



Strabismus 0927-3972/02/\$ 16.00

Strabismus – 2002, Vol. 10, No. 2,
pp. 95–105
© Swets & Zeitlinger 2002

Accepted 1 February 2002

**Neuroanatomical and neurophysiological
consequences of strabismus: Changes
in the structural and functional
organization of the primary visual cortex
in cats with alternating fixation and
strabismic amblyopia**

**PD Dr. Siegrid Löwel
Dr. Ralf Engelmann**

Forscherguppe 'Visuelle Entwicklung und Plastizität',
Leibniz-Institut für Neurobiologie, Magdeburg, Germany

Abstract In recent years, evidence has accumulated indicating that long-ranging neuronal connections within the primary visual cortex (area 17) mediate the influences of context and experience, possibly also those of expectation. After early onset strabismus, the layout of these connections is massively modified: in strabismic but not in normally raised cats, horizontal connections extend primarily between neurons activated by the same eye. As a possible consequence of the modified circuitry, neuronal synchronization between different ocular dominance domains is also massively reduced. Thus, the inability of strabismics to combine the signals arriving from the two eyes into a single percept may be caused by these structural and functional changes.

Strabismic amblyopia is also accompanied by significant modifications of intracortical associational interactions: corresponding to the psychophysical deficits, neurons driven by the normal eye displayed stronger synchronization of their responses than neurons dominated by the amblyopic eye.¹ These data demonstrated for the first time a clear neurophysiological correlate of strabismic amblyopia in area 17. They suggest that – similar to our observations in divergent squinters – at least some of the perceptual deficits of amblyopic patients are due to experience-dependent changes in intracortical circuitry. We analyze this question by combining optical imaging of intrinsic signals with 3-D reconstructions of neuronal circuitry.

Key words Strabismic amblyopia; alternating fixation; visual cortex; horizontal connections; intracortical connections; ocular dominance; orientation map; cats

*Correspondence and
reprint requests to:*

PD Dr. Siegrid Löwel
Forscherguppe 'Visuelle
Entwicklung und Plastizität'
Leibniz-Inst. für Neurobiologie
Brenneckestr.6
D-39118 Magdeburg
Germany
Tel.: +49-391-6263-637/619
Fax: +49-391-6263-648/638
E-mail: loewel@ifn-magdeburg.de
[http://www.ifn-magdeburg.de/
resgroups/rg4/rg4_home.jsp](http://www.ifn-magdeburg.de/resgroups/rg4/rg4_home.jsp)

Acknowledgements:

It is a pleasure for us to thank John M. Crook for his help in some of the experiments and for comments on the manuscript. We would also like to thank Thomas Dresbach for linguistic advice and Steffi Bachmann and Susann Becker for excellent technical assistance. Part of this work was supported by grants LO 442/5-1 and LO 442/5-2 of the DFG.

Introduction One of the basic features of visual scene analysis is to assemble the components of objects into a unified percept and to segregate them from background. Misalignment of the visual axes in early childhood leads to profound, often irreversible restrictions of adult visual performance. The visual acuity of the deviating eye is often dramatically reduced (strabismic amblyopia) and binocular functions, especially stereopsis, are reduced or not developed at all (stereo blindness).^{2,3} Strabismus is not only a common clinical symptom but also a well established model for developmental plasticity in the visual cortex because it is not actually the eyes that suffer but the cortex.

Because the images on the two retinae cannot be brought into register, strabismus eliminates correlated activity between the two eyes. If present in early life, this decorrelation leads to a breakdown of binocular convergence. As a consequence, the segregation of thalamocortical afferents into alternating ocular dominance columns is enhanced in area 17 of strabismic as compared with normal cats^{4,5} and visual cortical neurons become responsive almost exclusively to stimulation of either the left or the right eye.⁶ In strabismic cats, monocular visual stimulation induces 2-deoxyglucose (2-DG) labelled activity patterns extending in columns through all visual cortical layers, and these columns are in precise register with the thalamocortical afferents of the stimulated eye in layer IV.⁷

In recent years, evidence has accumulated indicating that long-ranging neuronal connections within the primary visual cortex (area 17) mediate the influences of context and experience, possibly also those of expectation. The following chapter focusses on squint-induced changes in the 'hardware' of these important computations, their functional consequences and on changes in the layout of functional cortical maps that are hypothesized to underlie the specific perceptual deficits of strabismics.

Functional architecture in area 17 of non-amblyopic squinters

In the visual cortex of the brain, long-ranging horizontal fibers interconnect regularly spaced clusters of cells and integrate information from widely distant points in the visual field (for a review, see Löwel and Singer⁸). Anatomical experiments in area 17 of divergently squinting cats revealed that cell clusters were driven almost exclusively from either the right or the left eye and that horizontal intracortical fibers preferentially connected cell groups activated by the same eye (Figure 1).⁹ Connections between cell groups driven by different eyes were dramatically reduced. In contrast, analysis of normally reared control animals provided no evidence for an eye-specific selectivity of horizontal connections. Since the horizontal network is rather homogeneous at birth,^{10,11} connections between cells that exhibit correlated activity are selectively stabilized. Thus, circuit selection depends on visual experience and the selection criterion is the correlation of activity, i.e. neurons that fire together wire together.

