

CENTRAL GATING OF DEVELOPMENTAL PLASTICITY IN KITTEN VISUAL CORTEX

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SUMMARY

1. In nine 4-week-old, dark-reared kittens we sutured one eye closed and rotated the other surgically. The kittens then grew up in a normally lighted animal colony with adequate room to play.

2. For about two weeks after surgery their visual-motor co-ordination did not differ from that of kittens with conventional monocular deprivation; then severe disturbance of visually guided behaviour became progressively more apparent until, after another two to three weeks, all the kittens stopped responding to most visual stimuli entirely. At that point their behaviour in an unfamiliar environment closely resembled that of binocularly deprived cats exposed to light for the first time.

3. Four weeks ($n = 3$) and 6 months ($n = 6$) after surgery, we examined the visual cortex with single-unit recordings, and with evoked potentials elicited by electrical stimuli and patterned lights. We obtained the single-unit recordings from 586 neurones of the striate cortex in both hemispheres, both ipsi- and contralateral to the deprived eye.

4. The single-unit recordings and the evoked potentials showed a clear relation between the kitten's abnormal visual behaviour and the functioning of the striate cortex. Only about half the normal percentage of cells responded to light, and most of those which did react had abnormal receptive field properties: they responded only sluggishly even when the light stimuli were aligned optimally.

5. We also evoked cortical potentials with phase alternating square wave gratings of variable contrast and spatial frequency. The amplitude of the potentials indicated that contrast-sensitivity was reduced at all spatial frequencies.

6. In the kittens tested 4 weeks after surgery, ocular dominance had shifted toward the open rotated eye but this shift was considerably less pronounced than in control kittens monocularly deprived for a comparable period of time.

7. In the kittens tested 6 months after surgery fewer cells than normal were binocular; ocular dominance had not shifted towards the open eye.

8. Numerous control experiments indicated that these abnormalities did not result from transitory immobilization of the eye alone nor from lesions of the retina or of the optic nerve.

We infer that a central mechanism prevents the inappropriate signals from the rotated eye from influencing the consolidation of central pathways.

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INTRODUCTION

Many studies indicate that the development of the striate cortex is normally guided by experience, and that the functioning of cortical neurones can be changed by manipulating visual experience (For a review see Barlow, 1975). These neuronal changes – in characteristics like ocular dominance and orientation preference – seem to consist of the selective stabilizing and weakening of excitatory connexions according to rules closely resembling those postulated by Hebb (1949) for adaptive synapses (Rauschecker & Singer, 1979, 1981; Singer, Freeman & Rauschecker, 1981).

However, the experiential factors which instigate these changes would have to be very carefully controlled. When a kitten is not fixating a target with both eyes but moving his head or eyes during orienting responses, for instance, then the retinal signals from the two eyes are not congruent and hence not suitable for developing binocular correspondence. There must be some controlling system able to evaluate the sensory signals in a more general behavioural context before they are allowed to induce changes in the cortical circuitry.

The first evidence for central control of developmental plasticity came from kittens made strabismic in both eyes (Singer, von Grünau & Rauschecker, 1979*b*). Such misalignment of the visual axes usually leads to disrupted cortical binocularity; but it turned out that the kittens that did *not* consistently fixate with either eye *did* maintain binocularity. A related finding has been reported recently for kittens in which both eyes were rotated (Crewther, Crewther, Peck & Pettigrew, 1980). Also in these animals cortical binocularity was maintained despite the incongruent activity patterns conveyed from the two eyes. To confirm the presence of a central control of developmental plasticity, in the present experiments we combined monocular deprivation with large-angle cyclotorsion. We suspected that a 90–180 deg rotation of the eye associated with reduced ocular motility would be so confusing that the kitten's brain could not adapt to it, so the normal shift in ocular dominance toward the open eye might fail to occur. This would be further evidence of a central control of plasticity, for if a Hebbian mechanism were the only control, then the normal shift would still occur: the retinal responses needed to start the mechanism would still be present. Some of these data have been published previously in a brief report (Singer, Yinon & Tretter, 1979*c*).

METHODS

Surgical eye rotation. In nine dark-reared, four-week-old kittens we sutured one eye closed and rotated the other surgically. In one additional kitten we left the unrotated eye open, to compare our results with previous electrophysiological studies of eye rotation (Blakemore, Van Sluyters, Peck & Hein, 1975; Yinon, 1975).

For surgery we anaesthetized the kittens first with ketamine chloride i.m. and then with Pentothal i.v. To rotate the eyes we severed the extraocular muscles, gently rotated the eyeball outward, then sutured the distal remnant of the rectus superior tendon to the intraorbital conjunctive tissue. Finally, we closed the eyelid temporarily to reduce the risk of infection and corneal damage. Later we determined the angle of rotation by comparing the orientation of retinal blood vessels on pictures of the fundus taken before and after the operation. A second comparison immediately before the neurophysiological tests showed that the angle of rotation was stable. Although we tried to minimize deviations of the visual axis, rotation always led to an additional 10–20 deg of strabismus. Table 1 lists the angle of rotation and survival time of each kitten.

After surgery we returned all kittens to their mothers and raised them in their familiar environment with room to play and an artificial day/night cycle of 16/8 h. As soon as they were permitted to do so (two days after surgery) the cats kept the rotated eye open. The pupillary light reflex could also be obtained readily.

Eye rotation always led to a transient immobilization of the rotated eye. But after a few weeks the eye muscles had apparently re-attached and small eye movements re-appeared. Movies taken during vestibular and optokinetic nystagmus showed that eye movements occurred with amplitudes between 10 and 15 deg, but that they remained abnormal (see below). Thus, in order to assess the

TABLE 1.

Kitten no.	Type of operation		Age at operation (weeks)	Age at recording (weeks)
	Left eye	Right eye		
633	rot. 70 deg	closed	4	8 weeks
1121	rot. 180 deg	closed	4	8
1122	rot. 90 deg	closed	4	8
67	rot. 180 deg	closed	3	5 months
68	rot. 100 deg	closed	4	6 months
96	rot. 150 deg	closed	4	5 months
1031	rot. 180 deg	closed	4	6 months
1033	rot. 180 deg	closed	4	6 1/4 months
1034	rot. 180 deg	closed	4	6 1/2 months
109	rot. 45 deg	open	5	12
631	immobilized (0 deg)	closed	4	9
632	immobilized (0 deg)	closed	4	4 months
9	open	closed	4	8
15	open	closed	4	8
1612	open	closed	4	8
C1	rot. 180 deg	closed	4	4 4/7 weeks (only histology)
C2	rot. 180 deg	closed	4	6 weeks (only histology)

effect of eye immobilization *per se* two control kittens had one eye closed and all extraocular muscles in the other eye severed but the eye ball left in its original position. In addition, all four distal muscle tendons were sutured to the orbital fringe. Care was taken to fix the eye in its normal resting position.

