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Schwann cells transplanted in the lateral ventricles prevent the functional and anatomical effects of monocular deprivation in the rat

(nerve growth factor/amblyopia/ocular dominance/lateral geniculate nucleus/neurotrophin)

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ABSTRACT **We investigated whether the transplant of Schwann cells prevents the physiological and morphological effects of monocular deprivation in the rat. On the day of eye opening in rats (postnatal day 14), we transplanted Schwann cells in the lateral ventricles and sutured the eyelids of one eye. After 20–30 days, at the end of the critical period for the visual system development, we analyzed the functional properties of visual cortical neurons. Spontaneous discharge, orientation selectivity, and receptive field size of visual cortical neurons in transplanted animals were in the normal range. Transplantation of Schwann cells prevented the detrimental effects of monocular deprivation on ocular dominance and binocularity of cortical neurons. Visual acuity of the deprived eye estimated by visually evoked potentials was also normal. Schwann cells derived from adult animals were as effective as those derived from neonates. The effects of Schwann cells on monocular deprivation were dependent upon the number of cells present in the transplant so that 10^6 Schwann cells were sufficient to prevent the effect of monocular deprivation, whereas 10^5 and 3.3×10^5 Schwann cells were ineffective, and 6.3×10^5 cells gave variable results. Shrinkage of the deprived lateral geniculate neurons was prevented by a transplant of 10^6 cells. In rats transplanted with hybridoma cells producing an antibody that functionally blocks nerve growth factor (NGF), we found that the effect of cotransplanted Schwann cells on monocular deprivation was partly counteracted. We conclude that transplantation of Schwann cells prevents both functional and anatomical effects of monocular deprivation, presumably acting through the production of NGF. We propose that transplants of Schwann cells could be a promising technique for clinical applications.**

Administration of neurotrophins has beneficial effects in animal models of Alzheimer disease (1–3), amblyopia (4), age-related deficits in learning and memory (5), and possibly Parkinson disease (6–8). However, neurotrophins do not cross the blood–brain barrier (9). This fact has prompted experiments on transplantation into the brain of biological producers of neurotrophic factors. In particular transplantation of Schwann cells (SC) and genetically modified fibroblasts in the central nervous system (CNS) have been shown to prevent the loss of axotomized cholinergic septal neurons and to promote their regeneration (10, 11). Moreover, SC transplanted into the eye partly prevent or delay the loss of retinal ganglion cells after optic nerve section (12). The effects of transplants have been tested primarily by neurochemical or anatomical techniques. In view of possible clinical applications of transplants, it is necessary that physiological tests also be carried out.

The present experiments investigate the response properties of visual cortical neurons of monocularly deprived rats (MD rats) that received transplants of SC into the lateral ventricles. In particular, we analyzed whether SC transplants can prevent the effects of monocular deprivation without altering the physiology of the visual system. The rationale for these studies is based on two previous observations: (*i*) the effects of monocular deprivation are prevented by infusion of nerve growth factor (NGF) into the lateral ventricle (4, 13–15), and (*ii*) SC are able to produce neurotrophic factors such as NGF and brain-derived neurotrophic factor (BDNF) (16, 17).

We found that transplantation of SC completely prevented the effects of monocular deprivation without causing any pathological effects in the hosts. In particular, SC do not cause any alteration in the physiology of the visual cortex.

MATERIALS AND METHODS

Primary Neonatal SC Cultures and Transplant Procedure. SC were prepared from neonatal rat sciatic nerves by the method of Brockes *et al.* (18) and were maintained in culture as described (19). In one preparation, Fluoro-Gold (Fluorochrome, Englewood, CO) was added to culture medium at 0.45 mg/ml to label SC (20); 24–48 hr after the explant, four different amounts of SC (10^5, 3.3×10^5, 6.3×10^5, and 10^6 SC) were suspended in the same volume of Hanks' solution and injected bilaterally in the lateral ventricles of postnatal-day-14–15 (P14–15) animals (under ether anesthesia) that first had been subjected to monocular deprivation (13). When radioactively labeled NGF was injected into the ventricles, radioactivity could be detected in the visual cortex. In two rats transplanted with 10^6 SC, we transplanted 2×10^6 αD11 hybridoma cells by the same procedure during the same surgical session. αD11 cells produce a monoclonal antibody against NGF that blocks NGF binding to its receptors (21). Hybridoma transplanted rats were treated with cyclosporin A as described (22). The transplantation of the parental myeloma line (P3U) or of another hybridoma line producing an irrelevant antibody (23) is known to have no effect on either the plasticity or the function of visual cortical cells (24).

