Embryonic Neurons Transplanted to Regions of Targeted Photolytic Cell Death in Adult Mouse Somatosensory Cortex Re-form Specific Callosal Projections

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In the neocortex, the effectiveness of potential transplantation therapy for diseases involving neuronal loss may depend upon whether donor neurons can reestablish the precise long-distance projections that form the basis of sensory, motor, and cognitive function. During corticogenesis, the formation of these connections is affected by tropic factors, extracellular matrix, structural pathways, and developmental cell death. Previous studies demonstrated that embryonic neurons and multipotent neural precursors transplanted into neocortex of mice undergoing photolytically induced, synchronous, apoptotic neuronal degeneration selectively migrate into these regions, where they differentiate into pyramidal neurons and accept afferent synaptic input. The experiments presented here assess whether embryonic neurons transplanted into regions of somatosensory cortex undergoing targeted neuronal death differentiate further and develop long-distance axons and whether this outgrowth is target specific. Neocortical neurons from Gestational Day 17 mouse embryos were dissociated, prelabeled with fluorescent nanospheres and a lipophilic dye (DiI or PKH), and transplanted into adult mouse primary somatosensory cortex (S1) undergoing apoptotic degeneration of callosal projection neurons. Donor neurons selectively migrated into and differentiated within regions of targeted neuronal death in lamina II/III over a 2-week period, in agreement with our prior studies. To detect possible projections made by donor neurons 2, 4, 6, 8, or 10 weeks following transplantation, the retrogradely transported dye fluorogold (FG) was stereotaxically injected into contralateral S1, ipsilateral secondary somatosensory cortex (S2), or ipsilateral thalamus. Ten weeks following transplantation, 21 ± 5% of the labeled donor neurons were labeled by FG injections into contralateral S1, demonstrating that donor neurons sent projections to this distant area, the original target of host neurons undergoing photolytically induced cell death. No donor neurons were labeled with FG injections into ipsilateral S2 or thalamus, and nearby targets of other subpopulations of neurons in S1. These data indicate that in the adult neocortex: (1) transplanted immature neurons are capable of extending long-distance projections between hemispheres through the mature white matter of the corpus callosum and (2) these projections are formed with specificity to replace projections by neurons undergoing synchronous degeneration. These experiments provide an experimental system with which to test factors affecting such outgrowth and connectivity. Taken together, these results suggest that the reconstruction and repair of cortical circuitry responsible for sensory, motor, or cognitive function may be possible in the mature neocortex, if donor neurons or precursor cells are provided with the correct combination of local and distant signals within an appropriately permissive host environment.

INTRODUCTION

Functional recovery from injury or degeneration in many regions of the mammalian central nervous system (CNS) would require the restoration of precise connections between populations of neurons that often are separated by long distances. The transplantation of embryonic or genetically engineered cells to replace degenerated neurons has been shown to ameliorate the effects of subcortical CNS injury and effect functional recovery in some systems (10, 26, 39, 58). In some models of CNS injury, this recovery can occur via the passive delivery or release of neurotransmitters or trophic substances. In many regions of the nervous system, replacement of neurotransmitters or trophic factors may not be sufficient to repair complex circuitry. Improved understanding of the limitations and control of axonal regeneration and connectivity with appropriate targets could have significant impact toward the repair of CNS injury. In the neocortex, the potential effectiveness of neural transplantation in the recovery of sensation, movement, or cognition may depend substantially on whether transplanted neurons or neural precursors can reestablish the precise regional and cellular projection patterns disrupted by neocortical pathology (4, 16, 17, 60, 62, 67, 68).
We have used an approach of targeted apoptotic neuronal degeneration, in which photolytic induction of cell death in neocortical neurons affects the migration and differentiation of transplanted neural precursors, to study the microenvironmental influences over migration and differentiation of embryonic neurons and multipotent precursors (47, 49, 66). In the present experiments, we use this system to investigate further whether embryonic neurons transplanted to neocortex with an appropriately permissive microenvironment are capable of making appropriate long-distance projections. With this approach (47, 50), a population of lamina II/III neocortical pyramidal neurons is retrogradely labeled with a photoactive chromophore, chlorin e6, and undergoes apoptotic degeneration when noninvasively exposed to appropriate long-wavelength light (65). Neuronal injury effected in this way is extremely selective, cell-type specific, and spatially defined. Only targeted neurons degenerate, while surrounding neurons, glia, and neuropil remain intact (47, 48).

