During CNS development, cell migrations play an important role, adding to the cellular complexity of different regions. Earlier studies have shown a robust migration of cells from basal forebrain into the overlying dorsal forebrain during the embryonic period. These immigrant cells include GABAergic neurons that populate the cerebral cortex and hippocampus. In this study we have examined the fate of other basal forebrain cells that migrate into the dorsal forebrain, identifying basal cells using an antibody that recognizes both early (dlx1/2) and late (dlx 5/6) members of the dlx homeobox gene family. We found that a subpopulation of cortical and hippocampal oligodendrocytes are also ventrally-derived. We traced the origin of these cells to basal multipotent stem cells capable of generating both GABAergic neurons and oligodendrocytes. A clonal analysis showed that basal forebrain stem cells produce significantly more GABAergic neurons than dorsal forebrain stem cells from the same embryonic age. Moreover, stem cell clones from basal forebrain are significantly more likely to contain both GABAergic neurons and oligodendrocytes than those from dorsal. This indicates that forebrain stem cells are regionally specified.

Whereas dlx expression was not detected within basal stem cells growing in culture, these cells produced dlx-positive products that are capable of migration. These data indicate that the developing cerebral cortex incorporates both neuronal and glial products of basal forebrain and suggest that these immigrant cells arise from a common progenitor, a dlx-negative basal forebrain stem cell.

Key words: CNS stem cells; oligodendrocytes; GABAergic neurons; telencephalon; cerebral cortex; basal forebrain; progenitor cells; cell fate; cell migration

The traditional view of cerebral cortical development, in which it arises solely from endogenous germinal zones, has been altered by recent studies demonstrating that some cortical cells originate in the basal forebrain (de Carlos et al., 1996; Anderson et al., 1997; Tamamaki et al., 1997; Tan et al., 1998; Lavdas et al., 1999; Wichterle et al., 1999; Anderson et al., 2001). The earliest migrating cells, starting at approximately embryonic day 12 (E12), appear to come from the medial ganglionic eminence (MGE) and migrate robustly into the ventricular (VZ), subventricular (SVZ), and intermediate zones (Lavdas et al., 1999; Wichterle et al., 1999; Anderson et al., 2001). A second migration starts at approximately E14, appears to come from the lateral ganglionic eminence (LGE), and has a more confined migratory route into the SVZ and VZ (Anderson et al., 1997, 2001). The homeobox gene *dlx* is expressed primarily by ventral cells and is functionally involved in their migration (Anderson et al., 1997). Many of the immigrant cells differentiate into GABAergic interneurons, however not all dlx-positive cells acquire this fate, and some remain in a mitotic state (Anderson et al., 2001). These findings encouraged us to assess the fate of other dlx-positive cells in the cortex. Because their destinations include developing white matter tracts, we examined whether some of the basal cells are of the oligodendrocyte lineage.

The idea of a basal (ventral) origin for forebrain oligodendrocytes is appealing given that in the spinal cord oligodendrocytes originate in the ventral VZ and migrate dorsally to colonize spinal white matter tracts (Orentas and Miller, 1996). Oligodendrocytes are stimulated to develop in the ventral region of the cord by sonic hedgehog (shh), and neuregulin, produced by notochord and floor plate (Pringle et al., 1996; Richardson et al., 1997; Orentas et al., 1999; Vartanian et al., 1999). In the brain, detection of the early oligodendrocyte markers PDGF-α receptor and plp/DM20 also suggests a few localized, primarily ventral sites of origin (Spaksky et al., 2000). In the early mouse forebrain, PDGFR-α expression is seen in the MGE and dorsal thalamus, and plp/DM20 is found in the basal plate of the diencephalon, zona limitans intrathalamica, caudal hypothalamus, entopeduncular area, amygdala, and olfactory bulb (Pringle and Richardson, 1993; Spaksky et al., 1998; Nery et al., 2001). Olig-1 and 2, basic helix-loop-helix genes expressed early in oligodendrocyte development are also found in these localized sites, preceded by shh expression (Lu et al., 2000; Zhou et al., 2000; Nery et al., 2001). Hence, the parallels between localized, shh-dependent ventral oligodendrocyte development in spinal cord and in brain are strong, making the idea of an analogous dorsal migration plausible.

