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On and off domains in primary visual cortex

Genderblind courtship in *Drosophila*

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On and off domains of geniculate afferents in cat primary visual cortex

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On- and off-center geniculate afferents form two major channels of visual processing that are thought to converge in the primary visual cortex. However, humans with severely reduced on responses can have normal visual acuity when tested in a white background, which indicates that off channels can function relatively independently from on channels under certain conditions. Consistent with this functional independence of channels, we demonstrate here that on- and off-center geniculate afferents segregate in different domains of the cat primary visual cortex and that off responses dominate the cortical representation of the area centralis. On average, 70% of the geniculate afferents converging at the same cortical domain had receptive fields of the same contrast polarity. Moreover, off-center afferents dominated the representation of the area centralis in the cortex, but not in the thalamus, indicating that on- and off-center afferents are balanced in number, but not in the amount of cortical territory that they cover.

Visual information is transferred from the retina to the visual cortex through two major functional channels that process local increments (on channel) or decrements (off channel) in light with respect to a mean background. Although on and off visual channels have been traditionally described as reversed versions of each other, increasing evidence indicate that they are very different. Even at the level of the retina, on- and off-center retinal ganglion cells can differ in receptive field size, response latency, amount of rectification in their synaptic inputs and their specific circuitry^{1,2}. Differences between on- and off-center retinal ganglion cells, either anatomical or physiological, have been reported in mice², rats³, dogs⁴ and humans⁵. Moreover, at early stages of development in ferrets, off-center retinal ganglion cells have higher firing rates than their on-center counterparts⁶.

On and off channels are not only quantitatively different, but they also segregate in different neuronal compartments, both at the level of the retina and the lateral geniculate nucleus (LGN). In the retina, on and off visual channels segregate in different sublayers^{7,8} and a similar sublayer segregation has been reported in the LGN of the macaque⁹, cat^{10,11}, ferret¹², tree shrew¹³ and mink¹⁴, with off-center afferents being found in layers and sublayers of the LGN closer to the optic tract than on-center afferents.

On- and off-center neurons converge for first time at the level of the primary visual cortex to build cortical receptive fields^{15–17}. However, although the same cortical cell can receive input from on- and off-center geniculate afferents, the number of afferents is rarely balanced: most cortical cells are either off-dominated or on-dominated. Therefore, although on- and off-channels converge at the same cortical

neuron, on-dominated and off-dominated cortical neurons could still segregate in the cortex. On- and off-center geniculate afferents have been found to cluster in ferrets¹⁸ and minks¹⁹. Moreover, in the tree shrew, a species where ocular dominance segregates in layers and not columns²⁰, on- and off-center geniculate afferents were also found to segregate in layers²¹.

Although the clustering of on- and off-center geniculate afferents could be interpreted as a rare specialization of the visual pathway in some animals, several computational models^{22–26} suggest that this type of segregation could be a general cortical feature that is closely related to the development of orientation columns. Paradoxically, although the main predictions from these models are based on data obtained from cat visual cortex, there is currently no evidence for clustering of on- and off-center geniculate afferents in the cat. Here, we provide evidence for such clustering and, by doing so, we support the notion that on- and off-channels segregation are important in cortical architecture. Some of these results were previously published in abstract form (Gordon *et al.*, *Soc. Neurosci. Abstr.* **19**, 333, 1993 and Jin *et al.*, *Soc. Neurosci. Abstr.* 436.12, 2006).

RESULTS

Evidence for segregation of on- and off-center geniculate afferents was obtained independently in two different laboratories and with two different techniques: by recording geniculate afferents from the muscimol-silenced cortex and by recording the current sinks generated by single geniculate afferents in the active cortex. Both techniques showed clustering of geniculate afferents with the same center sign (on-center

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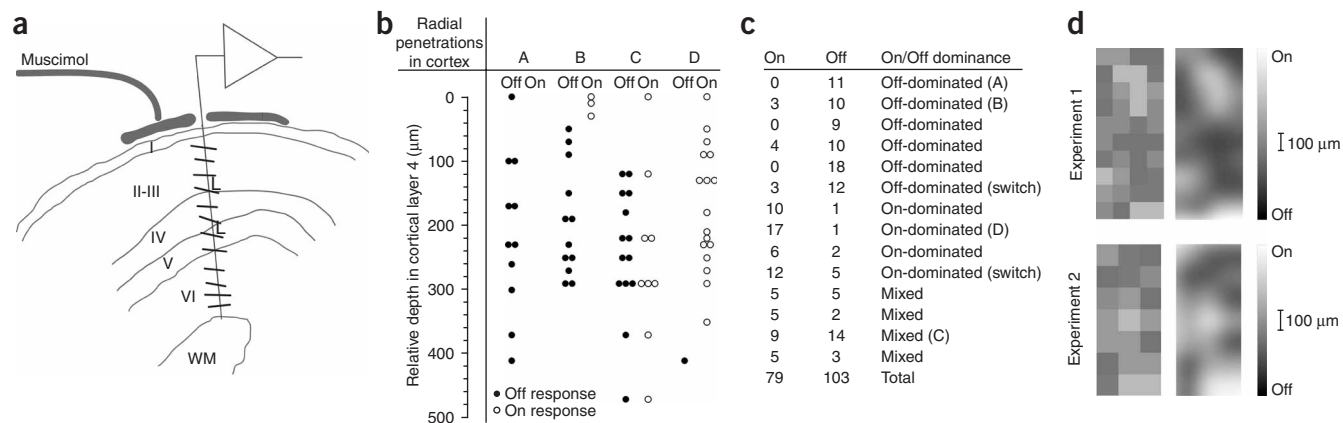