Theoretical considerations on the neuronal substrate of cognitive processes have led to the hypothesis that synchronous firing of neurons might play an important role in the processing of visual information (for a review, see Singer¹² and Engel et al.¹³) by binding activity of dis-

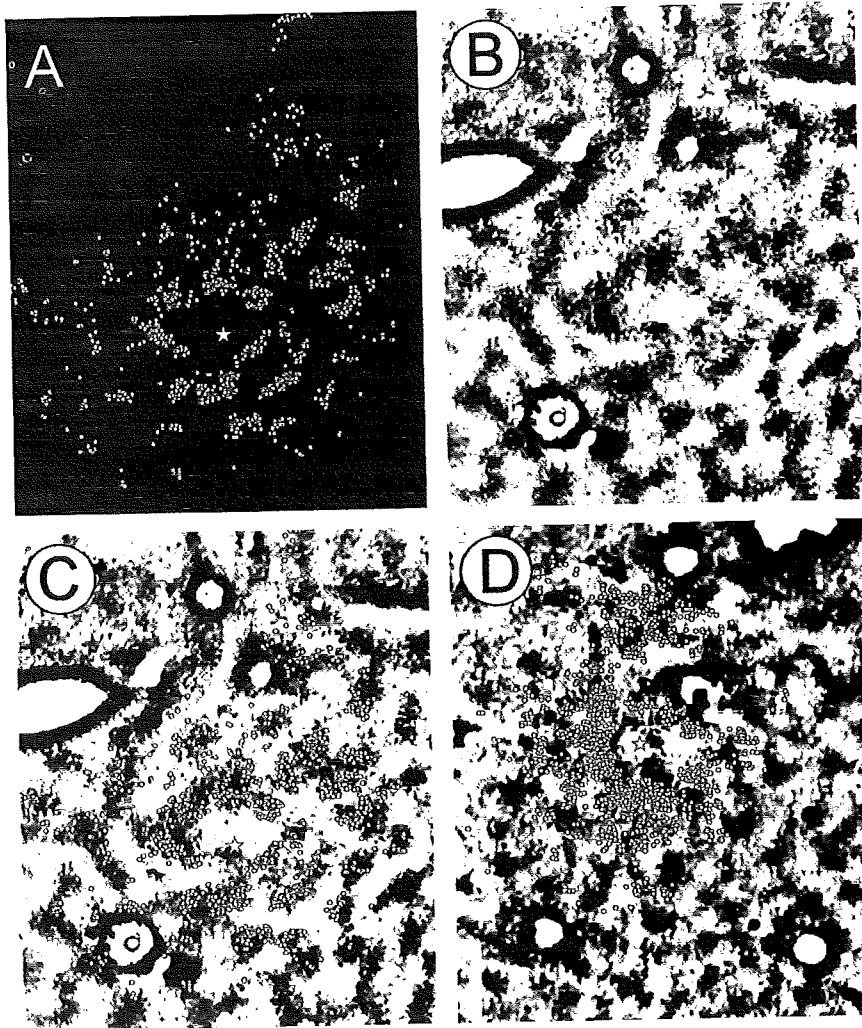


Fig. 1. In strabismic cats, horizontal intracortical fibers preferentially connect cell groups activated by the same eye. Topographic relations between ocular dominance columns and patchy intrinsic connections in the primary visual cortex of a strabismic (A–C) and a normally raised cat (D). (A) Distribution of retrogradely labelled cells after injection of a neuronal tracer into a right eye domain. White dots, the position of individual cells; asterisk, injection site. (B) 2-Deoxyglucose pattern showing the topography of ocular dominance territories in the region containing the retrogradely labelled cells in A. The black regions represent the domains of the right eye. (C) Superposition of A and B. Most of the retrogradely labelled cells are located in black regions (right eye domains) and thus in regions of the same ocular dominance as the injection site. (D) Superposition of ocular dominance domains (black regions) and retrogradely labelled neurons (white dots) in a normally raised cat. Note the absence of a systematic topographic relationship between the two patterns. Reprinted in modified form with permission from Löwel and Singer.⁹

tributed cortical neurons into coherent representations. If neuronal synchronization is really important for perceptual binding, then it should be modified in a predictable way in cases in which perception is modified. Psychophysical evidence indicates that strabismics are unable to combine the signals arriving from the two eyes into a single percept, even if these signals are made congruent by optical compensation of the squint angle.² In line with these perceptual capabilities, cross-correlation analyses in divergently squinting cats indeed revealed that neuronal synchronization was significantly reduced between cells dominated by different eyes while it was as frequent and strong as in normal cats between cells dominated by the same eye (Figures 2 and 3).¹⁴ Thus, squint not only cuts down horizontal fibers but also strongly reduces intracortical interactions between different ocular dominance domains.

By using optical imaging of intrinsic signals,¹⁵ it was shown that visual cortical activity patterns were different after left and right eye stimulation in both divergently and convergently squinting cats, so that both groups of animals displayed clearly segregated maps of left and

