

# Reestablishment of damaged adult motor pathways by grafted embryonic cortical neurons

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Damage to the adult motor cortex leads to severe and frequently irreversible deficits in motor function. Transplantation of embryonic cortical neurons into the damaged adult motor cortex was previously shown to induce partial recovery, but reports on graft efferents have varied from no efferent projections to sparse innervation. Here, we grafted embryonic cortical tissue from transgenic mice overexpressing a green fluorescent protein into the damaged motor cortex of adult mice. Grafted neurons developed efferent projections to appropriate cortical and subcortical host targets, including the thalamus and spinal cord. These projections were not a result of cell fusion between the transplant and the host neurons. Host and transplanted neurons formed synaptic contacts and numerous graft efferents were myelinated. These findings demonstrate that there is substantial anatomical reestablishment of cortical circuitry following embryonic cortex grafting into the adult brain. They suggest that there is an unsuspected potential for neural cell transplantation to promote reconstruction after brain injury.

One approach for overcoming the generally limited capacity of the mature CNS to regenerate axons or to generate new neurons in response to cell loss is the transplantation of embryonic neurons. This area of research has received a great deal of attention since the early 1970s<sup>1,2</sup>, mainly in the field of cell replacement therapy in Parkinson disease. In the cerebral cortex, point-to-point innervation of target areas by the transplant neurons is critical. Possible reconstruction of neocortical circuits following transplantation has been difficult to assess, mainly because of the technical limitation of distinguishing donor neurons and their axons from host tissue. Here, we have used a transgenic mouse line with an enhanced green fluorescent protein (GFP) cDNA under the control of a chicken  $\beta$ -actin promoter and cytomegalovirus enhancer<sup>3</sup>. In these mice, all cells in the brain express GFP, allowing for the characterization of the transplanted neurons and their axons in the nontransgenic host. The present series of experiments were undertaken to examine whether long-distance axonal growth can be achieved in the adult mouse following motor cortical damage by transplantation of embryonic day 14 (E14) mouse motor cortical tissue. We particularly focused on demonstrating specific reconstruction of damaged circuitry and appropriate reestablishment of synaptic contacts between the transplant and the host.

## RESULTS

Following lesion of the motor cortex in adult (4–6 months) C57BL/6 mice ( $n = 94$ ), we transplanted embryonic, GFP-expressing cortical tissue at E14 as described previously<sup>4</sup> (see Methods).

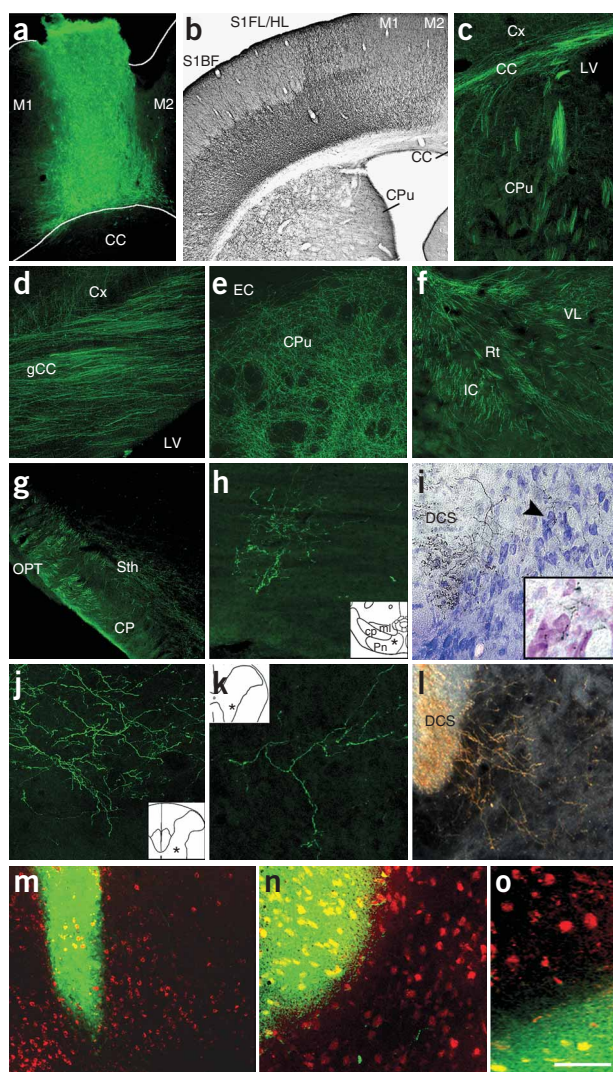
## Grafted neurons project to specific motor targets

