Age-Dependent Specification of the Corticocortical Connections of Cerebral Grafts

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To investigate further the factors involved in the development of cerebral cortical circuitry, we examined the organization of corticocortical connections of heterotopic grafts of fetal cortex placed into neonatal rat cortices. Presumptive perirhinal or sensorimotor areas of the cerebral wall were removed as slabs from embryonic day 12 (E12), E13, or E14 rats and transplanted heterotopically into either rostral perirhinal or sensorimotor cortical areas of neonatal rats. Two weeks later, the afferent cortical connections of the grafts were labeled by placing DilC₁₈-3 (Dil) into each transplant site. Both the E12 and E13 heterotopic transplants of presumptive perirhinal cortex contain mostly precursor cells. When these grafts are placed into sensorimotor cortex, callosal connections are formed primarily with the contralateral sensorimotor (Sml) area, the normal projection of Sml cortex. In contrast, the E14 heterotopic transplants of the perirhinal cortical wall, containing many more postmitotic neurons, developed contralateral connections with both sensorimotor and rostral perirhinal cortices. Thus, when precursor cells are transplanted heterotopically, by using E12/E13 donor tissue, the grafts receive projections that are similar to those of the host cortical area. When older cortical neurons, together with precursors, are transplanted into a heterotopic cortical area, by using E14 donor tissue, their cortical connections exhibit both host and original donor phenotypes. The data are consistent with our previous analysis of thalamocortical connections of grafts (Barbe and Levitt, 1992b) and suggest the existence of a cell–cell recognition system for thalamocortical and corticocortical circuit formation, whose mechanisms of action may be linked to the timing of neurogenesis.

[Key words: neural graft, sensorimotor cortex, perirhinal cortex, cell fate, cell specification, and cortical connections]

The cerebral cortex is organized into separate areas based on anatomical, neurochemical and functional differences. The precise organization of the cortex is achieved by the progressive steps of neurogenesis, migration of neurons to specific laminae

and axon elongation to appropriate targets. The time of origin of a neuron correlates strongly with both a neuron's final laminar position (McConnell, 1988; McConnell and Kaznowski, 1991) and its axonal connections (Caviness and Rakic, 1978; Lemmon and Pearlman, 1981; Jensen and Killackey, 1984; Katz, 1991; Schwartz et al., 1991). Although the mechanisms underlying the determination of the final pattern of cortical connections are unknown, the formation of specific projection patterns are probably based on a large number of factors, including molecular interactions that control laminar fate via local neuroepithelial factors (McConnell and Kaznowski, 1991), axonal guidance by pioneer axons (McConnell et al., 1989; Ghosh et al., 1990; Ghosh and Shatz, 1993), transient targeting (Stanfield and O'Leary, 1988), local positional cues (Fraser, 1980; Gierer, 1987; Walter et al., 1987; Harris, 1989; Stahl et al., 1990; Simon and O'Leary, 1992a, 1992b), diffusible factors used as intermediate guideposts (Tessier-Lavigne et al., 1988; Placzek et al., 1990), target chemotrophic and chemotropic molecules (Lumsden and Davies, 1983; Hankin and Lund, 1987; Heffner et al., 1990; Yamamoto et al., 1989, 1992; O'Leary et al., 1990; Baier and Bonhoeffer, 1992), chemoaffinity molecules (Sperry, 1963; Levitt, 1984), and selective elimination of temporary axonal projections (Stanfield et al., 1982, 1987; Chalupa and Killackey, 1989; O'Leary and Stanfield, 1989; Callaway and Katz, 1990).

Recent transplantation studies of cortical tissue have demonstrated an early commitment of neurons to express a specific molecular phenotype. In these experiments, the expression of the limbic system–associated membrane protein (LAMP), a marker of limbic cortex (Levitt, 1984; Horton and Levitt, 1988), was regulated in a complex fashion (Barbe and Levitt, 1991, 1992b). LAMP is a new member of the Ig-superfamily (Pimenta et al., 1993) and mediates adhesive interactions (Zhukareva and Levitt, 1993) that may be critical for the formation of some limbic circuits (Keller et al., 1989; Barbe and Levitt, 1992a). We found that there is a critical period for the specification of cortical neurons to express this molecular phenotype. Donor tissue from either LAMP-positive perirhinal or LAMP-negative sensorimotor regions, harvested at E12, when all cells are precursors, exhibit molecular features of the host milieu after grafting. Older grafts (E14 or E17), which have increasing proportions of postmitotic neurons, contain increasing numbers of neurons expressing a molecular phenotype faithful to their donor origin (Barbe and Levitt, 1991). Results of a second study suggest that this molecular specification correlates strongly with the thalamocortical connections of the grafted tissue (Barbe and Levitt, 1992b).

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Figure 1. Cytoarchitectural maps of the lateral (left) and medial (right) aspects of the cerebral cortical hemisphere, redrawn from Vogt and Miller (1981), Deacon et al. (1983), and Ziles et al., (1980), Paxinos and Watson (1986). FL, sensory and motor forelimb overlap area; HL, sensory and motor hindlimb overlap area. For other abbreviations, see Appendix.

In the present study, we have examined the pattern of ipsilateral and callosal projections into perirhinal and sensorimotor grafts from the host cortex. The experiments were designed to further investigate our hypothesis that certain phenotypic traits of neurons are specified early, and that the molecular phenotype of the neurons may help determine synaptic connections. Our present transplantation results show that the E14 heterotopically located perirhinal grafts form hybrid cortical connections that reflected both their donor origin and their new host locale, while E13 and E12 grafts form connections that reflect mostly their new host position.

Materials and Methods

Transplantation of fetal tissue to neonatal hosts. Fetal Sprague-Dawley rats (Harlan) were obtained from timed-pregnant dams. Plug date is considered to be E0. Dams were anesthetized with Nembutal (50 mg/kg body weight), and animals at embryonic day (E) 12, 13, and 14 were removed. Areas of the cerebral wall containing presumptive perirhinal and sensorimotor cortices were excised and prepared as described previously (Barbe and Levitt, 1991, 1992b). E12 and E14 correspond respectively to times before and after neurons initiate their commitment to the limbic molecular phenotype (Barbe and Levitt, 1991). Slabs of tissue were dissected out in ice-cold F12 media (GIBCO-Bethesda Research Labs, Gaithersburg, MD) with 6.5 mg/ml glucose, labeled with 0.002% fast blue (Sigma, St. Louis, MO) in media, rinsed and transplanted into newborn (P0) host rat pups, a time when corticocortical projections are still developing (Wise and Jones 1976, 1978; Ivy et al., 1979; Valentino and Jones, 1982; Hernit et al., 1990; Elberger, 1994). Transplants were placed heterotopically or homotopically into either sensorimotor or perirhinal cortex. Grafts into the sensorimotor cortex were placed in the rostral Sml area at the level of the septal end of the hippocampus. Grafts into the perirhinal cortex were placed into area 35, just caudal to the claustrum. Eighteen to 20 animals were grafted per group, totaling 151 animals. Two weeks after surgery, rat pups were perfused with 4% paraformaldehyde in 0.1M sodium phosphate buffer (pH 7.4). Brains were removed from the skull and postfixed by immersion for 24 hr. Control animals that had not received any transplants were also fixed for DiI labeling (see below).