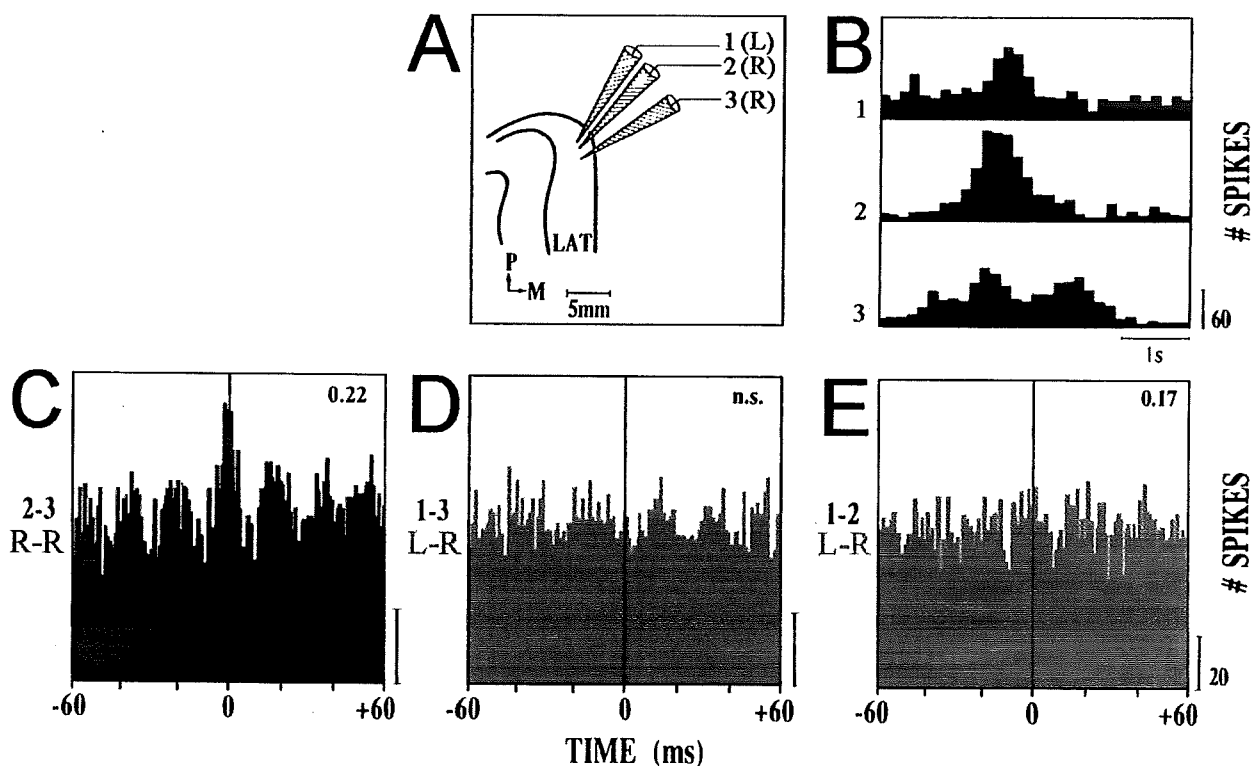


Fig. 2. Reduced neuronal synchronization between domains driven by different eyes in strabismic cats. (A) Position of the recording electrodes in area 17. Cells at electrode 1 responded exclusively to stimulation of the left (L), neurons at electrodes 2 and 3 to stimulation of the right (R) eye. (B) Peristimulus time histograms of neuronal responses at electrodes 1–3. (C, D, E) Cross-correlograms between the responses of the three cell groups. Synchronization was strong for cells with the same ocular dominance (2–3) (C) but weak (1–2) (E) or absent (1–3) (D) between cells with different ocular dominance. P, posterior; M, medial; LAT, lateral gyrus of the visual cortex; n.s., not significant. Reprinted in modified form with permission from König et al.¹⁴

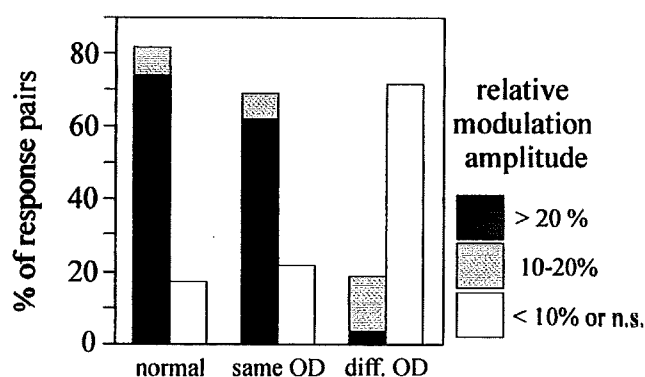


Fig. 3. Incidence and strength of response synchronization as a function of ocular dominance. The leftmost histogram shows data from normal cats. The middle histogram represents the correlograms of recordings from cell groups with the same ocular dominance (same OD), and the histogram to the right the correlation of cells with different ocular dominance (diff. OD). Black columns represent cases with strong synchronization, i.e. correlograms with a relative (rel.) modulation amplitude > 0.20; grey columns indicate weak correlations (rel. modulation amplitude between 0.10 and 0.20) and unfilled columns comprise correlation functions that did not show a significant modulation (n.s.) or had a relative modulation amplitude < 0.10. Reprinted in modified form with permission from König et al.¹⁴

