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STRABISMUS MODIFIES INTRINSIC AND INTER-AREAL CONNECTIONS IN CAT AREA 18

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Abstract—The development of long-range horizontal connections depends on visual experience. Previous experiments have shown that in area 17 of strabismic but not in normal cats, horizontal fibers preferentially connect cell groups driven by the same eye indicating that fibers between coactive neurons are selectively stabilized. To test whether this is a general organizing principle of intracortical long-range circuitry we extended our analyses to both intrinsic horizontal connections within area 18 and to inter-areal connections between areas 17 and 18. To this end, we visualized the functional architecture of area 18 by intrinsic signal imaging. Horizontal circuitry was labeled by injecting fluorescent latex microspheres into functionally identified domains. Additionally, domains sharing the same ocular dominance as the neurons at the injection sites were visualized by 2-deoxyglucose autoradiography to allow comprehensive labeling of functional domains in regions far from the injection sites. Quantitative analyses revealed that in strabismic cats, 72% of the retrogradely labeled neurons in area 18 and 68% of the neurons in area 17 were located in the same ocular dominance domains as the injection sites. In contrast, these numbers were 52% and 54% in normal animals. These data show that experience modifies both intrinsic connections within area 18 and inter-areal projections from area 17 to area 18 as has been previously described for intrinsic and callosal connections in area 17. This provides further evidence for the hypothesis that the correlation of activity is a major selection criterion for the stabilization of neuronal circuits during postnatal development.

Key words: area 18, strabismus, tangential connections, development, visual cortex.

Long-range tangential connections are a prominent feature of cortical circuitry (Fisken et al., 1975). In the mammalian visual cortex, they interconnect regularly spaced clusters of cells with similar functional properties such as preference for stimulus orientation or color (Ts'o et al., 1986; Ts'o and Gilbert, 1988; Gilbert and Wiesel, 1989; Gray et al., 1989; Hata et al., 1991; Malach et al., 1993, 1994; Kisvárday et al., 1997; Bosking et al., 1997; Schmidt et al., 1997). These horizontal pathways mediate inhibitory and excitatory effects from outside the classical receptive field and generate functionally coherent cell assemblies by synchronizing the activities of spatially distributed neurons (for a review see Löwel and Singer, 2002). Horizontal connections in the primary visual cortex (area 17) attain their adult specificity through experience-dependent mechanisms during the first 2–3 months after birth in cats (Luhmann et al., 1991; Callaway and Katz, 1991; Galuske and Singer, 1996; Löwel and Singer, 2002). Direct evidence for a selective stabilization of connections between cells exhibiting correlated activity was obtained in area 17 of strabismic cats. In strabismics, the optical axes of the two eyes are not aligned so that responses mediated by anatomically corresponding retinal loci are not correlated. As a consequence, squint accentuates the segregation of the geniculocortical afferents from the two eyes in layer IV (Shatz et al., 1977; Löwel, 1994), and most of the cells in area 17 become exclusively responsive to stimulation of either the left or the right eye (Hubel and Wiesel, 1965; König et al., 1993; Van Sluyters and Levitt 1980; Sengpiel et al., 1994). In addition, neuronal synchronization between different ocular dominance domains is severely reduced in strabismic cats compared with normal cats (König et al., 1993). Finally, tracing experiments revealed that cell clusters are driven almost exclusively from either the right or the left eye and that horizontal intracortical fibers preferentially connect cell groups activated by the same eye (Löwel and Singer, 1992; Trachtenberg and Stryker, 2001).

Thus, the development of long-range intracortical connections in cat area 17 depends on visual experience and horizontal fibers seem to be established and maintained preferentially between neurons with correlated neuronal activity (Löwel and Singer, 1992; Schmidt et al., 1997; Trachtenberg and Stryker, 2001).

To test whether this is a general organizing principle of intracortical long-range circuitry we extended our analyses to both intrinsic horizontal connections within area 18 and to inter-areal connections between areas 17 and 18. Interestingly, far less is known about the experience-dependent plasticity of neuronal circuitry in area 18, even though area 18 is one of the major output regions of area 17 (Scannell and Young, 1993). While the postnatal development of connections in area 18 is similar to that in area 17 (Price, 1986), their experience-dependent plasticity has not yet been studied in detail. Using optical imaging of intrinsic signals, we recently showed that in area 18 of strabismic cats, activity maps induced after stimulation of the left or the right eye with moving gratings are much more similar than in area 17 of strabismic cats and resemble those visualized in area 18 of normally raised animals (Schmidt and Löwel,

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Abbreviations: 2-DG, 2-deoxyglucose.

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2006a,b). Functional ocular dominance domains are thus not as clearly segregated in area 18 of strabismic cats as they are in area 17 (Löwel and Singer, 1992, 1993a,b).