Labeling with DiI. In order to examine the cortical connections of the transplant, a lipophilic fluorescent tracer, DiI₁₈-(3) [DiI; 1,1'-dioc-tadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (Molecular Probes, Eugene, OR); Godement et al., 1987], was inserted into the transplants or the identical areas of controls in the fixed brains to retrogradely label cortical neurons innervating the graft (efferent axons were also labeled but were not quantitated). The DiI was dissolved in 100% ethanol, precipitated by evaporation onto small pieces of pulled glass micropipettes and inserted into the transplant sites of the fixed brains. The DiI was also inserted into either the rostral Prh or Sml in age-matched nontransplanted control animals ($n = 4$ /group). Care was taken to keep the coated glass piece within the cortical wall itself without penetrating the underlying white matter. Brains were incubated at 37°C for 8–9 weeks in 4% paraformaldehyde fixative in 0.1 M sodium phosphate buffer, and were then sectioned on a vibratome into 200 μ m coronal slices. The sections were wet mounted onto slides and coverslipped with 80% glycerol in 0.1 M phosphate buffer. Fast blue and DiI at the transplant site were photographed, the coverslip removed and the sections counterstained with *m*-phenylenediamine in glycerol/phosphate buffer (Quinn and Webber, 1988). The sections were reexamined for integrity of the transplant, insertion site and depth of the DiI label in the transplant, the location of fibers extending from the grafts and location of retrogradely labeled neurons within the cortex.

Cytoarchitectural mapping and quantification. Only those brains with both well-integrated transplants and a DiI insertion site limited to the boundaries of the transplant (micropipette placed directly into the transplant site and glass not penetrating white matter) were used for analysis. While approximately 85% of the grafts survived well, only approximately 25% of the labeled grafts met both stringent criteria ($n = 3$ –7 per group). Labeled neurons were mapped according to laminar and area location in sequential coronal sections and were plotted onto maps depicting the entire medial and lateral surfaces of the cerebral hemisphere. The statistical differences between the mean number retrogradely labeled neurons in the areal locations of each experimental and control groups were evaluated with an analysis of variance procedure (ANOVA) followed by a Fisher PLSD test for significance. Additionally, the regions analyzed were categorized into groups based on the retrograde labeling patterns of the normal, nontransplanted brains: those regions that projected exclusively to the nontransplanted rostral perirhinal cortex, those regions that projected exclusively to the nontransplanted sensorimotor cortex, those regions that projected to both perirhinal and sensorimotor cortices in the nontransplanted animals. An additional category in the experimental animals included those cortical regions that contained retrogradely labeled neurons in the grafted brains only. The number of labeled neurons in each of these categories was compared using an ANOVA, as was the variance overall.

Figure 1 presents the medial and lateral surface reconstructions of cytoarchitectural parcellations of the different cortices employed throughout this study. Neocortical areas are delineated according to Ziles (1985, 1990) and Ziles and Wree (1985), Paxinos and Watson (1986), and Paxinos et al. (1991). In the following descriptions, Sml will be used to designate the first somatic sensory area, an area that includes part of the sensory-motor overlap zone (Hall and Lindholm, 1974; Donoghue et al., 1979, 1982; Sanderson et al., 1984). The term anterior cingulate cortex (ACg) refers to the anterior half of medial cortex composed of areas infraradiata (ACg2) and precentralis agranularis (ACg1) designated area 24 (Rose, 1929; Krieg, 1946; Guildin and Markowitsch, 1983), while posterior cingulate cortex (PCg; areas 23 and 29) refers to the posterior half of the medial cortex composed of areas retrosplenialis (PCg2) and agranularis (PCg1) (Rose, 1929; Krieg, 1946; Vogt and Miller, 1981; Guildin and Markowitsch, 1983; Ziles et al., 1986). Limbic areas were mapped according to criteria of Cechedo and Saper for the divisions of the insular association cortex as designated in the atlas by Paxinos and Watson (1986) and Guildin and Markowitsch (1983). The insular cortex (area 13), also known as the claustrum (Ziles et al., 1980), can be divided into agranular (AI) and granular (GI) portions (Guildin and Markowitsch, 1983). Posterior to the insular cortex is the perirhinal cortex (area 35; Krettek and Price, 1977; Ziles et al., 1980; Bayer, 1990), located just below the auditory cortex (Te1, Te3). The transplants in this study that were placed into perirhinal cortex (Prh or P) were placed into the rostral part of area 35. Cell plots were done in the all cortical areas between 0.20 mm rostral to bregma and 7.80 mm caudal from bregma. Cortical areas analyzed but not represented in Tables 1 and 2 are frontal motor area 1 (Fr1; area 1 of Krieg's 1946 map) cortex rostral to 0.2 mm rostral to bregma, frontal area 2 (Fr2), prepiriform cortex (pyr), dorsal endopiriform, ipsilateral forelimb (FL; DiI too dense), and entorhinal cortices. These latter areas with the exception of the ipsilateral FL were not included in the tables because the number of retrogradely labeled neurons present in these regions were very low to absent.

Results

Transplants were well incorporated within the host cortex. Fast blue labeling of the grafted cells allowed the determination of whether the DiI was placed within the graft site. Insertion of DiI

Table 1. Mean number of retrogradely labeled neurons present in the ipsilateral cortical areas analyzed following DiI labeling in the transplant or indicated area ($n = 3-7$ per group)

Group	Regions that project into Normal P ^a only				Regions that project into both P and Sm				Mean	Regions that project into Normal Sm only				Graft-dependent inputs			Total of category (means)
	ACg	AI	Prh	Mean ^b	Fr1 ^c	HL	SmI	SII		Te1	Te3	Oc	Mean	PCg	GI	Mean	
Normal P ^a	17	10	30 ^d	23.2*	3	23	105	98	57.2	0	0	0	0*	0	0	0	80.4
P → P	5	25 ^φ *	31 ^d	20.1*	11	43	116	84.3	63.5	5.5	2.75	0*	10.6	3	23.5 ^φ *	13.8 ^φ *	108
E12 Sm → P	2	14.5	62 ^d	34.3*	0	1*	30.5	77	26.6**	34	5.5	0*	12.6	0	10	4.8*	78.3
Normal Sm	0.5	0	0	0.17 ^φ	56	68	162 ^c	63	87	8	0.8	30 ^φ	13.1 ^φ	0	0	0	100.3
Sm → Sm	0	0	13.3	4.4 ^φ	15	13.6	181 ^c	22	51	3	0	23 ^φ	6	0	0	0	61.4
E14 P → Sm	13*	2.4	54.8*	23.5*	71.6 ^φ	33	202 ^c	156	112.3 ^φ	57.7	29* ^φ	20.1	35.6* ^φ	25* ^φ	7	16.1* ^φ	187.5
E13 P → Sm	0	0	1.3	0.4 ^φ	7.7	10.7	101 ^c	31	37.8	1	3	0*	1.1	0	0	0	39.3
E12 P → Sm	2	0	0	0.6 ^φ	49.5	1.3*	107 ^c	31	47.1	25	18.3	36.7 ^φ	26.9 ^φ	0	0	0	74.6