Primary Adult SC Cultures and Transplant Procedures. In six rats we transplanted SC taken from the sciatic nerves of adult rats. Adult rat SC were prepared from crushed sciatic

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Abbreviations: NGF, nerve growth factor; VEP, visually evoked potentials; LGN, lateral geniculate nucleus; HRP, horseradish peroxidase; MD rats, monocularly deprived rats; SC, Schwann cell(s); BDNF, brain-derived neurotrophic factor; CNS, central nervous system; P, postnatal day; deg, degree.

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nerves 10 days after injury as will be described elsewhere (G.F., unpublished data). Briefly, 2-cm lengths of crushed sciatic nerve were pooled into phosphate-buffered saline (PBS) and, after removal of the epineurium nerves, were teased into bundles, cut, and centrifuged for 5 min at 100 × *g*. They were suspended in Dulbecco's modified Eagle's medium (DMEM) containing 15% (vol/vol) fetal calf serum (FCS), 25 mM Hepes, dispase (Boehringer Mannheim, D) at 1.25 units/ml, collagenase (type I, Worthington) at 80 units/ml, hyaluronidase (Sigma) at 1 mg/ml, penicillin G at 0.5 unit/ml, and streptomycin (GIBCO) at 0.5 mg/ml and were incubated overnight at 37°C. After centrifugation, the resulting pellet was washed three times with DMEM containing 15% FCS and then resuspended in the same medium. Cells were plated into dishes, and after 48 hr the medium was replaced with DMEM containing 3% FCS. Cultures were fed three times per week. Under these conditions many bipolar, spindle-shaped cells were present after 30 days *in vitro*. These cells were immunopositive for S-100 and p75, two well-known markers for SC. Transplantation of these cells was performed as for neonatal SC.

Animals. Experiments were performed on 51 Long–Evans hooded rats. As in other mammals, neurons in the pigmented rat primary visual cortex have well-defined functional properties and show a high degree of binocularity (80% of visual cortical neurons of the Oc1b area). These characteristics of cortical cells develop gradually during the first weeks of postnatal life, and their correct development depends upon visual experience (25). Seven animals were reared normally while the other 44 (under ether anesthesia) were subjected to monocular deprivation by suturing the eyelids of one eye on the day of eye opening (P14–15). Of the MD rats, 34 were transplanted with SC, 2 were transplanted with SC together with αD11 hybridoma cells, 3 were transplanted only with αD11 as control, and 5 were untreated. After 20–30 days of monocular deprivation, electrophysiological and anatomical analyses were carried out.

The initial cell-recording experiments (16 of 51) were carried out blind, so that the experimenter was unaware of the transplant status of the animal.

Lateral Geniculate Nucleus (LGN) Neuron Shrinkage. Monocular deprivation induces shrinkage of deprived LGN neurons (14, 26). Injecting horseradish peroxidase (HRP) in one eye allows an easy identification of the ipsilateral and contralateral subfields of LGN (27). We measured the effects of monocular deprivation in the ipsilateral part of the LGN in untreated and SC-transplanted rats using a blind procedure. We anterogradely labeled retinal fibers with HRP (grade I, Boehringer Mannheim D), injecting 10–14 μl of HRP (30% in saline plus 2% dimethyl sulfoxide) in the nondeprived eye 24 hr after rats were perfused with saline followed by 1.25% glutaraldehyde/1% paraformaldehyde solution for 30 min and then by a 25% sucrose/0.1 M phosphate buffer solution. Brains were then cut with a liquid-CO_2 freezing microtome at 40 μm, and the HRP reaction product was revealed by using *p*-phenylenediamine/pyrocathecol as chromogens. Sections were subsequently counter-stained with cresyl violet (14, 29). Cells in the ipsilaterally receiving subfields of LGN showing a clear visible nucleus and nucleolus were drawn by means of a camera lucida. Drawings were fed into a computer, and soma size was measured by using an image analysis program.