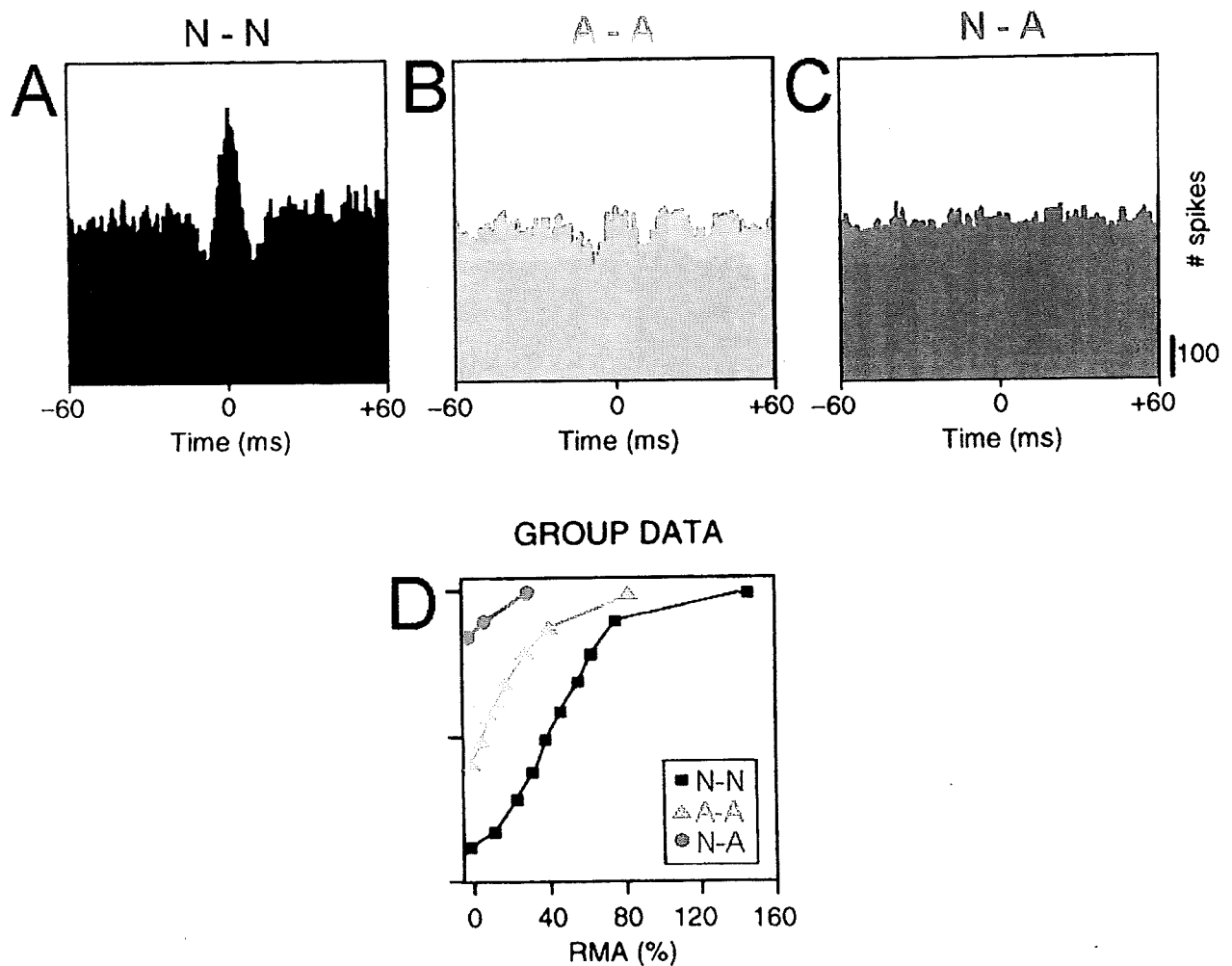


Fig. 4. Reduced neuronal synchronization between domains dominated by the amblyopic eye. (A, B, C) Cross-correlograms between pairs of recording sites driven by the normal (N-N) (A) or the amblyopic (A-A) (B) eye, or by two different eyes (N-A) (C). Synchronization was strong for cells driven by the normal eye (A), much weaker for A-A pairs and absent in an N-A pair. (D) Cumulative distribution of the relative modulation amplitudes (RMA) of cross-correlograms pooled over four animals. Reprinted in modified form with permission from Roelfsema et al.¹

right eye dominance domains in primary visual cortex.^{16,17} Furthermore, the combination of optical imaging with 3-D reconstructions of horizontal fiber networks revealed that convergent (non-amblyopic) squinters – as previously also shown for divergent squinters⁹ – have strongly reduced connections between the two sets of domains while the functional selectivity of the within-eye connections is rather normal.^{18,19}

We hypothesize that the inability of squinters to combine signals conveyed by the two eyes into a single percept is due to these structural and functional modifications of the neuronal architecture of the primary visual cortex.

Functional architecture in area 17 of amblyopic squinters While double vision is avoided by alternating fixation in non-amblyopic squinters, in strabismic amblyopes, only one eye takes part in fixation and the non-fixating eye is constantly suppressed from conscious experience. The resulting perceptual deficits of the deviating eye include a reduction of visual acuity (amblyopia), spatial distortions, crowding and a temporal instability of the visual scene.^{2,3} For years,

