

# Increased Neuronal Production, Enlarged Forebrains and Cytoarchitectural Distortions in $\beta$ -Catenin Overexpressing Transgenic Mice

Anjen Chenn and Christopher A. Walsh<sup>1</sup>

Department of Pathology, Northwestern University, Feinberg School of Medicine, Chicago, IL 60611-3008 and <sup>1</sup>Howard Hughes Medical Institute, Beth Israel Deaconess Medical Center and Department of Neurology, Harvard Medical School, Boston, MA 02115, USA

**$\beta$ -Catenin can function in the decision of neural precursors to proliferate or differentiate during mammalian neuronal development and may regulate cerebral cortical size by controlling the generation of neural precursor cells. Mice expressing high levels of a stabilized  $\beta$ -catenin transgene in neural precursors develop enlarged brains with expanded precursor populations, increased cerebral cortical surface area, and folds resembling sulci and gyri of higher mammals present at birth. Here we report the effects in adult mice expressing lower levels of the same stabilized  $\beta$ -catenin transgene in neural precursors. Adult transgenic animals develop enlarged forebrains with thin cerebral cortices with increased surface area, expanded subventricular zones with subcortical aggregations of neurons and enlarged, distorted hippocampi. The brains from transgenic mice also show apparent arrest of neuronal migration and dramatic disorganization of the layering of the cerebral cortex. These findings suggest that  $\beta$ -catenin can cause expansion of the precursor pool resulting in increased neuronal production and greater brain size and suggest a crucial role for  $\beta$ -catenin in neuronal migration and cortical lamination.**

## Introduction

What regulates cerebral cortical size? Humans possess an impressive repertoire of mental skills such as the abilities to read, write, and solve intricate problems. These skills are made possible by the cerebral cortex, the thin, layered sheet of neurons on the surface of the brain that underlies our most complex cognitive abilities. The cerebral cortex has undergone a disproportionate expansion in size during evolution, and this increase in the size of the cerebral cortex is thought to underlie the growth of intellectual capacity. The increased size of the cerebral cortex results primarily from a disproportionate expansion of the surface area of the cortex (Elias and Schwartz, 1969; Haug, 1987; Caviness *et al.*, 1995b; Finlay and Darlington, 1995; Rakic, 1995; Barton and Harvey, 2000; Clark *et al.*, 2001). It has been proposed that early changes in the ratio of symmetric (proliferative) versus asymmetric (neurogenic) divisions could influence the size of the cortical surface (Caviness *et al.*, 1995b; Rakic, 1995), but despite many hypotheses about how cortical size is regulated, few have been tested experimentally.

## $\beta$ -Catenin Regulation of Cortical Size

Recent studies have examined the role of  $\beta$ -catenin in the regulation of cerebral cortical size (Chenn and Walsh, 2002).  $\beta$ -catenin, an important component of adherens junctions (Cox *et al.*, 1996), interacts with proteins of the TCF/LEF family to transduce Wnt signals (Peifer and Polakis, 2000). Wnts (Parr *et al.*, 1993) and TCF/LEF family members (Oosterwegel *et al.*, 1993; Cho and Dressler, 1998) are expressed in the developing mammalian brain, and numerous studies support the role of Wnt signaling in cell fate regulation during brain development (McMahon and Bradley, 1990; Moon *et al.*, 1997; Galceran *et al.*, 2000; Lee *et al.*, 2000; Brault *et al.*, 2001).  $\beta$ -Catenin has been

implicated in many human cancers (Peifer and Polakis, 2000), including some bearing striking resemblance to neural precursors such as medulloblastoma (Huang *et al.*, 2000).  $\beta$ -Catenin is expressed highly in mammalian neural precursors, and  $\beta$ -catenin protein is enriched at adherens junctions at the lumen of the ventricle (Chenn and Walsh, 2002).

Embryonic transgenic mice expressing a stabilized form of  $\beta$ -catenin in neuronal progenitor cells developed grossly enlarged brains with greatly increased cerebral cortical surface area so extensive that the normally smooth cerebral cortex of the mouse formed convoluted folds resembling the gyri and sulci of higher mammals (Fig. 1) (Chenn and Walsh, 2002). The neural precursor population was markedly expanded in transgenic animals, and expression of different markers of cortical neuron populations indicated that despite the massive expansion of cortical surface area, transgenic precursors appeared to differentiate into young neurons in an approximately normal spatial pattern (Chenn and Walsh, 2002). Taken together, the expression studies suggested that over-activating  $\beta$ -catenin did not grossly disrupt the normal developmental sequence of neuronal differentiation, and the horizontal expansion of the cortical plate was a result of an increased number of proliferative precursor cells.