^a DiI was inserted into rostral perirhinal cortex only in age-matched nontransplanted animals.^b Mean number of retrogradely labeled neurons in category.^c Refers to caudal frontal cortex, area 1 only, an area directly lateral to ACg, medial to FL.^d Retrogradely labeled neurons are located caudal to transplant of DiI insertion site in rostral Prh cortex.^e Retrogradely labeled neurons are located in SmI area both rostral and caudal to transplant of DiI insertion site.* Group differs from Normal S, $0.05 > p \leq 0.001$.** Group differs from Normal P, $p = 0.05$.φ Group differs from Normal P, $0.05 > p \leq 0.001$.

into fetal cortical transplants placed into neonatal cortical areas resulted in retrograde labeling of neurons in both the ipsilateral and contralateral cortices. In our earlier analysis of thalamo-cortical projections, we found that tissue incubated for 6–7 weeks results in the same labeling patterns as seen with longer incubation periods. However, to insure complete labeling, we waited 8–9 weeks to analyze the distribution of DiI-filled cells. Grafts placed into the Sm host category varied in placement between SmI only or an area that overlapped both SmI and FL cortical regions. For quantitation, these grafts were treated as one type of transplant category. The corticocortical connections of the grafts corresponded in organization to the functional type of cortex previously determined by cell surface molecular characteristics (Barbe and Levitt, 1991).

Normal projections of perirhinal cortex and homotopic grafts

DiI insertion into the rostral Prh cortex of P14 nontransplanted control rats ($n = 4$) resulted in retrogradely labeled neurons in the Prh and ACg cortices, both limbic in function, and in two contralateral limbic regions. A diagram of a representative brain showing a typical DiI insertion site and the locations of retrogradely labeled neurons in this brain is shown in Figure 2. The mean number of labeled cells in the ipsilateral cortex is given in Table 1, while Table 3 shows that 28.9% of the projections into the rostral Prh cortex were from the ACg, AI cortices and the Prh cortex caudal to the insertion site. Also, 71.1% of the labeled neurons in this hemisphere were located in neocortical regions, the SmI, SII, HL, and the caudalmost part of Fr1. All of the cases had retrogradely labeled neurons in the ipsilateral

Table 2. Mean number of retrogradely labeled neurons present in the contralateral cortical areas analyzed following DiI labeling in the transplant or indicated area ($n = 3-7$ per group)

Group	Regions that project into Normal P ^a only			Regions that project into Normal Sm only			Graft-dependent inputs										Total of category (means)
	ACg	Prh	Mean ^b	SmI	FL	Mean	SII	Fr1 ^c	HL	Te1	Te3	Oc	PCg	AI	GI	Mean	
Normal P ^a	6.3	8.3	7.3*	0	0	0*	0	0	0	0	0	0	0	0	0	0	7.3
P → P	2.7	6.3	5.4*	0	0	0*	0	0	0	2.7	0	0	0	0	0	0.2	5.6
E12 Sm → P	4.3	7.7*	6*	0	0	0*	0	0	0	0	0	0	0	0	0	0	6
Normal Sm	0	0 ^φ	0 ^φ	14	1	7.5 ^φ	0	0	0	0	0	0	0	0	0	0	7.5
Sm → Sm	0	0 ^φ	0 ^φ	25.7	1	13.3 ^φ	1.3	0	3	0	0	0	0	0	0	0.5	13.8
E14 P → Sm	5.9	5.6*	5.7*	34 ^φ	3.7	18.3	23.1 ^φ *	35 ^φ *	33	0	0	2.1	3.9 ^φ	0	0	10.8 ^φ *	34.8
E13 P → Sm	0	0 ^φ	0 ^φ	6.7	0	3.3	0	1.7	0	0	0	0	0	0	0	0.2	3.5
E12 P → Sm	0	0 ^φ	0 ^φ	5.3	0.7	3	0	0.7	1	0	0	0	0	0	0	0.2	3.2

Note that no regions were found that projected into both P and Sm.

^a DiI was inserted into rostral perirhinal cortex only in age-matched nontransplanted animals.^b Mean number of retrogradely labeled neurons in category.^c Refers to caudal frontal cortex, area 1 only, an area directly lateral to ACg, medial to FL.* Group differs from Normal S, $0.05 > p \leq 0.001$.φ Group differs from Normal P, $0.05 > p \leq 0.001$.

Figure 2. Diagrams showing surface reconstruction of a representative brain that received a DiI injection into rostral perirhinal cortex at P14 (*large blackened area* indicates spread of DiI label from injection site). *Small dots* represent the distribution of retrogradely labeled cells in several neocortical and limbic areas of the lateral part of the ipsilateral cortex (*A*), the posterior cingulate cortex of the medial part of the ipsilateral cortex (*B*), the perirhinal cortex in the lateral part of the contralateral cortex (*C*), and the anterior cingulate cortex of the medial part of the contralateral cortex (*D*). One dot depicts up to six labeled neurons.

SmI and SII, one case lacked labeling in the HL, and another case lacked labeling in Fr1. The retrogradely labeled neurons in the ipsilateral SmI and SII areas were arranged in disjunctive columns, with the largest numbers in layers II and V; few labeled neurons were seen in layer IV. The DiI-labeled neurons in the ipsilateral ACg were arranged in lamina II, V–VI, and in all of the laminae of the AI and in the Prh cortex caudal to the insertion site. Tables 2 and 4 show that the only contralateral projections into the rostral Prh cortex were from the contralateral Prh and ACg cortices (100%), illustrating the lack of exuberance at this age. The heaviest concentration of labeled cells in these regions were located in laminae V–VI.

Grafts of E14 and E12 presumptive Prh cortex, placed homotopically into the neonatal rostral Prh host cortex ($n = 4$) had retrogradely labeled cells in the same areas as the Normal Prh controls as well as some additional regions. In order to facilitate defining a basic pattern of projections (e.g., limbic, nonlimbic) of each graft type, we grouped the areas containing labeled neurons into four categories (see Materials and Methods). The regions placed into these categories differ ipsilaterally from contralaterally, noted in Tables 1 and 2. The percentages of the mean number of labeled neurons in each category is compared to the sum of the category means (Tables 3 and 4). The homotopic Prh grafts received 18.6% of its ipsilateral inputs from the P-specific input category. The mean number of neurons in AI was significantly greater than that seen in the Normal Prh group ($0.05 > p \leq 0.001$), but the mean of the whole P-specific category did not differ significantly from the normal P control group

($p > 0.05$). The majority of the retrogradely labeled neurons, 58.8%, were in the P and Sm category (Fr1, HL, SmI, and SII; Table 3). Only two of the brains in this latter group had inputs from the ipsilateral HL and rostral Fr1 areas. In addition to inputs from the above categories, 9.8% of the retrogradely labeled neurons from the homotopic Prh grafts were mapped to the auditory cortices of two of the brains (the Sm-specific category), suggesting that the Te1 and Te3 cortices were at least partially included in the dissections of the perirhinal slabs used for grafting. There also were DiI-labeled neurons in the ipsilateral PCg in two of these brains and GI in three of the brains, these two cortical regions being part of the graft-dependent category (12.8%). Guildin and Markowitsch (1983) showed that PCg does project into certain parts of the perirhinal cortex, suggesting that some of our fetal Prh slabs contained these areas. Despite these minor differences, the overall variance of the mean number of neurons in all of the areal locations ipsilaterally was not significantly different between the homotopic Prh group and the Normal P control group ($p \geq 0.24$).