Embryonic cortical neurons selectively migrate into and differentiate within cortical areas undergoing photolytically induced neuronal degeneration in juvenile or adult mice (47, 66). Transplanted neurons or multipotent neural precursors (49) assume pyramidal morphologies, make local projections, receive synaptic contacts, and express neuronal antigens. These findings have suggested that upregulated developmental signal molecules within neocortex undergoing apoptotic neuronal degeneration can uniquely specify the differentiation of immature neurons and neural precursors well after these processes normally occur during corticogenesis. The microenvironment of neocortex undergoing this targeted cell death appears to provide a specific temporal and spatial sequence of cellular and molecular signals, not found in intact cortex, which guide neuronal cell differentiation after the development of the neocortex is complete. Observations from these prior studies led to the hypothesis that this sequence of signals within the altered microenvironment also might support long-distance axon outgrowth that is appropriate and specific to the restoration of interrupted pathways.

The present experiments assess: (1) whether embryonic neurons transplanted into regions of neocortex undergoing synchronous apoptotic neuronal degeneration can make long-distance projections and (2) whether these potential axons re-form the original or alternate projections. Results indicate that 6 to 8 weeks after their migration into areas of somatosensory cortex undergoing targeted degeneration, 21% of donor neurons made connections specifically with contralateral homotopic cortex, the original target of host neurons undergoing synchronous apoptotic cell death. Donor neurons did not project into ipsilateral S2 or ipsilateral thalamus, closer, alternate targets of other subpopulations of neurons in S1 (3, 15, 19, 30, 42, 43, 75–77). These data, as well as those from our previous transplantation studies (47, 49, 66), suggest that the mature neocortex undergoing selective apoptotic neuronal degeneration can reexpress signals that guide the development of immature neurons and the formation of specific projections to the original, denervated target.

A preliminary report of some of these experiments has been presented previously (36).

**MATERIALS AND METHODS**

The data from 36 C57B/6J mice are reported in this study. Embryonic neocortical cell suspensions from six separate dissociations were transplanted into mice from seven different litters. Callosal projection neurons in cortical lamina II/III of adult mouse primary somatosensory cortex (S1) were targeted for photolytic neuronal cell death, and Embryonic Day 17 neocortical neurons were transplanted into the spatially defined regions undergoing neuronal degeneration. In order to determine whether transplanted neurons specifically acquired the callosal phenotype or whether they projected to other targets as well, we injected the retrograde label fluorogold (FG) into homotopic contralateral S1, the original target of the degenerated host neurons, as well as into ipsilateral secondary somatosensory cortex (S2), or ipsilateral thalamus, nearby targets of other populations of intact host S1 neurons. We then investigated whether donor neurons intracellularly labeled prior to transplantation became labeled with FG, indicating the extension of axons to the target regions injected with FG.

Surgical procedures. Detailed chlorin e6 injection and laser exposure methods have been described previously (47, 66). Briefly, 2-week-old mice were deeply anesthetized with Avertin and the skull overlying primary somatosensory cortex (18, 54, 77) was removed. Glass micropipettes with tip diameters of 30–60 µm were used to microinject fluorescein latex nanospheres conjugated with chlorin e6 in 5 to 10 pressure injections spaced evenly throughout S1, delivering approximately 500 nl of label at each injection site. 5 injections were made at 100-µm increments, beginning 750 µm deep; following the most superficial injection, the micropipette was left in the neocortex for 1 min to minimize efflux. Following surgery, the bone was replaced, and pups were returned to their dams. Two weeks later, at 4 weeks of age, degeneration of approximately 65% of lamina II/III pyramidal neurons in S1 was induced within a spatially defined region approximately 800 µm in diameter (47, 66) by exposing the homotopic area in contralateral cortex through intact dura to light from a continuous-wave 674-nm near-infrared laser with custom optics (Candela), delivering a total incident energy...
of approximately 150 J/cm². Selective degeneration of S1 callosal projection neurons initiated by laser exposure at 4 weeks of age occurred over the following 3 to 4 weeks (47, 66).

