the local component of ECRF suppression. Type I cells also showed a fast onset of ECRF suppression, beginning with a slight delay (5.4 ± 1.0 ms, mean ± SEM) relative to the onset of the CRF response. The suppression latency was almost constant regardless of stimulus size, which showed no significant differences ($P = 0.83$, Kruskal-Wallis test). This result suggests that stimulation of a local ECRF close to the boundary of the CRF evokes a fast suppressive effect. In contrast, the suppression latency of type II cells dramatically changed in an inverse relationship with stimulus size ($P < 0.01$, Kruskal-Wallis test). As stimulus radius was increased from 2.5° to 10°, the suppression latency was shortened from 94.9 ± 14.5 (SEM) ms to 44.9 ± 3.4 ms (radius 2.5°, 3.3°, 5° vs. 10°, $P < 0.01$; 2.5°, 3.3° vs. 6.7°, 8.3, $P < 0.05$, Steel-Dwass test), and the delay of the onset of the CRF response was reduced from 58.6 ± 8.7 ms to 9.8 ± 2.4 ms. Type III cells exhibited a much longer suppression latency (109.3 ± 9.7 ms,
mean ± SEM), which was significantly longer than that of types I and II cells at any
stimulus size except for 2.5° (P < 0.01, Steel-Dwass test). The suppression latency was
delayed on average 70.9 ± 11.6 (SEM) ms from the onset of the CRF response.

Next, we quantified the spatial extents of the CRF radius, ECRF radius (the
distance from the center of the CRF to the outer edge of the ECRF), ECRF width (the
width between the outer edges of the CRF and ECRF), and their temporal dynamics.
The parameters “ECRF width” and “ECRF radius” provide important information for
validating the subcortical and cortical mechanisms from the spatial aspect, respectively.
If ECRF suppression in V1 is fully attributed to that in the LGN, the suppression of V1
cells should inherit the spatial property of the ECRF suppression of LGN neurons
whose CRFs constitute the margin but not the center of the CRF of the V1 cells. Thus,
the ECRF width should be similar between LGN neurons and V1 neurons. On the other
hand, if neurons with CRFs larger than V1 cells (Adesnik et al. 2012), including
neurons in higher order areas contribute to ECRF suppression in V1, the CRF radius of
those neurons should be similar to the distance from the center of the CRF to the outer
edge of the ECRF (ECRF radius) of V1 neurons.

Figure 6A demonstrates a typical example of the temporal change of the ECRF
size from the type I cell (Cell-1) depicted in Fig. 1D. Size tuning curves were
constructed using data of stimulus sizes equal to and larger than the CRF, where the
CRF radius was constantly 1.7° over time. The size tuning curves were fitted by a
Gaussian function. Arrows indicate the estimated outer boundary of the ECRF (ECRF
radius). In this cell, the ECRF size distinctively expanded from 60 ms after the stimulus
Figure 6B, C, and D show population data for the CRF radius, ECRF radius, and ECRF width plotted over time, respectively. The CRF radius did not temporarily change for type I and II cells, but shrank for type III cells (Fig. 6B: type I, $P = 0.07$; type II, $P = 0.92$; type III, $P < 0.05$, Friedman test). The CRF radius of type I and II cells was almost constant at about 2° until at least 150 ms after stimulus onset. Type III cells showed a significantly larger CRF radius in their early CRF response than type I and II cells ($P < 0.01$, Kruskal-Wallis test), a radius that then shrunk near the size of type I and II cells.

The spatial extent of the ECRF exhibited different temporal profiles from that of the CRF. Type I cells showed a significant temporal expansion of the ECRF radius and width (Fig. 6C and D, $P < 0.01$, Friedman test), especially from 30 ms through 120 ms (30 ms, 40 ms vs. 120 ms, 130 ms, $P < 0.05$, Steel-Dwass test). Thus, a transition from local suppression to global suppression occurred within 120 ms after stimulus onset. During that time, the ECRF radius increased from $4.52 \pm 0.40°$ (mean ± SEM) to $7.19 \pm 0.52°$, and the ECRF width from $2.03 \pm 0.32°$ to $4.08 \pm 0.50°$ (30 ms vs. 100 ms, 130 ms, $P < 0.05$, Steel-Dwass test). On the contrary, type II cells did not exhibit clear temporal changes in either the ECRF radius ($P = 0.10$, Friedman test) or ECRF width ($P = 0.06$, Friedman test). Compared with the early and local suppression of type I cells, these parameters in type II cells were considerably larger from the beginning of suppression (ECRF radius, $7.42 \pm 0.42°$; ECRF width, $5.13 \pm 0.43°$) and lasted throughout the time window analyzed. Both the ECRF radius and width were...
significantly different between type I and type II cells until 90 ms after stimulus onset 
($P < 0.05–0.01$, Wilcoxon test). The delayed ECRF suppression in type III cells also 
exhibited a large ECRF radius that did not change temporally. It should be noted that the 
ECRF radius and width were almost the same for all three types of V1 neurons after 130 
ms of the stimulus onset, suggesting that late and global components of ECRF 
suppression possess similar spatial properties in all V1 neurons.