To determine the pattern of projections developed by the transplanted GFP<sup>+</sup> neurons, we examined GFP labeling in different brain regions by immunohistochemistry (Fig. 1). The transplants filled the lesion cavity at 2 months postgrafting ( $n = 28$ ) and were well integrated in the host motor cortex (Fig. 1a). In all cases, a dense array of GFP<sup>+</sup> fibers was found in motor and sensorimotor cortices labeled similarly to control animals ( $n = 7$ ) that received injections of anterograde tracer (biotinylated dextran amine, BDA) into the motor cortex. We found the largest amount of GFP axonal labeling in layers VI–V, whereas more superficial layers IV–II showed less dense labeling (Fig. 1b and Supplementary Fig. 1 online). GFP<sup>+</sup> fibers extended through the ipsilateral corpus callosum to reach subcortical targets (Fig. 1c). GFP<sup>+</sup> callosal fibers (Fig. 1d) were traced to the contralateral hemisphere and contacted motor and somatosensory cortices. In all cases, GFP<sup>+</sup> fibers were present mainly ipsilaterally in the dorsolateral caudate putamen (Fig. 1e). In 16 of the 28 cases, we identified labeled fibers in the host thalamus, particularly in the ventrolateral and reticular nuclei (Fig. 1f). We also found labeled fibers in the internal capsule, cerebral peduncle and ventromedial part of the pontine nuclei (Fig. 1g,h).

One of the most notable features of this study was that labeled fibers were present in the spinal cord, (Fig. 1i–k). We detected GFP<sup>+</sup> axons ipsilaterally in the pyramidal tract, below the pyramidal tract decussation, in the contralateral corticospinal tract (Fig. 1i), and at cervical levels of the spinal cord (Fig. 1j,k) in 11 of 28 transplanted mice. This pattern of projection was similar in its distribution to that observed in

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**Figure 1** Distribution of GFP graft efferents toward host targets. (a) Transplant location in host M1 cortex. (b) DAB-nickel labeling of GFP<sup>+</sup> fibers in the cortex. A dense array of graft-derived axons was observed in the deep layers of host cortex, whereas less dense labeling was systematically found in the superficial layers. In the primary somatosensory area (S1BF), few fibers entered layer IV and then progressed radially to reach layer I. (c) GFP<sup>+</sup> fibers in cortex (Cx) and ipsilateral corpus callosum (CC). (d–h) GFP<sup>+</sup> fibers were found in the contralateral CC (d), caudate putamen (CPu, e), ventrolateral thalamic nucleus (VL, f), subthalamic nucleus (Sth), cerebral peduncle (CP, g), and pontine nuclei (Pn, h). (i) DAB-nickel labeling of GFP<sup>+</sup> fibers in the dorsal corticospinal tract (DCS). Sections were counterstained with cresyl violet to visualize motoneurons in the spinal cord (inset, high magnification of arrowhead in i, GFP<sup>+</sup> axonal terminal), bright-field illumination. (j,k) GFP<sup>+</sup> fibers reached C1 (j) and C8 (k) cervical levels. Insets in h, j and k show the section level, stars show the location of labeling. (l) Labeling in the corticospinal tract of normal mice following BDA injection into intact motor cortex, noncounterstained DAB-nickel material, dark-field illumination. (m–o) Following injection of retrograde tracer into the host VL (m), CPu (n) or pyramidal decussation (o), retrogradely labeled neurons were found in the host cortex and the transplant. Scale bars: a, 1 mm; b, 2 mm; c, e–g, 133  $\mu$ m; d, h, i, l, 67  $\mu$ m; j, k, n, 40  $\mu$ m; m, 500  $\mu$ m; o, 30  $\mu$ m.

### Circuit reconstruction is not a result of cell fusion

Several reports have previously described cell fusion between transplanted or newly generated cells and host cells. All of these reports, however, highlight the very limited quantitative aspect of this phenomenon that seems to implicate less than  $1 \times 10^{-6}$  cell<sup>5–7</sup>. More recently, it has been shown that photoreceptor precursors can integrate into the adult or degenerating retina, differentiate into rod photoreceptors, form synaptic connections and improve visual function. Notably, it was shown that these results are not the result of cell fusion between transplanted and host cells<sup>8</sup>. Nevertheless, we carried out four experimental procedures to investigate whether any cell fusion had occurred between the transplanted GFP cells and the host cells, which might have accounted for the projections that we observed.