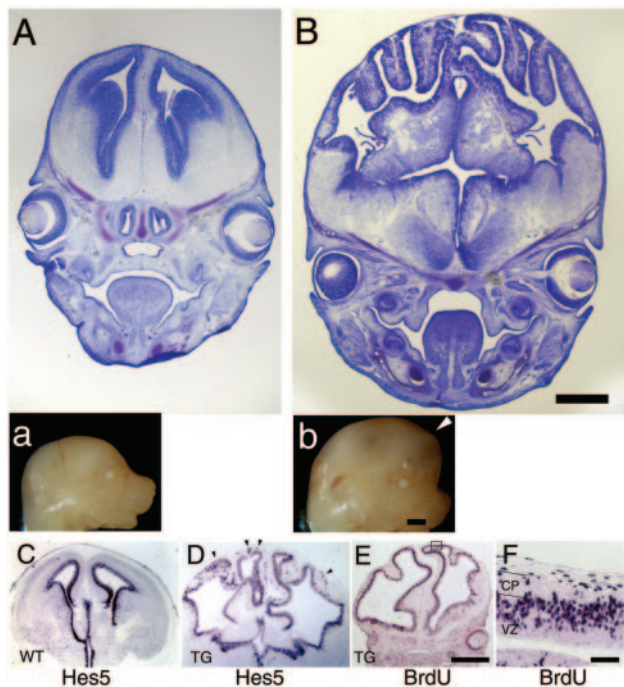
What caused the expansion in the progenitor pool? Enlargement of the precursor pool in transgenic brains can result from increased mitotic rates, decreased cell death, or changes in cell fate choice (whether to differentiate or to proliferate). Rates of cell division and cell death suggested that the progenitor cell population expansion was not caused by a simple effect of  $\beta$ -catenin on cell division, or by decreased apoptotic cell death. Instead, an approximate twofold increased proportion of transgenic precursors re-enter the cell cycle after dividing, when compared with wild-type neural precursors (Chenn and Walsh, 2002). Together, these studies suggested that  $\beta$ -catenin activation functions in neural precursors to influence the decision to re-enter the cell cycle instead of differentiate.

What are the long-term consequences of increasing the size of the neural precursor pool? Brain size may be highly regulated during development, with precise matching of neurons and targets. Cell death may compensate for excessive or unnecessary production of neurons. Our previous work did not address the phenotype of  $\beta$ -catenin transgenic animals after birth. Here, we examine the brains of adult transgenic animals overexpressing stabilized  $\beta$ -catenin in neural precursors at more modest levels, but still find remarkable changes in brain size and architecture.

## Materials and Methods

### Transgenic Mice

Standard molecular biology techniques were used to generate the transgenic constructs. The 5' UTR from the rat insulin II gene was amplified from embryonic rat genomic DNA using the primers



**Figure 1.** Enlarged brains and heads of  $\beta$ -catenin transgenic animals with horizontal expansion of precursor population. Mid-coronal section through the forebrain stained with cresyl violet of an embryonic day 15.5 wild-type littermate control (A) and comparable section of a transgenic animal (B) expressing a  $\Delta 90\beta$ -catenin–GFP fusion protein in neural precursors. The forebrain of transgenic animals is enlarged overall, with increased surface area and folding of the epithelial surface. Bar, 1 mm. Insets: Images of wild-type (a) and of transgenic (b) head reveals gross enlargement of the skull and forebrain vesicles protruding anteriorly (arrowhead) over the face of the embryo. Bar, 2 mm. (C, D) *In situ* hybridization for *Hes5* in comparable coronal sections through wild-type littermate control (C) and transgenic brain (D). *Hes5* is expressed in progenitor cells of in the ventricular zone of wild-type and transgenic brains. Additional areas of *Hes5*-expressing cells are located in ectopic regions away from the ventricular lumen in transgenic animals (arrowheads). Bar, 1 mm. (E, F) BrdU labeled cells in transgenic animals after 30 min exposure to BrdU. BrdU labels the same cells as the progenitor markers *Hes5* and *Hes1*. (F) Higher magnification image reveals overall organization of ventricular zone of transgenic animals is preserved, with S-phase progenitors occupying the outer half of the ventricular zone, similar to wild-type progenitors. Bar, 1 mm (E), 200  $\mu$ m (F). This figure is reproduced from Chenn and Walsh (Chenn and Walsh, 2002) with permission.

5'-TAAACGTCGACTAAGTGACCAGCTACAGTCGGAAA-3' and 5'-TGT-TAGGGCCCGTTGGAACAATGACCTGGAGA-3' and subcloned directly upstream of the  $\beta$ -catenin cDNA. Transgenic mice in the FVB/N strain were generated by transgenic mouse cores at the Children's Hospital Boston and Brigham and Women's Hospital.

#### Antibody Staining

Cryosections (10  $\mu$ m) were blocked with 5% donkey serum, 0.3% Triton X-100 in PBS for 30 min. Primary antibodies were diluted in blocking solution (1:2000, Research Diagnostics rabbit polyclonal). Peroxidase staining and counterstaining with Nuclear Fast Red using manufacturer's protocol (ABC kit, Vector) was used to visualize primary antibody binding. Silver staining and Luxol Fast Blue staining of paraffin-sectioned tissue was performed by the Children's Hospital Neuropathology Histology Service using standard techniques.