Fluoro-Gold Visualization. Rats were perfused with 4% paraformaldehyde/0.1 M phosphate buffer. Brains were removed, postfixed, and cryoprotected with 25% sucrose/0.1 M phosphate buffer. Brains were cut, and slices were mounted and observed with a fluorescence microscope.

Electrophysiology. We recorded single-cell activity from the primary visual cortex of normal rats, MD rats, and SC-treated MD rats. In the same session visually evoked potentials (VEP) from five normal, four MD, and five SC-treated MD rats were recorded in response to alternating gratings of optimal luminance and temporal frequency varying in contrast and spatial frequency. Animals were under urethane anesthesia (6 ml/kg of body weight; 20% in saline). Recordings were performed as described (4, 13). For single-cell recordings, location of the receptive field in the visual space, its size and organization, optimal stimulus orientation and direction of movement, ocular dominance class, and response type were determined for each cell according to standard criteria (30, 31). Only cells with receptive fields within the binocular visual field [>30 degrees (deg) nasal from the optic disk and in the upper visual field] were included in our sample. The mean receptive field position with respect to the vertical meridian was 12 ± 9 deg in normal rats, 10 ± 8 deg in untreated MD rats, and 11 ± 10 deg in SC-transplanted MD rats. VEP recordings were performed as described (4).

RESULTS

Transplantation of SC Does Not Cause Pathological Effects. The survival of SC was assessed in four rats transplanted with Fluoro-Gold-labeled SC. In these animals Fluoro-Gold labeled cells were found nearby the lateral ventricles and the injection sites at the end of the deprivation period—i.e., 30 days after transplantation. Labeled cells were not detected farther away than 1 mm from the ventricles or the injection sites (Fig. 1). The following observations suggest that SC transplants do not cause pathological alterations. (*i*) The behavior of treated animals (10^6 SC) and their weight were normal. The gross anatomy of the brain and the size of ventricles (Fig. 1) were indistinguishable from controls. (*ii*) The spontaneous activity (3.5 spikes per sec; $n = 50$ cells) and responsiveness to visual stimulation (12.5 spikes per sec; $n = 55$ cells) of visual cortical neurons were in the normal range both in terms of frequency and pattern of discharge (25). No unresponsive cells were found. Unresponsive cells in adult visual cortex are rare, although present in younger animals (25). (*iii*) The size and organization of receptive field of visual cortical neurons of transplanted rats [receptive field short axis; 6.35 deg (±2.3 deg, $n = 35$ cells)] were not significantly different from those of normal rats [6.1 deg (±1 deg, $n = 32$ cells)]. (*iv*) The number of nonorientation-selective cells was not increased by SC transplant [25.2% (±16.3%, $n = 7$ normal rats) of nonorientation-selective cells; 31.9% (±8.1%, $n = 6$ SC-transplanted MD rats) of nonorientation-selective cells]. (*v*) The visual acuity of transplanted rats, evaluated by VEP, was not different from that of normal adult animals [0.88 cycle/deg (±0.14 cycle/deg, $n = 3$ animals); $P > 0.05$].

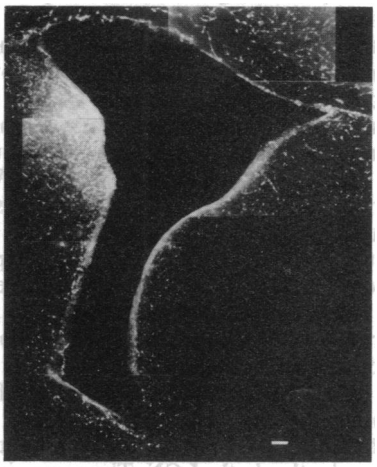

FIG. 1. Fluoro-Gold-labeled cells in the lateral ventricle of a P40 rat transplanted on P14 with 10^6 SC. (Bar = 300 μm.)