Spatiotemporal properties of ECRF suppression in the LGN

One possible neuronal mechanism underlying the ECRF suppression in V1 is a 
reduction of excitatory geniculocortical inputs owing to subcortical ECRF suppression. 
If this is the case, spatiotemporal profiles of ECRF suppression in V1 and LGN should 
resemble each other. To examine this point, we performed the size tuning test on LGN 
neurons (N = 17 cells) with basically the same stimulus conditions as V1.

Figure 7 represents an example of an X-type cell showing typical 
spatiotemporal patterns of ECRF suppression in the LGN. The cell’s CRF radius was 
0.7º, and the CRF stimulation caused a phasic and subsequent sustained response (red 
thick line in Fig. 7A). The CRF response was strongly suppressed to 57.8% with a 
grating patch slightly larger than the CRF (radius 2.0º) throughout the response period 
and was reduced from almost the beginning of the response. As the stimulus size was 
enlarged, additional suppression was not observed (43.14 – 60.14% of CRF response, 
52.16 ± 2.48%, mean ± SEM), suggesting that the suppression originates from the 
ECRF neighboring the CRF (local suppression). Like the local suppression of type I V1
neurons, the suppressive effect began quickly and lagged the CRF latency only slightly (Fig. 7C, 4.9 ± 0.7 ms, mean ± SEM), and the suppression latency was insensitive to stimulus size. However, unlike type I V1 cells, the spatial extents of the ECRF radius and width did not change temporally, and the ECRF width remained about 2º (Fig. 7D). These results suggest that the ECRF stimulation of LGN neurons evokes local suppression only.

The population data of the onset latency and the spatial extent of the ECRF suppression are presented with data of V1 neurons in Fig. 5 and Fig. 6, respectively (filled squares). To examine whether the ECRF suppression of LGN neurons is inherited to that of V1 neurons, we needed to compare the spatiotemporal properties of the ECRF suppression between the two areas. Since absolute values of CRF latency and size are longer and larger respectively in V1 than LGN, which reflects a hierarchical processing of excitatory inputs with spatial convergence from LGN to V1, the spatiotemporal properties of suppression relative to that of CRF responses were suitable for comparing the two areas without regard for inter-areal transmission delay or differential CRF size. Therefore, we mainly dealt with the difference between CRF latency and suppression latency (latency difference) and the ECRF width from the boundary of the CRF.

The population analyses of the LGN neurons are represented by the features of ECRF suppression of the neuron (Cell-9) shown in Fig. 7. The suppression latency slightly lagged the CRF latency (Fig. 5), a phenomenon similar to that of type I V1 neurons (LGN, 7.6 ± 0.8 ms; type I V1, 5.8 ± 0.7 ms, mean ± SEM). Moreover, the suppression latency of LGN neurons was only slightly sensitive to the stimulus radius.
(Fig. 5), which is also consistent with type I neurons. Thus, the temporal properties of ECRF suppression between LGN and type I neurons are similar, suggesting that ECRF suppression in the LGN originates from the local ECRF and is inherited to cortical neurons as local ECRF suppression. If this is the case, the ECRF width outside the boundary of the CRF should be similar between LGN and type I cells. As shown in Fig. 6D (filled squares, LGN; filled circles, type I), the ECRF width of both groups begins from the same values (LGN, 1.8 ± 0.4°; type I, 1.9 ± 0.3°, mean ± SEM). However, type I but not LGN cells gradually increased and deviated from the initial width over time, suggesting that the spatial property of the early ECRF component of type I cells reflects a geniculate origin that shifts to a cortical origin.