#### In Situ Hybridization

*In situ* hybridization was performed by the *in situ* hybridization core at Beth Israel Deaconess Medical Center. Non-radioactive *in situ* hybridization was performed as described (Berger and Hediger, 2001), using digoxigenin (DIG)-labeled cRNA probes. Probes were generated

from the following: *Reln* 0.7 kb from the 3' end of *Reln* cDNA sequence; *Hes5*: 992 bp full-length cDNA, *Hes1*: 708 bp of 5' end (*EcoRI*–*SmaI* fragment) of *Hes1* cDNA; *Tbr1*: 264 bp of 3' UTR (Bulfone *et al.*, 1995).

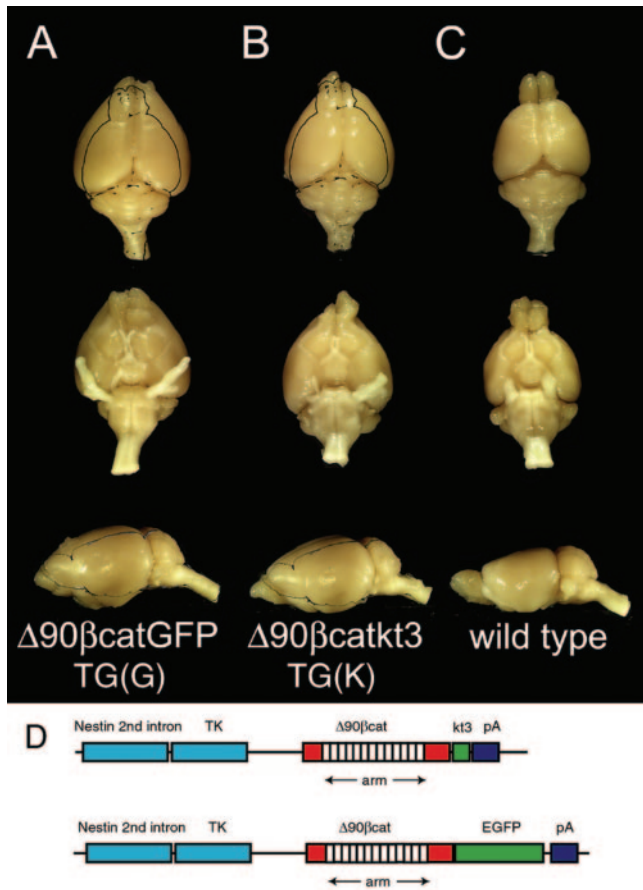
#### Results

Our previous work (Chenn and Walsh, 2002) showed that transgenic embryos expressing stabilized  $\beta$ -catenin in neural precursors at embryonic day 15.5 (E15.5) (Fig. 1), E17.5 and E19.5 (not shown) have grossly enlarged brains with a considerable increase in the surface area of the cerebral cortex without a corresponding increase in cortical thickness ( $n = 10$ ) (Fig. 1) (E15.5,  $n = 10$ ; E17.5,  $n = 6$ ; E19.5,  $n = 2$ ). The expression of a variety of markers specific for neuroepithelial precursors and differentiating neurons suggested that the neural precursor population was expanded in transgenic embryos (shown in Fig. 1: *Hes5* and BrdU). The results from embryonic transgenic animals suggest that  $\beta$ -catenin can regulate cerebral cortical size by controlling the generation of neural precursor cells.

#### Adult Brains from $\Delta 90\beta$ -Catenin Transgenic Mice Are Enlarged

The brains from the embryonic transgenic animals from this previous study were markedly abnormal, and although these animals were not examined postnatally, we anticipated that the cortical abnormalities observed would likely be incompatible with long-term survival. To examine the consequences of expanding the neural precursor pool on postnatal brain development, we generated transgenic mice using the same N-terminally truncated form of  $\beta$ -catenin fused at the C-terminal with green fluorescent protein (GFP) ( $\Delta 90\beta$ -catenin–GFP) used to generate embryonic transgenic mice (Chenn and Walsh, 2002), or fused at the C-terminal with the short epitope tag KT3. N-terminally truncated  $\beta$ -catenin no longer requires Wnt signaling for sustaining activity since it lacks key phosphorylation sites for GSK3 $\beta$  that normally target it for destruction in the absence of Wnts (Clevers and van de Wetering, 1997). This form of  $\beta$ -catenin is stabilized constitutively *in vivo*, and remains able to bind E-cadherin,  $\alpha$ -catenin, and activate transcription by binding with TCF/LEF co-factors (Clevers and van de Wetering, 1997; Barth *et al.*, 1997; Chenn and Walsh, 2002). The expression of  $\Delta 90\beta$ -catenin–GFP was driven by the enhancer element contained in the second intron of the nestin gene, which directs expression in CNS progenitor cells (Yaworsky and Kappen, 1999). To increase the likelihood that transgenic animals survive into adulthood, we modified the transgenic construct to express lower quantities of the transgene by omitting the rat Insulin II intron from the transgenic construct (Fig. 2D).