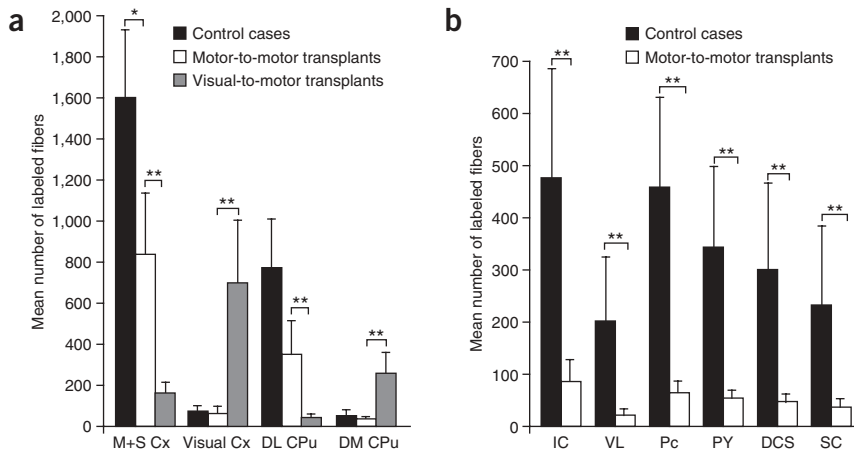
In the first experiment, we carried out fluorescence-activated cell sorting (FACS) analysis after staining the cells with propidium iodide<sup>9</sup>. The suspensions were analyzed with a Facscalibur flow cytometer (Becton Dickinson). We determined the DNA content of cell suspensions and always observed a single cell population in the transplant alone ( $99.97 \pm 0.01$ ,  $n = 6$ ) or in the transplant together with a very thin layer of adjacent cortex ( $99.98 \pm 0.01$ ,  $n = 3$ ). In addition, we carried out FACS and GFP immunohistochemistry in the same animals to determine the DNA content in cases where the transplant projected to the spinal cord. For this, 2 months following transplantation, we dissected out the GFP graft from the transplantation region and processed it for FACS. We then perfused the animals and processed the brains for GFP immunohistochemistry. We determined the DNA content of cell suspensions and always found a single cell population in the transplant ( $99.75 \pm 0.25$ ,  $n = 7$ ). Of the animals that we processed for FACS, 3 of 7 showed GFP<sup>+</sup> corticospinal axons.

Furthermore, to determine the cellular origin of retrogradely labeled neurons in the grafts, we transplanted motor cortex from female GFP<sup>+</sup> embryos into male recipient mice ( $n = 12$ ). Two and half months after transplantation, we injected a retrograde tracer (dextran, Alexa Fluor 568) into the host pyramidal tract decussation ( $n = 9$ ) or into the caudate putamen ( $n = 3$ ). We determined whether a Y chromosome was present in the host and graft nuclei by fluorescence *in situ* hybridization (FISH) using a CY3-labeled Y-chromosome DNA probe. We analyzed the GFP and retrograde labeling using laser scanning confocal microscopy on serial 0.5- $\mu$ m optical sections before the FISH procedure as previously described<sup>6</sup>. This was necessary, as the

control animals following BDA injection into the motor cortex (Fig. 1l). The percentage of labeled GFP<sup>+</sup> fibers compared with the control varied from ~50% in the motor and sensorimotor cortices and the dorsolateral CPu to 10–15% in the thalamus and the spinal cord (Fig. 2).

To confirm that transplanted GFP<sup>+</sup> neurons do contact target areas, we injected a retrograde tracer, cholera toxin (B subunit, CTB), into brain regions where GFP labeling was found. As expected, layer V neurons of the host motor cortex were labeled when we injected the retrograde tracer into the caudate putamen ( $n = 4$ ) or the pyramidal tract decussation ( $n = 4$ ). Layer VI neurons of the host motor cortex were labeled when the tracer was injected into the thalamus ( $n = 3$ ). In the transplant, GFP<sup>+</sup> cell bodies were also labeled when the tracer was injected in the various motor target regions. For instance,  $294 \pm 117$  GFP<sup>+</sup> cell bodies were labeled when we injected the tracer into the dorsolateral caudate putamen,  $53 \pm 22$  when we injected it into the thalamus and  $30 \pm 14$  when we injected it into the spinal cord (Fig. 1m–o).