It has been known that the strength of ECRF suppression depends on stimulus features such as orientation and spatial frequency (Akasaki et al. 2002; DeAngelis et al. 1994). We recently reported that the SF tuning properties of ECRF suppression dramatically changes with the time course of the response (Ishikawa et al. 2010). Therefore, it is possible that the orientation tuning property of ECRF suppression also changes with time. To examine this point, we tested two types of annular ECRF stimulus orientations, iso- and cross-orientation of the CRF grating, and examined the temporal dynamics of the orientation selectivity. We especially focused on the early time window (first 150 ms after stimulus onset), since that is most likely when a significant temporal change in the SF tuning property of ECRF suppression is likely to be observed (Ishikawa et al. 2010).

Figure 8 A, B, and E show PSTHs of the responses recorded from three single
neurons classified as type I, II and LGN neurons, respectively. In the type I V1 neuron (Cell-10), the early CRF response within 80 ms of the stimulus onset (pale gray zone in Fig. 8A) was suppressed regardless of the orientation of the ECRF stimulus. However, the subsequent response was suppressed more strongly by an iso-oriented ECRF than cross-oriented ECRF stimulation (uncolored zone in Fig. 8A). In contrast, the type II V1 neuron (Cell-11) exhibited orientation-selective suppression from the beginning of suppression (pale gray zone in Fig. 8B) that lasted until 150 ms (uncolored zone in Fig. 8B). Again, the iso-oriented ECRF stimulation inhibited more strongly the CRF response than the cross-oriented ECRF did. The same tendency was observed in the population PSTHs of the responses for type I (Fig. 8C) and type II cells (Fig. 8D). On the other hand, the ECRF suppression of the LGN neuron (Cell-12) was orientation-independent until 150 ms after stimulus onset (Fig. 8E). These type-specific characteristics for the orientation selectivity of the ECRF suppression were represented in the population averages as well (Fig. 8F). The orientation selectivity in type I V1 cells significantly developed from values close to LGN cells to those to type II V1 cells in an early time window (40–100 ms, \( P < 0.01 \), Freedman test). In contrast, type II V1 cells showed high OSI values throughout the analytical time period, and no significant difference was observed between any data points. Thus, the temporal dynamics of the orientation selectivity of the ECRF suppression was quite similar to that of the spatial extent of the ECRF suppression.

We considered whether the temporal dynamics of the OSI in type I V1 cells were artifactually produced by the response variability. However, the inter-trial
variability of the responses to the CRF + iso- or cross-oriented ECRF increased markedly from 30 to 40 ms after stimulus onset and remained stable from 40 to 60 ms (data not shown), while the OSI increased gradually from 40 to 90 ms (Fig. 8F), suggesting that the temporal development of the OSI cannot be explained by the response variability.

The orientation selectivity of the ECRF suppression of V1 neurons is possibly related with that of the CRF response. To examine this point, we measured the orientation selectivity of the CRF response as a Half-Width at Half-Height (HWHH) of the orientation tuning curves. No significant differences were observed in the HWHH among the three types of V1 neurons (type I ± SD (SEM), 33.6 ± 13.9 (2.6); type II, 34.6 ± 10.2 (2.1); type III, 32.3 ± 13.3 (4.0); p =0.37, Kruskal-Wallis test).

Laminar distribution of the three types of ECRF suppression in V1

Finally, we examined the relationship between the types of ECRF suppression and the laminar locations of thirty-two V1 neurons (Table 1), finding a clear laminar bias. In layer IV (granular layer), the geniculocortical recipient layer, all ECRF suppression was type I (5/5). Moreover, type II and III neurons were distributed in the supra- or infra-granular layers but not in layer IV. The laminar bias was statistically significant between the granular and the extragranular layers ($P < 0.05$, Tukey’s test for equality of proportions), suggesting that inter-areal and inter-laminar connections were responsible for the generation of the local and global ECRF components.
To elucidate the underlying mechanisms and functional roles of ECRF suppression in V1 of cat, we examined the temporal dynamics of spatial extents and orientation tuning properties of ECRF suppression in V1 and the LGN. Our results led to four major findings. First, the spatiotemporal profiles of ECRF suppression in V1 neurons are heterogeneous and vary from cell to cell. From this, we classified the cells into three types (I, II, and III) according to the existence or absence of early suppression and its spatiotemporal properties. Second, the size and orientation tuning property of ECRF suppression are not stable temporally, but change dynamically with the time course of the response even in a single V1 neuron (type I). Third, based on the spatial extent and onset latency of the ECRF suppression, we can conclude that there are three components of ECRF suppression in V1 neurons: 1) local and fast, 2) global and fast, and 3) global and delayed components. The local and global components were respectively less and more specific for ECRF orientation, and tend to be present in the thalamocortical recipient layer IV and other layers, respectively. Fourth, the ECRF suppression of LGN neurons showed fast onset, and was spatially narrow (local) and less specific to orientation throughout the response period. Furthermore, the features of the geniculate ECRF suppression match well with those of the local and fast ECRF component but not those of the global ECRF component of V1 neurons.