Furthermore, area 18 in cats receives direct input from the lateral geniculate nucleus and also major input from area 17. While area 17 predominantly receives input from X-cells, area 18 is dominated by input from Y-cells (Stone, 1983; Orban, 1984; Ferster, 1990a,b). Y-cells possess both larger receptive fields and larger thalamocortical afferent arbors terminating in layer IV compared with X-cells (Stone and Dreher, 1973; Harvey, 1980; Sherman and Spear, 1982; Humphrey et al., 1985a,b). This difference in input patterns between the two visual cortical areas might differentially affect the binocularity and binocular interactions of neurons in these areas and thereby also the eye specificity of both intrinsic and inter-areal connections. We therefore wondered whether strabismus would modify long-range intracortical connections in area 18 or whether functional selectivities for horizontal fibers would instead be similar between control and strabismic animals.

To this end, we first imaged the functional architecture of cat area 18 using intrinsic signal imaging (Schmidt and Löwel, 2006a,b), injected a retrograde tracer into functionally identified domains (Schmidt et al., 1997), visualized ocular dominance domains in addition by [^{14}C]-2-deoxyglucose (2-DG) autoradiography in awake animals (Löwel and Singer, 1992; 1993b; Löwel, 2002) to allow domains in regions far from the injection sites to be comprehensively labeled, and finally compared the distributions of retrogradely labeled neurons with the 2-DG-labeled functional domains (Löwel and Singer, 1992; Schmidt et al., 1997). Quantitative analyses were performed for both intrinsic horizontal connections within area 18 and for inter-areal connections between areas 17 and 18. Our results revealed that strabismus significantly modified both intrinsic horizontal connections within area 18 and inter-areal projections from area 17 to area 18.

EXPERIMENTAL PROCEDURES

Nine kittens from the colony of the Leibniz-Institute for Neurobiology in Magdeburg, Germany, were included in the present study. In six of the kittens, a divergent squint angle was induced surgically at postnatal day 17 or 18 (Löwel and Singer, 1992; Löwel et al., 1998; Roelfsema et al., 1994). Three normally raised cats served as controls. All animals were >8 weeks of age at the time of the imaging experiments.

All animal experiments were performed according to the German Law on the Protection of Animals and the corresponding European Communities Council Directive of November 24, 1986 (86/609/EEC). The experiments were designed to minimize the number of animals and their suffering.

Squint induction

The squint induction was carried out under anesthesia induced with an i.m. injection of ketamine (10 mg/kg, Ketanest®, Parke-Davis, Berlin, Germany) and xylazine hydrochloride (2.5 mg/kg, Rompun®, Bayer AG, Leverkusen, Germany). After incision of the conjunctiva, the tendon of the medial rectus muscle (divergent squint) was located and cut. In all strabismic animals, the angle of the resulting squint was measured repeatedly during early postnatal development using the corneal reflex method (Sherman, 1972; Olson and Freeman, 1978; von Grünau, 1979). To this end, the animals were manually restrained, several flashlight snapshots of the cats' heads were taken, and the ratio of the distance between the corneal reflexes over the distance between the pupils was determined from the photo prints. This ratio is a reliable indicator of eye alignment (Sherman, 1972; Sireteanu et al., 1993). The ratios of our divergent squinters were always below 0.93, and thus in the range of divergent squinters throughout the critical period (von Grünau, 1979; Sireteanu et al., 1993).

Optical imaging

To visualize the functional architecture of cat area 18, we used standard procedures for intrinsic signal optical imaging as detailed previously (Engelmann et al., 2002; Schmidt and Löwel, 2006a,b). In short, we used inhalation anesthesia (50% nitrous oxide/50% oxygen, supplemented with 0.8–1.2% halothane) throughout the experiment. The electrocardiogram, pulmonary pressure, end tidal CO_2 (3–4%), and rectal temperature (37–38°) were continuously monitored. To optically image area 18, a craniotomy was performed centered at Horsley-Clarke coordinate A7. Animals were stimulated monocularly with high-contrast square-wave gratings of four orientations (0°, 45°, 90°, and 135°) moving at a speed of two cyc/s with a spatial frequency of 0.15 cyc/degree at a distance of 25 cm from the eyes. The vascular pattern of the cortex was visualized at 546±10 nm (green), cortical activity maps at 707±10 nm (red). While imaging, the camera was focused 650–750 μm below the cortical surface. We used episodic stimulation during data acquisition (7 frames of 600 ms duration). We calculated "single condition maps" in which the images acquired during presentation of a particular stimulus were divided by the sum of all different stimulus conditions ("cocktail blank procedure"; see Bonhoeffer and Grinvald, 1993, 1996; Löwel et al., 1998; Engelmann et al., 2002).

Tracer injection

At the end of the optical imaging session, rhodamine- and fluorescein-conjugated latex microspheres ("red and green beads," Luma Fluor Inc. (Naples, FL, USA); Katz et al., 1984; Katz and Iarovici, 1990) were pressure-injected into functionally identified domains as detailed previously (Schmidt et al., 1997). Using the vascular pattern as a reference, all injections were placed into cortical regions that were strongly dominated by one eye and had unambiguous orientation preferences as determined by optical imaging (see Fig. 1). In addition, the locations of all injection sites were checked by superimposing the cell plots with the 2-DG autoradiographs (see below). The injections ranged from 200 to 400 μm in diameter.