Two weeks after laser exposure, at 6 weeks of age, suspensions of E17 neocortical cells, including rostral and caudal cortical areas, were labeled with a second type of latex nanosphere containing rhodamine (47) and with a lipophilic dye, Dil or PKH. In some early cases, neocortical cell suspensions were labeled with rhodamine nanospheres only. These nanospheres preferentially label neurons in vitro (47, 51), persist within neurons indefinitely, and do not secondarily label neurons of the host cortex (47, 66). The lipophilic dyes Dil and PKH label cellular membranes and outline cell somata and processes (2, 37, 38). Suspensions of primary neocortical cells prelabeled with nanospheres and lipophilic dye were transplanted through regions of S1 undergoing photolytically induced neuronal degeneration in 6-week-old mice, spanning neocortical lamina II/III through V. Micropipettes were lowered directly through the neuron-deficient regions to a depth of 500 µm and withdrawn over 200 µm at 50-µm intervals while injecting approximately 50 nl of cell suspension at each level (total 300 nl) to ensure a “tubular” distribution of cells across these laminae, thereby offering the donor neurons a laminar choice for potential migration and integration.

Fluorogold injections. A 2% solution of FG (Fluorochrome) in distilled water was injected via glass micropipettes with tip diameters of 40–60 µm. Two (n = 10), 4 (n = 6), 6 (n = 7), 8 (n = 2), or 10 (n = 11) weeks after neocortical transplantation, mice received FG injections into one of three target areas: S1, S2, or thalamus (areas determined according to the criteria in 18, 19, 30). Injections of FG into S1 contralateral to the transplant (n = 15) consisted of 8 to 12 pressure injections spaced evenly throughout S1 (Fig. 1a), delivering 500–750 nl of label. Injections into S2 (Fig. 1b) ipsilateral to the transplant (n = 10) consisted of 4 to 8 injections delivering 400–600 nl. Due to the angle of the S2 injection, FG also minimally labeled some regions surrounding S2 (areas 13, 14, and 41 according to 18). Injections into thalamus ipsilateral to the transplant (n = 11) consisted of 2 to 4 injections (Fig. 1c), delivering 200–300 nl. Injections into thalamus were aimed 45° relative to the vertical axis and introduced caudally, in order not to disrupt the cortical area containing the transplant or the white matter underlying it (19, 30). Coordinates were chosen and small injection volumes were used to minimize FG spread to adjacent white matter. The effective area of FG uptake within S1, S2, and thalamus extended up to a 500-µm radius surrounding the injection tracts due to diffusion of label. Regions with normal projections to these three target regions were examined for retrogradely labeled endogenous neurons.

![FIG. 1. Fluorogold injections were made into three potential target areas 2, 4, 6, 8, or 10 weeks after transplantation of embryonic neurons into regions of primary somatosensory cortex (S1) undergoing photolytically induced neuronal degeneration. (a) Unstained coronal section showing the homotopic S1 (outlined by dashed line) contralateral to the site of neural transplantation that was injected with FG (n = 15). Two of twelve FG injections are visible (arrows). (b) FG injections into the secondary somatosensory cortex ipsilateral to the transplant covered a wide area of parietal cortex (area outlined by dashed line; n = 10). The site of the transplant in S1 (asterisk) is in the section immediately rostral to the one shown. The area of effective FG uptake extends around the 8 injection sites throughout the region outlined, up to 500 µm in radius. One injection site is indicated (arrow). (c) FG injections into thalamus ipsilateral to the transplant (area outlined by dashed line; n = 11) was restricted to subcortical structures and involved most of thalamus. One of four injection sites is indicated (arrow). See text for details. Scale bar, 1 mm.]
host neurons to ensure that FG injections were correctly positioned. Following all surgical procedures, the excised skull was replaced, overlying skin was sutured closed, and mice were returned to the colony.