Transplantation of SC Prevents the Effects of Monocular Deprivation. The duration of monocular deprivation covered the entire critical period (from the day of eye opening, P14, until P35–45) of the rat (25). Monocular deprivation is known to induce a shift in the ocular dominance distribution of cortical cells toward the nondeprived eye (13), an impairment of visual acuity in the deprived eye (4), and shrinkage of deprived LGN neurons (14). All of these parameters were measured and found to be normal at the end of the deprivation period in SC-transplanted rats.

Ocular Dominance Distribution of Cortical Cells. The cumulative ocular dominance distribution of visual cortical neurons of seven normal and five MD SC-untreated rats is shown in Fig. 2 *A* and *B*. As described by Maffei *et al.* (13), monocular deprivation in the rat induces a dramatic shift of ocular dominance toward the nondeprived eye. To summarize the effect of monocular deprivation, an ipsilaterality index was attributed to each rat. The ipsilaterality index = (cells in ocular dominance classes 5–7)/(total number of responsive cells). The mean ipsilateral index for the normal animals was 0.11 ($\pm$ = 0.05, n = 7 animals), while for the MD animals it was 0.84 ($\pm$ = 0.165, n = 5 animals).

This shift was completely prevented by transplantation of 10^6 SC in five of six cases. In these five cases, the mean ipsilateral index, 0.07 ($\pm$ = 0.02, n = 5 animals) was not significantly different from that of normal animals ($P > 0.15$). In one animal the transplantation of SC was unsuccessful. The ipsilateral index, 0.85, was significantly different from that of normal animals ($P < 0.01$) and in the range of that of MD rats ($P > 0.1$) (Fig. 3). This failure can be due to several causes related to transplant techniques rather than to an insufficient efficacy of neonatal SC. For this reason we have separated the data of this unsuccessful transplant from the other five animals. In Fig. 2*C* we have superimposed the ocular dominance distribution of the successful cases (hatched columns) on the cumulative ocular dominance distribution including all of the data (open columns). Both distributions, and in particular the latter one, are very similar to that of normal animals. Fig. 2*D* shows that transplantation of 10^5 SC is insufficient to prevent the effect of monocular deprivation.

Effects of SC on Monocular Deprivation As a Function of the Number of Transplanted Cells. The results are shown in Fig. 3 where the ipsilateral index is reported as a function of the number of transplanted SC. Transplantation of 10^5 or 3.3×10^5 SC had little or no effect on monocular deprivation. Transplants of 6.3×10^5 SC gave variable effects, whereas 10^6 cells were sufficient to prevent the effect of monocular deprivation. Transplantation of an increasing number of SC is progressively more effective in preventing the shift of the ipsilaterality index toward the large values typical of MD animals.

Transplantation of 10^6 myeloma cells, which do not produce NGF or other neurotrophic factors, did not prevent the effects of monocular deprivation.

Visual Acuity of SC Transplanted Rats. As in other mammals (32) monocular deprivation in the rat (4) induces a strong reduction of visual acuity in the deprived eye. Visual acuity is taken as the highest spatial frequency of the stimulus that still evokes a VEP signal above noise level (33). Fig. 4 shows the mean visual acuity of normal rats, of the deprived eye of MD SC-untreated rats, and of the deprived eye of MD rats transplanted with 10^6 SC. In untreated rats, monocular deprivation reduces the visual acuity of the deprived eye by nearly a factor of 3 (t test; $P < 0.001$). The visual acuity of the deprived eye of SC-transplanted rats does not differ from that of normal animals (t test; $P > 0.05$).

Transplantation of SC Prevents the Anatomical Effects of Monocular Deprivation in the LGN. The soma size of neurons in the lamina of the LGN receiving ipsilateral inputs was measured in SC-untreated MD rats and in MD rats transplanted with different numbers of SC. Monocular deprivation caused a mean shrinkage of 20.6% $\pm$ 0.9% in deprived LGN neurons of SC-untreated MD rats (Table 1). A comparable amount of shrinkage is observed in rats transplanted with an insufficient number of SC. No shrinkage was observed in all four rats transplanted with 10^6 SC. In these rats, the mean soma size of neurons of the deprived and nondeprived LGN laminae was not significantly different (t test between the