2-DG autoradiography and histological procedures

One week after the tracer injections, we performed a 2-DG experiment in awake animals to label ocular dominance domains. To this end, a venous catheter was implanted under halothane anesthesia using an inhalation mask (2–4% halothane in a mixture of 70% nitrous oxide and 30% oxygen) as described previously (Rathjen and Löwel, 2000). After full recovery from anesthesia, one eye was occluded with a black contact lens and 2-deoxy-D-[U-^{14}C]glucose (Amersham, Freiburg, Germany) was injected i.v. at a dose of 3.7–4.2 MBq/kg in six strabismic cats and in three normal cats. The cats were allowed to move freely around the laboratory so that they received effective monocular stimulation. After 45 min, the animals received a lethal dose of pentobarbital (200 mg/kg) injected i.v. The visual cortices were flat-mounted (Freeman et al., 1987; Löwel et al., 1987) before the tissue was frozen on dry ice. To provide landmarks for later superposition, three holes were melted in the flat-mounts with warm needles. Sub-

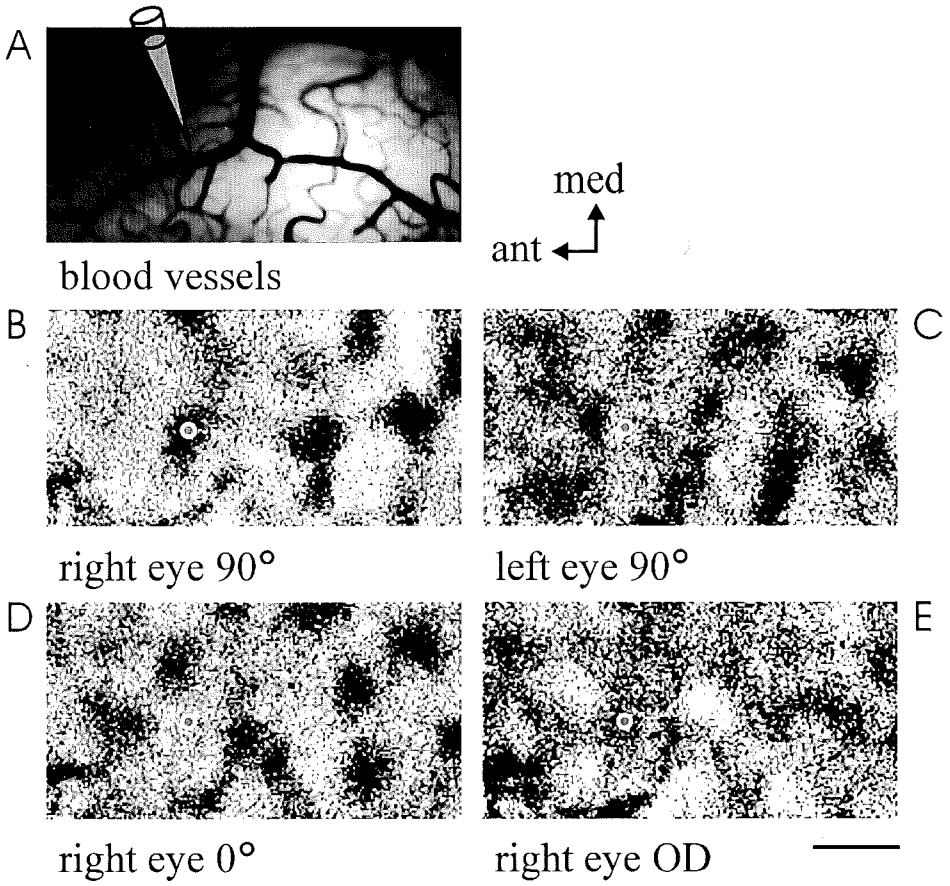

Fig. 1. Optical imaging of intrinsic signals guides tracer injections into functionally identified domains in the visual cortical area 18 of a strabismic cat. The animal was stimulated monocularly with oriented bars of 0° (horizontal), 45° and 90° (vertical), and 135° (0.15 cyc/degree, two cyc/s). (A) Blood vessel pattern of the imaged cortical region. (B, C) Cortical activation patterns evoked by visual stimulation of the right (B) or left eye (C) with moving vertical contours. (D) Activation pattern evoked by right eye stimulation with moving horizontal contours. (E) Contrast-enhanced map of ocular dominance domains of the same region of visual cortex as in A–D. The injection site of the retrograde tracer (beads) is indicated by a gray pipette in (A) and a gray dot in (B–E). In this example, the injection was targeted to a vertical orientation domain predominantly activated through the right eye (B). Note that the injection site is located in a rather inactive region when the left eye is stimulated with vertical contours (C) or when the right eye is stimulated with horizontal contours (D). Because the visibility of ocular dominance domains in optical imaging maps of cat area 18 is relatively weak (Schmidt and Löwel, 2006a,b), the contrast of the ocular dominance map in (E) had to be more enhanced than for the orientation maps in (B–D). Cortical coordinates: ant=anterior; med=medial. Scale bar=1 mm.

sequently, 25-μm-thick serial sections were cut parallel to the cortical surface, mounted on glass slides, dried on a hot plate and then exposed to X-ray film for 4–5 weeks (Löwel et al., 1987).