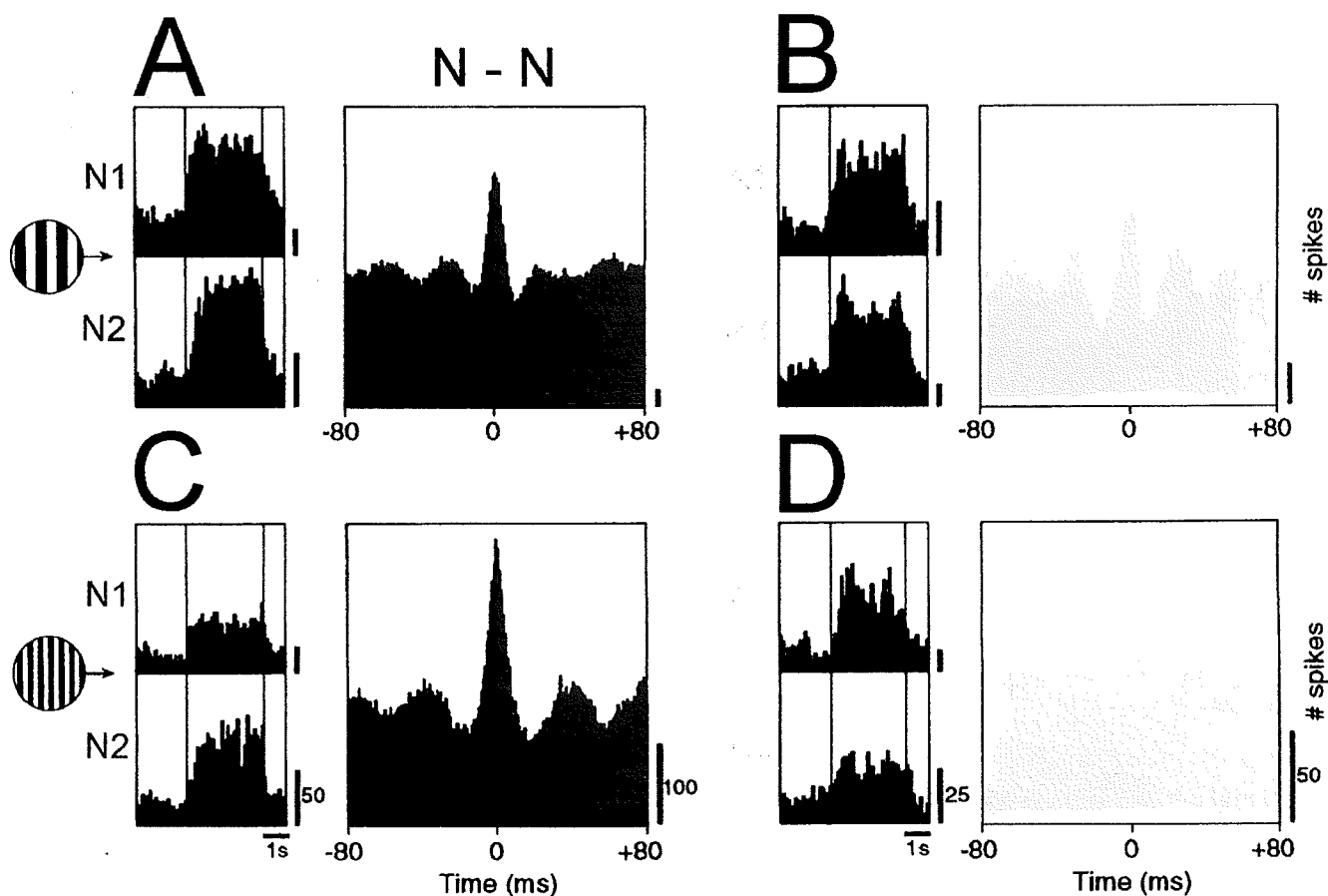


Fig. 5. Neuronal synchronization between cells driven by the amblyopic eye is especially weak at high spatial frequencies. Amplitudes and synchronization of responses to gratings of low (A, B) and high (C, D) spatial frequency for an N-N pair (A, C) and an A-A pair (B, D). Response histograms (left) illustrate that neurons at all recording sites clearly responded to both gratings. In contrast, response synchronization (cross-correlograms to the right) was severely reduced in the A-A pair at higher spatial frequency (compare B with D: the relative modulation amplitude of the central peak decreased from 80% for the coarse grating (B) to 30% for the fine grating (D)). Reprinted in modified form with permission from Roelfsema et al.¹

single cell recordings in squinting cats and monkeys have failed to disclose a clear relationship between the amblyopic deficits and modifications of neuronal response properties in the visual cortex.²⁰ Recently, however, a clear neurophysiological correlate of strabismic amblyopia has been demonstrated in the primary visual cortex. Roelfsema et al.¹ showed that strabismic amblyopia is accompanied by significant modifications of intracortical associational interactions: corresponding to the perceptual deficits, neurons dominated by the normal eye displayed stronger synchronization of their responses than neurons dominated by the amblyopic eye in cat area 17 (Figure 4). The interocular differences were highly significant and particularly pronounced for grating stimuli of high spatial frequency (Figure 5). These data suggested that the reduced synchronization between cells dominated by the amblyopic eye is due to abnormalities in the pattern of connections linking neurons driven from this eye. Thus, we hypothesize that – similar to our observations in divergent squinters – at least some of the perceptual deficits of amblyopic patients may actually be due to experience-dependent changes in intracortical circuitry.

We started to analyze this question by combining optical imaging of intrinsic signals with detailed 3-dimensional reconstructions of horizontal fiber networks in cats with behaviorally determined strabismic amblyopia. Our preliminary results are as follows.