To obtain a standard for the effects of short monocular deprivation (m.d.), three control kittens were raised in the dark in the same way as the experimental kittens but when they were 28 d old they received only monocular lid suture.

Finally, in another two kittens we examined the optic nerve histologically after one eye was rotated by 180 deg. After survival times of 4 and 14 d respectively they were killed and the brains perfused with Ringer solution followed by a phosphate buffered solution (pH 7.4) of 4% formaldehyde, 0.5% glutaraldehyde and 0.54% dextrose. After an appropriate post-fixation period the optic nerves were carefully dissected under the operation microscope and small blocks were post-fixed in 2% OsO₄ for 2 h. Subsequently, the blocks were dehydrated and embedded in Durcupan for the preparation of 2 μ m semi-thin sections.

Electrophysiological procedures. For the surgical preparation prior to recording the animals were anaesthetized with ketamine hydrochloride i.m. and additional pentobarbitone i.v. After all surgical procedures were completed the animals were paralysed with Flaxedil and anaesthesia was continued with nitrous oxide (70% N₂O, 30% O₂) supplemented by i.v. infusion of small doses of Nembutal 2 mg/kg.h. This assured a stable level of anaesthesia. The e.e.g. was characterized by low frequency high voltage activity with interspersed barbiturate spindles and did not

desynchronize with painful stimuli. E.e.g., body temperature, CO₂ concentration in the expired air, heart rate and renal as well as respiratory loss of fluid were monitored continuously, loss of fluid being replaced by infusion of a glucose-Ringer solution through an orally inserted gastric catheter.

For light stimulation the pupils were dilated with atropinesulphate and the nictitating membranes were retracted with Neosynephrine. After opaque contact lenses with artificial pupils, 2 mm in diameter, had been placed on the cornea, the refraction of the eyes was measured with a Rodenstock refractometer and corrected with spectacle lenses to be in focus with a white tangent screen at 1.5 m distance. The eyes were then inspected with a fundus camera and the retinal landmarks were plotted on the tangent screen.

Single units were recorded from striate cortex both ipsi- and contralateral to the rotated eye with micropipettes containing 1.5 M-potassium citrate. Operational amplifiers in the f.e.t.-equipped input stage allowed constant currents to be applied through the recording pipette, a procedure which proved extremely useful in activating silent cells. The recording tracks had an inclination of 30 deg to the cortical surface in order to minimize the sampling bias which would otherwise be introduced by the columnar organization of visual cortex. The location of the receptive fields of all analysed units was close to the horizontal meridian (± 5 deg) and did not exceed 15 deg eccentricity along the horizontal meridian.

Data acquisition. Since numerous cells responded only sluggishly even to optimally aligned light stimuli, response histograms were routinely compiled with a PDP 8 computer. In addition to the usual receptive field properties we rated the vigour of responses to optimally aligned stimuli in five classes. Responses which were only detectable after averaging were rated in class 1. Weak reactions, just detectable with conventional hand mapping, were rated in class 2. Clear and reproducible responses to hand-held stimuli were rated in class 3 and particularly vigorous responses in classes 4 or 5. We also tried to establish simple objective measures of responsiveness from signal to noise ratio or the absolute number of discharges but encountered many other classification problems. Therefore, we accepted the qualitative rating as a first step towards the assessment of a parameter that we consider crucial in deprivation studies. We have used it in several other investigations and have found very similar distributions of responsiveness for normal striate cortex.

In both eyes of all kittens with 180 deg eye rotation we measured the electroretinogram (e.r.g.) between silver ball electrodes placed on the limbus corneae and an indifferent needle electrode in the skin above the frontal sinus. The e.r.g. was averaged from fifty responses to brief stroboscopic light flashes delivered at intervals of 1 s.

In addition to responses to light cortical evoked potentials were studied in four cats after stimulation of the optic nerves using a method described in detail previously (Singer, 1977). To assure stable and reproducible cortical responses the mesencephalic reticular formation was stimulated before the stimulation of the optic nerve (Singer, Tretter & Cynader, 1976).

Contrast sensitivity measurements. In two kittens with long survival time we assessed contrast sensitivity from cortical-evoked potentials elicited by phase alternating square wave gratings. The procedures were identical to those described by von Grünau & Singer (1980). The visual stimulus subtended 39 deg \times 30 deg of visual angle. Monocular v.e.p.s were recorded for four spatial frequencies (0.15, 0.3, 0.7 and 1.3 c/deg) and six levels of contrast (38%, 22%, 8%, 4%, 2.5%, 1%). Overall luminance was kept constant in the photopic range at 11.5 cd/m². For each combination of spatial frequency and contrast, fifty full cycles with two reversals were averaged on two separate occasions. V.e.p. amplitudes were assessed from the first positive deflexion of the response. To determine the contrast threshold at each spatial frequency we calculated the regression line for v.e.p. amplitude *vs.* the logarithm of the contrast and extrapolated it to zero amplitude (Campbell, Maffei & Piccolino, 1973).

RESULTS

Behavioural observations

We tested the visual guided behaviour of all kittens repeatedly, without giving training on any particular task. The tests were similar to those described in detail by Van Hof (1977) and included: jumping, obstacle avoidance, tracking, reaching, placing and optokinetic nystagmus.

In all kittens with one eye rotated and the normal eye closed, post-operative development was similar and consisted of three phases. For about two weeks after

surgery the kittens developed like the kittens which were dark-reared and then monocularly deprived. They commonly blinked to flashes of light, withdrew from large approaching objects, attempted to track and to reach for small targets but as is typical for kittens of this age their tracking was unprecise and only rarely successful. At about six weeks of age this seemingly normal development ceased rather abruptly and the kittens started to display disturbed visuo-motor co-ordination: they showed spontaneous head-nystagmus, deviated sideways when running, would suddenly crawl backwards and circled stereotypically. After a few days these disturbances faded but at the same time the kittens appeared to rely less and less on visual cues. In their familiar environment spontaneous behaviour returned to normal but in a new environment it was obvious that they made little use of their open eye, but rather behaved very much like binocularly deprived animals immediately after eye opening. They continued to show the blink reflex but none of the above mentioned goal directed visuo-motor patterns developed properly. Tracking of small objects and visually guided placing could not be elicited at all.