We found that transgenic mice generated using this construct occasionally ( $n = 5$ ) survived to adulthood, albeit infrequently. We found that brains from surviving adult transgenic animals generated with this attenuated construct were also enlarged when compared with wild-type, age-matched control animals, although not as severely affected as the embryos expressing very high levels of  $\beta$ -catenin (Fig. 2A,B). Here, we describe a more detailed histologic examination of two individual brains from these rare surviving animals, 'K' and 'G,' generated with either the epitope-tagged  $\Delta 90\beta$ -catenin–kt3 transgene (K) or the GFP-tagged  $\Delta 90\beta$ -catenin–GFP transgene (G) and sacrificed at 15 months. Both brains were similarly enlarged, and the enlargement of the brains appears to be restricted to the forebrain; the remainder of the brain appeared unaffected. The skulls of the transgenic animals were also notably larger grossly. These findings suggest that modest increases in  $\beta$ -catenin activation in



**Figure 2.** Images of adult (15 months) transgenic mouse brains overexpressing a stabilized form of  $\beta$ -catenin, compared with a wild-type, age-matched control brain.  $\Delta 90\beta$ -catenin transgenic brains are enlarged compared with normal. (A)  $\Delta 90\beta$ -catenin–GFP transgenic brain and (B)  $\Delta 90\beta$ -catenin–kt3 transgenic brain compared with (C) wild-type age-matched control brain. The brain weights were 867 mg (A), 780 mg (B) and 551 mg (C), respectively. The increase in size of the brains is due primarily to expansion of the forebrain, and the remainder of the brains exhibit no overt abnormalities. In these two examples, the olfactory bulbs were damaged in dissection, but other enlarged transgenic brains examined had normal sized olfactory bulbs (not shown). (D) Transgenic constructs used to generate long-survival transgenic animals. Constructs removing the NH<sub>2</sub> terminal amino acids of mouse  $\beta$ -catenin are fused either to EGFP or the kt3 epitope tag. The constructs used for adult transgenic animals remove the intron from the rat insulin II gene from the construct used previously (Chenn and Walsh, 2002) and hence provide less intense overexpression of the truncated form of  $\beta$ -catenin.

neural precursors results in marked forebrain enlargement compatible, albeit infrequently, with long-term survival.

#### Anatomic Characterization of Adult $\Delta 90\beta$ -Catenin Transgenic Mice Are Enlarged

To determine the anatomical features and better characterize the cells of the enlarged brains of adult  $\beta$ -catenin transgenic mice, we examined sections through the forebrain. Low magnification images of coronal sections through the rostral, middle and caudal forebrain reveal that the transgenic brains are markedly enlarged and anatomically distorted. The enlargement of the forebrains is characterized by changes in multiple structures, including markedly increased size of the subventricular zone, dilation of the lateral ventricles, alterations in cortical surface area, lamination and organization, and abnormalities of hippocampal architecture.