The above data suggest that ECRF suppression in V1 consists of at least two distinct components with different spatiotemporal properties: one component is similar and attributable to the ECRF suppression in the LGN and the other is not. Therefore, we
conclude that multiple mechanisms, namely subcortical and cortical mechanisms, contribute to the local and global suppression in V1, respectively.

Origins of ECRF suppression in V1

The origin of ECRF suppression in V1 is unknown and controversial. Three possible sources have been proposed (see Smith 2006): (1) a reduction of geniculocortical inputs owing to ECRF suppression at subcortical levels (feedforward mechanism) such as the LGN (Alitto & Usrey 2008; Bonin et al., 2005; Jones & Sillito, 1991; Li & He, 1987; Naito et al., 2007; Ozeki et al., 2004; Sadakane et al., 2006; Solomon et al., 2002; Sun et al., 2004; Webb et al., 2005) or retina (Alitto & Usrey 2008; Enroth-Cugell et al. 1983; Li et al. 1991; Passaglia et al. 2001; Solomon et al. 2006); (2) intracortical circuitries in V1, including horizontal connections and intracortical inhibition (Anderson et al. 2001; Angelucci et al. 2002; Cavanaugh et al. 2002b; Das & Gilbert 1999; DeAngelis et al. 1994; Hashemi-Nezhad and Lyon, 2011; Haider et al. 2010; Levitt & Lund 2002; Ozeki et al. 2009; Sceniak et al. 2001; Walker et al. 1999, 2002); and (3) feedback projections from the extrastriate cortex that lead to an inhibition-dominant effect by driving inhibitory neurons or by changing the balance between excitation and inhibition in V1 (Angelucci et al. 2002, Angelucci & Bressloff 2006; Bair et al. 2003; Bullier et al. 2001; Levitt & Lund 2002; Ozeki et al. 2009; Schwabe et al. 2006). Our data argue that the three sources are not mutually exclusive. Rather, multiple mechanisms could contribute to the output of a single suppressive phenomenon for the reasons below.
ECRF suppression has been reported to lag CRF excitation anywhere from 0 to 60 msec (Ishikawa et al. 2010; Kneirim and Van Essen 1992; Lamme 1995; Zipser et al. 1996; Lee et al. 1998; Northdurft et al. 1999, 2000; Hupé et al. 1998, 2001a, b; Müller et al. 2003). In addition, we here discovered a much slower component which begins about 100 ms after stimulus onset in layers other than layer IV. Combined, our results argue that the large variation in time lag is due to distinct mechanisms attributable to the different visual areas or neuronal networks.

Like the CRF response, ECRF suppression in V1 also shows a clear specificity for stimulus features such as orientation, direction, spatial frequency, and temporal frequency (Akasaki et al. 2002; Allman et al. 1985, 1990; Gulyas et al. 1987; Hammond & Smith 1982, 1984; Hashemi-Nezhad and Lyon, 2011; Ishikawa et al. 2010; Kastner et al. 1997; Knierim & Van Essen 1992; Lamme 1995; Orban et al. 1987; Sillito et al. 1995; Webb et al. 2005; Zipser et al. 1996), and basically tunes to the preferred parameters of the CRF response. Such stimulus-feature specificity is thought to indicate a cortical mechanism. However, ECRF suppression tuned more broadly to the stimulus features than the CRF response of V1 neurons (DeAngelis et al. 1994; Durand et al. 2007; Ishikawa et al. 2010; Webb et al. 2005), suggesting a contribution by areas other than V1. Since most of those studies were done using drifting grating stimuli, ECRF suppression phenomenon likely emerge from a mixture of distinct suppressive mechanisms. Furthermore, the varying spatial properties of ECRF suppression observed at various stimulus conditions could reflect changes in the relative contribution of those mechanisms. Our results demonstrate that these multiple mechanisms are functioning
even in a single stimulus condition.