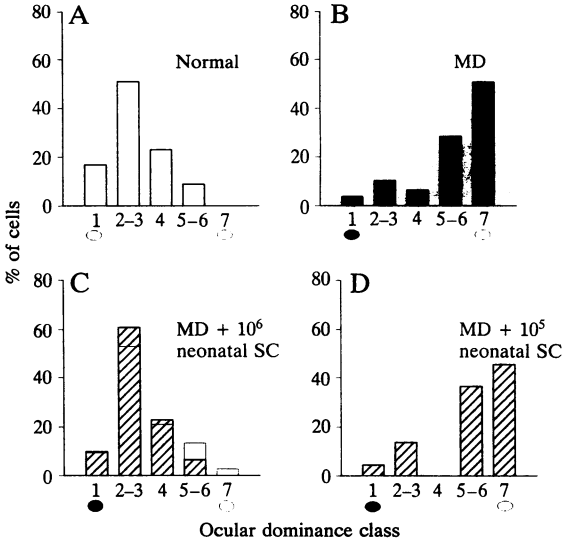

FIG. 2. Ocular dominance distribution of visual cortical neurons recorded in area Oc1b. (*A*) Data from seven normal rats (164 cells). (*B*) Data from five SC-untreated MD rats (108 cells). (*C*) Data from six MD rats transplanted with 10^6 SC (open columns, n = 115 cells), and data from the five of six successful cases (hatched columns, n = 100 cells). (*D*) Data from three MD rats transplanted with 1.2×10^5 SC (66 cells). ○, Nondeprived eye; ●, deprived eye. Ocular dominance distributions of visual cortical neurons of 10^6 SC-treated MD rats do not differ from those of normal (χ^2, $P > 0.05$) and are significantly different from MD rats SC-untreated or treated with 10^5 SC (χ^2, $P << 0.001$ for both). Ocular dominance distribution of 10^5 SC-treated MD rats is not different from that of untreated MD rats (χ^2, $P > 0.1$).

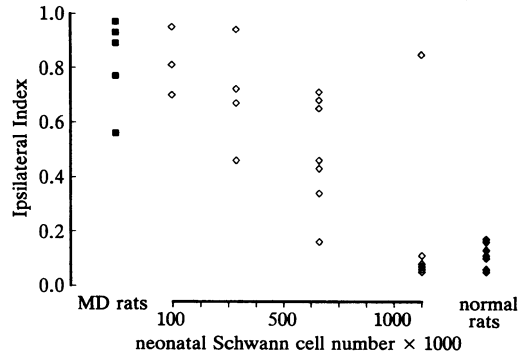

FIG. 3. The effect of SC on MD rats depends on the number of transplanted SC. ■, SC-untreated MD rats; ◇, SC-transplanted rats; ◆, normal rats. On the ordinate is reported the ipsilateral index. Ipsilateral index = (cells in ocular dominance classes 5–7)/(total number of responsive cells). Each point reports the result obtained in one rat. On the abscissa is reported the number of transplanted SC. No statistical difference is present between the mean ipsilateral indexes of MD SC-untreated MD rats and 10^5 SC-treated MD rats (t test, $P > 0.9$). A slight, but not significant (t test, $P > 0.2$), decrease in this index is present in 3.3×10^5 SC-treated MD rats. The mean ipsilateral index of 6.3×10^5 SC-treated MD rats is intermediate between SC-untreated MD rats and normal rats, being significantly different from both (t test, $P < 0.01$); 10^6 SC-treated MD rats have a mean ipsilateral index not significantly different from those of normals (t test, $P > 0.15$).

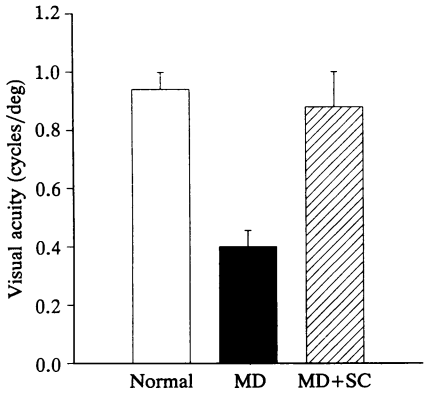

FIG. 4. SC at 10^6 prevent the reduction of visual acuity induced by monocular deprivation. Visual acuity was estimated by VEP for normal rats ($n = 5$), SC-untreated MD rats ($n = 4$), and 10^6 SC-treated MD rats ($n = 5$). In SC-untreated MD rats, the stimulated eye was the deprived eye. Monocular deprivation significantly reduces visual acuity of the deprived eye compared with normal eyes (t test, $P << 0.001$); 10^6 SC prevent this reduction (t test, $P << 0.001$), and the visual acuity of the deprived eye does not differ from that of a normal eye (t test, $P > 0.05$).

