et al., Eur. J. Neurosci. 11, 567 (1999); E. E. Smith, J. Jonides, R. A. Koepp, Cereb. Cortex 6, 11 (1996). However, activation has been consistently reported in this area when participants are asked to monitor or manipulate items in memory in tasks such as the self-ordered task [M. Petrides, B. Alivisatos, E. Meyer, A. C. Evans, Proc. Natl. Acad. Sci. U.S.A. 90, 873 (1993)] or search task [A. M. Owen, A. C. Evans, M. Petrides, Cereb. Cortex 6, 31 (1996)].


10. Statistical, Parametric Mapping [Wellcome Department of Cognitive Neurology, London, (1999). Statistical parametric maps, SPM(t)], were calculated for condition-specific effects within a general linear model. All modeled events and epochs were convolved by a canonical hemodynamic response function. Low-frequency changes in BOLD signal over time were modeled with discrete cosine functions of periodicity ranging from 400 to 2400 s. The data were temporally smoothed by a canonical hemodynamic response function and by intrinsic temporal autocorrelations modeled by a first-order autoregressive process. In addition, we included an autoregression of order 1 modeled by intrinsic temporal autocorrelations. To evaluate the contrast of working memory delay versus an equivalent period in control trials (memory versus nonmemory epochs), we calculated the best-fit percentage change in the BOLD signal (averaged across participants for each length of working memory delay) separately for each length of working memory delay (Fig. 3, C through D, and 4, C through D).


13. For any voxel, the sum of all modeled covariates multiplied by their empirically derived parameter es-
timates provides the best fit to the data for each participant. These fitted data were temporally re-
aligned to the start of working memory trials and averaged across participants for each length of working memory trial. These data were scaled to represent best-fitted percentage change in the BOLD signal (z axis) over time (x axis), separately for each length of memory (y axis) (Fig. 3, C through D and 4, C through D).


33. Normal anatomic space was defined according to J. Talairach and P. Tournoux, A Co-planar Stereotactic Atlas of the Human Brain (Thieme, Stuttgart, Germany, 1988), using the Montreal Neurological Institute template.


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**REPORTS**

**Generalized Potential of Adult Neural Stem Cells**

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The differentiation potential of stem cells in tissues of the adult has been thought to be limited to cell lineages present in the organ from which they were derived, but there is evidence that some stem cells may have a broader differentiation repertoire. We show here that neural stem cells from the adult mouse brain can contribute to the formation of chimeric chick and mouse embryos and give rise to cells of all germ layers. This demonstrates that an adult neural stem cell has a very broad developmental capacity and may potentially be used to generate a variety of cell types for transplantation in different diseases.

Multicellular organisms are formed from a single totipotent stem cell. As this cell and its progeny undergo cell divisions, the potential of the cells becomes restricted, and they specialize to generate cells of a certain lineage. In several tissues a stem cell population is maintained in the adult organ, and it may generate new cells continuously or in response to injury. The adult brain and spinal cord retain neural stem cells that can generate neurons, astrocytes, and oligodendrocytes (5–3).

Two stem cell populations have recently been identified in the adult central nervous system—ependymal cells (6) and subventricular zone astrocytes (7)—although it is not yet clear whether these two cell types represent independent populations or whether they share a lineage relationship (3, 8). These cells can be cultured as clonal cell aggregates referred to as neurospheres (9).

Most of the available data indicate that progeny produced by nervous system stem cells is limited to neural cell fates (1–5). It is, however, possible that the cellular fates generated by adult neural stem cells are restricted because of the limitations imposed on them by the particular environment in which they have been evaluated. In line with this, neural stem cells isolated from the adult forebrain were recently shown to be capable of repopulating the hematopoietic system and produce blood cells in irradiated adult mice (10). However, because this method of addressing the potency of neural stem cells fell within the limits of the hematopoietic system, their repertoire of progeny was still restricted. We have expanded the question of the differentiation potential of adult neural stem cells by exposing them to different inductive environments.