#### Subventricular Zone

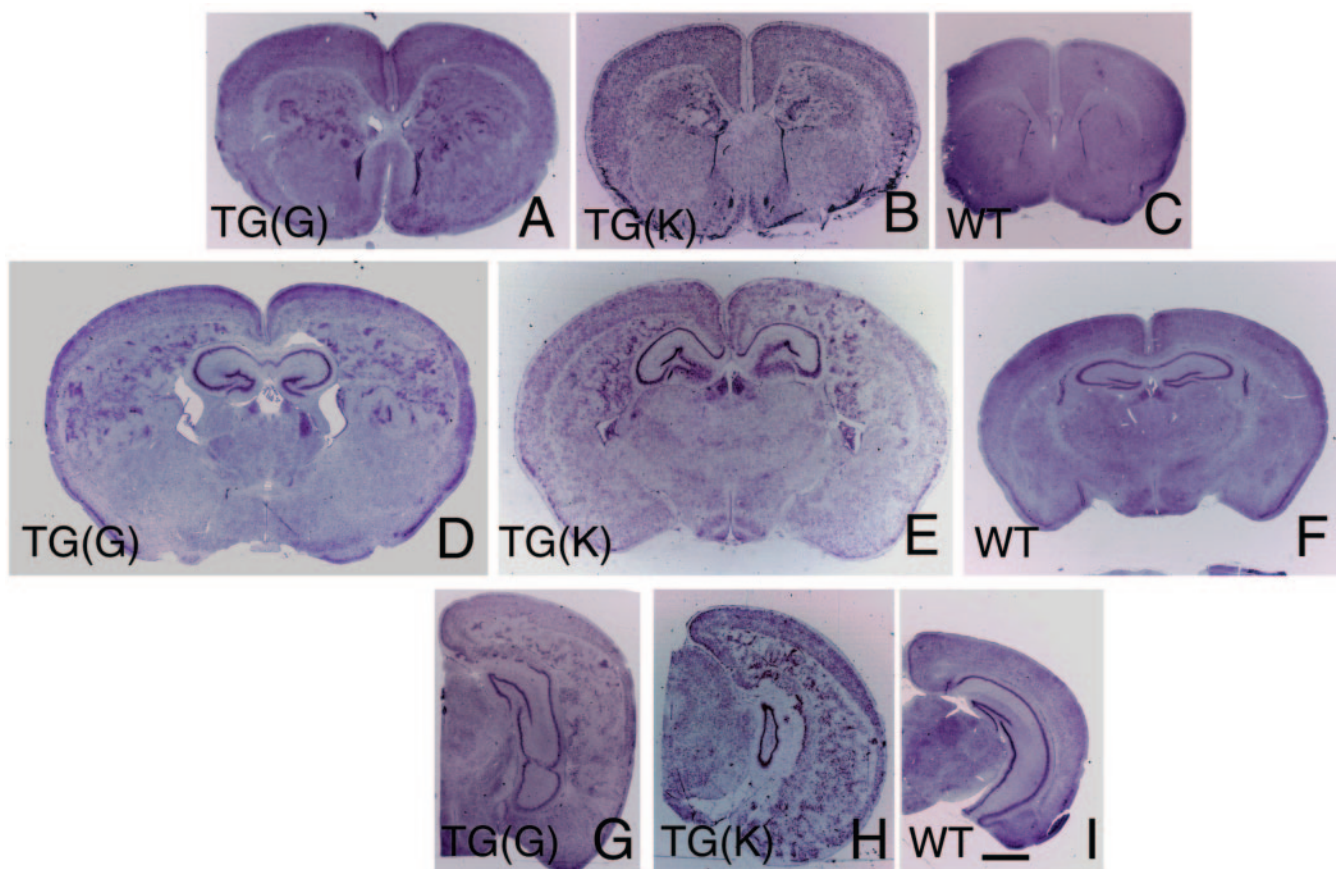
The most striking feature of the transgenic brains is the dramatic expansion of the size of the subventricular zone. Deep to the cerebral cortex, transgenic brains show large aggregates of neurons that are never seen in normal brains. These new neuronal ‘structures’ reside deep to the cerebral cortex, but apparently distinct from the underlying striatum (Figs 3 and 4), in a region similar to the position normally occupied by the subventricular zone. In some sections, these aggregations of neurons appears separate and distinct from the overlying cerebral cortex (Figs 3A,B and 4D,E,G) although, in other sections, neurons appear to coalesce together with neurons of the cortex (Figs 3D,E,G,H and 4H). For example, sections through the middle and caudal forebrain show marked distortion of normal cortical architecture by the appearance of these subcortical cells, which appear to crowd the space normally occupied by overlying cortex (Figs 3D,E,G,H and 5D,E), and many neurons invade into overlying cortex. These aggregations of neurons appear to be comprised of a wide variety of neurons, ranging in size from small to large. In other sections, the subcortical neurons appear to associate in streams or waves, resembling layers (Fig. 4A,B,I,J); in contrast, some neurons accumulated in distinct structures that resembled nuclei (Fig. 4C). Based on the location of these large expansive areas of neurons adjacent to the lateral ventricles, we interpret them as representing a greatly expanded subventricular zone in transgenic animals filled with heterotopic neurons that do not migrate properly. Prominent streams of smaller cells that resemble progenitors reside adjacent to the lateral ventricles, expanding the subventricular zone of the transgenic brains (Fig. 4A,C).

#### Ventricles

The lateral ventricles of the transgenic animals are dilated compared with wild type (Fig. 3D,G,H). This finding is consistent with the enlarged lateral ventricles observed in embryonic transgenic mice (Chenn and Walsh, 2002).

#### Cerebral Cortex

Sections through transgenic brains show that the cerebral cortex is increased overall in surface area, but often thinner than the cortex from comparable areas of wild-type brains (Figs 3 and 5, low power). The cortical folds induced by expressing high levels of the stabilized  $\beta$ -catenin transgene (Chenn and Walsh, 2002) were not evident in the brains from adult mice expressing lower transgene levels, since the cortical surface of the adult mice was smooth. The cortex in the adult transgenic mice appears relatively normal in thickness in rostral portions of the forebrain, but becomes more expansive in surface area and thinner in sections taken at intermediate rostral–caudal levels (Fig. 3D,E). Caudal sections through the cortex revealed that the posterior cortex is markedly reduced in thickness compared with wild-type cortex (Fig. 3G,H). The lamination pattern of the cortex appears quite normal in some areas, but strikingly abnormal in other areas. Especially rostral cortical regions showed large areas where the normal cortical layers were easily definable, although even in these areas the cortical layers were sometimes not as sharply definable as in normal mice (Figs 3A,B, 4D,E and 5B). In contrast, other areas of the transgenic cortex in the same brain were strikingly abnormal, even in regions adjacent to normal appearing regions of cortex (Fig. 5). Abnormal cortical regions ranged from thinner, slightly disorganized, and less cell dense (Fig. 5C), to grossly disorganized with no recognizable lamination and multiple neuronal