In this study we show that the dlx-positive immigrant cells from basal forebrain found in dorsal forebrain regions include oligodendrocyte lineage cells. A clonal analysis indicates that these oligodendrocytes originate from basal forebrain stem cells that also produce abundant GABAergic neurons but are themselves dlx-negative. Hence, these data identify a common progenitor for both neurons and glia that migrate from basal into dorsal forebrain during development.
MATERIALS AND METHODS

Animals, tissue dissociation, and cell culture

Timed-pregnant Swiss-Webster mice (Taconic, Germantown, NY) were used; the plug date is considered E0. Embryonic forebrain tissue was dissected and enzymatically dissociated in papain, then triturated gently and allowed to settle to produce a single cell suspension containing over 85% viable cells, as described previously (Qian et al., 2000). Single cells were plated at moderate density (30–40 cells/well) or at clonal density (1–5 cells/well) into poly-l-lysine (PLL)-coated Terasaki microwells in serum-free medium containing DMEM (Life Technologies, Rockville, MD) with B-27, N2 (Life Technologies), and 10 ng/ml basic fibroblast growth factor (Life Technologies). The cells were then incubated at 35°C, 5% CO2, 95% humidity. Optic nerves from postnatal day 5 (P5) mice were dissected and dissociated in papain (Wang et al., 1998) and plated into PLL-coated Terasaki wells in culture medium.

Immunopanning

P5 rat cortices were dissected and dissociated enzymatically using trypsin (Ingraham et al., 1999). After trituration, the dissociated cell suspension was passed through a mesh membrane to enrich for single cells. The cells were labeled with a mature oligodendrocyte marker, O1 antibody (a gift from Dr. Ken McCarthy, University of North Carolina, Chapel Hill, NC), which recognizes galactocerebroside, and plated on 35 mm Petri dishes precoated with secondary antibody (Jackson ImmunoResearch, West Grove, PA). After several washes, the galactocerebroside-expressing cells attached to the dishes were collected using a sheering buffer and plated into PLL-coated Terasaki microwells. Cells were stained live with O4 antibody (a gift from Dr. Anthony Gard, University of South Alabama, Mobile, AL), fixed acutely, and then stained for dlx.

Clonal analysis

Dissociated single cells from embryonic mouse cortex, LGE, or MGE were plated and cultured at clonal density in serum-free culture medium [B-27 plus N2 (Life Technologies) plus 10 ng/ml FGF-2] as described previously (Qian et al., 2000). Cells were observed in the inverted microscope and mapped every day for up to 12–13 d, with feeding every 2–3 d. Clones were then processed for immunohistochemistry, using live staining for O4 antibody and fixation with 4% paraformaldehyde before staining for other markers, including NG2, glutamic acid decarboxylase (GAD), β-tubulin III, RC2 (Developmental Studies Hybridoma Bank, Iowa City, IA) and Nestin (Developmental Studies Hybridoma Bank).

Immunostaining

Cryostat sections. Fixed embryos were frozen in O.C.T. TissueTek on dry ice. We cut 12 μm cryostat sections, then incubated them in a blocking solution of 0.1% Triton X-100 and 1% normal goat serum (NGS) in PBS for 15 min before staining for dlx and NG2.

Acutely isolated cells and cultured cells. After dissociation, cells were plated into culture wells for <1 hr for acute staining or cultured for a number of hours or days for later time points. Plated cells were washed with Dulbecco’s PBS with calcium and magnesium (CMPBS), fixed in ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (PBS) with calcium and magnesium (CMPBS), fixed in ice-cold 4% paraformaldehyde before staining for other markers, including NG2, glutamic acid decarboxylase (GAD), β-tubulin III, RC2 (Developmental Studies Hybridoma Bank, Iowa City, IA) and Nestin (Developmental Studies Hybridoma Bank).