Embryonic stem (ES) cells are totipotent and can be induced to differentiate into a variety of cell types when cultured as embryoid bodies (11). We reasoned that instructive signals for differentiation to diverse lineages must be present in these cultures. To evaluate the capacity of instructive signals from ES cells to guide the differentiation of neural stem cells, we cultured adult neural stem cells together with embryoid bodies. The neural stem cells, derived from ROSA26 mice (12), express β-galactosidase (β-Gal), enabling identification of their progeny by X-Gal histochemistry or with antibodies against β-Gal (13). Moreover, ROSA26 cells express the neurokinin resistance gene, which allowed us to later eliminate the G418-sensitive ES cells from the cocultures and specifically study the remaining resistant neural stem cell–derived...

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cells. When cultured with embryoid bodies, neural stem cell progeny was frequently found to display immunoreactivity for desmin (Fig. 1, A and B), an intermediate filament protein expressed by myocytes (14). Moreover, many of the neural stem cell–derived cells fused to form muscle cell–like syncytia and showed immunoreactivity to the myocyte-specific protein, myosin heavy chain (15) (Fig. 1, C and D). These cells did not display any signs of cellular transformation. Neural stem cells cultured under the same conditions, but in the absence of ES cells, did not express these markers, nor did they form syncytia. We did not find evidence for differentiation to endodermal cell fates by immunohistochemistry with an antibody against an endoderm-specific epitope [TROMA-1 (16)]. This observation is consistent with the fact that endodermal differentiation is rare in embryoid body cultures, in contrast to muscle cell differentiation, which is commonly observed. These data demonstrate that adult neural stem cell–derived cells can adopt a muscle fate in vitro. This finding is substantiated by reports of myocyte generation in the embryonic mouse neural tube as well as from a medulloblastoma cell line (17, 18).

To analyze the differentiation potential of adult neural stem cells in vivo, we assayed their ability to contribute to the formation of various tissues by introducing them into the early embryonic environment and observing the fate of their progeny. The lineage restriction of rodent neural crest cells was previously tested by transplantation to stage 18 chick embryos (19, 20). We injected adult mouse brain neural stem cells into the amniotic cavity of stage 4 chick embryos (21). We reasoned that this may allow some neural stem cells to integrate into the primitive ectoderm that faces the amniotic cavity and to become distributed in the definitive ectoderm, endoderm, and mesoderm during gastrulation (Fig. 2, A and B). The cells would then be exposed to various inductive environments in the different germ layers. Of the embryos that survived the injection of the neurospheres (26%), 24 out of 109 were chimeric and contained lacZ-positive cells derived from the adult neural stem cells. No chimeric embryos were derived from injections of dissociated neurosphere cells. The specificity of the X-Gal staining in chimeric embryos was supported by overlapping immunohistochemical labeling with antisera against β-Gal (21).

The murine origin of these cells was further confirmed by overlabeling immunoreactivity to the mouse-specific epitope H-2Kb (21) (Fig. 2F). In addition to the nervous system, where reproducibly a high degree of chimerism was seen, lacZ-expressing cells were frequently found in mesodermal derivatives such as the mesonephros and notochord, as well as in epithelial cells of the liver and intestine, which are of endodermal origin (Table 1 and Fig. 2). In the tissues containing lacZ-expressing cells, there was a mosaic pattern of labeling with a varying proportion of neural stem cell–derived cells (Fig. 2). The lacZ-expressing cells had indistinguishable morphology from surrounding host cells and expressed markers normally present in cells of the particular tissue, for example, Pax2 in mesonephric tubule cells (Fig. 2G) and keratin in epidermal cells (21).

To test whether a single neural stem cell may have the potential to generate progeny that can differentiate into various cell types, we established clonal cultures by transferring single cells from dissociated neurospheres to microwells with a micropipette (21). Neurospheres derived from single-cell cultures were tested in the chick assay and were found to have the same broad differentiation potential as described above, demonstrating that a single adult neural stem cell has the potential to generate progeny of various lineages. To specifically test the differentiation potential of an identified neural stem cell, we cultured cells from the lateral ventricular wall of animals that had received an injection of the fluorescent dye Dil (1,1’-dioctadecyl-3,3,3’,3’-tetramethyldifluorobenzocyanine perchlorate) (21). In such cultures, Dil-labeled neurospheres from ependymal cells (6) were isolated with a micropipette and injected into the amniotic cavity of chick embryos. These ependyma-derived neural stem cells showed
the same broad differentiation potential as described above (16).