Optokinetic nystagmus (o.k.n.) tested in a rotating drum with striped walls remained elicitable but within the resolution of measurements taken from films there was no indication of a reorganization. In three cats with long survival we opened the sutured eye one day before recording and tested o.k.n. monocularly; we prevented the non-stimulated eye from seeing the rotating stripes with a small mirror which also allowed us to inspect the eye-movements. When the normal eye was stimulated saccades in both eyes were conjugate and horizontal with respect to head co-ordinates, irrespective of the degree of rotation in the operated eye. When the rotated eye was stimulated movements in both eyes were again conjugate with respect to head co-ordinates. The direction of eye movements corresponded approximately to the direction of image shift on the retina of the rotated eye. Thus, in the cat with 100 deg rotation, horizontally moving stripes elicited jerky vertical movements in both eyes. Since later inspection revealed that the severed muscles had re-inserted where they were positioned in the orbit this suggests that the innervation pattern of the extraocular muscles following retinal stimulation had remained unaltered. However, the eye movements were jerky and lacked the regular beat of normal o.k.n., both of which suggest changes in the gain of the reflex.

The kitten in which the normal eye was left open developed differently and behaved very much like strabismic kittens that become amblyopic (Jacobson & Ikeda, 1979; Singer, Von Grünau & Rauschecker, 1980) and previously described kittens in which one eye was rotated and the other left open (Blakemore *et al.* 1975; Peck & Crewther, 1975; Peck, Crewther, Barber & Johannsen, 1979). With both eyes open visually guided behaviour was normal but the kitten used only the non-rotated eye for fixation. With the normal eye occluded, the kitten's performance became less accurate but still was considerably better than that of the kittens raised with only the rotated eye open. It displayed visually guided reaching, following and placing but these responses were less precise than those guided by the normal eye.

Receptive field properties

Kittens reared with one eye closed and the open eye rotated yielded somewhat different results after short (4–5 weeks) and long (5–6 months) survival times. Therefore, we will present separately results for the two groups.

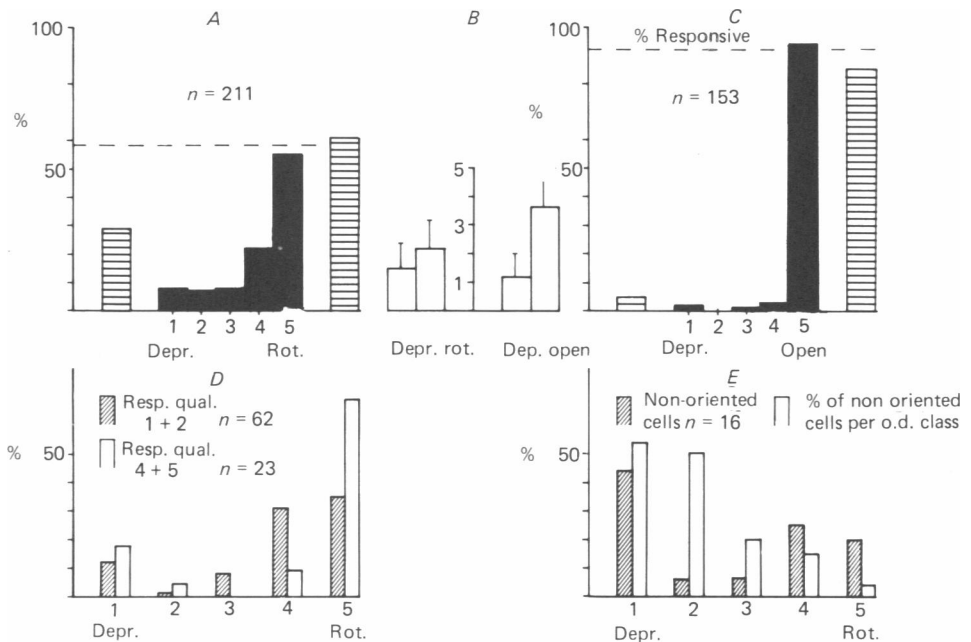


Fig. 1. Kittens with short survival time. *A*, ocular dominance (o.d.) distribution (filled columns), percentage of light reactive cells (dashed horizontal line) and percentage of cells excitable from either of the two eyes (hatched columns) from striate cortex of three kittens raised with one eye closed and the other rotated. The o.d. distribution is calculated in percent of the light reactive cells; the percentages of cells excitable from either of the two eyes are calculated from the sum of o.d. classes 1–4 and 2–5 and refer to the total sample of analysed cells ($n = 211$). O.d. classes 1 and 5 comprise cells driven exclusively from the deprived or the rotated eye, classes 2 and 4 refer to binocular cells with prevailing input from one eye and class 3 contains cells driven equally well from either eye. *B*, average indices for the quality of responses elicited from the two eyes in the experimental kittens (left pair) and in three kittens after conventional monocular deprivation (right pair). Vertical bars indicate standard deviations. *C*, o.d. distribution presented as in *A* from three control kittens that were monocularly deprived for the same period of time as the experimental kittens. *D*, o.d. distributions from experimental kittens calculated separately for cells with sluggish (hatched columns) and vigorous responses (open columns). *E*, o.d. distribution of the non-oriented cells (hatched columns) in experimental kittens. The percentage of non-oriented cells among the cells in a particular o.d. class is represented by blank columns.

Kittens with a short survival time (s.s.t.). We studied 211 neurones from striate cortex in the three s.s.t. kittens, 106 of them from the hemisphere contralateral to the rotated eye. Since recordings from the two hemispheres were similar, we have combined the data.

The functioning of the striate cortex was highly abnormal. Only 123 of the 211 cells (58%) could be driven by light. Most of the cells still reactive to light gave only weak and fatiguable responses. Moreover, most cells responded so inconsistently to stationary stimuli that it proved extremely difficult to determine whether the receptive field was simple or complex. On the scale of response vigour (see Methods)

the responses from the open rotated eye averaged only 2.2 ± 1.0 . This differs markedly from the average of 3.7 ± 0.9 found for responses to the open eye in the monocularly deprived (m.d.) control cats (Fig. 1B). In the χ^2 test the difference between the two distributions of vigour indices was highly significant ($P < 0.001$).

Another remarkable difference was that the deprived eye controlled many more neurones than it did in the m.d. control kittens (compare Fig. 1A and C). 38% of the responsive cells were still activated by the deprived eye and 28% had remained binocular. Correspondingly, the vigour indices for responses obtained from the deprived eye of experimental cats were higher than those for the deprived eye in the control m.d. cats (Fig. 1B), although they were still significantly ($P < 0.005$) lower than those for responses from the open rotated eye.

As shown in Fig. 1D and E changes in binocularity covaried with responsiveness and orientation tuning. The shift in ocular dominance towards the open rotated eye and the reduction of binocularity were more pronounced in the well than in the poorly responsive cells and neurones dominated by the open eye were more often orientation-selective than cells dominated by the closed eye.

The few cells ($n = 23$) that responded vigorously (vigour index 4 and 5) all had simple, narrowly tuned receptive fields, tended to be monocular ($n = 20$) and tended to be dominated by the open rotated eye ($n = 16$) (Fig. 1D). Of the remaining poorly responding cells 87% had oriented receptive fields but because of the sluggish reactions we did not attempt to assess precisely their tuning. It was, however, definitively broader than in normally reared cats, since responses to stimuli orthogonal to the preferred orientation were common. With four exceptions binocular cells had the same orientation and direction preference in the two eyes with respect to retinal co-ordinates. In those exceptions the receptive field was oriented in only one eye.