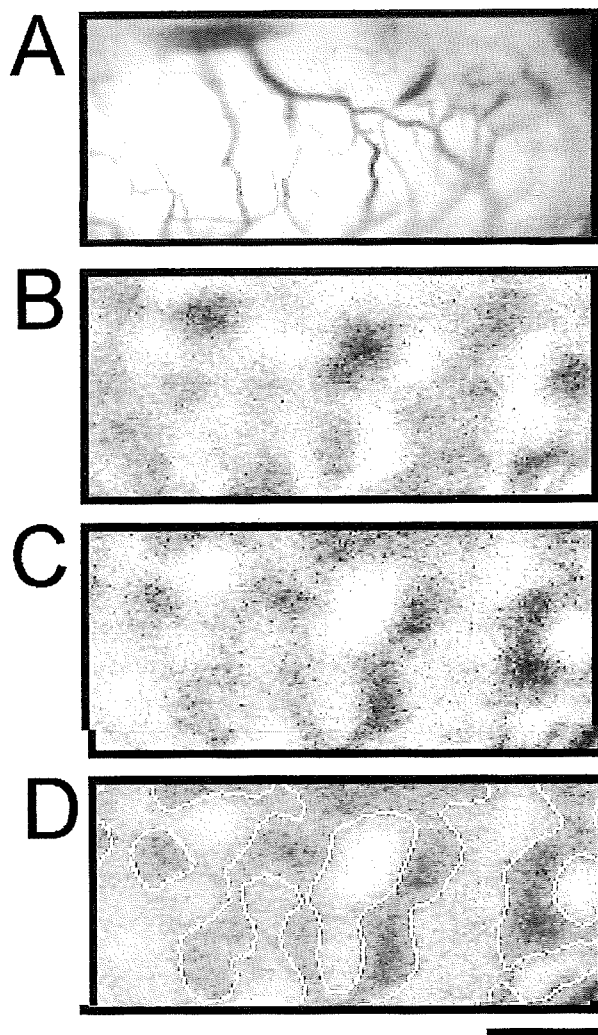


Fig. 6

Fig. 6. Segregated ocular dominance columns in area 17 of a cat with strabismic amblyopia. In the present example, cortical activity maps were induced by visual stimulation of the animal with moving square wave gratings of low spatial frequencies (0.25 and 0.5 cyc/deg) well below the behavioral threshold (3.0 cyc/deg for the normal and 1.5 cyc/deg for the amblyopic eye, as determined by using a modified jumping stand apparatus, see Katz and Sireteanu²⁵). (A) Blood vessel pattern of the imaged cortical area. Activation patterns for the normal (B) and amblyopic eye (C) are complementary: regions heavily activated by the amblyopic eye (dark regions in C, outlined in white in D) are only weakly activated by the normal eye (light grey regions in B) and vice versa. Scale bar, 1 mm.

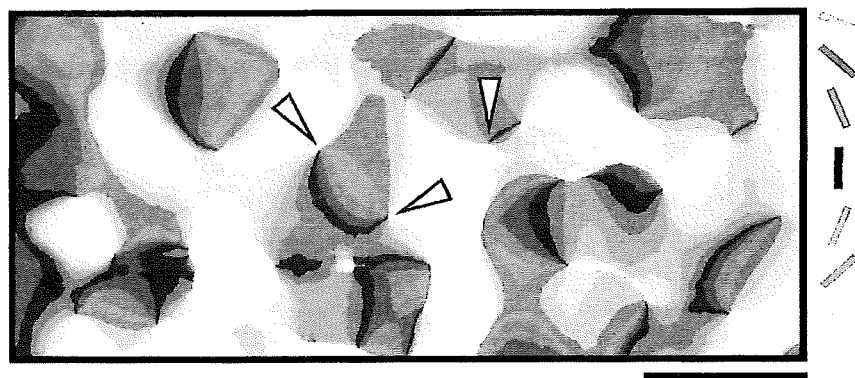
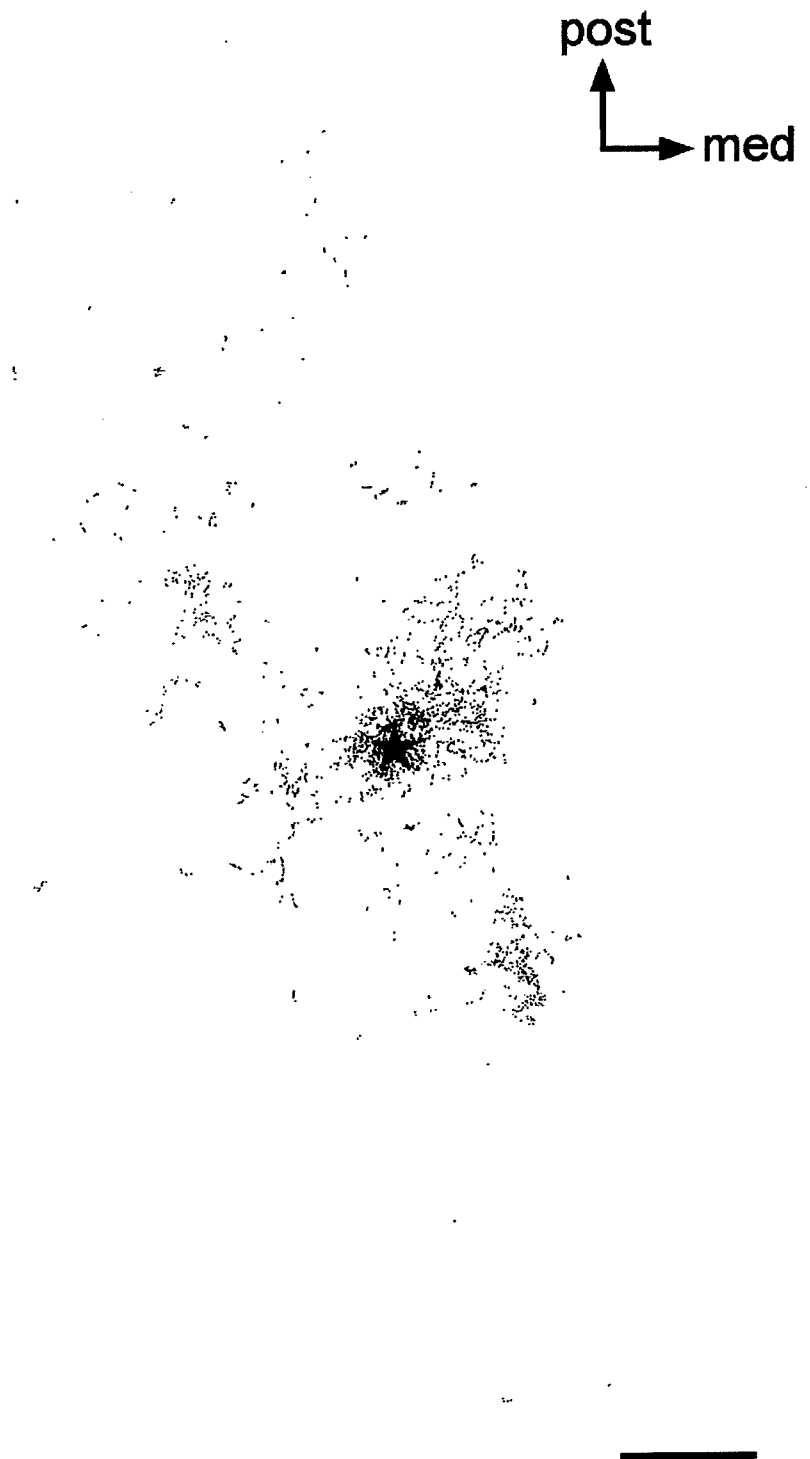