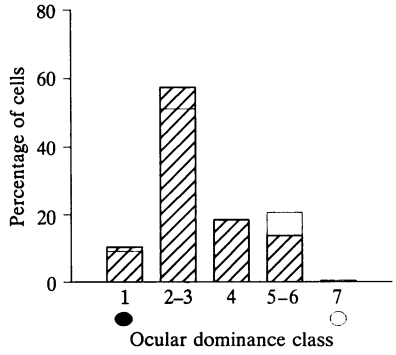

FIG. 5. The shift toward the ipsilateral, nondeprived eye induced by monocular deprivation is prevented in rats transplanted with 10^6 adult-derived SC. Data are from five MD rats transplanted with 10^6 SC (open columns, $n = 100$ cells) and from the four of 5 successful cases (hatched columns, $n = 86$ cells). ○, Nondeprived eye; ●, deprived eye. Ocular dominance distributions of cortical neurons of MD rats transplanted with 10^6 adult-derived SC are not different from those of normal rats (see Fig. 2*A*; χ^2, $P > 0.1$). The ocular dominance distributions of MD rats transplanted with neonatal-derived or adult-derived SC are not significantly different (χ^2 test, $P > 0.05$).

diameter distribution of deprived and nondeprived LGN neurons; $P < 0.05$).

Effects of Adult-Derived Schwann Cells. SC were taken from crushed sciatic nerves of adult rats 10 days after injury. SC (10^6 cells) were transplanted in five P14 MD rats. In four cases the transplantation was completely successful in preventing the shift of ocular dominance distribution induced by monocular deprivation (mean ipsilateral index is 0.13 ± 0.09, which is not significantly different from that of control animals; $P > 0.15$). In one case the transplantation was a complete failure (ipsilateral index = 0.75, which is not significantly different from those of MD SC-untreated rats; $P > 0.1$). The data are reported in Fig. 5. An extensive report of these results will appear elsewhere.

Effects of the Cotransplant of SC and Anti-NGF-Producing Hybridoma Cells. SC are known to produce several trophic factors, including NGF (16, 17, 34). We sought to determine whether the ability of SC transplants, in preventing the effect of monocular deprivation, is mediated by NGF. This problem was investigated by using the hybridoma cell line αD11. αD11 produces an antibody that is able to block NGF binding to its receptor (21). This antibody does not inhibit the biological activity of BDNF and NT-3 *in vitro* (A. Cattaneo, personal communication). Hybridoma cell transplant in the CNS as a means for counteracting the effects of NGF has been recently developed in our laboratory (24). The quantity of hybridoma cells necessary to counteract NGF *in vivo* was found to be on the order of 2×10^6 αD11. The effects of the transplants of hybridoma cells in the CNS are specific because transplants of parental myeloma cells or control hybridoma are ineffective (22, 24).

We cotransplanted two rats with 10^6 SC, which are sufficient to prevent the effects of monocular deprivation, and 2×10^6 αD11, which are sufficient to block NGF biological activity. We found that the transplantation of hybridoma cells counteracts at least in part the action of SC. Results are shown in Fig. 6. It can be noted that both distributions are shifted toward the nondeprived eye.

DISCUSSION

Our experiments have shown that transplants of SC hinder the effects of monocular deprivation; 10^6 SC prevent the shift in ocular dominance, impairment of visual acuity, and shrinkage of LGN neurons soma size. The repeatability of the transplant of SC was indeed very high, since only 2 of the 15 SC-treated animals were not successful. The reason for the failure of SC transplantation can be manifold. For instance, these could be an inability of SC to enter the brain because of cerebrospinal fluid and/or blood outflow during injection or to enhanced immune reaction due to a major tissue damage or a sensitized status of the animal (35).