Chimeric mice can be generated by aggregation of ES cells with morulae or injection into blastocysts, which are then implanted in pseudopregnant foster mothers (22). In addition, chimeric mice have been generated from blastocysts injected with adult hematopoietic stem cells (23). This study demonstrated that adult bone marrow–derived stem cells can integrate in the early embryonic environment, although only their potential to contribute to the hematopoietic system was assessed in this study (23). To test the ability of adult neural stem cells to contribute to the generation of tissues in the developing mouse embryo, dissociated cells from a neurosphere were aggregated with morulae and allowed to continue their development in vitro to the early blastocyst stage (21). Many lacZ-positive cells integrated into the trophectoderm layer, but only rarely were cells found in the inner cell mass of the developing blastocyst (Fig. 3A). More efficient contribution of neural stem cells to the inner cell mass was acquired by microinjecting the cells directly into early blastocysts. Aggregated morulae and injected blastocysts were transferred to foster mothers and allowed to develop until embryonic day 11. Analysis of lacZ expression in embryos by X-Gal histochemistry, immunohistochemistry, and reverse transcription–polymerase chain reaction (RT-PCR) (21) (Figs. 3 and 4) revealed that 1% (6 out of 600) of embryos derived from aggregation or injection of dissociated cells and 12% (11 out of 94) of embryos generated by injection of a neurosphere contained cells derived from adult neural stem cells. In all litters, most of the embryos completely lacked lacZ-expressing cells, and among the positive embryos, there was a varying degree of mosaicism. Chimeric embryos were of similar size as nonchimeric embryos and did not display any overt anatomical abnormalities (Fig. 3C). The specificity of the X-Gal staining in chimeric embryos was confirmed by overlapping immunohistochemical labeling with antisera against β-Gal (Fig. 4, A and B). In several embryos derived from injected blastocysts, extensive contribution of the ROSA26-derived neural stem cells was seen in the embryonic central nervous system, heart, liver, intestine, and other tissues (Figs. 3C and 4 and Table 1). lacZ-expressing cells expressed markers typical for the tissue in which they had incorporated, for example, desmin in heart, cytokeratin 20 in intestinal epithelium, and albumin in liver (Fig. 4). Perhaps the most striking indication that these cells may take on functions appropriate for the newly formed tissue was the finding of beating hearts of apparent normal anatomy in chimeric mouse embryos having a very large contribution of neural stem cell–derived cells in the heart. These data demonstrate that adult neural stem cells can integrate into the developing chick and mouse embryo, give rise to embryonic cells of various fates, and contribute to the generation of tissues and organs of all germ layers. Thus, the present study focuses on the integration and differentiation of adult neural stem cells into embryonic tissues and Table 1).

Fig. 3. Generation of chimeric mouse embryos. (A) X-Gal staining of a blastocyst developed in vitro from a morula aggregation experiment shows neural stem cell–derived cells (blue) in the inner cell mass. (B) RT-PCR detection of β-Gal mRNA in ROSA26-derived adult neural stem cells (+), amnion (a), head region (h), trunk (t), and caudal part (c) of a wild-type embryo (Control) and embryos generated by blastocyst injection of ROSA26 neural stem cells [injected, embryos other than shown in (C)]. Primers for the L19 gene were included in all reactions as an internal control. (C) X-Gal staining of an embryonic day 11 wild-type embryo (left) and a mouse embryo generated from a blastocyst into which adult neural stem cells were injected (right). Some endogenous staining is seen in the area of the otic vesicle in the wild-type embryo. Bars, 20 μm (A) and 1 mm (C).