Data analysis and image processing

The distributions of retrogradely labeled neurons, injection sites and landmarks were plotted with a Zeiss fluorescence microscope (Jena, Germany) and quantified as detailed previously (Schmidt et al., 1997). Five to seven sections from each hemisphere were analyzed quantitatively.

For data representation 2-DG autoradiographs were contrast-enhanced with image processing software (Adobe). The cell plots and 2-DG autoradiographs were superimposed with the aid of the plotted landmarks. For quantitative analyses, 50% of the pixels with the lowest gray levels were displayed black, the remaining 50% were displayed white (e.g. Fig. 2E; for details about image processing see Schmidt et al., 1997). Retrogradely labeled neurons were classified into two groups depending on their location on the 2-DG patterns: (1) neurons *within* and (2) neurons *outside* labeled domains. For quantification, cells in the respective domains were pooled over all plotted sections of one experiment (see Table 1).

RESULTS

Optical imaging of intrinsic signals in area 18

Using optical imaging of intrinsic signals, we recorded activity maps in area 18 of strabismic and control cats in 4.8 mm×2.7 mm large areas. Recordings were made both ipsilateral and contralateral to the squinting eye. An example of the blood vessel patterns of the imaged cortical areas is illustrated in Fig. 1A. In area 18 of control animals, visual stimulation of the left and right eye with moving gratings revealed patchy activity maps resembling those previously described in kitten area 18 (Gödecke and Bonhoeffer, 1996). As expected for normal animals, the layout of orientation domains visualized after left and right eye stimulation was rather similar. Surprisingly, the same result was obtained in area 18 of strabismic animals: unlike in our