Figure 1 Recording from geniculate afferents in the muscimol-silenced cortex. **(a)** Cortical recordings showing a radial alignment of a single electrode penetration in a cortical orientation domain, determined before application of muscimol. Cortical layers reconstructed from histology are indicated by Roman numerals I–VI (WM, white matter). The preferred orientations of cortical neurons recorded along the course of the electrode penetration are indicated by lines. Muscimol was applied to the surface of the cortex to silence cortical activity, and afferent receptive fields were plotted in layer IV between the two L's after 2 h, which show the centers of lesions made at the end of the experiment. **(b)** Four representative vertical penetrations through layer 4 are shown: two dominated by off-center afferents (A, B), one dominated by on-center afferents (D) and another one mixed (C). **(c)** Table showing all electrode penetrations, the number of afferents recorded in each (left) and the category assigned to each penetration (right). **(d)** Map showing the segregation of on- and off-center afferents obtained in two different experiments by making multiple single-electrode penetrations. Left, actual maps, luminance coded by the fraction of off afferents. Right, identical maps smoothed by a two-dimensional Gaussian to highlight the clustering of penetrations of like type.

or off-center) in the cat visual cortex. Moreover, recordings in the active cortex demonstrated that off-geniculate afferents dominate the cortical representation of the area centralis. These results are consistent with a segregation of on and off channels in the cat visual cortex and with some level of specialization of the off visual channel at the center of vision.

Geniculate recordings in muscimol-silenced cortex

A single electrode was introduced perpendicular to the cortex and carefully aligned in a single orientation column, as verified by recordings from multiunit activity at different cortical depths (**Fig. 1a**). After verifying the correct vertical alignment, the electrode was moved to the top of layer 4 and muscimol (50 mM) was infused into the cortical region surrounding the electrode to silence cortical activity ($74 \mu\text{l h}^{-1}$). After approximately 2 h of muscimol infusion, the electrode was systematically lowered into the brain to record from single geniculate afferents and to classify their receptive fields as either on- or off-center. **Figure 1b** shows four examples of cortical penetrations, three in which most of the geniculate afferents were of the same type (A, B and D) and one in which the afferents were mixed (C). We mapped 182 afferents in 14 penetrations, with each penetration containing 7–23 afferents. In 71% of the penetrations (10/14), more than 70% of the geniculate afferents had receptive field centers of the same sign (**Fig. 1c**; in two of these penetrations there was a clear switch point between off-dominated and on-dominated cortical regions), with only four penetrations showing a clear mixing of on- and off-center geniculate afferents.

In two additional cats, careful mapping with multiple evenly spaced penetrations by a single microelectrode revealed cortical regions that were dominated by either on- or off-center geniculate afferents that were ~ 200 – $300 \mu\text{m}$ in width (**Fig. 1d**). We compared the arrangement of these afferents with what would be expected from a random distribution using two different methods (see Methods). First, the distribution of penetrations classified by the fraction of afferents of a given center type was significantly different from a uniform distribution, by χ^2 -squared test ($P < 0.05$). Second, a Monte Carlo analysis of 10,000 randomly generated grids drawn from the experimentally derived distribution of penetrations demonstrated

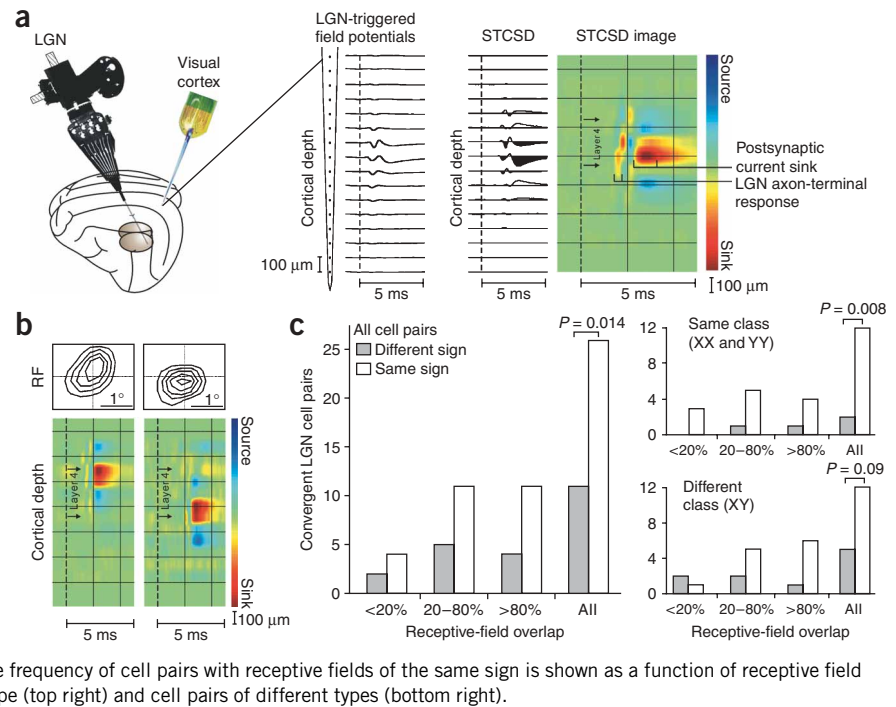
that the clustering of like penetrations was significantly higher than what would be expected for the larger grid (Experiment 1, **Fig. 1d**; $P < 0.001$). Owing to its size, clustering in the smaller grid approached, but did not reach, significance (Experiment 2, **Fig. 1d**; $P = 0.16$).