While inactivating a visual area by pharmacological drug application or cooling can dissect a single suppressive phenomenon into the suggested multiple mechanisms (Wang et al. 2010), these treatments themselves could influence the information processing. A simple alternative is temporal dissection, where one can examine the temporal dynamics of the spatial tuning properties of the ECRF suppression by using a stationary stimulus (Ishikawa et al. 2010; Knierim and Van Essen 1992; Li et al. 2000; Nothdurft et al. 1999). Since neurons in each visual area receive inputs from multiple sources with distinct spatiotemporal profiles, that is, lower and higher visual areas in the hierarchical processing of visual information, spatial properties of the ECRF are expected to change with time if different areas are involved. In fact, according to expectation, we found that the extent of the suppressive field and orientation selectivity of ECRF suppression change dynamically along the time course of the response. Similar temporal dynamics of ECRF suppression have been reported in previous studies (Knierim and Van Essen 1992; Nothdurft et al. 1999). Temporal dynamics of feature selectivity have also been observed in SF tuning, where the SF tuning of ECRF suppression in cat V1 changes from low-pass to band-pass along the time course of the response (Ishikawa et al. 2010). The early ECRF component with low SF preference in V1 matched well with the spatial and temporal properties of ECRF suppression in the LGN, but the late component with high SF preference did not. The above evidence, then, supports the idea that distinct neuronal populations with different spatial properties are involved at different times in ECRF suppression in V1.
Thus, spatiotemporal analysis enables us to predict when and where ECRF suppression occurs in intact neuronal circuits. We therefore considered the origin of the three components of the ECRF, with special interest in the temporal change of the spatial extent, as this can aid in anatomically estimating the neuronal circuitry implicated in the ECRF (Angelucci et al. 2002).

Neural substrates of each ECRF suppression component

Mechanisms of the local component in V1

Which neuronal circuits mediate local ECRF suppression? Several lines of evidence for the spatiotemporal properties of ECRF suppression suggest that the most probable neuronal mechanism for the local component of ECRF suppression in V1 is a reduction of geniculocortical (feedforward) input due to ECRF suppression at the subcortical level.

First, the spatial extent estimated as the ECRF width from the boundary of the CRF of the early suppression was small (about 1.8º) in type I V1 neurons, which agrees well with that of LGN neurons (about 1.7º). The ECRF width is a good measure for comparing LGN and V1 neurons, because a reduction of geniculate inputs from LGN neurons covering the border of the CRF in V1 neurons is thought to contribute to the ECRF of V1 (Jones et al. 2000). The similar ECRF width between type I V1 and LGN neurons strongly suggests the possibility that the local component is inherited from the LGN. Second, ECRF suppression in type I V1 neurons began on average 7 ms after the earliest CRF response, which corresponds to that of LGN neurons. Third, the onset
latency of ECRF suppression was not influenced by the ECRF size in either type I V1 or
LGN neurons. The fast and stimulus-size-independent onset of suppression can be
attributed to the spatially localized origin of ECRF suppression in the vicinity of the
ECRF. Fourth, the early component of ECRF suppression in type I V1 neurons was less
selective for stimulus orientation, which again corresponds well to that of LGN neurons.
Recently, Ozeki et al. (2009) reported that the orientation selectivity of the ECRF
suppression of the membrane potential response measured from layer 4 cells that have
monosynaptic connections with LGN cells is weak, but enhanced in those having
polysynaptic connections. This property strongly suggests that a smaller orientation
selective ECRF component is present in input layer cells, and orientation selective
mechanisms are added through cortical circuits. Fifth, all neurons in the thalamocortical
recipient layer (layer 4) were type I. Thus, the laminar bias of the local ECRF
suppression in addition to a good electrophysiological correspondence between type I
V1 and LGN neurons in terms of temporal and spatial properties of early ECRF
suppression supports the idea that the fast and local component of ECRF suppression in
type I V1 neurons originates from the ECRF at the subcortical level.

Mechanisms of the global component in V1

Temporal analysis of the global components indicated there exist distinct
subcomponents that have either a fast or delayed onset in the global ECRF component
of type II and III V1 neurons. The spatial extent of any global component (about 5° in
ECRF width) in V1 cannot be explained by the narrow ECRF width (about 1.8°) of
LGN neurons, suggesting the involvement of a cortical mechanism.