Thus, the transplants can develop and maintain a set of projections toward most of the cortical and subcortical targets normally contacted by motor cortex neurons including distant target areas such as the spinal cord.



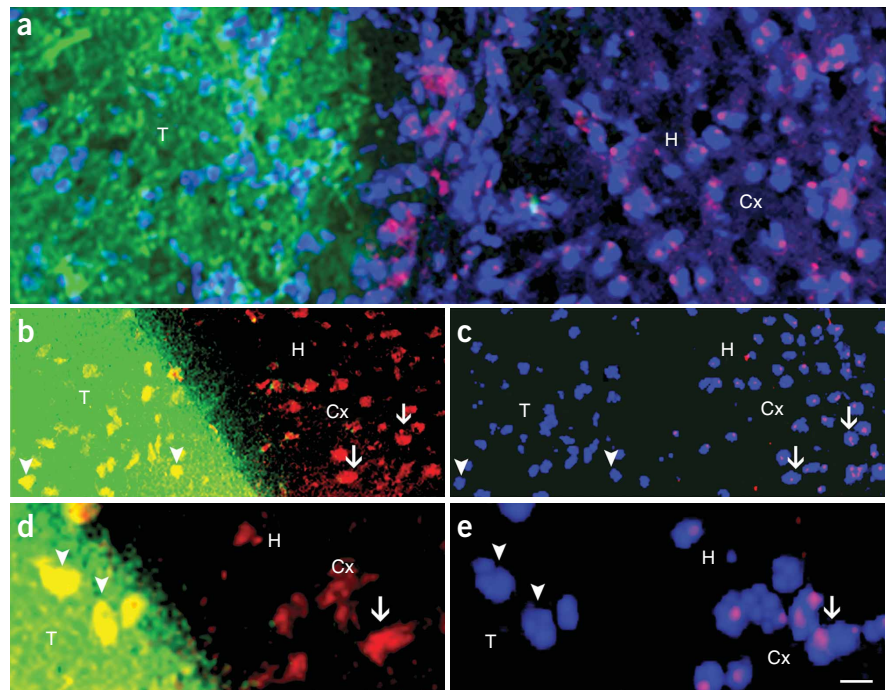
**Figure 2** Mean number of labeled fibers in control, motor-to-motor and visual-to-motor cases. For each group, fiber-labeling indexes were obtained by calculating the mean  $\pm$  s.d. total number of labeled fibers in each region. **(a)** Fiber number in ipsilateral motor and sensory (M+S) cortex, visual cortex, dorsolateral (DL) CPU and dorsomedial (DM) CPU. **(b)** Fiber number in the internal capsule (IC), ventrolateral thalamic nucleus (VL), peduncle cerebral (PC), pyramidal tract (PY), DCS and spinal cord (SC). Comparison between groups was carried out with the Mann-Whitney nonparametric test (\*  $P < 0.01$ ; \*\*  $P < 0.001$ ).

chromosome painting protocol abolishes fluorescence signals. Next, we carried out Y-chromosome FISH labeling followed by To-Pro3 nuclear staining on the same sections. In all 12 cases examined, we counted 554 GFP<sup>+</sup> cells and none showed a Y-chromosome FISH signal. In contrast, among the 621 Y-chromosome<sup>+</sup> cells, none were GFP<sup>+</sup> (Fig. 3a). We found retrogradely labeled neurons in the transplant in 4 of the 9 cases when we injected the tracer in the pyramidal tract decussation and in all 3 cases when we injected it in the caudate putamen. We quantified all labeled neurons within one series out of six sections. In the GFP<sup>+</sup> transplant, none of 168 neurons projecting to the caudate putamen (Fig. 3b,c) and none of the 57 neurons projecting to the spinal cord (Fig. 3d,e) contained a Y-chromosome signal.