**Figure 3.** Sections through  $\Delta 90\beta$ -catenin transgenic brains. Coronal sections through forebrains of ntk3 $\Delta 90\beta$ -catenin GFP transgenic (A, D, G), ntk3 $\Delta 90\beta$ -catenin ntk3 transgenic (B, E, H) versus wild-type mice (C, F, I). (A, B, C) Sections through rostral forebrain reveal relatively normal appearing overlying cortex superficial to large aggregates of neurons expanding the subventricular zone adjacent to the lateral ventricles. (D, E, F) Sections through mid-forebrain reveal similar accumulations of neurons in the subventricular zone. The cortical surface is thin and expanded; in areas, cells from the underlying subventricular zone invade the cortex. The lateral ventricles are enlarged, and the neuronal cell layer of the dentate gyrus is expanded. (G, H, I) Sections through caudal forebrain highlight more severe cortical thinning superficial to the expanded subventricular zone. Bar = 1 mm.

heterotopia (Fig. 5D,E). These neuronal heterotopia are found throughout the transgenic cortical plate, including the marginal zone. The composition of the cortical neuronal clusters appears similar to that observed in the subcortical subventricular zones, consisting of a variable mixture of small to large neurons. These abnormal aggregates of neurons do not appear to represent tumors however, since the cells appeared histologically to be well-differentiated neurons, and there was no evidence for necrosis, neovascularization, or excessive mitotic figures.

#### Hippocampus

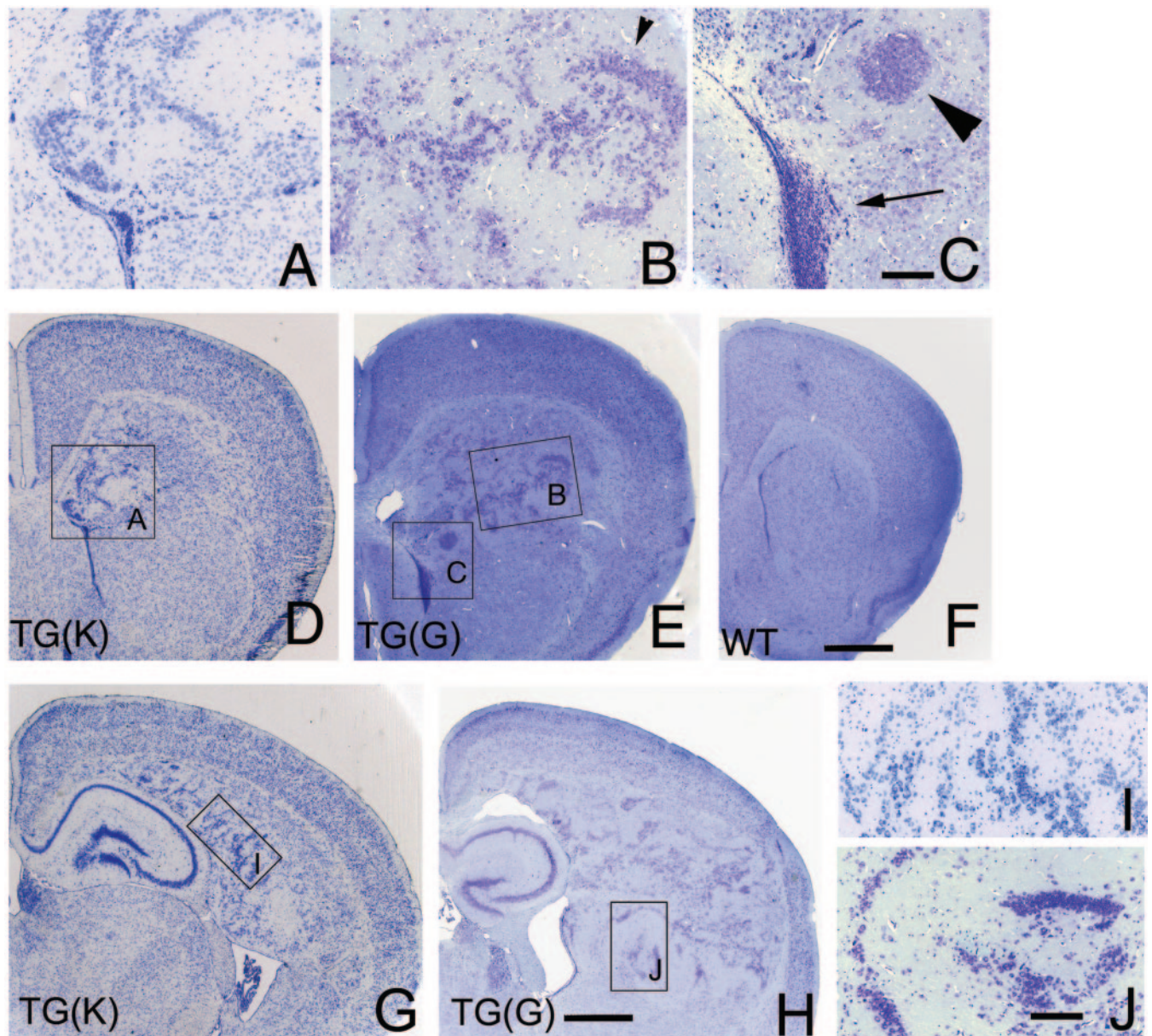
The hippocampi of the transgenic brains sectioned showed marked abnormalities as well. The anterior hippocampus of the transgenic mice was distorted and increased in dorsal-ventral dimension (Figs 3D,E and 5J-N). The layer of neurons comprising the dentate gyrus appears less well defined and thicker compared with wild type (Fig. 5J), and the normal smooth laminar structure of the dentate gyrus was distorted (Fig. 5G-K). In posterior regions (Fig. 3G,H) the architecture of the hippocampus was so distorted that it was difficult to identify corresponding regions in normal and transgenic animals. The CA fields, although not nearly as distorted as the dentate, showed a characteristic notching in a number of sections (Figs 3G and 5I,L).