The results are also clear contralaterally in the homotopic Prh grafted group compared to the Normal Prh animals (Tables 2, 4). Most of the retrogradely labeled cells (96.4%) in the contralateral cortex were located in the ACg and Prh cortices (the P-specific category). The only retrogradely labeled neurons in the contralateral graft-dependent category were located within one of the brains in the Te1 region. This brain had eight labeled cells in the contralateral Te1 cortex, 2 in the contralateral ACg, and 7 in the contralateral Prh cortex, strongly implying that this

Table 3. Percentage of mean number of retrogradely labeled neurons present in each category of ipsilateral cortical areas compared to total of category means

Group	Regions that project into Normal P only (ACg, AI, Prh)	Regions that project into both P and Sm (Fr1, ^a HL, SmI, SII)	Regions that project into Normal Sm only (Te1, Te3, Oc)	Graft-dependent inputs (PCg, GI)
Normal P	28.9	71.1	0	0
P → P	18.6	58.8	9.8	12.8
E12 Sm → P	43.8	33.9	16.1	6.1
Normal S	0.17	86.7	13.0	0
Sm → Sm	7.2	83.0	9.8	0
E14 P → Sm	12	59.9	19.0	8.6
E13 P → Sm	1	96.2	2.8	0
E12 P → Sm	0.8	63.1	36.1	0

^a Refers to caudal frontal cortex, area I only, an area directly lateral to ACg, medial to FL.

Table 4. Percentage of mean number of retrogradely labeled neurons present in each category of contralateral cortical areas compared to total of category means

	Regions that project into Normal P only (ACg, Prh)	Regions that project into Normal Sm only (FL, SmI)	Graft-dependent inputs (SII, FrI, ^a HL, Te1, Te3, OC, PCg, AI, GI)
Normal P	100	0	0
P into P	96.3	0	3.9
E12 Sm → P	100	0	0
Normal S	0	100	0
Sm → Sm	0	96.4	3.5
E14 P → Sm	16.4	52.6	28.6
E13 P → Sm	0	94.3	5.7
E12 P → S	0	93.8	6.2

Note that no regions were found that project into both P and Sm.

^a Refers to caudal frontal cortex, area 1 only, an area directly lateral to ACg, medial to FL.

one graft included the adjacent Te1 region as well as the rostral Prh cortex.

These labeling patterns are comparable to other studies showing that the rodent rostral perirhinal cortex receives ipsilateral connections from anterior and posterior cingulate (the latter depending on whether rostral or caudal perirhinal areas were labeled), caudal perirhinal, agranular insular and many neocortical cortices such as SII, and commissural connections from the contralateral rostral perirhinal and cingulate cortices (Deacon et al., 1983; Guildin and Markowitsch, 1983).

Normal projections of sensorimotor cortex and homotopic grafts

DiI was placed directly into the SmI area of three normal brains from aged P14 rats, and into both the FL and SmI of two normal brains. The labeling patterns were assessed as 1 group. The grafts were placed similarly, and thus were also assessed as 1 group (Tables 1–4). A drawing with the distribution of labeled cells in a representative brain is shown in Figure 3. The majority (86.7%) of the labeled neurons in the ipsilateral cortex were located in the category of regions that project into both the Normal Prh and Sm cortices; this category contains caudal Fr1, HL, SmI, and SII (Tables 1, 3). The ipsilaterally labeled neurons in this category were located in laminae II–III and V–VI in a columnar pattern. The ipsilateral FL was not included in the quantification because the extreme brightness of DiI in this region prevented the counting of cells in several of the brains. Labeled

neurons in the SmI cortex that extended caudal from the insertion site were able to be counted. The cortical regions that were labeled exclusively in the Normal Sm brains (Te1, Te3, and Oc) contained 13% of the labeled cells. There was also a very small number of labeled neurons (0.17%) in the ipsilateral ACg area of the P-specific category. The number of labeled neurons in this latter category was significantly less than the Normal Prh control brains ($p \leq 0.001$). Contralaterally, 100% of the labeled neurons were located in the SmI and FL cortices, with the majority in SmI. The location of even a few retrogradely labeled neurons in the contralateral FL area (Table 2) suggests that either the location of the DiI insertion site and/or the spread of DiI from the insertion site included an area in some of the brains that overlapped at least partially with the adjacent FL cortex.

Homotopic sensorimotor grafts into SmI ($n = 3$) gave labeling results that were similar to control Sm brains (Tables 1–4; $p > 0.5$). The vast majority of the ipsilateral cells, 83%, were located in the P and Sm category. The ipsilateral Sm-specific category contained 9.8% of the DiI-labeled neurons, with the majority of the labeled cells mapping to the Oc cortex. There were also inputs in one of the brains from the ipsilateral Prh cortex. The number of labeled neurons in the P-specific category was significantly lower in this homotopic Sm graft group than the Normal Prh control animals ($p \leq 0.04$) or the homotopic Prh graft group ($p \leq 0.03$). Contralaterally (Tables 2, 4), 96.4% of the inputs into the homotopic Sm grafts were from the Sm-specific category, primarily in the homotopic SmI region. There were

Figure 3. Diagrams showing surface reconstruction of a representative brain that received a DiI injection (large black area) into the primary sensorimotor cortex. Ipsilaterally, retrogradely labeled cells are present in patchy patterns in most of the neocortical areas mapped (A, B), but are present in the contralateral cortex only in the cortical area homotopic to the injection site (C). Note the lack of retrogradely labeled cells in this brain's cingulate cortices, both ipsilaterally and contralaterally, B and D, respectively. Symbols are as in Figure 2.

Figure 4. Diagrams showing surface reconstruction of a representative P14 brain that received a DiI injection (large black area) into a transplant (cross-hatched area and including blackened area) of a piece of E14 perirhinal cortex placed into the primary sensorimotor area of a neonate. DiI-labeled cells are present ipsilaterally in several neocortical areas such as SmI, SII, and auditory, as well as the associated insular cortices such as AI, GI and perirhinal (A), and in the cingulate and caudal frontal area 1 cortices on the medial surface of the ipsilateral hemisphere (B). In the contralateral side (C, D), retrogradely labeled cells are present in both several sensory-motor associated cortices as well as limbic areas such as the perirhinal and cingulate cortices. This distribution of labeled cells indicates a hybrid limbic and sensorimotor input pattern for this transplant group.

Figure 5. Photomicrographs from a brain that received an E14 perirhinal graft into the sensorimotor cortex of a neonate that was labeled with DiI 2 weeks after transplantation. *A*, Micrograph of the contralateral SmI showing retrogradely labeled cells in laminae III, V, and VI and labeled axons in laminae I, III, V, and VI. Note the columnar arrangement of labeled axons and cells, with few located in the cortical area in the right part of the field. *B*, Micrograph of the contralateral perirhinal cortex showing retrogradely labeled cells in laminae II and III (arrow).

also four labeled cells present in the contralateral SII cortex of one of the three brains analyzed, and nine labeled neurons in the FL region of another brain. There were no significant differences between the overall number and areal location of contralaterally labeled neurons in the homotopic Sm grafts compared to the Normal Sm control group ($p \geq 0.58$). As Table 2 shows, however, there were significant differences in the types of contralateral inputs into these grafts when compared to the Normal Prh control animals in the P-specific and Sm-specific categories, the former category being significantly less and the latter category being significantly more than labeled neurons in these categories in the Normal Prh labeled brains ($p \leq 0.001$ each).