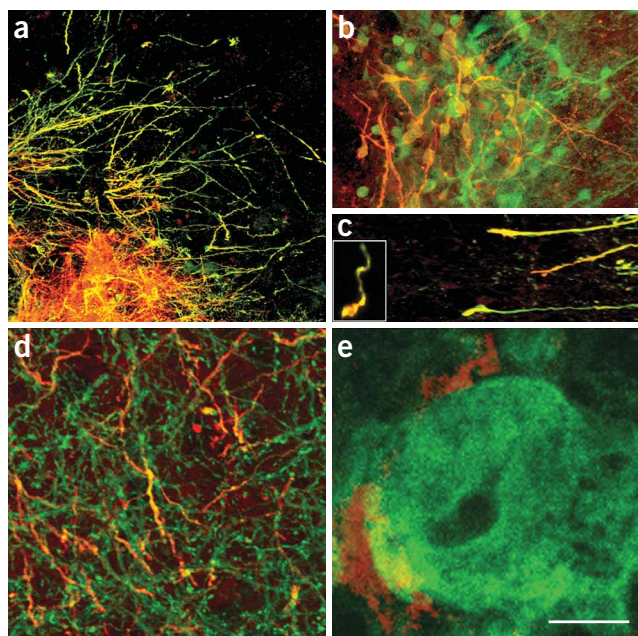
projections, these results rule out fusion between the remaining intact host neurons and transplanted GFP<sup>+</sup> neurons. Indeed, if fusion had occurred, we would have observed the same pattern of projections with visual and motor transplants.

In the last set of experiments aimed at investigating possible cell fusion between the transplant and the host, we examined whether transplanted neuronal cell bodies and processes express doublecortin (DCX), which would indicate that they are differentiating embryonic neurons and not mature fused neurons. DCX is a protein that is found in all populations of immature postmitotic migrating and

**Figure 3** FISH analysis and retrograde labeling on brain sections of adult male mice transplanted with female GFP embryos. **(a)** Host (H) cells showed Y-chromosome FISH labeling (red), but not transplanted (T) GFP<sup>+</sup> cells (green). Nuclei were labeled by To-Pro3 (blue). **(b)** Retrogradely labeled neurons were found in the host Cx (red) and the GFP<sup>+</sup> transplant (yellow) following tracer injection into host CPU. **(c)** FISH analysis on the same section showing that retrogradely labeled neurons of the host, but not of the transplant, contained the Y chromosome. **(d)** Retrogradely labeled neurons were found in the transplant (yellow) following tracer injection into host pyramidal decussation. **(e)** FISH analysis on the same section showing that retrogradely labeled neurons of the host, but not of the transplant, contained the Y chromosome. Arrowheads indicate the transplant GFP<sup>+</sup> neurons that were retrogradely labeled, but that did not contain the Y chromosome. Arrows indicate host retrogradely labeled neurons that also contained the Y chromosome. Scale bar: **a**, 35  $\mu$ m; **b,c**, 50  $\mu$ m; **d,e**, 15  $\mu$ m.



**Figure 4** Maturation indices of the transplant. (a–c) DCX expression in the graft and graft-derived axons. (a) GFP<sup>+</sup> neurons (green) and their processes co-expressed DCX (red). (b) High-power images showing GFP<sup>+</sup> soma and processes co-expressing DCX. (c) GFP<sup>+</sup> fibers expressing DCX in the thalamus at 15 d postgrafting. Insert, GFP<sup>+</sup> fiber expressing DCX in the spinal cord. (d) Double-labeling of GFP (green) and PLP (red) showed several myelinated GFP<sup>+</sup> fibers in the cortex. (e) Host synaptophysin terminals (red) were detected in the immediate vicinity of GFP<sup>+</sup> cell body (green). Scale bar: a, 22  $\mu$ m; b, 30  $\mu$ m; c, 15  $\mu$ m; d, 20  $\mu$ m; e, 10  $\mu$ m.



differentiating neurons, and is expressed throughout the period of corticogenesis, but not in adulthood<sup>13</sup>. At 4–8 d postgrafting, the vast majority of GFP<sup>+</sup> neurons and their leading axons, which had growth cone-like endings, expressed DCX ( $n = 7$ ; Fig. 4a–c). DCX<sup>+</sup> neurons were not colabeled with markers of mature neurons such as NeuN and MAP-2 (data not shown). DCX expression decreased gradually in the transplant and their process, and disappeared 20 d postgrafting ( $n = 3$ ).

Taken together, this set of experiments rules out fusion, directly and indirectly, as an explanation for the point-to-point massive reconstruction of cortical circuitry.