Recordings from geniculate afferents in active cortex

Recordings from geniculate afferents in the active cortex were obtained with spike-triggered current source–density analysis (STCSD²⁷). Neuronal activity was simultaneously recorded with 16 vertically arranged electrodes in the primary visual cortex (NeuroNexus Technologies) and seven independently movable electrodes in the LGN (7-Channel Thomas Recording array, Thomas Recording), all in precise retinotopic alignment (**Fig. 2a**, left). Well-isolated spikes from each geniculate cell were used to trigger average field potentials that were recorded at each of the 16 cortical sites, and the field potentials were then used to calculate the current sink generated by each geniculate afferent in the cortex (**Fig. 2a**, middle). The current sink generated by each single geniculate afferent was focused in layer 4 (and sometimes also in layer 6) and had a triphasic time course that corresponded to the spike recorded from the geniculate axonal terminal in the cortex, a synaptic delay and a post-synaptic sink (**Fig. 2a**, right). The time course and depth profile of these single-afferent current sinks were virtually identical to those previously described for thalamic afferents in the somatosensory cortex²⁷.

Because several geniculate cells were simultaneously recorded, it was possible to identify pairs of geniculate cells that generated significant current sinks at the same cortical domain (see Methods for significance criteria). **Figure 2b** shows an example of one such pair of cells with off-center receptive fields that were classified as Y (left) and X (right) on the basis of their linearity of spatial summation²⁸. Consistently with the anatomy^{29–31}, the current sink generated by the Y cell (left) showed a shorter presynaptic conduction delay and was located more superficially in layer 4 than the current sink generated by the X cell (right). Similar to the cell pair illustrated in this example, 70% of the geniculate cell pairs converging at the same cortical domain had receptive fields of the same sign, either both off or both on ($P = 0.014$, $n = 37$, χ^2 -square test; **Fig. 2c**).

Figure 2 Recording from geniculate afferents in active cortex. (a) Simultaneous recordings from single cells in the LGN and local field potentials in the visual cortex. Well-isolated spikes from a single geniculate cell were used as triggers to obtain spike-triggered field potentials for each cortical channel. The time of the geniculate spike is indicated by the vertical dashed lines in the depth profiles. The second spatial derivative of these field potentials, which is directly proportional to the current density at a point, was estimated by current source–density analysis. The result from this STCSD analysis is shown through the depth of the cortex as individual traces and a colorized image. (b) Example of two geniculate cells that generated current sinks at the same cortical domain and had overlapping receptive fields of the same sign. The cell on the left was of Y type and the one on the right of X type. As expected from previous anatomical studies²⁹, the Y cell had faster conduction velocity and projected higher in layer 4 than the X cell. The time of the geniculate spike is indicated by the vertical dashed lines in the depth profiles. (c) 70% of the geniculate cell pairs converging at the same cortical domain had receptive fields of the same sign ($n = 37$, $P = 0.014$, χ -square test). The frequency of cell pairs with receptive fields of the same sign is shown as a function of receptive field overlap for all cell pairs (left), cell pairs of the same type (top right) and cell pairs of different types (bottom right).



These results led us to conclude that on- and off-center geniculate afferents are not homogeneously distributed in the cat primary visual cortex. This conclusion is further supported by another result from STCSD analysis: off-center geniculate afferents are over-represented at the center of vision in the cortex. Although the number of current sinks generated by on-center and off-center afferents was balanced in the visual periphery (>5 degrees of eccentricity), 74% of the measured current sinks were generated by off-center geniculate afferents (19/26, $P = 0.018$, χ -square test, data obtained from nine cats; **Fig. 3a**) near the cortical representation of the area centralis (0–5 degrees of eccentricity). This off-dominance at the area centralis did not seem to be a result of a larger number of off-center geniculate afferents. In fact, on-center afferents seemed slightly more numerous than off-center afferents in the thalamus, although the difference was not significant (60 versus 42, $P = 0.07$, χ -square test; **Fig. 3b**).

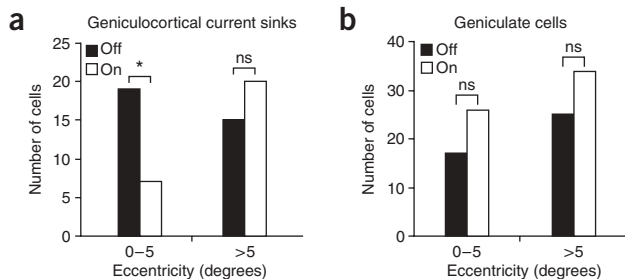


Figure 3 Off-center geniculate cells dominate the representation of the area centralis in cat visual cortex. (a) Near the cortical representation of the area centralis (<5 degrees eccentricity), current sinks generated by off-center geniculate afferents were more frequently found than current sinks generated by on-center geniculate afferents ($P = 0.018$, χ -square test, data obtained from nine cats). This difference was not found outside of the area centralis. (b) Recordings from LGN showed that on- and off-center geniculate cells are balanced in number at two different eccentricity ranges. Significance was assessed with a χ -square test (ns, not significant; * indicates $P = 0.018$).