Tissue preparation. Four days after FG injections, mice were deeply anesthetized with Avertin, then transcardially perfused with heparinized saline followed by 2% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Brains were postfixed overnight in the same fixative and blocked, and serial coronal sections through the region of cellular transplantation and FG injections were cut on a vibrating microtome at a thickness of 40 µm. Sections were processed for fluorescence and immunocytochemistry and observed with a Zeiss microscope equipped with epifluorescence and Nomarski DIC optics.

Cell quantification. In each mouse, three sections through the site of transplantation in the region of targeted degeneration and donor neuron migration were used for analysis. Each section was examined at low magnification for initial assessment of transplant position and at higher magnification for identification of individual labeled neurons. High-magnification examination was used to identify donor neurons by morphologic criteria using Nomarski DIC, process labeling by DiI or PKH fluorescence, and the presence of lysosomal fluorescence from neuron-selective nanoparticles. Fluorescence examination was performed using custom-fabricated narrow bandpass filter sets that ensured specificity to the identification of fluorogold, fluorescein, and rhodamine intracellular fluorescence without crossover between nanosphere fluorescence wavelengths. Donor neurons in lamina II/III were counted in sample fields in each section approximately 100–250 µm away from the injection tract using a 100× high numerical aperture objective. After donor neurons were identified, the fields were examined for FG fluorescence and the number of retrogradely labeled donor neurons was determined. Sample fields, 250 µm in diameter, represent approximately 20% of the total area in each section into which donor neurons migrated and differentiated.

Immunocytochemistry. As further confirmation of the neuronal phenotype of donor neurons identified by morphologic criteria, tissue sections were reacted for microtubule-associated protein 2 (MAP-2; 46) and neuron-specific enolase (NSE). Tissue sections were reacted in 0.1 M PBS and incubated in primary antibody (gift of Dr. K. Kosik, Brigham and Women’s Hospital and Harvard Medical School) diluted 1:500 in 0.1 M PBS with 1% goat serum for 30 min. After several washes in PBS, sections were incubated in goat anti-mouse IgG conjugated to peroxidase (Vector; diluted 1:50 in PBS) for 1 h and treated with a Vectastain ABC kit. Sections reacted for NSE were pretreated with a solution of 0.25% Triton X in 50 mM Tris-buffered saline (TBS) for 30 min and blocked with 10% rabbit serum and 0.1 M lysine in TBS for 1 h. Following several washes in TBS, sections were incubated for 1 h at room temperature in NSE primary antibody (Polysciences) diluted 1:500 in TBS, washed several times before incubation in rabbit anti-rat IgG conjugated to peroxidase (Vector; diluted 1:50 in TBS) for 1 h, and treated with a Vectastain ABC kit.

RESULTS

Projections 2 to 8 weeks following transplantation. Two weeks following their implantation into primary somatosensory cortex of 6-week-old adult mice undergoing neuronal degeneration, Embryonic Day 17 neurons migrated specifically into areas of selective cell death in lamina II/III. Examination of transplanted donor neurons by DiI or PKH labeling, and by Nomarski DIC optics, indicated that many in lamina II/III displayed pyramidal morphologies: 20- to 25-µm-diameter cell bodies, prominent apical dendrites, long axons extending toward white matter, large nuclei, and prominent nucleoli. At this early time following transplantation, no donor neurons were labeled by injections of FG into contralateral S1 (n = 4; 0 of 119 neurons), ipsilateral S2 (n = 3; 0 of 74 neurons), or ipsilateral thalamus (n = 3; 0 of 67 neurons). However, many DiI- or PKH-labeled donor neurons within the plane of a single section extended axons toward underlying white matter approximately 100–150 µm in length (Fig. 2).