The labeling patterns in both Normal Sm and the homotopic Sm grafted animals are comparable to previous degeneration and retrogradely transported horseradish peroxidase studies, reporting that the sensorimotor area of the adult rat receives commissural connections from the contralateral sensorimotor cortices, and ipsilateral connections from a wide variety of neocortical areas (Wise and Jones, 1976, 1978; Akers and Killackey, 1978; Vogt and Miller, 1981; Donoghue and Wise, 1982).

Heterotopic E14 perirhinal grafts placed into neonatal sensorimotor cortices

The E14 perirhinal transplants that were included in the final analysis were placed into three closely related locations: (1) three grafts located exclusively in the SmI region, (2) one graft located exclusively in the FL region, and (3) three grafts that overlapped into both SmI and FL cortices. The results of all seven are tallied as one group in Tables 1 and 3 and will be discussed following a summary of the results of inputs into the separate graft placement types. In type A, DiI labeling resulted in retrogradely labeled cells in several sensorimotor and limbic areas both ipsilaterally and contralaterally. Figure 4A–D is a representative diagram of type A's placement site and labeling pattern. The ipsilateral inputs into these grafts were as follows: 60% were located in the P and S input category, 7.3% in the P-specific category, 18% in the Sm-specific category, and 15% in the graft-dependent category. The contralateral inputs into the type A grafts were 36.6% in the P-specific category, 33% in the Sm-specific category (only one cell was located in the FL region of one of these three brains, the remainder being located in SmI) and 28.5% in the graft-dependent category. Retrogradely labeled

E and *F*, Photomicrographs from a E14 Prh into SmI representative brain. *E*, Micrograph of the contralateral posterior cingulate area showing retrogradely labeled cells in lamina I and III (long arrows). Short, wide arrows indicate boundary between posterior cingulate cortex and caudal motor cortex (Fr1). *F*, Ipsilateral posterior cingulate gyrus of same brain showing retrogradely labeled cells in lamina III and V (long arrows) and labeled axons branching in laminae I, III, V, and VI. Short, wide arrows indicate boundary between posterior cingulate and caudal Fr1 cortex. Symbols are as in Figure 2.

Figure 6. Representative diagrams (A–D) of a brain that received an E13 perirhinal transplant into the sensorimotor cortex of a neonate (transplant site indicated by cross-hatching). DiI was inserted into the transplant on P13 (black area in transplant site). Ipsilateral lateral (A) and medial (B) surfaces of the hemispheres show retrogradely labeled neurons in SmI and II and auditory cortices. (C) The contralateral hemisphere has retrogradely labeled cells in the homotopic SmI area only. (D) No labeled cells are evident on the contralateral medial surface. Micrographs (E and F) from another representative brain show retrogradely labeled cells in the contralateral SmI cortex (E). The white matter tracts are located in the lower left-hand edge of the micrograph. Note that in the contralateral SmI cortex that the retrogradely labeled cells and fibers are arranged into patchy columns. F, No retrogradely labeled cells are present in the contralateral posterior cingulate cortex (PCg). *w*m, white matter. Symbols are as in Figure 2.

cells were located in laminae I–III in the contralateral PCg cortex (Fig. 4E) and in laminae I, III and V of the ipsilateral PCg cortex (Fig. 4F). There may have been labeled neurons in lower layers of the contralateral posterior cingulate cortex as well, but the DiI labeling of crossing commissural fibers was too bright to distinguish labeled neurons in this area. DiI-labeled cells in the contralateral SmI of these grafts were located in lamina III, V–VI (Fig. 5A). The labeled cells in the contralateral Prh cortex were scattered throughout the laminae; Figure 5B shows labeled cells in the upper laminae of this cortex.

In type B of E14 P into Sm graft placement, the retrogradely labeled cells on the ipsilateral side are present primarily in SmI, SII, HL, FL, Te1, with fewer neurons located in cingulate and insular cortices. Contralateral inputs were from both cingulate regions, the Prh, SmI, FL, HL, and FrI cortices. In type C of graft placement, the ipsilateral inputs were as follows: 53.6% were located in the P and Sm input category, 13% in the P-specific category (only one of the brains had labeling in ACg, 5 cells out of 60 total in that hemisphere), 16.5% in the Sm-specific category and 17% in the graft-dependent category. The contralateral inputs into these grafts were 8.6% in the P-specific

category, 66.8% in the Sm-specific category and 24% in the graft-dependent category, the labeling within this latter category being located in the regions SII, HL, FrI, and PCg.

When one considers all seven E14 Prh into Sm brains together (Tables 1, 3), approximately 60% of the ipsilateral DiI-labeled neurons are located in the P and Sm category, 12% in the P-specific category, 19% in the Sm-specific category, and 8.6% in the remaining graft-dependent input category. Table 1 shows that the number of labeled neurons in the P-specific input category was significantly higher than the same category in the Normal Sm control group, but are very similar to the mean number of labeled neurons in these areas of the Normal Prh animals. The mean number of labeled neurons is high in the three other input categories, significantly higher than the Normal Prh control group in the P and Sm input category, and significantly higher than both control groups in both the Sm-specific and the graft-dependent categories. Overall, the mean number of the ipsilaterally labeled neurons in the E14 P into Sm grafted animals was higher than the Normal Prh control group ($p \leq 0.01$) and the Normal Sm control group ($p \leq 0.02$). The higher number of inputs into these E14 grafts suggests that an exuberant num-

Figure 7. Representative diagrams (A–D) of a brain that received a transplant of E12 presumptive perirhinal cortical wall into the sensorimotor cortex at P0. DiI was inserted on P13. Ipsilateral lateral (A) and medial (B) cortical hemispheres show retrogradely labeled cells in SmI area around the transplant site only. Contralateral lateral (C) and medial (D) cortices have labeling only in the homotopic SmI area. Representative micrographs (E, F) of another brain from the same group show retrogradely labeled cells in the contralateral SmI cortex (E) in laminae I–III. No retrogradely labeled cells are present in the contralateral posterior cingulate cortex (F). Symbols are as in Figure 2.

ber of inputs are being maintained, but which are lost in the E13 and E12 P into Sm grafts (see Tables 1, 3). The presence of labeled cells in the PCg in four of the brains suggests that the dissections of these brains may have included caudal Prh cortex in addition to the rostral Prh cortex, since the caudal perirhinal cortex has been shown to receive inputs from that region (Guil-din and Markowitsch, 1983). However, these inputs were not in the E13/E12 P into Sm grafts from the PCg cortex (presumably dissected in a similar manner), suggesting perhaps a maintenance of exuberant inputs ipsilaterally into the E14 P into Sm grafts.