### Graft maturation indices

During normal development, most axons derived from the cortex are myelinated by 28 d after birth<sup>14</sup>. We examined myelination of transplant-derived axons 2 months after grafting with antibodies raised against the proteolipid protein (PLP,  $n = 9$ ). Approximately 30% of the GFP<sup>+</sup> fibers were also PLP<sup>+</sup> in the cortex and striatum (Fig. 4d). To investigate the level to which transplanted neurons integrate into cortical circuitry, we assessed synaptic contacts between transplant and host. We found the presynaptic vesicle protein synaptophysin in host terminals in the immediate vicinity of the transplant GFP<sup>+</sup> cell bodies and neurites (Fig. 4e). These results were further confirmed by electron microscopy studies. Indeed, we have found that host neurons developed axo-somatic and axo-dendritic contacts onto GFP<sup>+</sup> transplant neurons ( $n = 5$ ) (Fig. 5a) and conversely, GFP-labeled terminals formed synaptic contacts with host cortical, striatal and thalamic neurons (Fig. 5b–d). In addition, we have also shown newly generated myelin in GFP<sup>+</sup> axons (Fig. 5e,f).

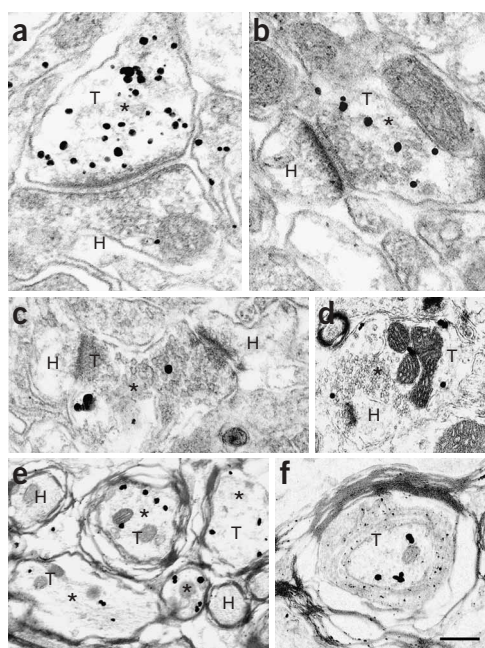
The formation of synaptic contacts between host and transplant, and myelination of transplant fibers provide evidence of substantial integration of the transplanted neurons in the host cortical circuitry.

### DISCUSSION

This study is the first demonstration of point-to-point reconstruction of damaged motor circuits in the adult brain. This reconstruction was achieved by grafting fetal cortical cells from GFP transgenic mice into the previously damaged cortex of wild-type adults. Our data indicate (i) that transplanted embryonic motor cortical neurons were capable of extending long-distance projections in the mature host brain to appropriate cortical and subcortical targets, (ii) that these projections were formed with specificity, as the topographical distribution in each structure seems similar to that found in normal animals, (iii) that some of these projections were myelinated, (iv) that transplant and host established reciprocal synaptic contacts, and (v) that graft-mediated appropriate reconstruction of damaged motor circuitry required grafting of homotopic donor cells.

### Cortical efferents reconstruction

The potential effectiveness of neural transplantation therapy critically depends on whether transplanted neurons can reestablish the disrupted regional and cellular projection patterns<sup>15</sup>. In this regard, possible outgrowth from grafts of embryonic motor cortex placed into the damaged motor cortex of adults is subject to controversy. Indeed, reports on graft efferents have varied widely, from no efferent projections<sup>16</sup> to sparse, or at best, moderate innervation<sup>17–20</sup>. In only a few



**Figure 5** Synaptic contacts between the transplant and the host. GFP immunoreactivity was detected by immunogold reaction at the electron microscopic level. (a) Electron micrographs showing axo-dendritic contacts between host and transplanted neurons. (b–d) GFP-labeled terminals formed synaptic contacts with host cortical (b), striatal (c) and thalamic neurons (d). Note that in c, a single GFP<sup>+</sup> axon terminal was in contact with two host dendrites. (e,f) Electron micrographs showing myelination of transplant-derived and host fibers in the internal capsule. Asterisks show GFP<sup>+</sup> elements. Scale bar: a,b,d,f, 1  $\mu$ m; c, 1.5  $\mu$ m; e, 2  $\mu$ m.

cases have investigators reported a somewhat extensive innervation of some host brain targets following selective lesion procedures inducing synchronous degeneration of cortical projection neurons<sup>21,22</sup>. None of these reports, however, have examined the complete efferent projections from the graft to the appropriate host target sites. Our findings on possible reconstruction of motor cortex efferents by embryonic grafts following aspirative lesion are consistent with previous observations obtained with newly born cells arising from endogenous precursors<sup>23,24</sup>. Indeed, using a somewhat different approach that did not include transplantation, a previous report showed that a few (0–7) endogenous precursor cells in the cortex could differentiate into cortico-spinal neurons 56 weeks following apoptotic degeneration of cortico-spinal motor neurons<sup>24</sup>. However, massive graft projections to the host cortex, caudate putamen, and to a lesser extent, the thalamus and spinal cord, as presented here have never been previously reported, for any type of lesion, graft or species considered.