Consistent with the off-dominance revealed in the current sinks, cortical simple cells near the area centralis were more frequently off-dominated than on-dominated. We analyzed the spatial receptive fields from pairs of neighboring cortical simple cells that were simultaneously recorded with the same electrode tip within ~ 5 degrees of the area centralis (using standard, sharp electrodes for extracellular recording; **Fig. 4**). The spatial arrangement of neighboring receptive fields was very diverse (**Fig. 4a**); some cell pairs had their receptive fields in counterphase (in pairs 1 and 2, the off-subregion of one simple cell overlapped the on-subregion of the other cell), whereas others did not (in cell pairs 3 and 4, the off-subregions overlapped). However, in contrast to their diversity in spatial phase³², most simple receptive fields were similar in that they were off-dominated (**Fig. 4b**). Seven of the eight simple receptive fields shown in **Figure 4a** had dominant off-subregions, and, on average, pairs of off-dominated simple cell subregions were sixfold more frequent than pairs of simple cells with on-subregions ($P = 0.004$, χ -square test).

How can there be more off-dominated simple cells in the area centralis (**Fig. 4b**) if the number of on- and off-center geniculate afferents is approximately the same (**Fig. 3b**)? To answer this question, we measured the horizontal extent of the postsynaptic current sinks generated by individual on- and off-center geniculate afferents in the primary visual cortex. First, we identified a single geniculate afferent that generated a substantial current sink in a given cortical region and then we made multiple, closely spaced electrode penetrations to estimate the horizontal dimension of the current sink (see Methods for details). Although these experiments were technically demanding, we managed to obtain a sample large enough to make some relevant comparisons ($n = 19$ geniculate afferents). As would be expected from the distribution of synaptic boutons from single geniculate afferents (**Fig. 5a,b**, blue), our measurements showed that the strength of the main single-afferent current sink was normally distributed (**Fig. 5a**, red) and that the current sinks generated by X geniculate afferents covered smaller horizontal distances than the current sinks generated by Y geniculate afferents (**Fig. 5b**, red). Furthermore, as would be

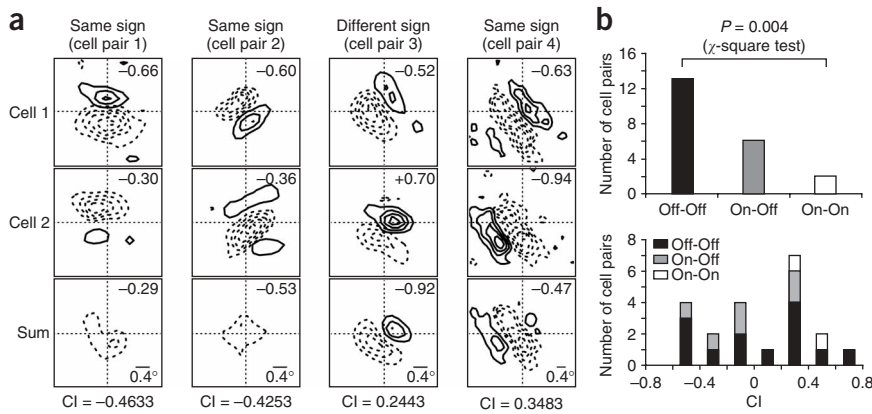


Figure 4 Most cortical simple cells are off-dominated in the cortical representation of the area centralis. **(a)** Examples from four pairs of neighboring simple cells (each cell pair was recorded from the same electrode). Each column shows the receptive fields for each cell (top and middle) and the receptive field sum at the bottom. The top left corner of each panel provides a subregion-strength ratio calculated as (sign) \times (non-dominant response)/(dominant response), where the sign is -1 when the dominant subregion is off and 1 when the dominant subregion is on (notice that most cells in this example have negative ratios, which correspond to off-dominant subregions). The correlation index at the bottom of the column was calculated by cross-correlating the two spatial receptive fields. **(b)** Top, cell pairs with off-dominated receptive fields were more frequently found than cell pairs with on-dominated receptive fields ($P = 0.004$, χ^2 -square test). Bottom, correlation indices (CIs) from all pairs of simple cells studied.

expected from the low density of the synaptic boutons at the periphery of the geniculate axonal arbor^{29,33}, the horizontal distance covered by the single-afferent current sink was smaller than the average distance covered by the geniculate axonal arbors (Fig. 5a,b; the average difference in Fig. 5b could also reflect incomplete sampling of some single-afferent sinks, see Methods).