Kittens with a long survival time (l.s.t.). In the six cats which survived for 4–5 months we recorded 375 cells, 195 from the striate cortex ipsilateral to the rotated eye. Since, as in the s.s.t. kittens, there was no consistent difference between the two hemispheres, we will present the data together. The mean distance between successively recorded neurones varied from 60 to 110 μm in the six cats and was not significantly different from values in the other groups. Only 52% of the cells could be driven and the majority of the reactive cells gave weak and sluggish responses. The mean vigour indices of responses from the open rotated and the closed eye were 2.3 ± 0.95 and 1.88 ± 0.8 , respectively. The value for the closed eye is slightly higher than in the s.s.t. kittens and the difference between the two eyes is no longer significant. Only 10% of the cells responded vigorously to light (vigour index 4 and 5) compared to 23% in the s.s.t. kittens.

The most important finding in the l.s.t. group is the absence of a shift in ocular dominance towards the open rotated eye. We will present the data from the six kittens together since the ocular dominance distributions were very similar and did not vary systematically with the differences in degree of eye rotation (Fig. 2A). Of 197 responsive cells the open rotated eye drove 132 neurones and the deprived eye, 143 cells. The percentage of binocular cells is markedly reduced compared to normal cats but still surprisingly high (40%) considering that these cats had only monocular visual experience.

As in the s.s.t. group there was a relation between ocular dominance, the

vigorousness of responses and orientation tuning, the fraction of cells with vigorous responses and oriented receptive fields being higher among neurones dominated by the open rotated eye (Fig. 2C and D). Most of the few vigorously responding cells (quality index 4 and 5, $n = 20$) were again monocular ($n = 17$) and driven by the open rotated eye ($n = 13$). The four monocular cells connected to the deprived eye were sharply tuned for orientation had simple receptive fields. Of the three binocular cells with vigorous responses only one had an orientation preference but was broadly

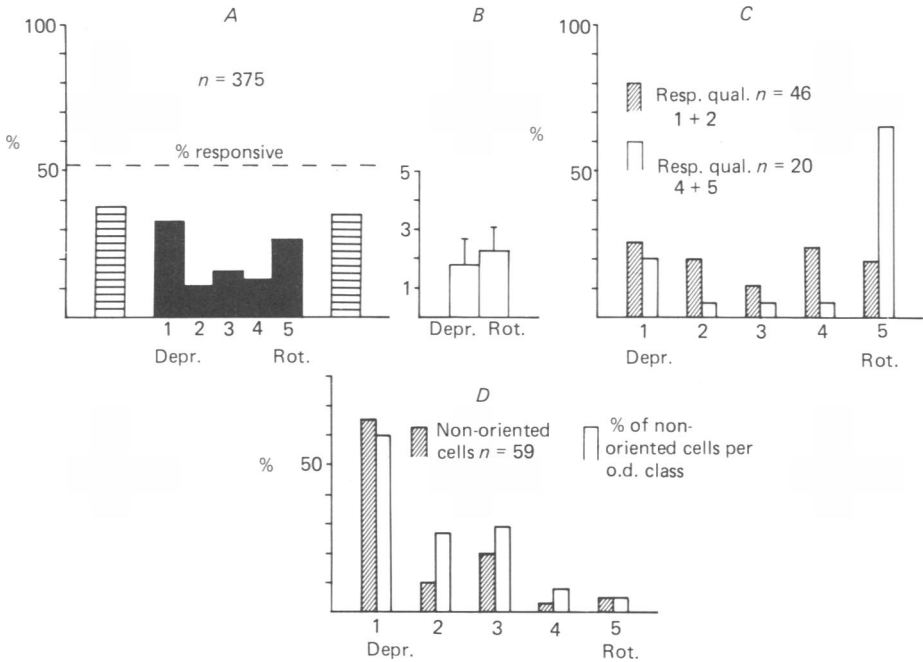


Fig. 2. Kittens with long survival time. *A*, o.d. distribution (filled columns) of striate cortex neurones of six kittens raised with one eye closed and the open eye rotated. The hatched columns refer to the percentage of cells excitable from the two eyes in relation to the total sample of analysed cells ($n = 375$). The horizontal dashed line indicates the percentage of light-reactive cells. *B*, average indices for the quality of responses obtained from the two eyes. *C*, o.d. distributions for cells with sluggish (hatched columns) and vigorous (open columns) responses. *D*, o.d. distribution of non-oriented cells (hatched columns) and percentage of non-oriented cells among neurones in the respective o.d. classes (open columns).

tuned (± 45 deg). Altogether, the percentage of cells with an orientation preference was lower (70%) than in the s.s.t. group (87%) and with the exception of the well responding simple cells orientation tuning was poor. As in the s.s.t group, the orientation and direction preferences of binocular cells were always virtually identical for the two eyes when they were related to retinal landmarks.

As described previously (Singer, Tretter & Yinon, 1979*a*), there was also a bias in the distribution of orientation preferences in kittens whose eyes were rotated by 180 deg. Among the binocular cells only 16% preferred orientations close to the

vertical (67–112 deg) while 60% preferred orientations close to the horizontal (157–22 deg). Among the monocular cells no such bias was detected.

The kitten with one eye rotated and the normal eye open. This kitten was included as a link to previous studies and as our own control for the physiological differences that distinguish this preparation from the kittens raised with one eye rotated and the other closed. As would be expected from the behavioural differences, the results from the kitten (109) with the normal eye open differed markedly from those of the other

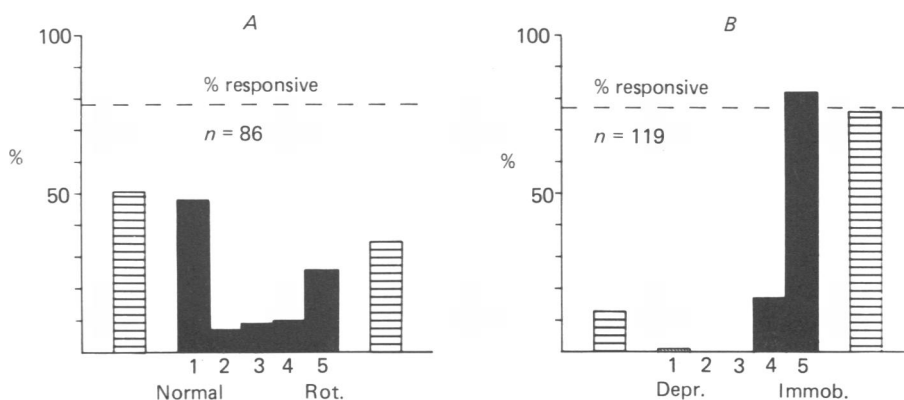


Fig. 3. O.d. distributions from kitten 109 with one eye rotated and the other open (A) and from two kittens with one eye immobilized and the other closed (B). Filled columns, o.d. distributions; hatched columns, percentage of cells excitable from the two eyes relative to the total sample of analysed cells; horizontal dashed lines, percentage of light reactive cells.