Similar results were obtained when FG injections were delayed for up to 8 weeks after transplantation. Four (n = 6), 6 (n = 7), or 8 (n = 2) weeks after their transplantation into these regions, transplanted donor neurons had migrated, differentiated into pyramidal morphologies, and extended locally visible axons, but they were not labeled by FG injections into contralateral S1 (n = 7; 0 of 253 neurons). Remaining endogenous host neurons labeled with fluorogold were observed interspersed with donor neurons following contralateral S1 injections (20–50 FG-labeled host neurons per 4.9 × 10⁴ µm²), further confirming that FG injections were correctly located to retrogradely label callosal neurons surrounding the transplantation site. Donor neurons were not labeled by FG injections into ipsilateral S2 (n = 4; 0 of 113 neurons) or ipsilateral thalamus (n = 4; 0 of 90 neurons).

These results show that up to 8 weeks after embryonic neurons were transplanted into regions of S1 undergoing targeted degeneration, or 6 weeks after completing migration into these regions, donor neurons did not extend projections to the contralateral S1, the original target of degenerating host neurons, nor to...
ipsilateral S2 or thalamus, closer targets of other, intact endogenous neurons in S1.

Projections 10 weeks following transplantation. Ten weeks following neuronal transplantation, 21 ± 5% (± SEM) of the donor neurons (Fig. 3; n = 4; 6 of 23, 26%; 9 of 27, 33%; 3 of 22, 14%; 5 of 43, 11%) in neocortical lamina II/III were retrogradely labeled via their callosal projections by FG injections into contralateral S1, a distance of 6 to 8 mm from the transplant site (Figs. 4 and 5). Remaining endogenous host neurons interspersed with donor neurons were also labeled from contralateral S1 injections (Fig. 5d; 20–50 FG-labeled host neurons per 4.9 × 10^4 µm^2). Donor neurons were not labeled by FG injections into S2 (n = 3; 0 of 100 neurons) or thalamus (n = 4; 0 of 131 neurons) in the hemisphere ipsilateral to the transplant (Fig. 3). In contrast, endogenous host neurons in lamina II/III and deep-layer host neurons, respectively, were labeled by FG injections in these two locations, confirming correct positioning of the FG injections.

Fluorogold labeling of donor neurons was visible as intracellular granular fluorescence and light cytoplasmic fluorescence (Figs. 4b and 5c). Fluorogold-labeled donor neurons examined under high magnification Nomarski DIC optics displayed extensive dendritic processes and somata typically 20–25 µm in diameter (Fig. 4d). Approximately 90–95% of transplanted neurons identified morphologically expressed the neuronal antigens MAP-2 and NSE, further confirming the neuronal projections by neurons grafted after photolysis.
neuronal identity indicated by transplanted donor neuron morphology. Donor neurons stained positively for these markers whether or not they were retrogradely labeled with FG. MAP-2 staining of donor neuron somata and dendritic processes (Fig. 6) support observations made under Nomarski DIC optics of donor neuron cell body size and of the extent of processes.

These results indicate that donor neurons extend long-distance projections selectively into the contralateral SI, or the immediately underlying white matter, 8–10 weeks after their transplantation into areas of ongoing synchronous neuronal degeneration, or 6–8 weeks after migrating into these regions. The projections are appropriate, and they partially restore the original projections made by degenerating host SI neurons. Donor neurons do not send axons into ipsilateral S2 or thalamus, closer targets of other subpopulations of neurons in SI.