Notably, when we compared the current sinks generated by on- and off-center geniculate afferents within 5 degrees of the area centralis (Fig. 5c), the horizontal distances of the current sinks were, on average, 1.8-fold larger for off-center than on-center geniculate afferents (675 versus 364 μm , $P = 0.03$, Mann-Whitney test; in each of these groups, there was a tendency for X afferents to cover smaller distances than Y afferents, although the difference did not reach significance; $X_{\text{on}}/Y_{\text{on}}$, 283/475 μm , $P = 0.122$; $X_{\text{off}}/Y_{\text{off}}$, 600/800 μm , $P = 0.463$). Moreover, although the average strengths of off and on postsynaptic current sinks were not significantly different within the entire sample [off, 113 $\mu\text{V mm}^{-2}$; on, 141 $\mu\text{V mm}^{-2}$; $n = 55$, $P = 0.425$, Mann-Whitney test), we did find a significant correlation between the strength

more contiguous cortical penetrations were considered to be in the same cluster if all of the receptive fields obtained from multiunit cortical activity in the different recording sites of the silicon probe were dominated by the same sign. By using this criterion, we were able to measure five clusters (four off, one on) with an average cluster width of $340 \pm 89 \mu\text{m}$. The average cluster width obtained with measurements from cortical multiunit activity is a reasonable approximation of the 200–300- μm cluster width estimated from afferent recordings in the muscimol-silenced cortex (Fig. 1d).

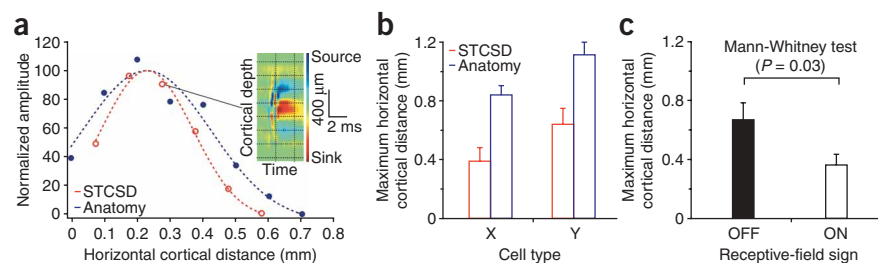
DISCUSSION

We have demonstrated that on- and off-center geniculate afferents are clustered in layer 4 of the cat visual cortex, as in the ferret, mink and tree shrew^{18,19,21}. The clusters are horizontally arranged (Fig. 1) and the probability of finding two geniculate afferents with the same receptive field sign in a vertical electrode penetration is ~ 0.7 (Figs. 1c and 2c). We also demonstrate a dominance of off-center geniculate afferents at the cortical representation of the area centralis.

Figure 5 Off-center geniculate afferents cover more cortical territory than on-center geniculate afferents. **(a)** Cortical territory covered by a single X geniculate afferent estimated as the number of synapses (blue, taken from ref. 29) and as the strength of the single-afferent current sink measured in multiple penetrations with a 16-channel silicon probe (red). The colored inset shows the depth profile of the single-afferent current sink measured in one of the penetrations.

(b) Maximum distance covered by single X and Y geniculate afferents, estimated from the anatomical reconstruction of single afferents (blue, taken from ref. 29) and from measurements of single-afferent current sinks (red). Error bars show one s.d. Note that the average Y/X distance ratio obtained with the two measurements was similar (anatomy, 1.4; physiology, 1.6); however, the measurements of current sinks were more restricted in cortical distance, probably because the density of synaptic boutons is very low at the periphery of the axonal arbors and because some current sinks may have been under sampled (as a result of the limited number of penetrations used to measure horizontal distance).

(c) The current sinks generated by off-center geniculate afferents can be recorded at larger cortical distances than the current sinks generated by on-center geniculate afferents ($P = 0.03$, Mann-Whitney test, $n = 19$). The sample contains 8 off-center geniculate afferents (3 X, 4 Y and 1 nonclassified cell) and 11 on-center geniculate afferents (6 X, 4 Y and 1 nonclassified cell).



These results provide support for cortical models that predict a partial segregation of on- and off-center geniculate afferents in the cat visual cortex^{22–26} and they support the possibility that on and off segregation could be a general feature of cortical organization and orientation maps. Although the segregation of on- and off-center geniculate afferents remains to be demonstrated in the primate, some indirect observations are consistent with a possible segregation. For example, there is evidence for clustering of spatial phase in primate area V1 (ref. 34) and there is also evidence that blue-on and blue-off afferents are partially segregated at the top of layer 4 in primates³⁵. On the other hand, a recent study demonstrated a lack of clustering for on- and off-center geniculate afferents in the rabbit, an animal that has no cortical orientation columns (Stoelzel *et al.*, *Soc. Neurosci. Abstr.* 503.7, 2006). The precise relationship between on and off segregation and the organization of orientation maps predicted by some models^{22–26} has not yet been demonstrated. However, to our surprise, we did find evidence for the off-dominance predicted by one of these models in the adult primary visual cortex²³.

The clustering of on- and off-center geniculate afferents could be explained by initial inhomogeneities in the cortical distribution of the two types of afferents that could later be accentuated by Hebbian mechanisms²². Hebbian mechanisms could also explain the dominance of off-center geniculate afferents, as these afferents have been shown to dominate retinal and cortical activity at early stages of development. In the ferret retina, off-center retinal ganglion cells fire more than on-center retinal ganglion cells during the second to third week of postnatal development⁶. And in the kitten cortex, the majority of cortical neurons (76%) generate only off-responses early during the second postnatal week³⁶. The predominance of off-cortical responses at early stages of development could allow off-center geniculate afferents to have a competitive advantage and to dominate a larger cortical territory than on-center geniculate afferents. Unfortunately, there is currently no anatomical data available that could be used to compare axonal arbor sizes for on- and off-center geniculate afferents in the area centralis²⁹. Differences in the extent of axonal arborizations have been reported for cells of different type in the cat (for example, Y axonal arbors are twice as large as X arbors²⁹) and for cells that receive input from different eyes in the tree shrew (for example, ipsilateral eye axons are, on average, threefold larger than contralateral eye axons³⁷). However, the intracellular filling of single geniculate afferents is a difficult technique that so far has provided cell samples that are too small to make relevant comparisons within specific eccentricity ranges^{29–31}. However, the intracellular fillings clearly demonstrate that single axonal arbors cover horizontal distances in the cat (700–1,300 μm) that are much larger than the size of the axonal clusters described here ($\sim 300 \mu\text{m}$). The difference is likely a result of the density of synaptic boutons and axonal branches, which is highest at the very center of the axon terminal, and it may be ‘undetectable’ just a few hundred μm from the center^{29,33}.