Fig. 4. Contribution of adult neural stem cells to the formation of mesodermal and endodermal tissues in the mouse. (A and B) Adjacent cross sections through the abdominal cavity of a low-degree embryonic day 11 chimeric embryo, displaying X-Gal labeling (A) and β-Gal immunoreactivity (B) in epithelial cells of the stomach (s) and intestine (l). (C) Immunoreactivity for the epithelial marker cytokeratin 20 in lacZ-expressing adult neural stem cell–derived cells (D). (E) Cross sections of a highly chimeric embryo labeled with an antibody against β-Gal, demonstrating abundant contribution of adult neural stem cell progeny in liver and heart. (F) Neural stem cell–derived cells in the heart (β-Gal immunoreactivity, green) of another chimeric embryo also show immunoreactivity for the myocyte marker desmin (red). In the liver, neural stem cell progeny (X-Gal labeling, blue) are immunoreactive for the hepatocyte-specific protein albumin (red) (G). A detail from (G) is shown at higher magnification in the inset. Arrow in (G) indicates the right lung bud. Bars, 100 μm (B and E), 50 μm (D and F), and 10 μm (G).
is intriguing because the adult neural stem cells can differentiate along this lineage after transplantation to irradiated adult mice (10). It is well established from ES cell studies that the strain background of the ES and host cells have a large influence on the degree of chimerism in specific tissues and that certain strain combinations result in no or very low chimerism in certain organs (24). Thus, although the ES cells are totipotent, they do not display their full potential in certain situations. In line with this, it appears that the neural stem cells may have a broader differentiation potential than revealed in the chick and mouse embryo assays.

In addition to the demonstration of blood cell generation by neural stem cells (10), there have recently been indications that other stem cell populations may not be restricted to generating cell types specific for the tissue in which they reside. Thus, marrow stromal cells transplanted to the brain can generate astrocytes (25). Moreover, hematopoietic stem cells can give rise to myocytes, and muscle progenitor cells can generate blood cells (26, 27). Together with the data presented here, these studies suggest that stem cells in different adult tissues may be more similar than previously thought and perhaps in some cases have a developmental repertoire close to that of ES cells.

References and Notes

13. Neurosphere cultures were established as described [6, 21].
16. D. L. Clarke et al., data not shown.
21. A detailed description of methods and additional figures can be found at Science Online at www.sciencemag.org/feature/data/1047254.shl .
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Table 1. Frequency of chimerism in various tissues in chick and mouse embryos containing neural stem cell progeny.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Chick (%)</th>
<th>Mouse (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forebrain</td>
<td>71 (17/24)</td>
<td>53 (9/17)</td>
</tr>
<tr>
<td>Midbrain</td>
<td>71 (17/24)</td>
<td>53 (9/17)</td>
</tr>
<tr>
<td>Hindbrain</td>
<td>71 (17/24)</td>
<td>53 (9/17)</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>96 (23/24)</td>
<td>59 (10/17)</td>
</tr>
<tr>
<td>Epidermis</td>
<td>79 (19/24)</td>
<td>65 (11/17)</td>
</tr>
<tr>
<td>Notochord</td>
<td>96 (23/24)</td>
<td>94 (16/17)</td>
</tr>
<tr>
<td>Mesonephric epithelium</td>
<td>92 (22/24)</td>
<td>71 (12/17)</td>
</tr>
<tr>
<td>Mesonephric mesenchyme</td>
<td>92 (22/24)</td>
<td>71 (12/17)</td>
</tr>
<tr>
<td>Somites</td>
<td>71 (17/24)</td>
<td>71 (12/17)</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>38 (9/24)</td>
<td>59 (10/17)</td>
</tr>
<tr>
<td>Lung epithelium</td>
<td>45 (9/20)</td>
<td>59 (10/17)</td>
</tr>
<tr>
<td>Stomach epithelium</td>
<td>83 (20/24)</td>
<td>82 (14/17)</td>
</tr>
<tr>
<td>Stomach wall</td>
<td>83 (20/24)</td>
<td>71 (12/17)</td>
</tr>
<tr>
<td>Intestinal epithelium</td>
<td>96 (23/24)</td>
<td>76 (13/17)</td>
</tr>
<tr>
<td>Intestinal wall</td>
<td>83 (20/24)</td>
<td>65 (11/17)</td>
</tr>
<tr>
<td>Liver</td>
<td>92 (22/24)</td>
<td>94 (16/17)</td>
</tr>
</tbody>
</table>

and raises many intriguing questions regarding the long-term commitment and survival of these cells.

Although we reproducibly found neural stem cell progeny in various organs in chick and mouse embryos, other tissues contained no lacZ-expressing cells. For example, we did not detect any contribution to the hematopoietic system in the models we used. This