**DISCUSSION**

The main finding of the present experiments is that embryonic neurons transplanted through regions of adult primary somatosensory cortex undergoing targeted degeneration of callosal projection neurons can selectively replace lost host neurons and form similar, long distance projections to the contralateral primary somatosensory cortex. The callosal projections extended by transplanted neurons are appropriate; they extend toward the targets of endogenous callosal projection neurons undergoing apoptotic degeneration. Donor neurons do not extend axons into the ipsilateral second-

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**FIG. 4.** Ten weeks after being transplanted into regions of SI undergoing targeted photolytic degeneration, or 8 weeks after completion of migration into these regions, donor neurons in lamina II/III are callosally labeled only by FG injections into contralateral SI. (a) Donor neurons (outlined by small arrows) labeled with rhodamine nanospheres display intralysosomal granular fluorescence (long arrow). (b) The same field as in a observed for fluorogold fluorescence. One of the two donor neurons is retrogradely labeled with FG (asterisk), visible as distributed granular fluorescence and light labeling of the neuronal cytoplasm. (c) The same field as in a and b observed under a custom narrow bandpass filter set for fluorescein nanosphere fluorescence, further confirming the donor origin of these neurons by the absence of fluorescein nanospheres used for chlorin 6 targeting. A remaining endogenous host neuron (outlined by small arrowheads) is visible in the lower right corner. (d) The same field observed using Nomarski DIC optics. Small arrows outline the same donor neurons in a, b, and c. The arrowheads point to the dendrite of the lower neuron. The transplant site is approximately 250 µm to the left of these donor neurons. Ventral is toward the bottom of the photomicrographs. Scale bar, 50 µm.

**FIG. 5.** Subpopulations of donor and endogenous neurons are retrogradely labeled by fluorogold injections into contralateral SI 10 weeks following transplantation. (a) Camera lucida drawing indicating the site of neuronal transplantation (solid line) spanning lamina II/III through V in SI and the region of lamina II/III shown in photomicrographs b–d demonstrating rhodamine, fluorescein, and FG fluorescence at higher magnification. Scale bar, 1 mm. (b) Region indicated by box in a (box larger than to scale) observed for rhodamine nanosphere fluorescence. Donor neurons with FG retrograde labeling (outlined by arrowheads) in c and without FG retrograde labeling (large arrows) are distributed throughout the area interspersed with remaining endogenous host neurons (outlined by small arrows and indicated by long arrow). (c) Same field as in b observed for FG fluorescence. Two donor neurons (outlined by arrowheads) and the host neuron indicated in d (outlined by small arrows and indicated by long arrow) are retrogradely labeled by FG injections into contralateral SI. Other donor neurons remain unlabeled. (d) Same field as in b and c observed for fluorescein nanosphere fluorescence. The host neuron from b and c (outlined by small arrows and indicated by long arrow) is doubly labeled with FG. Scale bar in b, c, and d, 50 µm.
ary somatosensory cortex or ipsilateral thalamus, which are alternate, nearby targets of other host S1 neurons.

The present data confirm and extend previous findings in motor cortex that donor neurons and neural precursors migrate selectively into regions of neocortex undergoing targeted photolytic degeneration in lamina II/III, extend local projections, and differentiate morphologically and antigenically (47, 49, 66, and unpublished observations). This migration and differentiation occurs long after the ages at which these events normally take place during neocortical development. Taken together, these results suggest that: (1) neocortex undergoing targeted cell death in adult mouse can reexpress early developmental signals that guide the migration and differentiation of transplanted immature neurons and (2) these signals permit specific re-formation of disrupted neocortical cytoarchitecture and selective long-distance projections. Further, they provide evidence that embryonic neurons transplanted into regions of the mammalian brain undergoing apoptotic neuronal degeneration without significant inflammation or gliosis can support axon extension and, under appropriately permissive conditions, that this outgrowth can be directed specifically toward the partial restoration of damaged pathways.