The cortical site of origin of the transplant is critical for the reconstruction of normal neuronal circuitry, as visual grafts do not seem competent to replace the degenerating motor pathways. Our results here, together with our previous findings<sup>12,19</sup>, strongly suggest that already committed embryonic neurons are able to specifically recognize molecular cues still maintained in the adult brain or reexpressed following the lesion. Thus, appropriate reconstruction of damaged circuitry in the adult brain may require grafting of homotopic donor cells.

### Functional consequences

Several studies, including our own, have examined the functional outcome of embryonic cell transplantation following cerebral cortex lesions in adult rats<sup>10,25,26</sup>. In some cases, partial recovery was reported<sup>27–31</sup>, even though no evidence of substantial neural circuit reconstruction was provided. In a previous study, we have shown partial recovery of skilled forelimb reaching after transplantation of fetal cortical tissue in adult rats with motor cortex lesion<sup>29</sup>. Furthermore, it has been shown that transplanted cells are electrophysiologically and metabolically responsive to host stimulation<sup>32–34</sup>. Our present observations therefore provide an anatomical substrate that might account for the functional recovery reported in some studies after grafting.

Taken together, our findings open new possibilities for cell-based therapies of brain injury and disease. This is of particular interest given that cortical circuits have been implicated in several neurodegenerative diseases such as Huntington disease, Alzheimer disease and amyotrophic lateral sclerosis.

### METHODS

**Animals.** Housing of the animals and all animal experimental procedures were carried out in accordance with the guidelines of the French Agriculture and Forestry Ministry (decree 87849) and of the European Communities Council Directive (86/609/EEC). All efforts were made to reduce the number of animals used and their suffering.

**Transplantation.** Adult (4–6 months) C57BL/6 mice ( $n = 94$ ) were used as recipients, and the transplantation procedure was carried out as described previously<sup>4</sup>. Briefly, we anaesthetized the animals with avertin (250 mg per kg of body weight) and the motor cortex was aspirated from approximately 0.5–2.5 mm rostral to the Bregma and from 0.5–2.5 mm lateral to the midline, with the corpus callosum left intact. Immediately after the lesion, we removed E14 fetuses from transgenic mice that ubiquitously expressed GFP, dissected out fragments of motor cortex, and deposited the fragments into the host lesion cavity. Care was taken to maintain the original dorso-ventral and antero-posterior orientations of the cortical fragments during the transplantation procedure.

**Brain harvesting.** We injected the mice with a lethal dose of avertin and we immediately perfused them with a physiological saline solution, followed by either 4% paraformaldehyde in phosphate buffer for antibody staining or 1% paraformaldehyde for FISH. We then removed the brains and the spinal cord and cryoprotected them in a 30% sucrose solution overnight. Thick tissue sections (40  $\mu\text{m}$ ) were obtained for antibody staining on a sliding microtome (RM2145, Leica). Thin sections (12  $\mu\text{m}$ ) were obtained for FISH on a cryostat (CM3050, Leica) and mounted on super frost slides (Menzel-Glaser).

**Antibody staining.** We stained the floating sections after pre-incubation for 1.5 h at room temperature (21–23 °C) with antibodies for GFP (1:1,000, mouse or rabbit; Molecular Probes), DCX (1:100, guinea pig; Abcam), PLP (1:50, rat; gift from B. Zalc, Institut National de la Santé et de la Recherche Médicale U-495, Paris), or synaptophysin (1:1,000, mouse; Chemicon) overnight at 4 °C. We then incubated the sections in appropriate secondary antibodies for 1.5 h at room temperature (21–23 °C). The blocking solution contained 3% BSA and 0.3% Triton X-100. We incubated one series of GFP sections with a biotinylated secondary antibody (1:250; Vector Laboratories) and then incubated the sections with an avidin-biotin horseradish peroxidase complex (ABC Elite Kit; Vectastain). Sections were then treated with a solution containing 0.05% 3,3'-diaminobenzidine (DAB; Sigma), 0.01% H<sub>2</sub>O<sub>2</sub> and 1.5% nickel ammonium sulfate in 0.1 M acetate buffer (pH 6). In control animals, we delivered BDA (10% in 0.1 M phosphate buffer; Molecular Probes) for 10 min into the motor cortex as previously described<sup>25</sup>. BDA was revealed with an avidin-biotin horseradish peroxidase complex as described above.