#### Amygdala

Rostral portions of the amygdala (Fig. 4E) appeared to have grossly normal cell distribution, although the nuclear architecture was not analyzed in detail. In contrast, posterior parts of the amygdala (Fig. 3G,H) showed complete disruption of the normal nuclear architecture and pattern of cell distribution so that the normal amygdaloid nuclear structure could not be defined, and borders between abnormal hippocampal cells and abnormal amygdaloid cells could not be clearly established.

#### Anatomic Summary

These studies suggest that  $\beta$ -catenin activation in neural precursors can cause a massive distortion of forebrain architecture apparently due to an increase in neuronal number in the adult forebrain. In addition to resulting in increased surface area of the cortex, the expansion of neuronal populations appears to underlie the expansion of the subventricular zone neuronal populations in the forebrain deep to the cerebral cortex. The appearance of these neurons shows few features characteristic for malignancy such as nuclear pleomorphism, abundant mitotic figures, and necrosis, or neovascularization. Perhaps reflecting a developmental gradient, caudal regions of the brain are more markedly distorted than anterior regions. Finally, consistent with embryonic expansion of the precursor pool, the lateral ventricles of adult transgenic animals are enlarged, with regions



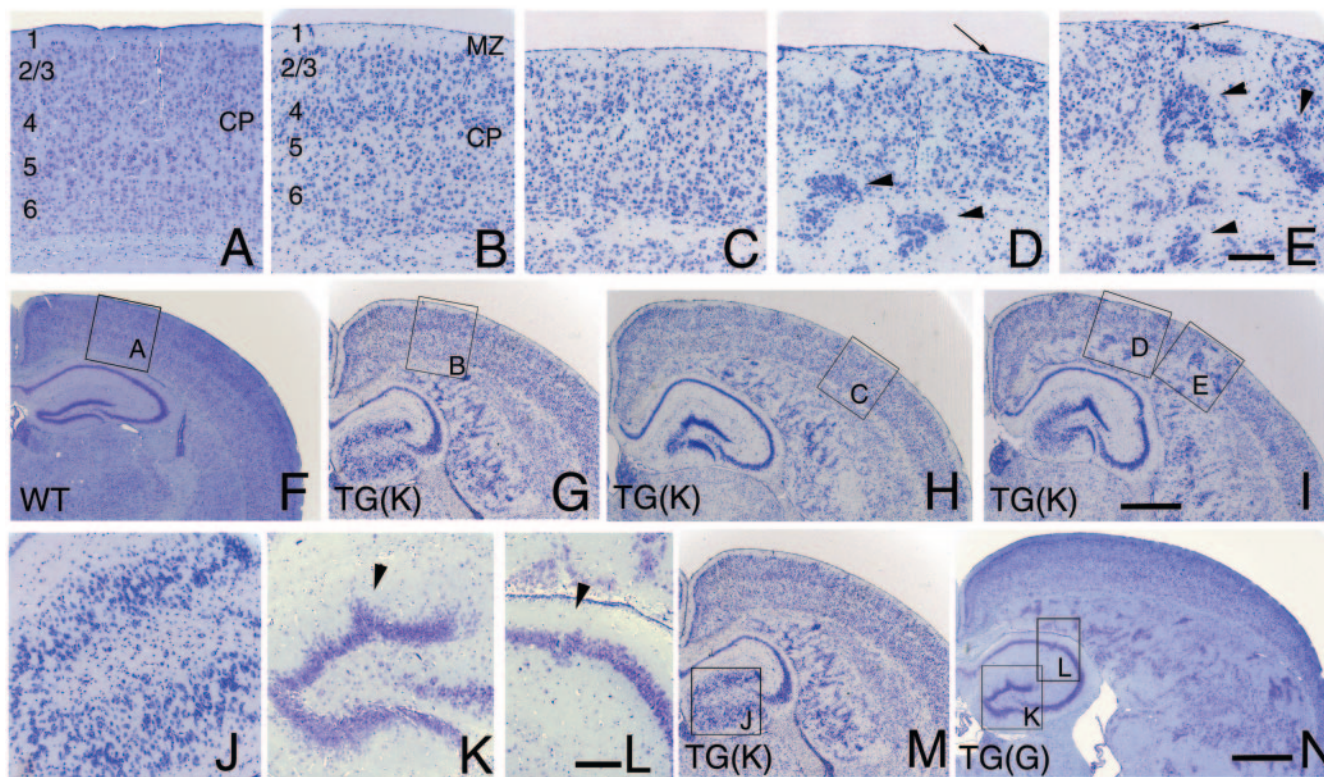
**Figure 4.** Images of rostral and mid forebrain in  $\beta$ -catenin overexpressing transgenic mice. Subventricular zone in rostral forebrain is greatly expanded (*D, E*). Higher power images (*A, B, C*) reveal a variety of neuronal morphologies, including small precursor-like cells localized adjacent to ventricle (arrow, *C*), medium and larger neurons in laminar structures (arrowhead, *B*), and tight clusters (arrowhead, *C*). Mid-forebrain sections reveal the expanded subventricular zone evident in low power (*G, H*). The cortex in *G* appears morphologically normal, with all cortical layers distinct and normal in thickness (higher power in Fig. 5). In contrast, cortex in *H* is thin in areas, and disorganized. Higher power of subventricular zone (*I, J*) reveals streams of variably sized neurons in linear aggregates that sometimes resemble the layering pattern of the hippocampus and dentate gyrus. Bar = 1 mm (*D, E, G, H*) and 200  $\mu$ m (*A-C, I, J*).