The finding that the area centralis of cats is off-dominated, together with the pronounced on and off asymmetries demonstrated in human psychophysical studies^{38–42}, raises the possibility that the primate fovea may also be off-dominated. Because the fovea is the area with the highest spatial resolution in the retina, an off-dominated fovea would explain why visual acuity is preserved in the absence of on-visual responses in both human³⁸ and nonhuman primates⁴³, and would also explain why recordings from visually evoked potentials in humans demonstrate finer spatial resolution in off than in on visual pathways⁴¹. Notably, studies measuring Landolt C resolution demonstrate finer visual acuity in dark backgrounds than light backgrounds^{39,40}, perhaps because light scatter becomes an important factor in tasks that require the detection of small gaps between lines.

The demonstration that on and off geniculate afferents segregate in cat primary visual cortex has major implications for the understanding of cortical receptive field construction and topographic mapping. Models and circuitry mechanisms that are based on the assumption that each cortical position has a balanced representation of on and off visual responses will have to be revised. This assumption has been commonly used, for example, to estimate the number of thalamic afferents per cortical column⁴⁴, the number of thalamic inputs per cortical cell⁴⁵ and the contribution of specific neuronal circuits to the summed population activity of cortical domains⁴⁶. The results reported here support the relevance of models of cortical development that predict segregation of thalamic afferents by center type^{22–26}, and suggest that such segregation is a key feature that should be evaluated in any future modeling efforts.

METHODS

Recordings from muscimol-silenced cortex. Extracellular recordings from single geniculocortical afferents were obtained from the muscimol-treated primary visual cortices of 12 adult cats. Cats were anesthetized with ketamine (20 mg per kg of body weight) and acepromazine (0.2 mg per kg), followed by thiopental sodium (25 mg ml⁻¹ solution administered as indicated to maintain slow waves and spindles on electroencephalography). Tracheotomy was carried out and the animals were intubated and ventilated with 2:1 mixture of N₂O:O₂. The animal was then paralyzed with gallamine triethiodide (10 mg per kg h⁻¹ through an intravenous femoral catheter). Temperature, electrocardiogram and expired CO₂ were monitored to ensure adequate anesthesia and ventilation. Pupils were dilated with 1% atropine sulfate and the nictitating membranes retracted with 2% neosynephrine. The eyes were refracted and fitted with contact lenses to focus on a tangent screen placed 125 cm in front of the animal. The skull and dura overlying area 17 were removed. These procedures were approved by the University of California, San Francisco Animal Care and Use Committee.

In ten of these animals, radial electrode penetrations were made with custom-fabricated, 1.5–2.0 M Ω resistance, resin-coated etched tungsten micro-electrodes into area 17. This resistance range was found to be well-suited for recording of individual geniculocortical afferents, as well as for well-isolated individual cortical neurons. Signals were amplified and filtered, and a window-discriminator was used to characterize spikes from multiple neurons and/or single geniculocortical afferents. The principal difficulty in this experiment was the alignment of the microelectrode to pass exactly radially through area 17 near the curved crown of the lateral gyrus; 1–14 exploratory penetrations, differing in inclination in the coronal plane and in lateromedial position, were made at slightly different anteroposterior positions until perfect radial alignment was obtained, as judged by the constancy of orientation preference of the cortical cells encountered as the electrode was advanced throughout the depth of the cortex from layers 2 to 6 (Fig. 1a). The electrode was then withdrawn to the top of layer 4 where it remained in place while 50 mM muscimol was perfused onto a layer of gelfoam set upon the pial surface and covered with agarose (as in described in ref. 47). After 2 h of perfusion at 74 $\mu\text{l hr}^{-1}$, the cortical cells were no longer active and the perfusion was stopped. Action potentials arising from geniculocortical afferents (as verified in pilot experiments by their ability to follow rapid geniculate stimulation) were recorded throughout the depth of layer IV, as verified later by histological examination. The receptive field and center-type of each afferent were determined by using stimuli presented by hand onto the tangent screen. In four of these animals, the process was then repeated in the opposite hemisphere; a total of 14 radial penetrations through area 17 were studied.

In two additional animals, muscimol was applied directly to the cortex without first recording from cortical cells. Subsequently, a series of near-radial penetrations spaced at 100 μm in a rectangular grid was made in the posterior part of area 17; the receptive fields and center types of the first four afferents encountered in each penetration were ascertained.