Fig. 7

Fig. 7. Orientation preference ('angle') map of the same piece of cortex as illustrated in Figure 6. Cortical activity maps were induced by visual stimulation of the left and right eye with low spatial frequency gratings (see legend of Figure 6). The preferred orientation for every region of the imaged cortex is greyscale-coded according to the scheme on the right side of the figure. Note the pinwheel-like organization of orientation domains: there are numerous singularities in the map (arrowheads) around which all grey-levels (orientations) appear once. Scale bar, 1 mm.

Optical imaging of the functional architecture of area 17 revealed that monocular iso-orientation domains had a patchy appearance and were different for the left and right eye, so that segregated ocular dominance domains could be visualized (Figure 6) (see also Löwel et al.¹⁶). Importantly, at spatial frequencies well below the cats behavioral

Fig. 8. Horizontal connections in strabismic amblyopes are long-ranging and patchy. Topography of long-range intrinsic connections visualized after a biocytin injection into area 17. The injection site is marked with a black asterisk. Each black dot corresponds to a single bouton. Scale bar, 0.5 mm.



threshold, there were no differences in activity levels between domains activated by the normal and amblyopic eye. Our optical imaging data thus give no indication of a disadvantage of the amblyopic compared to the normal eye for analyzing low spatial frequency gratings,

corresponding to the perceptual capabilities. In contrast, at higher spatial frequencies, where visual performance is impaired, the activity levels of the amblyopic eye are reduced compared to those of the normal eye.²¹

Iso-orientation domains exhibited a pinwheel-like organization (Figure 7) as previously described for normally raised^{22,23} and non-amblyopic exo- and esotropic cats,^{16,17} indicating that the basic structure of orientation preference maps is not obviously disturbed by strabismic amblyopia.

Injections of the tracer biocytine (see, for example, Kisvárdy et al.²⁴) into optically visualized and electrophysiologically characterized sites in area 17 revealed that horizontal connections extending from domains driven by the amblyopic eye are both long-ranging and patchy (Figure 8). The functional specificity of the horizontal network and possible differences in layout and selectivity compared to connections extending from domains activated by the normal eye are the subject of current investigations.

Summary & conclusions

1. In the primary visual cortex (area 17) of strabismic cats, neurons become responsive almost exclusively to stimulation of either the left or the right eye while in normally raised animals, the majority of neurons are binocularly driven.
2. The segregation of thalamocortical afferents into alternating ocular dominance columns is enhanced in area 17 of strabismic as compared with normal cats.
3. In area 17 of strabismic animals, activity patterns induced after stimulation of the left or the right eye are clearly different, whereas activity patterns induced through the two eyes in normal animals are nearly identical.
4. In area 17 of strabismic cats, long-ranging horizontal (intracortical) fibers preferentially connect cell groups activated by the same eye, while there is no evidence for an eye-specific selectivity of the horizontal network in normally raised animals. Visual experience thus influences the development of the long-ranging intracortical circuitry and the selection criterion for the selective stabilization of pathways is correlated activity: 'neurons that fire together wire together'.
5. In area 17 of strabismic cats, neuronal synchronization between cells located in different ocular dominance domains is massively reduced, while it is as frequent and strong as in normal cats between cells dominated by the same eye.
6. Taken together, the domains of the left and right eye in area 17 of strabismic cats are clearly segregated both structurally and functionally. The modified structural and functional architecture of area 17 may therefore underlie the perceptual deficits observed in strabismics, i.e. the inability to combine signals arriving from the two eyes into a single percept.
7. It remains to be determined whether modified horizontal connections are also responsible for reduced neuronal synchronization between cells dominated by the amblyopic eye and thus for the perceptual deficits of patients with strabismic amblyopia.