Contralaterally, the results are clearer. The E14 P into Sm grafts receive a hybrid mixture of commissural inputs. Tables 2 and 4 show that the E14 P into Sm grafts received inputs from both the P-specific and the Sm-specific input categories, 16.4% and 52.6%, respectively. In the Normal Prh only input category, five of the seven grafts received projections from the contralateral ACg cortex while six of the brains had input from the contralateral Prh cortex. The mean number of labeled neurons in this category was significantly higher than the Normal Sm control animals. The number of labeled neurons in the graft-dependent category of inputs also was significantly higher than both control groups, possibly due to the dissection technique, placement of the graft, or exuberance again. The sensorimotor and

limbic type inputs contralaterally in this graft group could also be due to a maintenance of some of the developmentally normal exuberant cortical collaterals that have already died back in the control animals (Wise and Jones, 1976, 1978; Ivy et al., 1979; Valentino and Jones, 1982), resulting in a hybrid cortex in terms of connectivity.

Heterotopic E13 perirhinal grafts into neonatal sensorimotor cortex

While the E14 grafts exhibited a mixture of input patterns, the inputs to the E13 grafts were more straightforward, appearing more like normal Sm. There were retrogradely labeled neurons primarily in the SmI cortex both ipsilaterally and contralaterally. A drawing with the distribution of labeled cells in a representative brain is shown in Figure 6A–D, and the results are summarized in Tables 1–4. DiI labeling of the graft site resulted in many ipsilaterally located, retrogradely labeled neurons (96.2%) in the P and S category of inputs. There were also four cells labeled in one of the brains in the ipsilateral Prh cortex (the P-specific input category). The number of labeled neurons in this latter category was significantly lower than the Normal Prh control animals ($p \leq 0.001$), as well as the E14 P into Sm and E12 Sm into P groups. The auditory areas in the Sm-specific category also projected into these grafts; however, the mean

Figure 8. Representative diagrams (A–D) of a brain that received a transplant of E12 presumptive sensorimotor cortical wall into the perirhinal cortex at age P0. DiI was inserted on P13. Retrogradely labeled cells are present in the ipsilateral lateral hemisphere (A) and are located in both neocortical areas (SI, SII, Tel, and Te3) and mesocortical and archicortex cortical areas (piriform cortex and perirhinal cortex caudal to the rostrally placed graft). B, Medial view shows retrogradely labeled cells in the anterior cingulate cortex. C, The contralateral hemisphere shows retrogradely labeled cells in the homotopic perirhinal cortex only. D, No labeled cells are present in the medial part of the contralateral hemisphere. E, Micrograph

number of labeled neurons in this category was not significantly different than the Normal Sm controls. The neurons in the ipsilateral neocortical areas were present in lamina II–III, and V, a normal distribution pattern for ipsilaterally projecting corticocortical neurons. The retrogradely labeled cells present in the ipsilateral Prh cortex were located throughout all the laminae of that area, again a normal distribution pattern compared to the controls. Retrogradely labeled cells were not present in the ipsilateral ACg, PCg or Oc cortices.

Again, in contrast to the E14 grafts heterotopic grafts that receive inputs from both contralateral sensorimotor and limbic regions, the E13 P into Sm grafts received 94.3% of their contralateral inputs from the SmI cortex (Tables 2, 4), one of the regions in the Sm-specific category. As in the control cases, the contralaterally located, retrogradely labeled neurons in the SmI cortex were arranged in columns and patches and were situated mostly in laminae II–III and V–VI of the host cortex (Fig. 6E). One of the brains also had five labeled neurons in the contralateral graft-dependent region Fr1 (6.2% of all the inputs). There were no contralateral inputs from the P-specific category (Tables 2, 4; Fig. 6F), as there were in the Normal Prh controls and the E14 P into Sm graft group.

Heterotopic E12 perirhinal grafts into neonatal sensorimotor cortex

This graft category, like at E13, resulted in projection patterns most like Normal Sm. The E12 P into Sm grafts were placed primarily in the SmI cortex ($n = 3$ analyzed). A representative diagram (Fig. 7) shows the graft site and the location of retrogradely labeled neurons. The results are summarized in Tables 1–4. Most of the ipsilateral afferents (63.1%) arose from the P and Sm input category. The majority of these neurons were located in the SmI area caudal to the transplant, although labeled neurons were also located in SII, HL, and Fr1 in most of the brains. An additional 36% of the inputs into this graft type were from the Sm-specific category. In the ipsilateral P-specific category, there were two or three labeled neurons/brain that mapped to the ACg cortex. The number of labeled neurons in this latter category of ipsilateral inputs into the grafts were significantly lower than those to Normal Prh control animals ($p < 0.004$), as well as the E14 P into Sm and E12 Sm into P graft groups. As expected for grafts with Sm-like features, there was a significantly higher number of ipsilateral inputs from the Sm-specific category of inputs when compared to the Normal Prh control group, but no significant differences were found overall between this graft group ipsilaterally when compared to the Normal Sm control animals ($p \geq 0.73$).

The E12 heterotopically located perirhinal grafts received contralateral afferents mostly from homotopic SmI cortical region (Fig. 7C,E) and FL (Fig. 7C), with a few retrogradely labeled neurons mapping to the HL and motor areas Fr1 (Table 2) in some of the brains. No labeled cells were present contralaterally in the limbic PCg (Table 2, Fig. 7F) or Prh cortices. The laminar arrangement of all the contralaterally labeled cells in SmI was normal, located in layers II–III, and V–VI (Fig. 7E). As seen ipsilaterally, there were no significant differences between this graft group from the Normal Sm control animals in

any of the categories or overall ($p > 0.94$). The number of labeled neurons in the Prh-specific category of inputs was significantly lower than the Normal Prh control animals ($p \leq 0.001$), as well as the E14 P into Sm, E12 Sm into P, and homotopic P graft groups.

Heterotopic E12 sensorimotor grafts into neonatal perirhinal cortex

The E12 sensorimotor into rostral perirhinal grafts ($n = 3$ analyzed) received many inputs similar to the control rostral Prh cortex (Tables 1–4). A representative brain is shown in Figure 8A–D. These grafts received many ipsilateral inputs from both the P-specific (43.8%) and the P and Sm (33.9%) categories. The number of ipsilateral inputs in these two categories to this graft type were significantly greater than the Normal Sm control animals, $p \leq 0.02$ for the P-specific category, and $p \leq 0.05$ for the P and Sm category. In addition to the inputs from the above categories, 16.1% of the DiI-labeled neurons are located in the auditory cortices of the Sm-specific category and 6.1% in the GI cortex of the graft-dependent category. The presence of inputs in the auditory cortex could be due to the dissection technique of the E12 presumptive Sm cortex. The input from GI cortex is not inappropriate, as it is part of the same insular association cortex that includes the perirhinal cortex. There were no significant differences in either the categories or overall in the number of neurons or areal locations in this graft type when compared to the Normal Prh control animals (Table 1).

Retrogradely labeled neurons are located contralaterally in only the limbic Prh and ACg cortices of the P-specific input category following placement of E12 sensorimotor grafts into host perirhinal cortex (Tables 2, 4). Figure 8E shows a DiI-labeled cell located in the contralateral perirhinal cortex as well as DiI-labeled axons. Contralaterally, this graft type has very different inputs from the Normal Sm controls in both the P-specific and the Sm-specific categories (Tables 2, 4). Also, the number of neurons in the P-specific category were significantly higher in the E12 Sm into P graft group than the E13 P into Sm, the E12 P into Sm, and the homotopic Sm graft groups.