**FACS.** Two months after grafting, we dissected out the transplant alone or the transplant ( $n = 13$ ) with a thin layer of the adjacent cortex ( $n = 3$ ), and a cell suspension of isolated nuclei for the DNA ploidy analysis was prepared as previously described<sup>9</sup>. The suspensions were analyzed with a Facscalibur flow cytometer (Becton Dickinson).

**FISH analysis.** As a first step, we examined cortical sections (12  $\mu\text{m}$ ) for the presence of retrogradely labeled neurons (dextran, Alexa Fluor 568, Molecular Probes) in the GFP transplants and the host cortex, and we scanned 0.5- $\mu\text{m}$  optical sections using a scanning confocal microscope (FV1000; Olympus). The position of the labeling was recorded with respect to the corners of the slide, to locate the exact position of the GFP and retrograde labeling after FISH procedure. The FISH protocol was modified from a previous description<sup>35</sup>. Briefly, the sections were microwaved in 10 mM sodium citrate buffer (pH 6.0, 10 min at 850 W), allowed to cool, and rinsed with 2 $\times$  SSC for 5 min. Then the sections were treated with pepsin (0.005%) at 37 °C for 7 min, rinsed for 1 min in 2 $\times$  SSC, rinsed briefly in distilled water, dehydrated in ethanol, and then air-dried. The slides were denatured at 65 °C for 2 min in 70% formamide in 2 $\times$  SSC, quenched in ice-cold ethanol, and air-dried. We denatured the Cy3-labeled whole-mouse Y-chromosome paint solution (15 ng  $\mu\text{l}^{-1}$ ; Cambio) at 65 °C for 10 min and then held it at 37 °C for 60 min. Paints were applied to sections, which were then coverslipped and incubated in a dark humidified chamber overnight at 37 °C. Washes were carried out in 2 $\times$  SSC, then in 50% formamide in 1 $\times$  SSC at 45 °C for 5 min. The nuclei were stained with To-Pro3 (1/3,000; Molecular Probes), and the sections were coverslipped with Vectashield (Vector Laboratories).

**Electron microscopy.** For immunogold detection, we incubated the sections in goat antibody to rabbit IgGs conjugated to ultra-small gold particles (0.8 nm, 1:100; Aurion) as previously described<sup>36</sup>. The immunogold signal was intensified using a silver enhancement kit (HQ silver; Nanoprobes). Serial ultra-thin immunostained sections were cut with a Reichert ultracut S (Leica) and were contrasted with lead citrate and observed in a Tecnai electron microscope (Philips).

**Data analysis.** The patterns of distribution of GFP<sup>+</sup> fibers were plotted with the aid of a camera lucida. For each animal, we carried out quantification of the fibers on five sections for each area, using a high-magnification objective (20 $\times$ ). For each group (control, motor-to-motor and visual-to-motor), fiber-labeling indexes were obtained by calculating the mean  $\pm$  s.d. total number of labeled fibers. We examined immunofluorescence-treated sections with a BX60 microscope equipped with fluorescein and rhodamine filters. The number of

retrogradely labeled neurons in the host and the transplant was quantified using a high-magnification objective (20 $\times$ ). We selected sections and photographed them with a confocal laser scanning microscope setting (FV1000; Olympus). We carried out comparisons of fiber numbers in motor-to-motor versus control and motor-to-motor versus visual-to-motor groups by using the Mann-Whitney U test for nonparametric samples.

Note: Supplementary information is available on the Nature Neuroscience website.

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#### AUTHOR CONTRIBUTIONS

A.G. carried out most of the experiments, designed and carried out the study, sought funding, and wrote the manuscript. L.P. carried out the FISH studies, B.D. carried out the electron microscopy studies, A.C. carried out the analysis on the confocal microscope, F.M. carried out the FACS analysis. M.R. initially designed the study and is now retired. M.J. designed and supervised the study, sought funding, and wrote the manuscript.

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