RESULTS

The basal cell marker dlx is expressed in oligodendrocyte progenitor cells in embryonic mouse dorsal forebrain white matter

Four dlx genes (dlx 1, 2, 5, and 6) are found in the CNS where they show restricted patterns of expression in ventral forebrain from early stages, indicating that they play a role in differentiation of this region. Dlx 1 and 2 have almost identical expression patterns, being low in the telencephalon at E10, and increasing rapidly in the basal region so that by E12 they are readily detectable in the LGE and MGE, while remaining at extremely low levels in the overlying cerebral cortex (Bulfone et al., 1993; Porteus et al., 1994; Anderson et al., 1997, 2001; Liu et al., 1997; Eisenstat et al., 1999). Dlx 1 and 2 are strongly expressed in progenitor cells, initially in the VZ, and then in the SVZ. Later, as the cells mature, dlx1 and 2 stimulate expression of dlx 5 and 6 in the same cells, now located in the SVZ and in the mantle zone (Liu et al., 1997; Eisenstat et al., 1999). To quantitate the percentage of dlx-positive cells in the developing mouse cerebral cortex and basal forebrain, we enzymatically dissociated forebrain cells and stained them acutely using an antibody that recognizes dlx 1, 2, 5, and 6 (Panganiban et al., 1997). Fifty-five percent of acutely dissociated LGE cells at E14 are dlx-positive, compared with <1% of cortical cells. Using this antibody we can now trace the development of dlx-positive cells for a longer period than using antibodies that detect dlx1/2 alone. Previous reports of dlx1/2 expression revealed a few cells apparently entering the cerebral cortex from basal forebrain areas. In coronal sections of E13–E14 mouse forebrain stained using the pan-dlx antibody, prominent streams of dlx-positive cells from the basal forebrain deep into the cerebral cortex are visible (Fig. 1). One main stream can be tracked into the marginal zone, and the other into the intermediate zone. This clear distribution of dlx-positive cells apparently streaming from basal to dorsal regions provides an image consistent with previous reports documenting basal cell migration into the cortex (de Carlos et al., 1996; Anderson et al., 1997, 2001; Tamamaki et al., 1997; Tan et al., 1998; Lavdas et al., 1999; Wichterle et al., 1999). Because some of the dlx-positive cells were entering regions known to develop into white matter, we decided to examine whether any of the dlx-positive cells contribute oligodendrocytes to cortical white matter tracts. By E18, as shown in Figure 2, dlx-positive cells were detected in three major areas of developing dorsal white matter: subcortical white matter, corpus callosum, and fimbria. In contrast, no dlx-negative cells were detected in the optic tract in the ventral, diencephalic region. The number of dlx-positive cells in these regions of developing white matter was quantified by double labeling the dlx-stained sections with DAPI, which labels cell
nuclei. Dlx-expressing cells comprised 5–13% of total cells present in sections of these three areas of dorsal forebrain white matter at E18, but were absent from the optic tract at this age.

To examine whether these dlx-positive cells were in the oligodendrocyte lineage, we first double-immunolabeled E18 sections with NG2, a surface marker expressed on early oligodendrocyte progenitor cells (Dawson et al., 2000). Double-labeled cells were visible in the emerging white matter tracts (Fig. 3). To quantify the double-labeled population, E18 forebrain tissue was enzymatically dissociated to single cells, which were stained acutely (Fig. 4). Seventeen percent of cortical and 44% of striatal NG2-positive cells at E18 were dlx-positive. Given that essentially all of the dlx-positive cells that are detected in the cerebral cortex are believed to migrate from basal areas (Anderson et al., 1997, 2001), these data suggest that some of the early oligodendrocyte progenitor cells originate from the basal forebrain.

A subpopulation of postnatal cortical oligodendrocytes expresses the basal cell marker dlx

To determine whether these dlx-positive oligodendrocyte progenitor cells developed into mature oligodendrocytes, we examined dlx expression in acutely isolated O1-immunopanned oligodendrocytes from P5 rat cortex. O1 antibody, which recognizes galactocerebrosid, labels primarily postmitotic oligodendrocytes (Warrington and Pfeiffer, 1992). As shown in Figure 5, a substantial fraction, nearly 40%, of O1-immunopanned cells expressed the basal cell marker dlx. In contrast, none of the O4-positive or O1-positive oligodendrocytes obtained from the P5 optic nerve expressed dlx. These data indicate that basal-derived oligodendrocyte progenitor cells contribute substantially to the mature oligodendrocyte population in dorsal white matter, migrating into the subcortical white matter, the corpus callosum, and into the fimbria. Because not all basal cells express dlx and because dlx expression is transient, declining with development (Porteus et al., 1994), it is possible that we might have underestimated the contribution of basal oligodendrocytes to dorsal white matter tracts. The fact that no dlx was detectable in optic nerve shows that dlx is not a general marker of developing oligodendrocytes, and that dlx-positive oligodendrocyte lineage cells do not migrate into this region of white matter. These observations suggest that just as basal-derived GABAergic neurons can migrate dorsally into cerebral cortex and even into hippocampus (Anderson et al., 1997; Pleasure et al., 2000), some basal-derived oligodendrocyte lineage cells can migrate long distances into dorsal white matter tracts, including the hippocampal white matter.