kittens and closely resembled those described previously for such cats (Blakemore *et al.* 1975; Yinon, 1975; Crewther *et al.* 1980). Of eighty-six cells sampled from the two hemispheres, 78% responded to light and most of those responses appeared normal, with only seven cells showing no orientation preference. Binocularity was reduced to an extent found in squinting cats (Hubel & Wiesel, 1965; Singer *et al.* 1979, 1980): only 26% of the cells responded to stimulation of either eye. Moreover, the ocular dominance distribution was asymmetric. In both hemispheres the contralateral eye was dominant but much more so in the hemisphere contralateral to the normal eye, so that there was an overall bias in favour of this eye (Fig. 3A). Also in this kitten the orientation and direction preferences in binocular cells were usually the same in both eyes when related to retinal co-ordinates. However, in two cells the direction preferences in the two eyes were identical with respect to head co-ordinates. In agreement with the results of Crewther *et al.* (1980), these two cells had several features in common: they were driven equally well from both eyes, were located in infragranular layers and had large receptive fields (3–4 deg in diameter) which were positioned on the horizontal meridian at about 4 deg eccentricity. They responded well to small moving spots and showed a marked direction preference, responses to movements in the 0-direction being virtually absent.

Controls with immobilized eyes. The two kittens with one eye closed and the other immobilized were allowed to survive for 5 weeks and 3 months, respectively. By the

time of recording small eye movements had reappeared in both animals and upon course testing their visuo-motor behaviour appeared normal. In the kitten with a short survival time the percentage of cells responding to light was somewhat lower (71 %) than in cats with conventional monocular deprivation (93 %) but in the cat with a long survival time it was close to normal (87 %). In both cats the large majority of neurones had become monocular and were excitable only from the open eye (Fig. 3B). Most responses to light were vigorous and the selectivity for stimulus orientation as well as the polar distribution of orientation preferences appeared normal. Since we did not determine tuning quantitatively from response histograms, however, we could have missed small differences from normal cats.

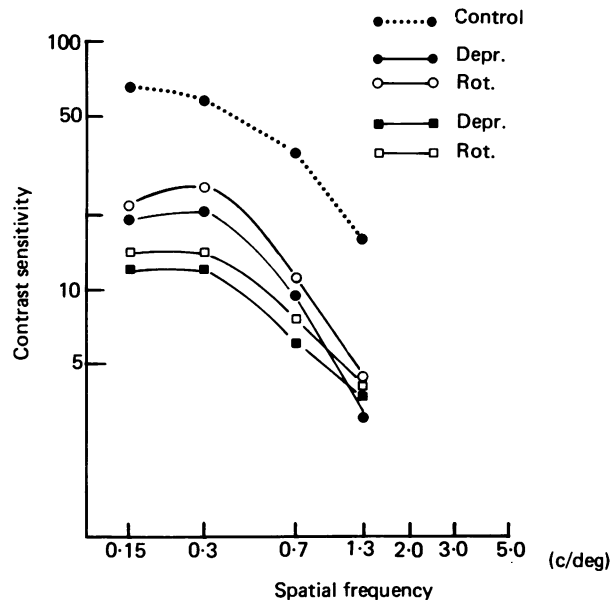


Fig. 4. Contrast sensitivity functions determined from evoked potentials elicited with phase alternating gratings. The control is from the normal eye of a six-month-old strabismic kitten and is in the range characteristic for normal adult cats. The pairs indicated by circles and squares are from experimental animals with long survival time (1031, 1034), filled and open symbols referring to responses from the deprived and the rotated eyes, respectively. Logarithmic scales are used for spatial frequency (abscissa) and contrast sensitivity (ordinate).

Contrast sensitivity

We used v.e.p.s to measure the contrast sensitivity of two kittens with a long survival time after eye rotation and monocular deprivation. These contrast sensitivity functions are plotted in Fig. 4 and are compared with the contrast sensitivity of the normal eye of a strabismic kitten examined with identical techniques in a previous study (Singer *et al.* 1980). This control is in the range characteristic for normal adult cats. In both kittens with the open eye rotated contrast sensitivity was markedly