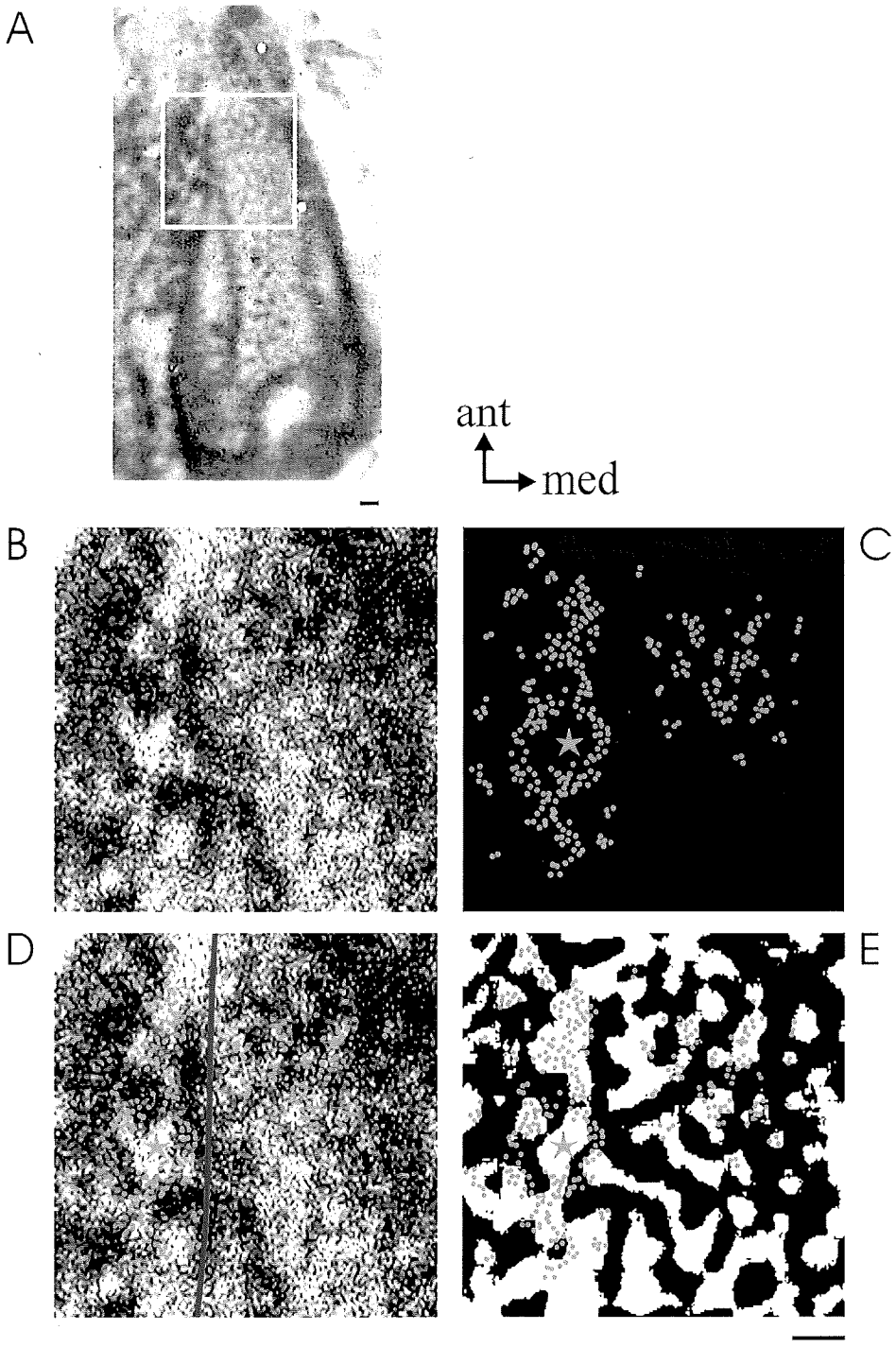

Fig. 2. Topographic relations between intrinsic and inter-areal connections and ocular dominance domains in areas 17 and 18 of a strabismic cat. The 17/18 border is indicated by the red line in D. (A) Layout of 2-DG-labeled ocular dominance domains of the right eye. 2-DG autoradiograph of a flat-mount section from the left hemisphere. (B) Enlarged detail of the 2-DG autoradiograph from the boxed region in A, located at the border between areas 18 (left) and 17 (right). (C) Distribution of retrogradely labeled cells within the same region illustrated in B. Neurons labeled with green beads are represented by *green dots*; the injection site in area 18 is indicated by the *green asterisk*. Note that there are labeled neurons in both areas 18 (left) and 17 (right) (area 18: $n=239$; area 17: $n=109$). (D) Superposition of B and C with the help of the three landmarks. (E) Superposition of the labeled neurons with the contrast-enhanced 2-DG labeled ocular dominance domains to quantify the cell distributions. Note that the green beads were injected into a left eye domain and that the majority of the retrogradely labeled neurons are localized in the weakly labeled domains of the left eye in both areas 18 and 17. Abbreviations: ant=anterior, med=medial. Scale bar=1 mm.

Table 1. Quantification of the distribution of retrogradely labeled neurons inside and outside the 2-DG-labeled ocular dominance domains in areas 18 and 17 of strabismic and control cats

Cat	Color of injection[a]	Weight [g]	Age [weeks]	Squint angle	Injection size [μm^2]	Analyzed sections [*n*]	OD of injection site	Labeled cells in area 18 [*n*]	Same OD in area 18 [*n* (%)]	Labeled cells in area 17 [*n*]	Same OD in area 17 [*n* (%)]
Intrinsic and inter-areal connections in strabismic animals											
S1	Green	1150	11	0.90	300×400	5	Left	2661	2291 (86.1)	427	303 (71.0)
S1	Red	1150	11	0.90	250×400	5	Right	657	323 (49.2)		
S2	Green	730	8	0.91	300×300	6	Left	238	169 (71.0)	389	221 (56.8)
S2	Red	730	8	0.91	400×300	6	Right	137	83 (60.6)		
S3	Green	740	9	0.87	200×150	7	Left	137	115 (83.9)		
S3	Red	740	9	0.87	400×200	7	Right	219	162 (74.0)	108	86 (79.6)
S4	Green	1050	12	0.89	300×400	5	Left	4589	3368 (73.4)	1357	862 (63.5)
S5	Green	1150	14	0.90	100×200	5	Left	1249	883 (70.7)	75	50 (66.7)
S6	Green	1370	12	0.88	200×150	5	Left	776	578 (74.5)		
Mean								1185	71.5%		67.5%
S.E.M.								±503	±3.7%		±3.8%
S.D.								±1510	±11.2%		±8.5%
Intrinsic and inter-areal connections in control animals											
N1	Green	1100	14		200×200	5	Left	715	340 (47.6)	1340	749 (55.9)
N2	Green	1100	12		150×200	5	Left	1603	907 (56.6)	1213	661 (54.5)
N2	Red	1100	12		100×100	5	Right	371	187 (50.4)	413	208 (50.4)
N3	Green	1560	12		200×200	5	Left	790	431 (54.6)		
Mean								870	52.3%		53.6%
S.E.M.								±261	±2.0%		±1.6%
S.D.								±522	±4.1%		±2.9%

Cell numbers of all analyzed sections per hemisphere were summed. The difference in cell distributions between strabismic and control animals was very significant in area 18 ($P=0.007$, two-tailed *t*-test) and significant in area 17 ($P=0.037$, two-tailed *t*-test). OD, ocular dominance; same OD, number of labeled neurons in domains of the same OD as at the injection site of the tracer; squint angle, the ratio of the distance between the corneal reflexes over the distance between the pupils as a measure of eye alignment.

[a] Color of the fluorescent microspheres used for tracer injection.

previous observations in area 17 of strabismic animals (Löwel et al., 1998; Engelmann et al., 2002), functional ocular dominance domains were not clearly segregated and could be visualized only when the contrast was significantly enhanced (Fig. 1E) (Schmidt and Löwel, 2006a,b).