Basal oligodendrocytes arise from multipotent stem cells that produce both neurons and glia

Previous studies in murine spinal cord and cerebral cortex have shown that early in development oligodendrocytes arise from multipotent stem cells, cells that also make neurons. These cells produce neuronal progeny first and glia later. Hence, restricted oligodendrocyte progenitor cells are rare early in development and become abundant at later times (Levison and Goldman, 1997; Rao, 1999; Rogister et al., 1999; Qian et al., 2000). To understand more about the progenitor cells in the basal forebrain that produce oligodendrocytes, we performed a clonal analysis at different embryonic ages.

The E11–E14 basal forebrain is composed primarily of dividing progenitor cells. Like the cortical germinal zone, the basal forebrain germinal zone is composed of different types of progenitor cells, including restricted progenitors that produce solely neurons or glia and multipotent stem cells that produce both neurons and glia (Temple, 1989; Reynolds et al., 1992; Birling and Price, 1998). By plating single progenitor cells from basal forebrain in a standardized culture environment and following their division and differentiation in vitro, we could assess whether the cells that produced oligodendrocytes were restricted to that lineage or whether they were multipotent. Single cells from E11.5–E13.5 basal forebrain were plated at clonal density in Terasaki wells, and clones were followed for 5–12 d. The clones were then stained for NG2 or O4 to label oligodendrocyte lineage cells and for β-tubulin III to label neuronal progeny. When clones derived from E11.5 cells were analyzed by this method, we found that 95% of basal forebrain (LGE) progenitors that gave rise to oligodendrocytes were multipotent stem cells that produced both neurons and glia, whereas only 5% were restricted progenitor cells that generated solely glia. By E13.5, however, 50% of the oligodendrocyte-generating progenitor cells were multipotent stem cells, whereas 50% were restricted glioblasts (Fig. 6). These data suggest that at early stages, multipotent stem cells are the main source of oligodendrocytes in the basal forebrain region and that they produce restricted glial progenitor cells that begin to predominate at later stages, as shown previously for spinal cord and cerebral cortex. We conclude that oligodendrocytes found in dorsal white matter that express the basal marker dlx originate from basal forebrain multipotent stem cells.

Stem cells from basal forebrain preferentially generate GABAergic neurons and oligodendrocytes

Given that both GABAergic neurons and oligodendrocytes migrate into the cerebral cortex and that the oligodendrocytes arise from basal stem cells, we asked whether the basal stem cells were a common precursor for these two types of cells. Hence, we examined the neuron and glial content of clones derived from single progenitor cells from E12–E14 cortex, LGE and MGE using the marker GAD to identify GABAergic neurons (Fig. 7).

Comparing the types of neuron produced by the progenitor populations as a whole, we found that basal forebrain progenitor
cells produced significantly more GABAergic neurons than cortical progenitor cells: 91 ± 5% of total neurons developing from E12–E14 MGE progenitor cells were GAD-positive, compared with 75 ± 5% from LGE and only 31 ± 9% from cortex (Fig. 7B). This is consistent with in vivo studies showing 80–90% of neurons in the basal ganglia are GABAergic, compared with 20–40% of neurons in the cortex (Hendry et al., 1987; Smith and Bolam, 1990; Graybiel, 1990; Parnavelas, 1992; Kita, 1993).