Data analysis of muscimol-silenced cortex. To determine the likelihood of obtaining these results by chance, a Monte Carlo analysis was conducted. Simulated experiments were constructed from the same number of penetrations and the same number of afferents per penetration as the actual experiments. They were thus identical, except that afferents were drawn at random from a

population of on- and off-center afferents in the proportions observed. A cluster index, $(1 - ((\text{number of switches in center type}) / (\text{number of afferents} - \text{number of penetrations}))) \times 100$, that expresses the probability that two successive afferents were of the same center type was calculated for each simulated experiment and compared with the cluster index of the actual experiment. Fewer than 1 in 100,000 simulated experiments generated a cluster index that was higher than that obtained from the actual data.

Two types of analysis were used to determine the significance of the patchy appearance of the grids. First, the distribution of penetrations with given proportions of center-type or eye-specific afferents was compared with that expected from randomly distributed afferents. Grids from both differed significantly from the expected binomial distribution for both categories (χ -squared test, $P < 0.05$). Second, a Monte Carlo analysis generated 10,000 grids that were identical to the actual data, except that the locations of the penetrations in the grid were randomized. A difference score was devised that would be at a minimum when all like penetrations were grouped together. The larger grid (Fig. 1d, Experiment 1) proved to be significantly patchy ($P < 0.01$); a lower difference score was rarely generated. The smaller grid (Fig. 1d, Experiment 2) was more clustered than four out of five of those drawn from a random population, but this difference was not deemed to be significant ($P \approx 0.16$).

Recordings from active cortex. In the recordings from the active cortex, cats were initially anesthetized with ketamine (10 mg per kg, intramuscular) followed by thiopental sodium (induction: 20 mg per kg, intravenous; maintenance: 1–2 mg per kg h^{-1} , intravenous; additional doses supplemented as needed). At the end of the surgery, animals were paralyzed with Norcuron (0.2 mg per kg h^{-1} , intravenous) and respired through an endotracheal tube. Temperature, electrocardiogram, electroencephalogram, pulse oximetry, indirect arterial pressure and expired CO_2 were monitored throughout the experiment. Pupils were dilated with 1% atropine sulfate and the nictitating membranes retracted with 2% neosynephrine. Eyes were refracted, fitted with contact lenses and focused on a tangent screen. All procedures were carried out in accordance with the guidelines of the US Department of Agriculture and approved by the Institutional Animal Care and Use Committee at the State University of New York, State College of Optometry.

Electrophysiological recordings and data acquisition. In the recordings from the active cortex, the recorded signals were amplified, filtered and collected by a computer running Plexon (Plexon). The field-potential recordings in visual cortex were filtered between 3 Hz and 2.2 kHz, and sampled continuously at 5 kHz. The spike recordings in both cortex and LGN were filtered below 250 Hz and above 8 KHz, and sampled at 40 kHz every time a spike crossed a threshold. The spike discrimination was carried out on-line and more rigorously off-line. LGN recordings were performed with a matrix of seven independently moveable electrodes made of quartz-insulated platinum-tungsten filaments (80- μm fiber, 25 μm of metal core, 1 μm at tip, impedance values from 1.5–6.0 $\text{M}\Omega$). Cortical recordings were obtained with a Silicon probe (NeuroNexus Technologies) with 16 different recording sites that were arranged vertically and separated by 100 μm from each other. Each recording site had an area of 703 μm^2 (26.5 μm in diameter, impedance of $< 0.6 \text{ M}\Omega$). The extracellular recordings from cortical simple cells illustrated in Figure 4 were obtained with standard sharp electrodes aimed at layer 4 (layer 4 was identified on the basis of electrode depth and the strong multiunit ‘hash’ generated by geniculate afferents).

Visual stimulation. In the recordings from the active cortex, visual stimuli were shown on a 20-inch monitor (Nokia 445Xpro, Salo) and generated either with Visionworks (Vision Research Graphics, frame rate of 120 Hz) or with an AT-vista graphics card (Truevision, frame rate of 128 Hz). We used white noise to map receptive fields by reverse correlation analysis. The white noise consisted of an m-sequence of checkerboards with 16×16 pixels (0.45–0.9 degrees), with each checkerboard being presented for 15.5 ms. The linearity of spatial summation of the geniculate cells was measured with full field–contrast reverse gratings. We used at least two different spatial frequencies that were higher than the optimal (usually 0.55 cycle per degree and 1.1 cycle per degree) and each spatial frequency was presented at eight different phases and repeated at least eight times per spatial phase at 0.4 Hz. Geniculate cells were classified in X or Y on the basis of this linearity test²⁸. For a small number of cells, visual responses

were strongly suppressed with full-field gratings (< 5 spikes per 50-ms bin); these cells were labeled as ‘nonclassified’.

STCSD analysis. The current sinks generated by individual geniculate afferents in the cortex were measured as follows²⁷. First, the spikes from well-isolated geniculate afferents were used as triggers to average the cortical field potentials that were simultaneously recorded at the 16 cortical sites (the spikes were obtained either under white noise stimulation or spontaneous activity). The second spatial derivative of these local field potentials is directly proportional to the current density at a point. Because the potential can only be sampled at finite intervals through the depth of the cortex, the second derivative is approximated by a finite-difference equation⁴⁸:

$$(\delta^2\Phi/\delta z^2) = [\Phi(z + n\Delta z) - 2\Phi(z) + \Phi(z - n\Delta z)] / (n\Delta z)^2,$$

where Φ is the field potential, z is the coordinate perpendicular to the layers, Δz is the sampling interval (100 μm) and $n\Delta z$ is the differentiation grid ($n = 2$). The differentiation grid 2 is equivalent to spatial smoothing and allows for the reduction of high spatial frequency noise. The results of this finite-difference equation, applied to each of the 16 field-potential recordings, are the current sinks and sources generated by a single geniculate afferent through the depths of the cortex, referred here as STCSD.