The anatomical and physiological properties of intrinsic long-range horizontal connections within V1 appear to explain the spatial wideness and high specificity of the orientation of ECRF suppression (Hirsch and Gilbert 1991). The axon collaterals of pyramidal cells in supragranular layers extend to several millimeters in both cat (Gilbert and Wiesel 1983; Kisvárday et al. 1997; Martin and Whitteridge 1984) and monkey (Blasdel et al. 1985; Fisken et al. 1975; Fitzpatrick et al. 1985; McGuire et al. 1991; Rockland and Lund 1983) and connect neurons in columns that have like orientation preference (Gilbert and Wiesel 1989; T’so et al. 1986), suggesting an orientation-dependent long-distance interaction. In fact, through horizontal connections, neuronal activity propagates more than 10 mm along the surface distance of V1 in cat (Bringuier et al. 1999), which corresponds to about 13° of the visual angle and is sufficiently larger than the far ECRF (about 8° in radius) seen here.

Horizontal connections can explain the delayed global component but not the fast global component that begins less than ten milliseconds after the onset of the CRF response. This is because the propagation velocity (0.1–0.2 m/s) through the horizontal projection is too slow to evoke fast suppression (Bringuier et al. 1999; Grinvald et al. 1994; Nelson and Katz 1995; Slovin et al. 2002; Tucker and Katz 2003). Since a far ECRF (about 8°) corresponds to about a 6 mm cortical tangential distance in cat (Payne 1991), far ECRF suppression should be delayed about 60 ms if this effect is mediated by the horizontal projection. Additionally, horizontal connections cannot explain the shortening of the onset latency of the ECRF suppression that occurred with enlarging
the stimulus size (Fig. 1, cell B and Fig. 5). If by increasing the stimulus size more
distant V1 neurons begin to contribute to ECRF suppression through horizontal
connections, the added suppressive effect should be delayed in a distance-dependent
manner, which means the effect cannot contribute to the shortening of the onset latency
of ECRF suppression.

It would seem that the potential neuronal substrate for the global component is
a feedback projection from higher order cortical areas to V1, because the appearance of
the global component (type II and III cells) shows a laminar bias toward the
supragranular and infragranular layers of V1, which themselves mainly receive inputs
from higher order areas. Additionally, feedback projections fulfill the spatial and
temporal constraints of both fast and delayed global ECRF components. The feedback
connections are made by fast-conducting axons (Girard et al. 2001) whose velocities
range between 2 and 6 m/s, which is about 10 times faster than those of the horizontal
connections. Accordingly, reversible inactivation of monkey MT was seen to reduce the
strength of ECRF suppression induced by a moving background in area V1 (Hupé and
et al. 1998; Bullier et al. 2001; Bardy et al. 2009) with almost no time lag to the CRF
response (Hupé et al. 1998, 2001a, b). We also found that fast global ECRF suppression
was only slightly delayed, just 10 ms on average after the CRF response, and no delay
was observed in a certain population of neurons, which agrees with the 5–10 ms
difference in interareal response latencies (Maunsell 1986; Raiguel et al. 1989).

The receptive fields of area 18 cells are wider by approximately a factor of
three than those in area 17 cells (McLean et al. 1994; Pernberg et al. 1998). Therefore,
feedback inputs from the spatial extent with radii no less than 7.5° (mean CRF radius 2.5° × 3) could be expected to return from area 18. Furthermore, the CRF radius of area 20 and PMLS neurons is known to be more than 10°. Thus, the spatial extents of the feedback inputs from higher-order areas correspond to or sufficiently cover that of the global component (8° in radius) of V1 cells.

The feedback projection from cells having a CRF larger than V1 neurons seems to explain well the stimulus-size-dependent shortening of the onset latency of ECRF suppression. When the CRF of a V1 neuron is stimulated, the stimulus is smaller than the CRF of higher-order neurons, which causes not only a weak response but also a slow onset of the response. Enlarging stimulus size beyond the CRF of V1 neurons allows higher-order neurons to fire more vigorously and rapidly, causing more strong and rapid feedback to V1. Correspondently, Smith et al. (2006) found that the shorter onset latency of suppression is proportional to the strength of suppression.

However, even if corticocortical feedback or long-range horizontal projections are involved in global ECRF suppression, it remains unknown how their inputs lead to suppressive modulation of the CRF response of V1 neurons. Recent studies demonstrated that both excitatory and inhibitory inputs to V1 neurons are reduced during ECRF suppression (Ozeki et al. 2004, 2009), suggesting that network activity as a whole is reduced. Moreover, Ozeki et al. (2009) reported that ECRF stimulation induces a transient increment of the inhibition in the V1 network. The sudden imbalance between excitation and inhibition acted as a trigger to reduce network activity.