We then examined the stem cell clones within the dorsal and basal forebrain progenitor cell populations (Fig. 7). Of the total E13 cells plated, the percentage of stem cell clones generated under these conditions was similar for all three regions, with a slight increase in frequency from basal to dorsal: 5.7% for MGE, 8.3% for LGE, and 11.6% for cortex. Within stem cell clones, the proportion of GAD-positive neurons decreased from basal to dorsal areas: 85% of the neurons in MGE clones were GAD-
positive, compared with 46% for LGE and 42% for cerebral cortex (Fig. 7C). Basal stem cells from E13 MGE and LGE were significantly more likely (1.5-fold) to contain both GAD-positive neurons and oligodendrocytes than stem cells from E13 cortex (Fig. 7D). Thus, although LGE stem cell clones only contained 46% GAD-positive neurons, similar to the proportion for cortical stem cell clones, they were more likely than cortical clones to contain both these cell types. Taken together, these data indicate that basal forebrain progenitor cells are primed to make GABAergic neurons and oligodendrocytes.

To examine the dlx expression within basal stem cell clones, we stained developing clones growing in serum-free medium supplemented with FGF2 for dlx and cell-type-specific markers (Fig. 8). After 5 d, stem cell clones were identified as rapidly growing clones that contained /H9252-tubulin III-positive neuronal progeny, NG2-positive glial progenitor cells, and dividing stem cells that are negative for these markers but positive for the progenitor markers nestin and RC2. These criteria have been shown to characterize stem cell clones in these cultures (Davis and Temple, 1994; Qian et al., 2000) (our unpublished observations). The clones were then examined immunohistochemically for dlx expression. In all cases, dlx expression overlapped with the differentiation markers used, /H9252-tubulin III and NG2, whereas progeny that were negative for these markers did not stain for dlx, suggesting basal stem cells are dlx-negative.

DISCUSSION
A basal origin for dorsal forebrain oligodendrocytes
Our observation of numerous dlx-positive cells that coexpress early and mature oligodendrocyte markers in developing forebrain white matter indicates that ventral tissue may normally be a substantial source of dorsal forebrain oligodendrocytes. This augments previous findings of localized ventral sites of oligodendrocyte origin in the forebrain (Thomas et al., 2000; Nery et al., 2001). Why was an oligodendrocyte fate not noted previously for cells migrating from basal populations? Dlx1/2 has not been shown to overlap with oligodendrocyte markers, however the antibody we used recognizes both the early ventral markers dlx 1/2 and the later markers dlx 5/6, which appear in more mature ventral cells (Anderson et al., 1997; Liu et al., 1997; Eisenstat et
This may have allowed us to colocalize dlx expression with the later-appearing oligodendrocyte markers. Although dlx1/2 knock-out animals clearly have reduced GABAergic cells in the cerebral cortex, an influence on oligodendrocyte production might have gone undetected because the mutants die around birth, before the major onset of oligodendrocyte begins (Anderson et al., 1997).

The specific site of origin of dlx-positive dorsal forebrain oligodendrocytes is not clear: whether LGE, MGE, or from more caudal CNS sites that express this marker. In the Nkx2.1 mutant mouse, in which MGE is converted to LGE, there is a dramatic loss of oligodendrocytes (Sussel et al., 1999; Nery et al., 2001), suggesting that MGE is a more significant source of oligodendrocytes in vivo than LGE. Our observation that dlx-positive cells are not found in the optic tract suggests that migrations of oligodendrocyte progenitor cells are regulated: hence, specific tracts may acquire oligodendrocytes from particular regional sources. Whether all dorsal forebrain oligodendrocytes arise from basal forebrain is not clear. In culture, isolated stem cell clones derived from the cerebral cortex from as early as E10 make abundant oligodendrocytes, even without addition of sonic hedgehog (Qian et al., 2000). However, whether they do so in vivo is not known. We did find a small percentage of NG2-positive cells from E18 mouse cerebral cortex that expressed Pax 6, which labels largely dorsal forebrain areas (Stoykova et al., 1996) (data not shown), suggesting that some cortical oligodendrocytes might be produced from endogenous dorsal stem cells. More extensive studies with specific regional markers should ascertain the specific origin of oligodendrocytes in different white matter tracts. These data suggest that white matter may be regionally chimeric, and thus imply a structural basis within white matter tracts that has not been appreciated previously.