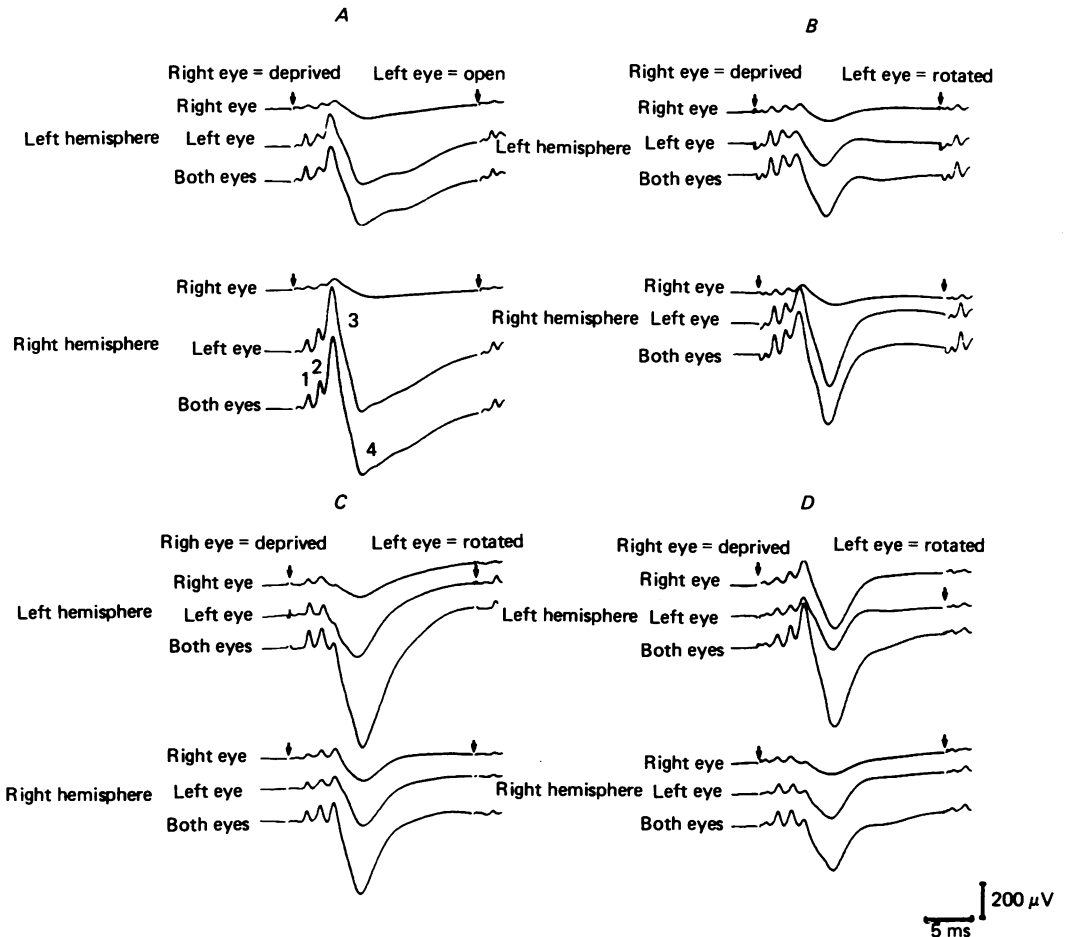


Fig. 5. Cortical evoked potentials elicited by stimulation of the optic nerves. All responses are obtained with conditioning stimulation of the mesencephalic reticular formation to obtain a stable level of excitability and are averaged from twenty stimulus presentations. The optic nerve stimuli were applied as double shocks separated by 20 ms and are indicated by arrows. In all records positive potentials are upward. *A*, control cat with conventional monocular deprivation. Numbers 1-4 on the bottom trace indicate the response components referred to in the text. *B*, responses from kitten 1121 with short survival time. *C* and *D*, responses from kittens 1031 and 1033 with long survival time.

reduced at all spatial frequencies tested and equally so in the deprived and the rotated eyes (Fig. 4). Extrapolation of the curves to 100% contrast suggests that visual acuity was also probably reduced.

Controls for lesions of the optic nerve

Responses to electrical stimulation. To determine whether the effects of eye rotation might result simply from lesions of the optic nerve, we examined cortical field potentials elicited by electrical stimulation of the optic nerves. If such was the case

the presynaptic component of the potentials elicited from the rotated eye should be abnormal. Moreover, previous investigations have shown that field potential analysis can be used as a complementary method to single cell analysis for the assessment of ocular dominance (Singer, 1977; Blakemore & Hilman, 1977). Since, as in normal cats (Mitzdorf & Singer, 1980), the response was smaller in the ipsilateral than in the contralateral hemisphere, we only compared responses from the same side. Comparison of control m.d. cats, cats with the open eye rotated, and cats with the open eye immobilized confirmed the results obtained from single unit recording. In the s.s.t.

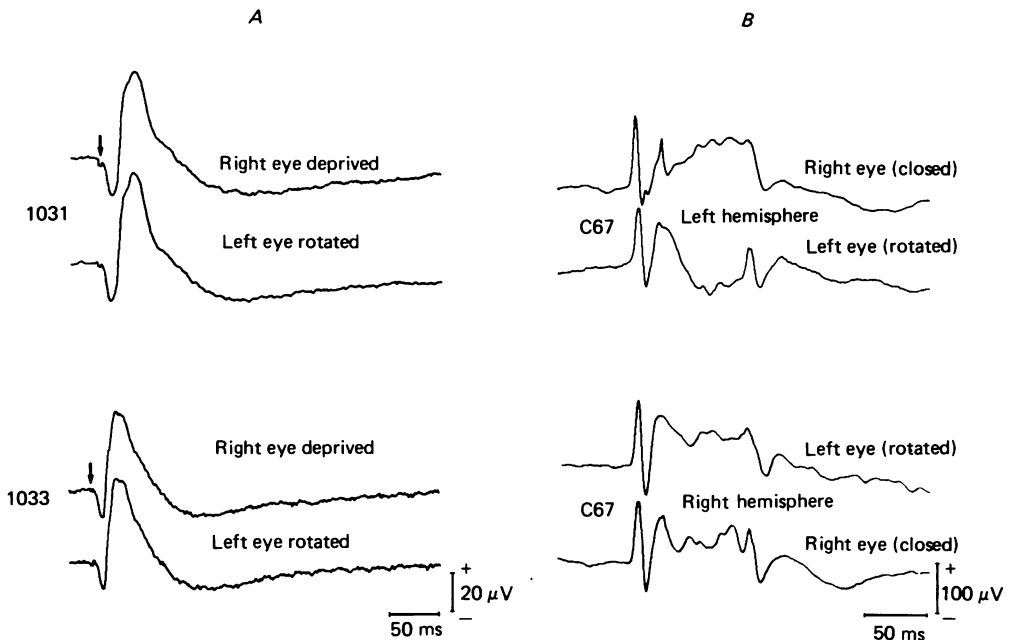


Fig. 6. Electroretinograms (*A*) and flash evoked cortical potentials (*B*) from kittens with long survival time.

group the amplitude of the response evoked by the deprived eye was reduced but less than in the control m.d. cats (Fig. 5). In the l.s.t. cats the difference in amplitude between responses evoked by the rotated and the deprived eye was consistently smaller than in s.s.t. cats and on occasions barely detectable. In both s.s.t. and l.s.t. cats the presynaptic components (no. 1 in Fig. 5*A*) of the responses evoked by the rotated eye were either equal to or larger than those evoked by the deprived eye. These findings suggest that eye rotation had not caused any major damage to the optic nerve.

That conclusion was also supported by an examination of components which reflect mainly mono-, di- and polysynaptic activity in cortical layers IV, III and II, respectively (Mitzdorf & Singer, 1979). As exemplified in Fig. 5 the shape of these components was normal when evoked potentials were elicited from either eye. However, as would be expected from the decrease in excitability of the visual cortex of the cats with cyclotorsion, in those cats the post-synaptic response components

(2-4) were relatively small in relation to presynaptic peak 1 when compared to the m.d. controls.

Electroretinograms and flash evoked potentials. The responses evoked by electrical stimulation of the optic nerves do not allow one to determine whether eye rotation had caused damage to the retina prior to the ganglion cell level. Therefore, we recorded electroretinograms (e.r.g.s) and cortical evoked potentials after flash stimulation from the rotated and deprived eyes of the l.s.t. group. In none of the animals could we detect any consistent difference between the two eyes in either the e.r.g. responses or the cortical evoked potentials (Fig. 6).

Histology of the optic nerves in two control kittens with 180 deg eye rotation. We examined ten semi-thin ($2\ \mu\text{m}$) sections from each nerve with the light microscope under oil immersion. In none of the sections did we detect any difference between the nerves of the deprived and the rotated eye. In all sections we detected a small fraction of dark, fully stained fibres, which were perhaps more frequent close to the edge of the nerve but which did not follow any other obvious pattern of distribution. We counted the numbers of dark fibres in a sample of 400 fibres taken from four randomly selected areas of each nerve. The percentage of dark fibres ranged from 0.4 to 0.8% and was about the same in nerves of normal and rotated eyes.