Summary of results

The ipsilateral and contralateral inputs of the heterotopic transplants of E12 and E13 grafts are very characteristic of their new host locale, while those inputs of the E14 grafts exhibited hybrid features, both of their original and new environment. Contralaterally, the input patterns are very similar in the P-specific category when comparing the Normal Prh, Homotopic Prh, and E12 Sm into P groups. The E14 P into Sm graft group also has labeled neurons in this category. The similarity in the Sm-specific input category is striking between the Normal Sm, homotopic Sm, and the three heterotopic perirhinal graft groups. The only graft group that has commissural inputs from both of these categories of regions is the E14 P into Sm, suggesting that it has a hybrid mixture of sensorimotor and limbic functional inputs. This graft group also has significantly more ipsilateral connections with the Prh, ACg, and AI regions than the Normal Sm, homotopic Sm, and E13/E12 heterotopic Prh graft groups,

←

from another brain illustrates one retrogradely labeled neuron (arrow) and several labeled fibers in the contralateral perirhinal cortex. Symbols are as in Figure 2.

in addition to a large number of inputs from the sensorimotor-type cortices ipsilaterally.

Discussion

In our studies of cerebral cortical grafts, we have presented results suggesting that there is an early, restricted period of fetal development in which cortical cells can exhibit marked plasticity in the expression of specific phenotypic traits (Barbe and Levitt 1991, 1992b; Levitt et al., 1993). Certain features of these cells may be altered by extrinsic factors in the new host milieu through E13, after which (E14) the donor transplanted tissue begins to express certain features characteristic of its cortical origin in addition to its new host environment. This conclusion is based on the analysis of three phenotypes: (1) the expression of the limbic cortical marker protein LAMP (Levitt, 1984; Horton and Levitt, 1988; Barbe and Levitt, 1991), (2) the organization of thalamocortical projections (Barbe and Levitt, 1992b), and (3) in the present study, corticocortical projections.

Developmental timing and commitment to corticocortical phenotypes

In the present study, we show that the local cues of the host newborn rat cortex are capable of mediating the differentiation of precursor cells in the E12 cortical grafts. The heterotopic transplants of E12 tissue exhibit many afferent corticocortical connections that reflect those typical of the new host locale (although there still appears to be some nonspecific inputs ipsilaterally at this time point of study of the grafts). Because the cerebral wall at E12 contains almost exclusively progenitor cells (Hicks and D'Amato, 1963; Bayer and Altman, 1991), the data suggest that the precursors are exposed to cues in the new area that are able to affect a sequence of differentiative events, including the final commissural projection patterns of the daughter cells (and at least partially the ipsilateral projection patterns). Heterotopic transplantation of E14 tissue produces grafts exhibiting mixed, or hybrid corticocortical connections. The hybrid phenotype is abnormal, reflecting features of both the donor origin and the new host locale. This complex result was also obtained in our analysis of the molecular phenotype and thalamocortical projection patterns of similar transplants (Barbe and Levitt, 1991, 1992b), and in the analysis of efferents of occipital grafts placed into host Sm regions (Ebrahimi-Gaillard et al., 1994). We believe the formation of a mixed phenotype in our studies, normally not seen, is probably due to the extended period of time, E13–E17, over which neurons are generated in the rostral and lateral cortices (Hicks and D'Amato, 1968; Altman and Bayer, 1990). Thus, at E14, the lateral cerebral wall contains both precursor cells and postmitotic neurons in the process of migration to the cortical plate. In contrast, E17 cortical neurons exhibit a more homogenous molecular phenotype after transplantation (Barbe and Levitt, 1991). Our previous and present findings support our hypothesis that the specification of the cerebral cortex is due to an interaction of both genetic and external cues that act upon cells in a time-dependent fashion (Levitt et al., 1993), restricting their commitment to express specific phenotypes over an extended period of development. While the transplant data shows that E12 precursors clearly have not irreversibly committed to a specific phenotype, and thus are amenable to regulation by extrinsic cues, cell culture results (Ferri and Levitt, 1993) suggest that there is some fating of the progenitors to express a limbic phenotype. These experiments reveal that precursors from presumptive perirhinal and sensori-

motor cortices, when grown in culture under identical defined conditions, will differentiate into LAMP-positive and negative neurons, respectively. Our and other recent analyses of the connectivity and molecular features of cortical grafts (Cohen-Tanoudji et al., 1994; Ebrahimi-Gaillard et al., 1994), the cell culture data (Ferri and Levitt, 1993), behavioral analysis of grafts (Barth and Stanfield, 1994), and other reports of early differences in the cerebral wall (Arimatsu et al., 1992; Dehay et al., 1993; Kennedy and DeHay, 1993), strongly suggest that regional differences in the wall begin to emerge early (at least by E14 in our studies) and lead to the parcellated cerebral cortex hypothesized by Rakic (1988). Our studies suggest that these ventricular zone cells can be influenced by local environmental cues that move the population through specific periods of developmental restriction (Parnavelas et al., 1991; Levitt et al., 1993).

Response of grafted cortical cells depends on age of cells as well as phenotype examined

There are numerous factors involved in the determination of neuronal function and formation of cortical circuitry, each of which helps define the connections between a neuron and its targets. Target recognition and synaptogenesis are achieved through complex mechanisms (Sperry, 1963; Meinertzhagen, 1973, 1974; Trujillo-Cenoz and Melamed, 1973; Tomlinson and Ready, 1987; Grenningloh et al., 1991; Götz et al., 1992; Kunes et al., 1993). In the mammalian cerebral cortex, the areal and laminar positions of a cortical neuron are generally predictive of where that neuron will send an axonal process (Jones, 1981). The time of origin of the neuron defines the laminar position (Angevine and Sidman, 1961; Rakic, 1974; Bayer and Altman, 1991) and the commitment to reside in a specific cortical layer (McConnell, 1985, 1988; McConnell and Kaznowski, 1991). The laminar cortical and subcortical projection patterns are subject to a wide variety of control mechanisms (Price and Blake-More, 1985; Killackey, 1990; Katz, 1991; Yamamoto, 1992; O'Leary and Koester, 1993). For example, the host environment can modulate the projection patterns of layer V neurons in E17–E18 parietal and visual cortex transplanted heterotopically (Stanfield and O'Leary, 1985; O'Leary and Stanfield, 1989). Afferent thalamocortical fibers are able to control the expression of some features of cellular organization, such as the barrels in somatosensory cortex (Woolsey and Wann, 1976; Schlaggar and O'Leary, 1992). While these phenotypes appear highly plastic at later stages of fetal or postnatal rat development, when they are still forming, our transplantation data have focused on phenotypic features that develop earlier. Are these data consistent with each other? Suggestions have been made that the early commitment by some cortical neurons to the limbic phenotype is due to a phylogenetically conserved parcellation of certain regions (O'Leary, 1989; Walsh, 1993). While the limbic system in general is considered highly conserved, association cortical areas, which include some of the limbic regions, change as dramatically in primates, where they are much more highly evolved, as do primary sensory and motor regions (Bayer, 1990; Killackey, 1990; Sur et al., 1990; Kaas, 1993). We have posed an alternate explanation to that of early developmental divergence of the different cortical divisions (Levitt et al., 1993). Occipital cortical grafts, from E17–E18 donors, showed an ability to respond to somatosensory environmental cues by forming barrels (Schlaggar and O'Leary, 1992). From our analysis of corticocortical and thalamocortical projections of limbic and sensorimotor grafts, we would predict that the visual cortex grafts, even

at E17, might contain hybrid populations. At E17–E18, 50–70% of the layer IV neurons have been generated in visual cortex (Miller, 1988), a consequence of the fact that the rodent cortex develops in a rostrocaudal and lateromedial gradient. Functional analyses of occipital transplants to rostral cortex indicate that indeed only a subpopulation of grafted neurons may be responding to the new environment, since somatosensory electrophysiological responses (Castro et al., 1991) and mediation of sensory behavior recovery by the occipital grafts (Barth and Stanfield, 1994) fail to occur. Our analysis of corticocortical connections of heterotopic grafts, with the tissue harvested at three different developmental ages, suggests that transplants containing a higher percentage of precursor cells will have a better chance of exhibiting phenotypic features most similar to the new host locale, and thus a better chance at possibly mediating meaningful functional recovery.