Multipotent stem cells may be the basal source for GABAergic interneurons and oligodendroglia that migrate into the cerebral cortex

Previous studies in developing spinal cord and cerebral cortex indicate that oligodendrocytes arise from multipotent stem-like cells that also generate neurons (Williams et al., 1991; Levison and Goldman, 1997; Register et al., 1999; Qian et al., 2000). There are no indications of restricted oligodendrocyte progenitor cells present at very early times: these only arise later after they have been generated from stem cells. Our studies indicate that the same scenario applies to the LGE and MGE; perhaps it is general for the entire CNS. Hence, dlx-expressing oligodendrocytes found in the cerebral cortex come originally from basal stem cells.

The fact that LGE and MGE stem cell clones usually contained both GABAergic neurons and oligodendrocytes suggests that this stem cell may be a common precursor for both of these cell types that migrate from localized sites of origin. In the Nkx2.1 mouse, there is a dramatic loss of both oligodendrocyte lineage cells and GABA cells (Sussel et al., 1999; Nery et al., 2001). This could reflect disruption in the formation or differentiation of a common precursor for interneurons and oligodendrocytes; it would be interesting to examine the stem cell population in these mutants.
Alternatively, it is possible that although basal stem cells are a source of both GAD-positive neurons and oligodendrocytes, the GAD-positive neurons produced by these cells remain in the basal forebrain, whereas GAD-positive neurons made by other types of basal progenitor cells migrate into the cortex. This seems unlikely given that basal stem cells make GAD-positive neurons that express dlx, a protein that is necessary for migration into dorsal areas. Moreover, when we made time-lapse recordings of basal forebrain stem cells, we noted that they generated very motile neuronal and glial progeny, so that it was more difficult to follow lineage patterns from these clones than from dorsal clones (data not shown). This high motility, which has been described for basal forebrain cells previously (Wichterle et al., 1999), is consistent with the idea that basal stem cells generate progeny that migrate.

These studies indicate that stem cells from dorsal and basal forebrain areas are different, with the most prominent differences seen between MGE and cerebral cortex; for example MGE stem cells make twice as many GAD-positive neurons. These differences may indicate intrinsic heterogeneity among stem cell populations, either because of regional differences or developmental stage. One interpretation of these data is that ventral and dorsal signals act on stem cells to make them generate particular, region-appropriate cell types. Hence, basal forebrain stem cells are biased early in development to generate GAD-positive neurons that predominate in basal forebrain CNS areas. By allowing these
they can generate abundant oligodendrocytes (Register et al., 1999; Naft-Oumesmar et al., 1999; Cao et al., 2001; Akiyama et al., 2001). Does this reflect regulation of a GABAergic or oligodendrocyte fate decision, and if so what factors might direct the fate choice?

Our data indicate that forebrain stem cells do not express dlx. Within stem cell clones, dlx was always found in the later progeny of stem cells—β-tubulin III-positive neurons, NG2-positive glial progenitor cells, or O4-positive lineage cells—but not in the cells that lack these differentiation markers, which include the stem cells. In acute staining of E14 basal forebrain cell suspensions, 55% of LGE cells were dlx-positive; the remaining 45% dlx-negative cells could accommodate the stem cell population. If as we suspect, stem cells are dlx-negative, then proliferating dlx-positive cells that have been seen in vivo in cortical germinal zones around the time of birth (Anderson et al., 2001) may turn out to be dividing oligodendrocyte progenitor cells that exist in these areas throughout life (Levison et al., 1999) rather than stem cells.

The mechanism whereby oligodendrocyte-lineage cells migrate into the overlying cortex is not clear. Glial progenitor cells are highly migratory and disperse widely within the cortical SVZ (Kakita and Goldman, 1999). Given that dlx is functionally involved in GABAergic neuron migration (Anderson et al., 1997), it may well play a similar role in the migration of oligodendrocyte lineage cells. Interestingly, dlx-positive GABAergic cells first appear in the cerebral cortex before dlx-positive glial lineage cells are detected. Cortical stem cells produce neurons first and glial cells later (Qian et al., 2000); perhaps a similar timing mechanism operates within basal stem cells to regulate the time of production and migration of neurons and glia destined for the cerebral cortex. In conclusion, these data indicate that basal forebrain stem cells generate both neuronal and glial progeny that migrate widely within the forebrain. Elucidation of the mechanisms by which these multipotent cells are specified to make GABAergic neurons or oligodendrocytes will be of central importance for understanding forebrain development and maintenance.

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