Increasing the inhibitory inputs may be mediated by a specific type of inhibitory neuron.
such as somatostain-expressing inhibitory neurons (Adesnik et al. 2012). Since feedback connections from higher order visual areas (Mignard and Malpeli 1991; Wang et al. 2000, 2007; Huang et al. 2004, 2007; Bardy et al. 2009) and horizontal connections (Ts’o et al. 1986; Hirsch & Gilbert 1991; Yoshimura et al. 2000) in V1 have been reported to mostly enhance neural activity, it is reasonable to think that the excitatory inputs from these projections imbalance the excitation and inhibition in the V1 network circuit.

There remains controversy about the sources and underlying neuronal mechanisms of ECRF suppression, as the spatial and temporal properties of ECRF suppression have been seen to vary widely. The present study found that even when the ECRF is stimulated by a single parameter, multiple ECRF mechanisms contribute to an apparently single suppressive phenomenon, which helps explain the high variability reported in different studies.
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Figure legends

Fig. 1. Histological reconstruction of electolitic lesions and examples of two V1 simple cells showing different time courses of stimulus-size tuning. A–C: Micrograms of three serial sections stained with cytochrome oxidase and electrolytic lesions (white arrows). D, E: PSTHs of responses (mean ± SEM) to stationary sinusoidal grating patches with optimal grating parameters (Cell-1, orientation: 240°, SF: 0.4 cpd, contrast: 80%; Cell-2, orientation: 135°, SF: 0.2 cpd, contrast: 60%) and varying stimulus sizes. The CRF response of each neuron is drawn as thick red (D) and magenta lines (E); the other colored lines are responses to stimuli larger than the CRF size (CRF + ECRF). F, G: Time courses of ECRF suppression obtained by subtracting the CRF response from CRF + ECRF responses bin-by-bin (differential PSTHs). Note the difference in the size-dependency of early ECRF suppression between Cell-1 and Cell-2 in the pale yellow zone. The early CRF response was suppressed almost equally for all ECRF sizes in Cell-1 (D, F) and proportionally to ECRF size in Cell-2 (E, G).

Fig. 2. Types I and II are classified according to the degree and onset latency of near ECRF suppression.

Relationship between “% near ECRF effect” (abscissa) and “Onset delay of near ECRF suppression” (ordinate) of type I (filled circles) and type II (open circles) cells. Two cell populations were clearly distinguished by these parameters reflecting the degree and onset latency of near ECRF suppression. The population characterized by the local and fast component and the global and delayed component of near ECRF suppression was
classified as type I and type II, respectively. Data points indicated by numbers correspond to cells in figures 1, 4, 7, and 8.

**Fig. 3. Average population responses of neurons classified as type I or II.**

A, B, E, F: PSTHs of responses (mean ± SEM) to stimuli with varying sizes were normalized by the maximal PSTH value of the CRF response of each neuron. The normalized PSTHs were gathered with respect to cell type and neuron CRF size, and then averaged to generate respective average population PSTHs. A and E show average PSTHs constructed from type I neurons with a CRF size of 1.7° (n = 11) and 2.5° (n = 7), respectively, and B and F from type II neurons with a CRF size of 1.7° (n = 6) and 2.5° (n = 11), respectively. C, D, G, H: Differential PSTHs were calculated for each cell from the normalized PSTHs and then gathered and averaged the same way as average PSTHs. C, D, G, and H are the average differential PSTHs of the neurons used in A, B, E and F. Note that type-specific characteristic features of ECRF suppression are clearly represented in the pale yellow zones.

**Fig. 4. Typical time courses of CRF response and ECRF suppression for cell types I, II, and III.**

A–C: A, B, and C show two example cells for cell type I, II, and III, respectively. Thick black, thin red, and thin blue lines indicate visual responses (top, PSTHs, mean ± SEM) and response suppression (bottom, differential PSTHs) evoked by stimulation of the CRF, CRF + near ECRF, and CRF + large ECRF, respectively. Characteristic features of
each cell type are observed in gray areas (0–80 ms) of the PSTHs and differential PSTHs. The orientation, SF, and contrast of the grating stimuli presented were 270°, 0.3 cpd, and 80% for Cell-3; 300°, 0.5 cpd, and 80% for Cell-4; 120°, 0.3 cpd, and 80% for Cell-5; 240°, 0.3 cpd, and 80% for Cell-6; 90°, 0.6 cpd, and 80% for Cell-7; and 90°, 0.3 cpd, and 60% for Cell-8, respectively.