Layout of ocular dominance domains in areas 17 and 18

Monocular visual stimulation of awake strabismic cats produced isolated patches of increased 2-DG uptake in areas 17 and 18 as described previously (Löwel and Singer, 1993b; Löwel, 1994). In area 18, ocular dominance domains were more widely spaced compared with area 17, both in strabismic and control animals (Fig. 2A; Löwel, 1994). In area 18, 2-DG patterns were essentially similar between normal and strabismic animals, a result consistent with our recent optical imaging studies (Schmidt and Löwel, 2006a,b). 2-DG labeled ocular dominance domains in area 18 extended in columns through the entire cortical thickness as they did in area 17 (Löwel and Singer, 1993b; Löwel, 1994).

Topography of intrinsic and inter-areal connections

Fig. 2 shows an example of the horizontal intrinsic and inter-areal projections in areas 17 and 18 of a strabismic cat, after an injection of green beads into a left eye domain (pale gray domain; Fig. 2A, B, D) in area 18. Intrinsic connections in area 18 extended up to 5 mm in anterio-posterior direction and up to 3 mm in medio-lateral direction. Inter-areal connections, i.e. retrogradely labeled neurons in area 17 were found up to 8 mm from the injection site in area 18 (Fig. 2C–E). Typically, a strip about 1–2 mm wide at the border between areas 17 and 18 was devoid of labeled neurons (Fig. 2C). The distribution of retrogradely labeled neurons in area 17 had a patchy appearance while regions containing labeled neurons in area 18 were larger, more confluent and less patchy (Fig. 2).

Ocular dominance selectivity of intrinsic horizontal connections in area 18 and inter-areal projections from area 17 to area 18

To quantify the topographic relationship between the patterns of retrogradely labeled neurons and 2-DG-labeled ocular dominance domains, the 2-DG autoradiographs were subjected to a thresholding procedure to obtain sharp boundaries between active and inactive regions (Fig. 2E; see also Schmidt et al., 1997). Comparing neuron distributions with the corresponding 2-DG autoradiographs revealed that after tracer injections in area 18 of strabismic cats, retrogradely labeled neurons were preferentially located in the same ocular dominance domains as at the injection site (Table 1 and Fig. 2). In the example illustrated in Fig. 2, the tracer injection was targeted to a non-labeled left eye domain (light gray respectively white domain in Fig. 2D resp. 2E) and the retrogradely labeled neurons were preferentially located within the territories of the same, left eye (light gray resp. white regions in Fig. 2A, B, D resp. E).

Quantitative analyses of all tracing experiments revealed that in strabismic animals, on average 71.5±11.2% (mean±S.D., n=9 hemispheres) of the retrogradely labeled cells in area 18 and 67.5±8.5% (mean±S.D., n=5 hemispheres) of the labeled cells in area 17 were located in the same ocular dominance domains as the neurons at the injection sites. In contrast, in control animals, 52.3±4.1% (mean±S.D., n=4) of the labeled cells in area 18 and 53.6±2.9% (mean±S.D., n=3) of the cells in area 17 were found in the same ocular dominance domain as at the injection site (see Table 1). The difference between control and strabismic animals was significant in both area 18 (P=0.007, two-tailed t-test) and area 17 (P=0.037, two-tailed t-test).

All animals were >8 weeks of age at the time of the imaging experiments, and the strabismic cats had >5 weeks of modified visual experience. According to Trachtenberg and Stryker (2001), 7–14 days of strabismic experience are sufficient to produce a saturating effect on the ocular dominance distribution of intrinsic horizontal connections; thus all strabismic animals included in the present study should have reached maximal eye selectivity of their connections. Nevertheless, to exclude age-dependent effects we also tested the significance of the results when the two youngest animals (S2 and S3) were excluded from the analyses. In this case, the difference in cell distributions between control and strabismic animals remained significant in both area 18 (P=0.034, two-tailed t-test) and area 17 (P=0.008, two-tailed t-test) and the average percentages of labeled cells were nearly identical to the previous analyses: in area 18, 70.8±13.4% (mean±S.D., n=5 hemispheres) and in area 17, 67.1±3.8% (mean±S.D., n=3 hemispheres) of the retrogradely labeled cells were located in the same ocular dominance domains as the neurons at the injection sites. Furthermore, although the absolute numbers of labeled cells varied considerably in different animals—as has been observed in previous studies (Löwel and Singer, 1992; Schmidt et al., 1997)—the average numbers of labeled cells in area 18 of strabismic and control animals were quite similar: 1185±1510 (mean±S.D., n=9 hemispheres) in strabismic and 869±522 (mean±S.D., n=4 hemispheres) in control animals. The difference in the number of labeled cells was not significant (P=0.698, two-tailed t-test).

These data show that in strabismic cats, both horizontal intrinsic connections within area 18 and inter-areal projections from area 17 to area 18 preferentially interconnect territories served by the same eye. In contrast, in controls, retrogradely labeled neurons were equally distributed in both right and left eye domains and no evidence for an eye-specific preference of the two types of cortical connections was observed in areas 18 or 17. This agrees with previous evidence that in normally raised cats, horizontal connections are related to orientation but not to ocular dominance domains (Gilbert and Wiesel, 1989; Kisvárday et al., 1997; Schmidt et al., 1997; Schmidt and Löwel, 2002; Buzás et al., 2006).