The transplanted SC do not induce pathological effects as supported by the following observations: general health, gross behavior, and weight of treated animals were in the range of controls. The development of functional properties

Table 1. SC prevent the shrinkage of deprived LGN neurons of MD rats

Transplanted SC, no. $\times 10^{-6}$	Nondeprived LGN lamina		Deprived LGN lamina		
	No. of neurons	Mean diameter, μm	No. of neurons	Mean diameter, μm	% of shrinkage
1	200	13.1	202	13.2	−0.5
1	131	13.2	157	12.9	2.31
1	167	14.6	157	14.2	2.15
1	75	13.1	78	12.8	2.34
0.33	94	13.6	88	11.3	20.4
0.1	139	14.1	113	11.5	18.4
0	155	13.40	150	10.76	19.9
0	127	14.20	130	11.20	21.5
0	140	14.70	133	11.75	20.0

Percentage of shrinkage = [(mean diameter of the neurons of nondeprived lamina − mean diameter of the neurons of deprived lamina)/mean diameter of the neurons of the nondeprived lamina] × 100.

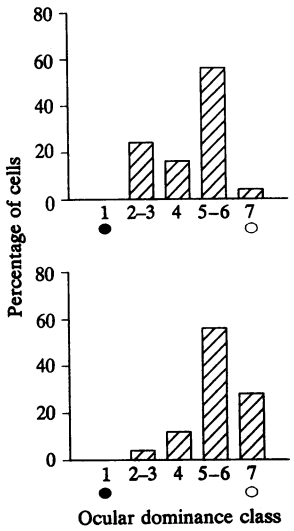

FIG. 6. Ocular dominance distribution of single rats transplanted with 10^6 SC and 2×10^6 hybridoma cells. ○, Nondeprived eye; ●, deprived eye. Both distributions are significantly different from that of normal rats and of SC-treated MD rats (χ^2 test, $P < 0.01$). The two distributions are clearly shifted toward the ipsilateral nondeprived eye.

of the visual cortex seems to be normal in the transplanted animals. These properties are immature at the time (P14) of SC injection (25), and they are found as in normal adult animals at the time (P45) of cell recording. In addition, the action of SC seems to be specific because (*i*) the effect of SC is dose-dependent and (*ii*) intraventricular transplants of other cells like αD11 hybridoma cell line do not prevent monocular deprivation.

As to the mechanism of action of SC, a possible hypothesis suggested by our results is that the effect of SC is mediated by neurotrophic factors. This hypothesis is supported by studies showing that infusion of NGF in the ventricles prevents the effects of monocular deprivation (13, 15) and that SC produce NGF, BDNF, and other neurotrophic factors *in vitro* (17, 36) and *in vivo* (16). This property is probably maintained when SC are transplanted into the CNS as suggested by the recent observation that a considerable amount of NGF was found to be present in pieces of sciatic nerve transplanted into the CNS (28). Our results showing that transplants of hybridoma cells producing antibody against NGF counteract, at least in part, the effect of SC suggests that the SC action could be partly mediated by NGF. Different neurotrophins could also play a relevant role. There remains open the possibility that SC exert their action indirectly through activation of neurotrophic factor production in other cell types.

As to the site of action of NGF or other neurotrophic factors produced by SC, subcortical structures or the visual cortex are equally likely. We only know that the injection of radiolabeled NGF in the lateral ventricle can reach the visual cortex and that the local application of NGF is effective in preventing the effects of monocular deprivation. The mechanism of action of NGF in preventing the effects of monocular deprivation is not yet clear, and this argument has been discussed extensively in other papers (13).

Our results indicated that SC transplants are good candidates for clinical applications. An obstacle in this approach is the reaction of the immune system to the transplant. The effect of SC on monocular deprivation and the persistence of Fluoro-Gold-labeled cells 1 month after the transplant do not exclude reactions of the immune system at later times. Moreover, clinical application is generally performed in adults where the probability of rejection is greater. One way of overcoming this difficulty is suggested, however, by results presented here and in our unpublished data showing that adult-derived SC are as effective as neonatal-derived SC. Currently we are also investigating autologous transplants of SC in adult animals.

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