Our estimates indicate that the spatial specificity of STCSD is $< 150 \mu\text{m}$. First, spike-triggered field potentials recorded in the barrel cortex fall sharply (85% amplitude reduction) when measured in a neighboring barrel that is just 150 μm away (see Figs. 6 and 7 in ref. 49; the barrel cortex is ideal for these measurements because most thalamic axons are completely restricted to one barrel). Second, theoretical measurements indicate that the amplitude of a current sink falls sharply in $< 150 \mu\text{m}$ when measured laterally from its border (Fig. 3 in ref. 50). Note that $< 150 \mu\text{m}$ is an overestimate because the theoretical measurements assume a flat distribution of currents, whereas the distribution of synaptic boutons is approximately Gaussian (Fig. 5 and ref. 29). Notably, experiments making multiple closely spaced penetrations in cat visual cortex indicate that STCSD does not overestimate the size of the geniculate arbors and that it can reproduce quite closely the ratio of cortical distance covered by Y/X axonal arbors (1.4 (ref. 29) versus 1.6 according to STCSD, Fig. 5).

The STCSD generated by a single geniculate afferent in the cortex had a fast axonal component (0.8–2.1 ms after the geniculate spike), followed by a synaptic delay (~ 0.5 ms) and a postsynaptic sink (defined here as the first 1 ms following the synaptic delay). A STCSD sink was considered significant if it met three criteria. First, the amplitudes of both the STCSD axonal component and postsynaptic sink were larger than 40 $\mu\text{V mm}^{-2}$. Second, there was a gap (zero-crossing) between the axonal component and the postsynaptic sink. Third, the signal-to-noise ratio was greater than 5 (signal-to-noise ratio, postsynaptic sink/baseline; baseline, current sink amplitude 1 ms before the axonal component). This set of criteria proved to be highly reliable in identifying geniculate axons that were located in the immediate neighborhood of the electrode (in each of 37 cases in which an STCSD passed these criteria, cross-correlation analysis between the geniculate single unit and the cortical multiunit recorded with the silicon probe revealed a narrow peak displaced from zero, indicating the presence of the geniculate axon and/or monosynaptic connected cells in the immediate vicinity of the electrodes).

It should be noted that the STCSD generated by a single geniculate afferent is very different from the STCSD generated by pairs of neighboring afferents (multi-afferent STCSD). In the multi-afferent STCSD, the axonal component is wider, sometimes has multiple peaks, and there is no gap between the axonal component and the postsynaptic sink (because neighboring afferents do not have identical axonal conduction times, spike waveforms and depth profiles). In that sense, the main criterion to identify a single-afferent STCSD (presence of a synaptic gap) is very similar to the criterion used to identify a well-isolated unit in an extracellular recording (presence of a refractory period).

The fields generated by single thalamic afferents are not homogeneous in the horizontal dimension and, therefore, our measurements of current density violate the assumption of an ‘infinite, homogeneous volume conductor’⁴⁸. However, all studies that use current source–density analysis violate this assumption to some extent or another, because all brain regions are finite horizontally and stimuli do not generate uniform fields. With respect to the current investigation, an important question concerns how far the probe

recording sites 'see' horizontally relative to the horizontal extent of the currents that are generated. Geniculate arbors in the cat are generally 0.7–1.3 mm in horizontal extent and the distribution of synaptic boutons in the main arbor clump is roughly Gaussian²⁹; our procedures of retinotopic alignment strive to place the recording probe near the center of this arborization. Because a previous study in the barrel cortex showed a very steep drop in the strength of spike-triggered field potentials when recording from sites misaligned by a single barrel (150 μm)⁴⁹, the distance that our probes see horizontally is likely to be small relative to the extent of the geniculate arbor (see also Fig. 5a,b). Moreover, the main purpose of this study was not to make precise, absolute measures of synaptic (or axonal) currents, but to identify axons projecting in the neighborhood of our probe. For these reasons, the violation of the homogeneity assumption is unlikely to have a substantial influence in our results.

The laminar location of the current sinks generated by single geniculate afferents was estimated from the location of the current sink generated by full-field stimuli (white-black sequences presented at 0.5 Hz and repeated 100 times). In the cat, these stimuli generate strong current sinks in the cortical layers that receive the densest geniculate input (layers 4 and 6), and these sinks can be used as references to identify the laminar location of the recordings. The horizontal diameter of the current sink was measured as the maximum cortical distance between significant current sinks. These measurements were obtained after making multiple cortical penetrations (mean, 7; range, 4–10), separated by 100–200 μm from each other, along the anteroposterior axis of the lateral gyrus (each penetration was aimed at the very center of the gyrus). The strength of the current sink was measured at the peak of the sink, within 1 ms following the synaptic delay.

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AUTHOR CONTRIBUTIONS

J.A.G., E.S.R. and M.P.S. designed, executed and analyzed the studies of muscimol-silenced cortex. J.Z.J., C.W., C.-I.Y., H.A.S. and J.-M.A. designed, executed and analyzed the studies of the active cortex. All authors participated in the writing of the manuscript.

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