Fig. 5. Onset latency of ECRF suppression in relation with stimulus size.
Onset latencies of ECRF suppression of each cell type in V1 and the LGN. Data are reported as mean ± SEM. Those at the far right side are onset latencies of the CRF responses. Symbols indicate significant differences between type III cells and type I and II cells (* *p < 0.01), and between type I cells and type II cells (##p < 0.01).

Fig. 6. Temporal changes of spatial extents of the CRF and ECRF.
A, Temporal changes of size tuning curves of a type I V1 cell (Cell-1 in Fig. 1). Data for stimulus sizes equal to and larger than the CRF are plotted and fitted by a Gaussian function. Arrows indicate ECRF radii. B, C, and D show population data for the time courses of the CRF radius, ECRF radius, and ECRF width, respectively. The parameters were obtained from the fits of the size-tuning curves of the responses in a 50 ms bin width 10 ms intervals. Data are reported as mean ± SEM. Symbols indicate significant differences between type III cells and type I and II cells in B (* *p < 0.01), between type I cells and type II cells (*p < 0.05, * *p < 0.01) in C and D, and between type II cells and type III cells (#p < 0.05) in D.
Fig. 7. Example of an X-type cell showing typical spatiotemporal profiles of ECRF suppression. A and B show the PSTHs (mean ± SEM) and differential PSTHs, respectively, of the responses of an X type LGN cell. Conventions are the same as in Fig. 1. C: Onset latencies of ECRF suppression and CRF response. D: Temporal changes of the spatial extents of the CRF radius, ECRF radius, and ECRF width. Note that the spatiotemporal features of the ECRF suppression in the LGN neuron are the localization and fast onset observed in the pale yellow zones of A and B, which matches well with those of the local component in type I V1 neurons. The localized suppression continues throughout the visual stimulation, which also resembles the weak but sustained component in type I V1 neurons. The orientation, SF, and contrast of the grating stimuli presented were 0°, 0.2 cpd, and 80%, respectively.

Fig. 8. Temporal dynamics of orientation selectivity of ECRF suppression. A, B, E: Example of PSTHs (mean ± SEM) of type I (A, Cell-10) and type II (B, Cell-11) V1 neurons, and a LGN neuron (E, Cell-12) that all show typical temporal dynamics in the orientation selectivity of ECRF suppression. C, D: population PSTHs (median with error bars of quartile deviation) of type I (C) and type II (D) cells. Iso- and cross-oriented ECRF stimuli (Iso, red; cross, blue) suppressed early responses (0-80 ms, pale gray) to the same extent in type I (A and C) and LGN (E) neurons, but differently in type II (B and D) neurons where the suppression was stronger for the iso-oriented ECRF stimulus. After 80 ms of stimulus onset, the suppressive effects of the iso- and
cross-oriented ECRF stimuli became more differentiated in both types of V1 neurons, but remained constant in the LGN neuron. 

F: Temporal changes of the orientation selectivity index (OSI). The orientation selectivity significantly changed from low to high in the early time window (40–100 ms) in type I V1 cells ($P < 0.01$, Freedman test; * $P < 0.05$, ** $P < 0.01$ vs. 40 ms, Wilcoxon signed rank test), but was constant in type II V1 cells at higher selectivity and in LGN cells at lower selectivity. The orientation, SF, and contrast of the grating stimuli presented at the CRFs were 15°, 0.3 cpd, and 80% for Cell-10, 125°, 0.5 cpd, and 80% for Cell-11, and 0°, 0.2 cpd, and 80% for Cell-12, respectively.
Figure 1 (Shimegi et al.)
Figure 2 (Shimegi et al.)

Onset delay of near ECRF suppression (ms)

% near ECRF effect
Figure 3 (Shimegi et al.)
Figure 4 (Shimegi et al.)
Figure 5 (Shimegi et al.)
Figure 6 (Shimegi et al.)
Figure 7 (Shimegi et al.)
Figure 8 (Shimegi et al.)
Table 1  Laminar distribution of V1 cells

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<th>type III</th>
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**Percentage within type**

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**Percentage within layer**

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**Recording depth (μm): Median and Range**

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Parenthesis in the “number of cell” indicates the number of simple cells.
* p < 0.05, Tukey’s test for equality of proportions