DISCUSSION

The main results of the present study are that both horizontal connections within visual cortical area 18 and inter-areal connections from area 17 to area 18 are modified in their functional selectivity by strabismus: while in strabismic cats, connections preferentially extend between domains driven by the same eye, this preference is absent in normally raised controls animals.

Methodological considerations

To quantitatively analyze of the functional selectivity of the cortical connections, we applied a thresholding procedure as introduced by Schmidt et al. (1997) that restricted 2-DG-labeled domains activated by the same eye to 50% of the cortical surface, assuming that left and right eye domains occupy on average the same amount of cortical surface area.

Since we were interested in analyzing both intrinsic and inter-areal connections in the same animals we based our superpositions on the 2-DG rather than the optical imaging maps because only the 2-DG technique allows cortical activity patterns in both areas 17 and 18 to be simultaneously visualized.

Furthermore, superimposing 2-DG-labeled ocular dominance domains with the patterns of labeled neurons allowed cell locations and columnar patterns in the same flat-mount sections to be directly correlated, circumventing problems related to distortion and shrinkage of sections attributable to histological procedures.

Layout of functional domains

As previously described in both control and strabismic animals, ocular dominance domains in area 18 were larger than in area 17 (Shatz et al., 1977; Cynader et al., 1987; Diao et al., 1990; Löwel, 1994). No obvious differences were noted between the 2-DG or optical imaging patterns evoked by stimulating either the deviated or the nondeviated eye. This is consistent with electrophysiological evidence from strabismic cats that suggests that neurons driven by the normal and deviated eyes have similar and normal orientation tuning (Hubel and Wiesel, 1965; Freeman and Tsumoto, 1983; Kalil et al., 1984; Sengpiel et al., 1994).

Intrinsic and inter-areal connections

Our quantitative data indicate that horizontal intrinsic connections within area 18 are modified by visual experience as described previously for intrinsic connections in area 17 and for callosal connections (Löwel and Singer, 1992; Schmidt et al., 1997). In area 18 of strabismic cats, on average 72% of the labeled neurons were located in territories sharing the same ocular dominance as the neurons at the injection site while in control animals no ocular preference of the cortical circuitry was detected (52%). Thus, although marked differences in the layouts of ocular dominance maps exist between areas 17 and 18 of strabismic cats (Schmidt and Löwel, 2006a,b), our data indicate that strabismus induces a similar degree and basic type of plasticity in horizontal intracortical connections in area 18 as in area 17. In addition, inter-areal connections from area 17 to area 18 are modified by visual experience in a similar fashion as the intrinsic horizontal connections. These findings are compatible with the notion that the selection criterion for stabilizing horizontal intrinsic connections is the correlation among the responses of interconnected neurons.

In area 17 of strabismic animals, cell clusters were driven almost exclusively by either the left or the right eye and horizontal intracortical fibers preferentially connected cell groups activated by the same eye (Löwel and Singer, 1992). Our new data suggest that the functional preference of the horizontal fibers seems to be similar in areas 18 and 17 of strabismic animals although the distribution of labeled cells tends to be slightly less selective in area 18 compared with area 17. There is also a significant difference in the functional preferences of inter-areal projections from area 17 to area 18 between control and strabismic cats: in strabismic animals, about 68% of the retrogradely labeled neurons in area 17 are located in isoocular domains, while in controls this is only 54%. Consistent with our data, it was previously shown that in normally raised cats, inter-areal projections from area 17 to area 18 originate from cell clusters receiving binocular input from the ipsi- and contralateral eye (Price et al., 1994a,b).

Thus, both intrinsic horizontal fibers within area 18 and inter-areal projections to area 18 of strabismic cats preferentially connect domains driven by the same eye and thus are similar in functional selectivity as previously described for both intrinsic (Löwel and Singer, 1992) and callosal connections in area 17 of strabismic cats (Schmidt et al., 1997). This is surprising given the recent observation that enhanced ocular dominance segregation does not occur in area 18 of strabismic cats because optically recorded activity maps resemble those obtained in control animals (Schmidt and Löwel, 2006a,b). Thus although the functional architecture of area 18—as visualized with intrinsic signal imaging—is rather similar in strabismic and control animals (Schmidt and Löwel, 2006a,b), the functional selectivity of the horizontal intrinsic circuitry is significantly different: there is an eye specificity of the horizontal fibers in strabismic but not in normally raised animals. Several reasons may account for these results.