These data, in combination with those of our prior experiments, support the hypothesis that the specific loss of neurons induces the reexpression of signals that

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**FIG. 6.** Transplanted neurons stain positively for the neuronal marker MAP-2. (a) High-magnification view of donor neurons (outlined by small arrows) in lamina II/III labeled with rhodamine nanospheres approximately 250 µm away from the transplantation site. (b) The same field as in a observed for FG fluorescence. The same three donor neurons outlined in a (small arrows) are retrogradely labeled by FG injections into contralateral S1. (c) The same field as in a and b observed for MAP-2 antibody staining. Two of the same three donor neurons from a and b retrogradely labeled with FG stain positively for MAP-2 in this focal plane. The donor neuron at the bottom of the photomicrograph (small arrows; asterisk) is cut in the plane of section and shows staining in its soma as well as its apical dendrite (arrowheads). The middle neuron (small arrows, double asterisk) shows MAP-2 staining only in its soma in this focal plane, while the topmost neuron is further out of the plane of section and does not show MAP-2 staining. Small arrows point to the locations of same donor neurons in the three panels. Ventral is toward the bottom of the photomicrographs. Scale bar, 50 µm.
enable donor neurons to migrate and differentiate. The selective migration and differentiation of transplanted embryonic neurons occurs only in spatially defined regions of neocortex undergoing specific neuronal loss. Embryonic neurons transplanted into intact neocortex, into neocortex lesioned with kainic acid, or into intact regions of neocortex at a distance from regions undergoing targeted degeneration fail to migrate or differentiate (47, 49, 66). Data from transplantation studies of Sotelo and coworkers using the Purkinje cell degeneration (PCD) mutant mouse are consistent with these results. Embryonic neurons transplanted into PCD cerebella devoid of Purkinje neurons specifically migrate into and differentiate within the molecular layer of the adult host cerebellum (31, 32, 69–72). However, similar transplants into normal intact cerebellum, or into PCD mutants at a stage when Purkinje cells have not yet disappeared, fail to migrate or differentiate (72). Together, these data support the interpretation that in the adult CNS, the synchronous apoptotic degeneration of neurons can activate the expression of microenvironmental signals that are permissive for migration and differentiation of transplanted embryonic neurons long after these processes normally occur during development.

It has been previously demonstrated that embryonic neurons transplanted into the neonatal brain as blocks of tissue or as cell suspensions are able to survive, receive input, and extend projections to a wide variety of targets. Embryonic neurons placed into lesion cavities in the sensorimotor cortex of rats receive projections from sensorimotor cortex, thalamus, claustrum, hypothalamus, locus coeruleus, zona incerta, and median raphe (16, 17) and extend projections to host sensorimotor cortex and thalamus (25, 68), regions with normal afferent and efferent connections with sensorimotor cortex. Similarly, embryonic neurons placed into the cortex of newborn rats (20, 21, 27, 28, 60, 62, 73) extend and receive projections to and from a number of their normal targets. Embryonic neurons from frontal cortex transplanted into motor/sensory cortex of adult rats similarly can form connections with their normal targets in the host thalamus (34). In the present study, we add to these earlier findings by demonstrating that embryonic neurons transplanted into an adult mouse brain with selective neocortical neurodegeneration can survive and extend specific long-distance projections. Using an experimental paradigm in which a specific subpopulation of cortical neurons is induced to undergo apoptotic degeneration, we demonstrate that the projections made by transplanted neurons are not only directed toward normal targets, they are selectively directed toward the original projection area of the cortical neurons that underwent selective degeneration. No projections were observed to two alternate, normal targets of other subpopulations of neurons in S1. This suggests that, under appropriate conditions, the adult neocortex can reexpress signals that guide the survival and differentiation of immature neurons and direct the extension of axons to specific targets, restoring elements of normal neocortical connectivity.