DISCUSSION

Behavioural consequences of cyclotorsion

Rotating the eyes introduces a mismatch between retinal co-ordinates and other sensory-motor representations. Rather than reorganize these, our kittens simply stopped processing visual signals. This is at odds with two other studies which found extensive behavioural adaptation after cyclotorsion (Peck & Crewther, 1975; Peck *et al.* 1979). We think the discrepancy comes from four differences in experimental procedure: (1) we usually rotated the eye farther; (2) we closed the other eye so no normal signal was available for comparison (except in one control kitten, whose rotated eye did function); (3) we never forced our animals to use the rotated eye, they received no training on visual discriminations, and they grew up in the same restricted environment they came from; (4) we rotated the eye later than in the directly comparable study of Peck *et al.* (1979), so our cats also recovered ocular motility later and only toward the end of the critical period. The study of Hein, Vital-Durand, Salinger & Diamond (1979) demonstrated that eye movements are required for the development of visuo-motor co-ordination. It must be assumed, therefore, that the development of 'compensatory' strategies also requires ocular motility. Hence, retarded restitution of eye movements could readily account for the absence of functional recovery in our cats. The normal development of the control kittens with one eye closed and the other immobilized but not rotated is not incompatible with this interpretation. In contrast to Hein's study, where immobilization was permanent due to sections of the oculomotor nerves, eye movements recovered in our case, although to a limited extent. This restricted motility has apparently been sufficient for the development of normal visuo-motor co-ordination but not for the acquisition of compensatory strategies.

The neurophysiological consequences of neglect

Although the kittens neglected the rotated eye, signals from it obviously affected the development of the visual cortex: in the short-lived kittens ocular dominance was biased toward the open eye; in all kittens there was a relationship between ocular dominance, orientation selectivity, and the vigour of responses; and after 180 deg rotation the orientation preferences were biased (see also Singer *et al.* 1979). Yet the rotated eye did not influence the visual cortex in the same way as the open eye of monocularly deprived cats. In the short-lived kittens the rotated eye dominated many fewer cells than the open eye of monocularly deprived cats, and in the long-lived kittens ocular dominance was symmetrical. In several respects our kittens resembled binocularly deprived cats rather than monocularly deprived ones (Wiesel & Hubel, 1965; Buisseret & Imbert, 1976; Singer & Tretter, 1976; Watkins, Wilson & Sherman, 1978). As in b.d. preparations a large number of cortical cells were unresponsive, and most of the responsive ones functioned abnormally. As in visually inexperienced cats (Buisseret & Imbert, 1976; Fregnac & Imbert, 1978) the few normal cells tended to be monocular and to have simple receptive fields. The results of this functional analysis are in good agreement with data from morphometric examinations of cell growth in the l.g.n.d. (L. Goetz & W. Singer, in preparation). In all A-laminae cell growth was retarded as is the case in binocularly deprived kittens (Kalil, 1978) and the differences between laminae connected to the open and the closed eye were much less pronounced than in cats with conventional monocular deprivation.

As proposed previously (Blakemore & Van Sluyters, 1975) and supported by recent experimental evidence, interocular competition, acquisition of orientation selectivity and normal responsiveness appear all to depend on changes in excitatory transmission that follow the Hebbian rules (Rauschecker & Singer, 1979, 1981; Singer *et al.* 1981). According to functional criteria particular subsets of converging pathways increase their efficiency and become consolidated while others weaken. All abnormalities in the functional state of striate cortex in the present preparation can thus be accounted for by the absence or substantial reduction of these experience-dependent selective modifications of excitatory transmission.

Evidence for central factors

It is unlikely that some peripheral lesion was responsible for the absence of experience-dependent consolidation. None of the tests revealed any major defect in the retina or the optic nerve and minor peripheral damage would be unlikely to so dramatically impair visually-guided behaviour (Jacobson, Eames & McDonald, 1979). Nor is it likely that the abnormalities resulted from unstable retinal images, since the control kittens with the one eye immobilized developed normally. We suspect, therefore, that some central mechanism capable of perceiving the gross mismatch between retinal co-ordinates and other sensory-motor maps has blocked reactions to retinal signals from the rotated eye.

This interpretation can also account for the sequence of behavioural changes and the differences between the groups with short and long survival. The delayed onset of disturbed visuo-motor co-ordination suggests that cyclotorsion was obviously not conflicting initially, maybe because of late maturation of pathways required for

visuo-motor integration or because of the late re-appearance of small eye movements which may be required for the detection of faulty eye position. The central mechanism appears to have suppressed signals from the rotated eye only when they interfered with behaviour and correspondingly results from the s.s.t. group show that the rotated eye had started to dominate cortical pathways. Later visuo-motor behaviour became abnormal and results from the l.s.t. group show that the rotated eye lost its domination. A likely morphological correlate of the renewed equilibration of the ocular dominance distribution in the l.s.t. group is that cell growth in the deprived l.g.n. laminae while being retarded initially continued beyond the eighth week even in the deprived laminae (L. Goetz & W. Singer, in preparation).

There are a number of similarities between cats reared with one eye rotated and strabismic cats that develop amblyopia (Jacobson & Ikeda, 1979; von Grünau & Singer, 1980; Singer *et al.* 1980). In both cases retinal signals are available, but inappropriate and in both the inappropriate signals lose control of visual behaviour. The resulting disturbances of experience dependent development are similar: excitability of the cortical neurones is lowered, contrast sensitivity and visual acuity remain poor and visuo-motor co-ordination is impaired. In strabismic amblyopia interocular inhibition appears to selectively eliminate signals which would lead to diplopia (Sireteanu & Fronius, 1981; Sireteanu, Fronius & Singer, 1981). Since the cats we studied had the unrotated eye closed, some other process must have regulated the influence of retinal signals on striate cortex.

Wherever this central control is mediated, our results demonstrate that the developing brain screens retinal signals and allows only selected segments to induce changes in cortical organization. In line with the introductory remarks about the possible functional significance of developmental plasticity such a tight control of input dependent modifications appears to be necessary to prevent accidental and inappropriate sensory signals from inducing changes in circuitry that would be inadequate and – because irreversible – particularly dangerous.