Constraints on graft connectivity

Our previous analysis of thalamocortical connections of tissue transplanted to perirhinal or somatosensory regions of the newborn rat brain indicated that different regions of the cortex are homogeneous early during development and that certain extrinsic factors in a host newborn cortical region are able to influence innervation patterns. For example, transplanting E12 presumptive sensorimotor tissue into neonatal limbic cortex resulted in the invasion of extensive limbic thalamic afferents (Barbe and Levitt, 1992b). It is interesting to note that more than 85% of the neurons in this heterotopic graft type expressed a limbic-associated molecule, LAMP (Barbe and Levitt, 1991). The E14 sensorimotor transplants, when placed into perirhinal cortex, contained a low percentage of LAMP-positive neurons, but axons from the somatosensory ventrobasal complex failed to grow into these grafts. We suggested that this was due to the inability of the ventrobasilar thalamic axons in a neonatal rat, which normally are growing through the dorsal part of the internal capsule, to detect the presence of and grow toward tissue with sensorimotor-type molecular characteristics in the aberrant ventrolateral location. Upon entering the internal capsule, thalamic axons show little tendency to grow in the wrong direction (DeCarlos et al., 1992; Erzurumlu and Jhaveri, 1992). The limbic thalamic fibers from lateral dorsalis that project to frontal and anterior cingulate cortices (Domesick, 1969) and the somatosensory ventrobasal axons course under the region of all the grafts placed into the sensorimotor cortex. This location provides the thalamocortical afferents with direct access to the transplants. In the present study, the host contralateral perirhinal, anterior cingulate and somatosensory fibers have crossed the corpus callosum and some axons have entered the cortex at the time of transplantation (Wise and Jones, 1976; Ivy et al., 1979; Valentino and Jones, 1982; Jensen and Altman, 1992). All these contralateral corticocortical axons pass under the grafts placed into SmI cortex, thus providing them access to the transplants. In contrast, somatosensory callosal projections, like the somatosensory thalamocortical axons, do not course underneath the perirhinal cortex. Because of this, we limited our present analysis of corticocortical projections to grafts placed dorsally in somatosensory cortex, except for the very early E12 Sm into P transplants, in which we are able to manipulate the molecular phenotype of most of the cells. Here, limbic corticocortical projections were formed between the host and E12 Sm graft placed into the perirhinal location. We did perform a few E14 somatosensory transplants into perirhinal cortex, but retrogradely labeled callosal

neurons were never seen, even with labeling times up to 10 weeks (data not shown). Again, we believe this is due to spatial constraints, preventing the somatosensory fiber system from growing toward the ventrolateral wall of the cerebral hemisphere, and the lack of recognition of the target cells.

In the present study, not all the afferents projecting into the grafts precisely match normal perirhinal or primary somatosensory cortical connections. For example, heterotopic grafts of E14 tissue from the rostral perirhinal cortex, placed into host sensorimotor cortex, receive connections from not only the contralateral and ipsilateral anterior cingulate cortex, but also from the posterior cingulate cortex and sometimes the granular insular cortex. Guildin and Markowitsch (1983) showed that the rostral perirhinal cortex is part of the associative insular cortex in the rat, this area receiving projections from posterior cingulate, anterior cingulate cortices and granular and agranular insular cortices. Variations in the inputs into these grafts suggest some inconsistencies in our dissection techniques, so that grafts could include more caudal perirhinal cortex, and others, the rostral perirhinal cortex, which is part of a heterogeneous associative cortex. There does appear to be exuberance of the ipsilateral inputs into the E14 perirhinal grafts, probably due to the young age of the hosts at the time of analysis (P14), making interpretation difficult. However, when one compares the contralateral inputs of E14, E13, and E12 perirhinal tissues when transplanted into sensorimotor cortex, it becomes clear that the connections of the E14 grafts retain organizational features of their original areal location in the embryo as well as develop features characteristic of their new host locale. The E14 heterotopic perirhinal transplants have axonal projection patterns from both contralateral homotopic sensorimotor areas and contralateral limbic areas. The younger grafted tissue (E12 and E13) receives contralateral projections only from sensorimotor cortical areas. The limbic commissural inputs into the E14 P into Sm grafts may be exuberant inputs that have been maintained specifically into these grafts. We have begun analysis of grafts surviving long term (P65), using the same transplantation paradigm. These studies reveal thus far that the hybrid connections seen in the E14 heterotopically located Prh grafts are maintained into adulthood, with the presence of both sensorimotor and limbic thalamic and corticocortical inputs (Barbe, unpublished results). These results are buttressed by studies showing that the mature, restricted pattern of commissural projections in the rat is established between P7 and P15 (Wise and Jones, 1976; Ivy et al., 1979; Ivy and Killackey, 1981; Valentino and Jones, 1982), as well as a recent study by Ebrahimi-Gaillard et al. (1994), in which occipital cortex transplanted into frontal cortex projected to host targets in a hybrid pattern that was characteristic of both donor and host cortical regions.

In conclusion, short term survival of aged E14 grafts, which contain over 50% LAMP expressing neurons (Barbe and Levitt, 1991), receive cortical as well as thalamic (Barbe and Levitt, 1992b) inputs that are hybrid in nature. Perhaps the early expression of LAMP in limbic cortical neurons, at or after neurons become postmitotic (Barbe and Levitt, 1991; Ferri and Levitt, 1993), reflects a specific developmental path taken by neurons along which the expression of other limbic characteristics will follow. Thus, the fating and ultimate commitment of neurons to this molecular phenotype may be important in determining the connectivity and function of cerebral cortical neurons.

Appendix

Abbreviations

ACg1,2	anterior cingulate cortex parts 1, 2
AI	agranular insular cortex
cc	corpus callosum
Ent	entorhinal cortex
FL	forelimb
Fr1	frontal motor cortex area 1
Fr2	frontal motor cortex area 2
GI	granular insular cortex
HL	hindlimb
Oc or Occ	occipital cortex
P or Prh	perirhinal cortex
PCg1	agranular posterior cingulate cortex
PCg2	granular posterior cingulate
Prh	perirhinal cortex
Pyr	piriform cortex
SII	secondary somatosensory cortex
Sm	primary sensorimotor cortex, also known as Par1, SI, and primary somatosensory area
SmI	primary sensorimotor cortex
Te1,2,3	auditory cortex, area temporalis
th	thalamus

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