The experiments reported here reinforce the idea that axon extension is one developmental process that is substantially subject to epigenetic control. In the present experiments, 21% of transplanted embryonic neurons extended callosal projections to appropriate targets in the contralateral S1. However, these callosal projections were not observed until 10 weeks after transplantation, or 8 weeks after neurons migrated into their final positions. By contrast, during normal development, callosal projection neurons extend axons into the opposite cortex 1 to 1½ weeks after the neurons are generated (40, 41, 56, 57). It is possible that embryonic neurons transplanted into adult neocortex take longer to innervate their targets than their counterparts during corticogenesis simply because the distances separating projection and target neurons are greater in adult animals. Neurons transplanted into adult CNS cannot use the guidance pathways present during development that facilitate the establishment of distant projections (5, 6, 9, 12, 23, 24, 44, 45, 52). In addition, the signals influencing axon extension may be expressed for a limited time and/or a limited distance (72). In some cases, neurons supplied with an appropriate microenvironment can innervate appropriate local or distant targets (1, 8, 35, 80, 81). Purkinje neurons, by contrast, rarely send out axons into their targets despite being in a host microenvironment that is permissive for their appropriate migration and morphological differentiation (71, 72). In other cases, transplanted neurons can extend axons toward their targets only if they are placed close to their targets in spinal cord (29, 55) or adult hippocampal formation (33, 80).

Observations of axon outgrowth by embryonic donor neurons transplanted into adult animals indicate that axon outgrowth inhibitors present in mature CNS (7, 11, 13, 14, 61, 63, 64) do not have an absolute inhibitory effect. One explanation proposed to account for this observation is that transplanted embryonic neurons may not yet have receptors for myelin-associated inhibitors (78). Alternatively, differences in lesion paradigms used in the various transplantation experiments may account for reported differences in the observations of axon outgrowth. Experiments using nonspecific, invasive lesions with inflammation and gliosis reported little or no axon extension (61, 64) raising the possibility of mechanical barriers to axonal outgrowth (53, 59). Studies using lesions that caused minimal trauma and gliosis reported significant axon extension (22, 78, 79). It is possible that the glial response to more inflammatory injury, in addition to the presence of inhibitory
molecules on oligodendrocytes, adds to the inhibition of axon extension by mature neurons in adult mammalian CNS.

Our data further suggest that in adult neocortex, degenerating neurons and their target neurons can induce the reexpression of signals guiding axon outgrowth that is specifically directed toward the reinnervation of appropriate targets. Embryonic neurons transplanted into regions of S1 in adult mice undergoing neuronal degeneration send projections into the contralateral S1, the target of host neurons undergoing synchronous apoptotic cell death, in preference to the other two alternative targets examined. No projections were observed into ipsilateral S2 or into ipsilateral thalamus, nearby targets of intact host neurons, although it is possible that such projections could arise later, or in substantially smaller numbers, than those detected in S1. Similarly, in previous reports of successful axonal extension, the outgrowth is at least partially target specific. In the hippocampal formation of mature rodents, transplants of embryonic tissue send projections into lesioned entorhinal zones that are restricted to appropriate terminal areas (80, 81). Human neurons transplanted into cortex (74), into ventricle (74), or into a point along the pathway (78, 79) of the lesioned nigrostriatal system in adult rats extend axons toward nigral targets that can travel along white matter tracts of the medial forebrain bundle and internal capsule for distances of up to 20 mm (78, 79) and which seemingly avoid areas that are not normal targets of nigral neurons. Our data and these other reports provide evidence that neurons transplanted into regions of the mammalian brain undergoing neuronal degeneration without significant inflammation or gliosis can support axon extension and, under appropriately permissive conditions, that this outgrowth can be directed toward the partial restoration of damaged pathways.

In conclusion, the present experiments indicate that in the mature mammalian neocortex following selective degeneration of a subpopulation of neurons: (1) transplanted immature neurons can form long distance projections and (2) these projections can be formed with specificity. These results support the hypothesis that synchronous apoptotic cell death can induce the reexpression of signals that guide highly specific and directed axonal outgrowth by embryonic neurons transplanted into regions of ongoing cell death. Hence, given an appropriately permissive host microenvironment, axon outgrowth by transplanted embryonic neurons may be able to specifically restore the pathways that are lost as a result of neuronal death and, in doing so, could potentially restore the elements of sensory, motor, or cognitive functions associated with these pathways. Elucidation of the mechanisms underlying this directed, specific axon outgrowth and factors affecting it will be of significant value in further understanding limitations and control of regeneration relevant to a variety of CNS neurodegenerative diseases and injuries.

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