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REFERENCES

- BARLOW, H. B. (1975). Visual experience and cortical development. *Nature, Lond.* **258**, 199–204.
- BLAKEMORE, C. & HILMAN, P. (1977). An attempt to assess the affects of monocular deprivation and strabismus on synaptic efficiency in the kittens visual cortex. *Exp. Brain Res.* **30**, 187–202.
- BLAKEMORE, C. & VAN SLUYTERS, R. C. (1975). Innate and environmental factors in the development of the kitten's visual cortex. *J. Physiol.* **248**, 663–716.
- BLAKEMORE, C., VAN SLUYTERS, R. C., PECK, C. K. & HEIN, A. (1975). Development of cat visual cortex following rotation of one eye. *Nature, Lond.* **257**, 584–586.
- BUISSERET, P. & IMBERT, M. (1976). Visual cortical cells: their developmental properties in normal and dark reared kittens. *J. Physiol.* **255**, 511–525.
- CAMPBELL, F. W., MAFFEI, L. & PICCOLINO, M. (1973). The contrast sensitivity of the cat. *J. Physiol.* **229**, 719–731.
- CREWTER, S. G., CREWTER, D. P., PECK, C. K. & PETTIGREW, J. D. (1980). Visual cortical effects of rearing cats with monocular or binocular cyclotorsion. *J. Neurophysiol.* **44**, 97–118.

- FREGNAC, Y. & IMBERT, M. (1978). Early development of visual cortical cells in normal and dark-reared kittens: relationship between orientation selectivity and ocular dominance. *J. Physiol.* **278**, 27-44.
- GRÜNAU, M. VON & SINGER, W. (1980). Functional amblyopia in kittens with unilateral exotropia: II. Correspondence between behavioural and electrophysiological assessment. *Exp. Brain Res.* **40**, 305-310.
- HEBB, D. O. (1949). *The Organization of Behaviour*. New York: John Wiley & Sons.
- HEIN, A., VITAL-DURAND, F., SALINGER, W. & DIAMOND, R. (1979). Eye movements initiate visual-motor development in the cat. *Science, N.Y.* **204**, 1321-1322.
- HUBEL, D. H. & WIESEL, T. N. (1965). Binocular interaction in striate cortex of kittens reared with artificial squint. *J. Neurophysiol.* **28**, 1041-1059.
- JACOBSON, S. G. & IKEDA, H. (1979). Behavioural studies of spatial vision in cats reared with convergent squint: is amblyopia due to arrest of development? *Exp. Brain Res.* **34**, 11-26.
- JACOBSON, S. G., EAMES, R. A. & McDONALD, W. I. (1979). Optic nerve fibre lesions in adult cats: pattern of recovery of spatial vision. *Exp. Brain Res.* **36**, 491-508.
- KALIL, R. (1978). Dark rearing in the cat: effects on visuomotor behaviour and cell growth in the dorsal lateral geniculate nucleus. *J. comp. Neurol.* **3**, 451-467.
- MITZDORF, U. & SINGER, W. (1979). Excitatory synaptic ensemble properties in the visual cortex of the macaque monkey: a current source density analysis of electrically evoked potentials. *J. comp. Neurol.* **187**, 71-84.
- MITZDORF, U. & SINGER, W. (1980). Monocular activation of visual cortex in normal and monocularly deprived cats: an analysis of evoked potentials. *J. Physiol.* **304**, 203-220.
- PECK, C. K. & CREWTER, S. G. (1975). Perceptual effects of surgical rotation of the eye in kittens. *Brain Res.* **99**, 213-219.
- PECK, C. K., CREWTER, S. G., BARBER, G. & JOHANNSEN, C. F. (1979). Pattern discrimination and visuomotor behaviour following rotation of one or both eyes in kittens and adult cats. *Exp. Brain Res.* **34**, 401-418.
- RAUSCHECKER, J. P. & SINGER, W. (1979). Changes in the circuitry of the kitten's visual cortex are gated by post-synaptic activity. *Nature, Lond.* **280**, 58-60.
- RAUSCHECKER, J. P. & SINGER, W. (1980). The effects of early visual experience on the cat's visual cortex and their possible explanation by Hebb synapses. *J. Physiol.* **310**, 215-239.
- SINGER, W. (1977). Effects of monocular deprivation on excitatory and inhibitory pathways in cat striate cortex. *Exp. Brain Res.* **30**, 25-41.
- SINGER, W., FREEMAN, B. & RAUSCHECKER, J. (1981). Restriction of visual experience to a single orientation affects the organization of orientation columns in cat visual cortex: a study with deoxyglucose. *Exp. Brain Res.* **41**, 199-215.
- SINGER, W. & TRETTER, F. (1976). Receptive-field properties and neuronal connectivity in striate and parastriate cortex of contour-deprived cats. *J. Neurophysiol.* **39**, 613-630.
- SINGER, W., TRETTER, F. & CYNADER, M. (1976). The effect of reticular stimulation on spontaneous and evoked activity in the cat visual cortex. *Brain Res.* **102**, 71-90.
- SINGER, W., TRETTER, F. & YINON, U. (1979a). Inverted vision causes selective loss of striate cortex neurons with binocular, vertically oriented receptive fields. *Brain Res.* **170**, 177-181.
- SINGER, W., VON GRÜNAU, M. & RAUSCHECKER, J. (1979b). Requirements for the disruption of binocularity in the visual cortex of strabismic kittens. *Brain Res.* **171**, 536-540.
- SINGER, W., VON GRÜNAU, M. W. & RAUSCHECKER, J. (1980). Functional amblyopia in kittens with unilateral exotropia: I. Electrophysiological assessment. *Exp. Brain Res.* **40**, 294-304.
- SINGER, W., YINON, U. & TRETTER, F. (1979c). Inverted monocular vision prevents ocular dominance shift in kittens and impairs the functional state of visual cortex in adult cats. *Brain Res.* **164**, 294-299.
- SINGER, W., TRETTER, F. & YINON, U. (1982). Evidence for functional plasticity in the visual cortex of adult cats. *J. Physiol.* **324**, 239-248.
- SIRETEANU, R. & FRONIUS, M. (1981). Naso-temporal asymmetries in amblyopic vision: consequence of long-term interocular suppression. *Vision Res.* **21**, 1055-1063.
- SIRETEANU, R., FRONIUS, M. & SINGER, W. (1981). Binocular interaction in the peripheral visual field of human squint and anisometric amblyopes. *Vision Res.* **21**, 1065-1074.
- VAN HOF, J. (1977). Visual field measurements in monocularly deprived and normal cats. *Exp. Brain Res.* **30**, 353-368.

- WATKINS, D. W., WILSON, J. R. & SHERMAN, S. M. (1978). Receptive-field properties of neurons in binocular and monocular segments of striate cortex in cats raised with binocular lid suture. *J. Neurophysiol.* **41**, 322–337.
- WIESEL, T. N. & HUBEL, D. H. (1965). Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *J. Neurophysiol.* **28**, 1029–1040.
- YINON, U. (1975). Eye rotation in developing kittens: the effect on ocular dominance and receptive field organization of cortical neurons. *Exp. Brain Res.* **24**, 215–218.