References

- 1 Roelfsema PR, König P, Engel AK, Sireteanu R, Singer W. Reduced synchronization in the visual cortex of cats with strabismic amblyopia. *Eur J Neurosci.* 1994;6:1645–1655.
- 2 Duke-Elder S, Wybar K. *System of Ophthalmology. VI. Ocular Motility and Strabismus.* London, U.K.: Kimpton, 1973.
- 3 Von Noorden GK. *Binocular Vision and Ocular Motility. Theory and Management of Strabismus.* St. Louis, MO: C.V. Mosby Co., 1990.
- 4 Shatz CJ, Lindström S, Wiesel TN. The distribution of afferents representing the right and left eyes in the cat's visual cortex. *Brain Res.* 1977;131:103–116.
- 5 Löwel S. Ocular dominance column development: Strabismus changes the spacing of adjacent columns in cat visual cortex. *J Neurosci.* 1994; 14:7451–7468.
- 6 Hubel DH, Wiesel TN. Binocular interaction in striate cortex of kittens reared with artificial squint. *J Neurophysiol.* 1965;28:1041–1059.
- 7 Löwel S, Singer W. Monocularly induced 2-deoxyglucose patterns in the visual cortex and lateral geniculate nucleus of the cat: II. Awake animals and strabismic animals. *Eur J Neurosci.* 1993;5: 857–869.
- 8 Löwel S, Singer W. Experience-dependent plasticity of intracortical connections. In: Fahle M, Poggio T, editors. *Perceptual Learning.* Cambridge: MIT Press, 2002: in press.
- 9 Löwel S, Singer W. Selection of intrinsic horizontal connections in the visual cortex by correlated neuronal activity. *Science.* 1992;255: 209–212.
- 10 Callaway EM, Katz LC. Emergence and refinement of clustered horizontal connections in cat striate cortex. *J Neurosci.* 1990;10: 1134–1153.
- 11 Katz LC, Callaway EM. Development of local circuits in mammalian visual cortex. *Annu Rev Neurosci.* 1992;15:31–56.
- 12 Singer W. Neuronal synchrony: A versatile code for the definition of relations? *Neuron.* 1999;24:49–65.
- 13 Engel AK, Roelfsema PR, Fries P, Brecht M, Singer W. Role of the temporal domain for response selection and perceptual binding. *Cerebral Cortex.* 1997;7:571–582.
- 14 König P, Engel AK, Löwel S, Singer W. Squint affects synchronization of oscillatory responses in cat visual cortex. *Eur J Neurosci.* 1993;5: 501–508.
- 15 Bonhoeffer T, Grinvald A. Optical imaging based on intrinsic signals: The methodology. In: Toga A, Mazziotta JC, editors. *Brain mapping: The methods.* San Diego, CA: Academic Press, 1996; 55–97.
- 16 Löwel S, Schmidt KE, Kim D-S, Wolf F, Hoffmann F, Singer W, Bonhoeffer T. The layout of orientation and ocular dominance domains in area 17 of strabismic cats. *Eur J Neurosci.* 1998;10: 2629–2643.
- 17 Engelmann R, Crook JM, Löwel S. Optical imaging of orientation and ocular dominance maps in area 17 of cats with convergent strabismus. *Vis Neurosci.* 2002;19:1–11.
- 18 Engelmann R, Crook JM, Kisvárdy ZF, Löwel S. Connectivity patterns in area 17 of convergently squinting cats (Abstract). *Soc Neurosci Abstr.* 2001;27:285.1.
- 19 Engelmann R, Crook JM, Kisvárdy ZF, Löwel S. Functional specificity of horizontal intracortical circuitry in the primary visual cortex (area 17) of convergently squinting cats (Abstract). In: Elsner N, Kreutzberg GW, editors. *The Neurosciences at the Turn of the Century. Proceedings of the 4th Meeting of the German Neuroscience Society 2001 (28th Göttingen Neurobiology Conference).* Stuttgart-New York: Thieme, 2001;607.
- 20 Von Noorden GK. Amblyopia in humans and clinical relevance of animal models. In: Lennerstrand G, Von Noorden GK, Campos EC, editors. *Strabismus and Amblyopia.*

- New York: Plenum Press, 1988; 169–171.
- 21 Schmidt KE, Galuske RAW, Singer W. Orientation maps of amblyopic cats at different spatial frequencies (Abstract). *Soc Neurosci Abstr.* 1999;25:1423.
 - 22 Bonhoeffer T, Kim D-S, Malonek D, Shoham D, Grinvald A. Optical imaging of the layout of functional domains in area 17 and across the area 17/18 border in cat visual cortex. *Eur J Neurosci.* 1995;7: 1973–1988.
 - 23 Hübener M, Shoham D, Grinvald A, Bonhoeffer T. Spatial relationships among three columnar systems in cat area 17. *J Neurosci.* 1997;17: 9270–9284.
 - 24 Kisvárdy ZF, Toth E, Rausch M, Eysel UT. Orientation-specific relationship between populations of excitatory and inhibitory lateral connections in the visual cortex of the cat. *Cerebral Cortex.* 1997;7: 605–618.
 - 25 Katz B, Sireteanu R. Development of visual acuity in kittens: A comparison between jumping stand and Teller acuity card test. *Clin Vis Sci.* 1992;7:219–224.