It is conceivable that anesthesia may have an effect on the visualization of ocular dominance columns with optical imaging in area 18. It has been shown previously that monocularly induced 2-DG patterns in area 17 differ between awake and anesthetized cats (Löwel and Singer, 1993a,b). In controls, monocular stimulation induced cortical 2-DG activation patterns that are in register with the termination sites of afferents of the stimulated eye only in awake animals. These data suggested the existence of a mechanism which restricts cortical activation after monocular stimulation to territories that are in register with the afferents from the stimulated eye. This mechanism appears to be effective only when the animals are awake and actively exploring their environment, suggesting an active inhibitory process, perhaps related to mechanisms of se-

lective attention (Löwel and Singer, 1993b). However, in area 17 of strabismic cats, anesthesia did not prevent the visualization of clearly segregated ocular dominance domains with both 2-DG autoradiography (Löwel and Singer, 1993b) and intrinsic signal imaging (Löwel et al., 1998). Thus, if anesthesia effects were the major reason for the apparent absence of enhanced segregation of ocular dominance domains in strabismic area 18, one would have to assume that anesthesia acts differentially on visual cortical areas 17 and 18 which is unlikely.

Interestingly, electrophysiological recordings have revealed differences between areas 17 and 18 of strabismic cats that may underlie the observed differences in ocular dominance map layout in these areas. In particular, there seem to be differences in inhibitory interactions. While binocular excitatory convergence onto single cells is markedly reduced in both areas 17 and 18 of strabismic cats, many apparently monocular cells in area 18 have been shown to display substantial binocular interactions when both eyes are stimulated together (Bishop, 1973; Cynader and Regan, 1978; Cynader et al., 1984 and Chino et al., 1988). Cynader et al. (1984) have found that binocular facilitation in area 18 was as marked in the population of cells studied in strabismic cats as it was in controls. In sharp contrast, strabismus virtually abolished the disparity-specific binocular interactions in area 17 that are such a distinctive feature of normal striate neurons, but left pronounced, nonspecific interocular suppression in the majority of cells (Sengpiel et al., 1994). Quantitatively, less than 10% of all neurons from area 17 showed any enhancement of the monocular response and only 5% exhibited genuine facilitation (Sengpiel et al., 1994). A number of other studies agree on the fact that the most prominent aspect of cortical physiology in strabismics is the high prevalence of suppressive binocular interactions both in cat area 17 (e.g. Ohzawa and Freeman, 1986; Chino et al., 1994; Sengpiel et al., 1994) and in monkey V1 (e.g. Smith et al., 1997; Zhang et al., 2005). Thus, in area 17, the majority of cells showed interocular suppression while substantial binocular interactions are still present in area 18 of strabismic cats. These differences in binocular interactions could increase the likelihood of common activation of visual cortical neurons specifically in area 18 of strabismic cats thus making segregation of ocular dominance columns more difficult.

The most salient difference between visual cortical areas 17 and 18 is that area 18 in cats, unlike V2 in monkeys, receives direct input from the lateral geniculate nucleus and major inputs from area 17. While area 17 predominantly receives X-cell input area 18 is dominated by Y-cell input (Stone, 1983; Orban, 1984). Y-cells possess both larger receptive fields and larger thalamocortical afferent arbors compared with X-cells (Stone and Dreher, 1973; Harvey, 1980; Sherman and Spear, 1982; Humphrey et al., 1985a,b) which might influence the degree of decorrelation between left and right eye inputs in the two areas. In addition, although the present results indicate an eye specificity of the 17–18 inter-areal projections in strabismic animals (on average, 67.5% of the retrogradely labeled neurons are located in the same ocular dominance domain as the neurons at the injection site) quite a number of projections target domains of opposite ocularity. In contrast to area 17 of strabismic animals, in which ocular dominance domains are clearly segregated and afferent input from the two eyes is decorrelated, the increased binocular interactions in area 18 (mediated by both the inter-areal input from area 17 and possibly also intrinsic connections) could lead to more similar neuronal activity maps after stimulation of the left and the right eye consistent with our optical imaging data (Schmidt and Löwel, 2006a,b).

Why then do intrinsic horizontal connections within area 18 nevertheless preferentially connect neurons driven by the same eye? Our present data show that in strabismic cats, ocular dominance domains in area 18 receive input preferentially from domains driven by the same eye in area 17 (in addition to their thalamic input). In contrast, in controls, ocular dominance domains in area 18 receive input from both left and right eye territories in area 17. Afferent information in area 18 of strabismic cats is thus strongly biased for ocular preference and therefore could convey the correlation of neuronal activity between same eye territories that is necessary to selectively stabilize intrinsic horizontal fibers during early development. Interestingly, the eye selectivity of these intrinsic connections is slightly smaller (72% iso-eye) than in area 17 (80–90% iso-eye; Löwel and Singer, 1992; Trachtenberg and Stryker, 2001) which could be due to their less eye-specific input which could in turn increase the correlation of responses in different ocular dominance domains in strabismic area 18 compared with area 17. Thus the experience-dependent changes of long-range horizontal connections in area 18 and of inter-areal connections between areas 17 and 18 support the hypothesis that the number of connections between two sites in the cortex reflects the strength of previous co-activation of these two sites: neurons wire together if they fire together (Löwel and Singer, 1992) and neurons that fail to sync fail to link (Mark Bear, personal communication).

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