of the anterior subventricular zone containing substantially increased numbers of small cells morphologically resembling precursors.

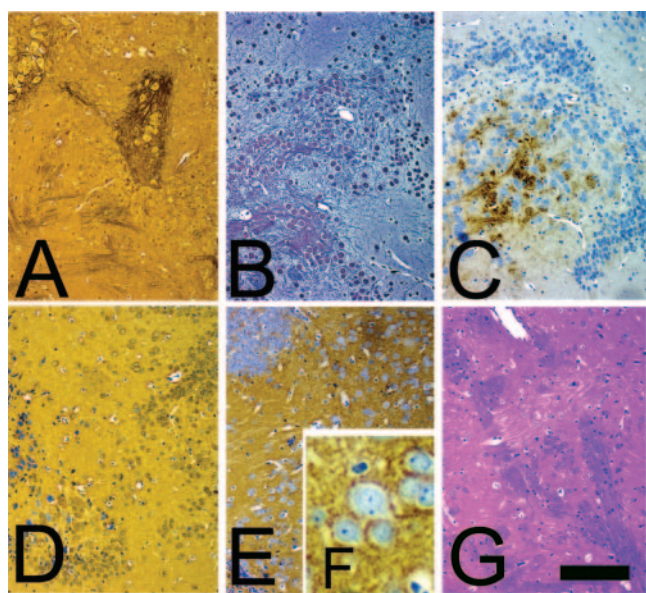
#### **Characterization of Subcortical Neurons**

Higher magnification examination revealed that the expanded subcortical subventricular zone in the transgenic brain is filled with excess cells that have the histological appearance of well-differentiated neurons, and many of the cells are relatively large with abundant Nissl substance. To confirm the neuronal identity of cells in adult transgenic animals, we stained sections through transgenic forebrains with a number of commonly used histological stains and found that most of the cells in the transgenic

brains are differentiated neurons (Fig. 6). Bielschowsky silver staining (Fig. 6*A*) and Bodian silver stains (Fig. 6*B*) for neurofilaments support the morphologic identification of cells in transgenic brains as neurons. Immunohistochemical staining (dark brown) for neurofilaments provides further support the new cells are neuronal (Fig. 6*C*), and staining with a monoclonal antibody to neuron-specific enolase (NSE) shows strong staining (brown) in similar cells (Fig. 6*D*). Synaptophysin staining reveals the presence of strong punctate staining adjacent to the cell bodies of many neurons in the transgenic animals, suggesting that synaptic connections are made to the neurons (Fig. 6*E, F*). Finally, Luxol Fast Blue staining highlights myelin in cellular regions of transgenic brains (Fig. 6*G*), although it is



**Figure 5.** Cortical and hippocampal abnormalities in  $\beta$ -catenin overexpressing transgenic brains. Compared with wild-type cortex from a similar rostral–caudal plane (A), the cortex of transgenic animal K is highly variable in composition. In mildly affected areas (B), cortical layers were definable but less precise than normal in some cases. In other areas (C), the cortex was thinner than normal but with some remaining lamination definable. In the most severely affected areas (D, E) cortical architecture was severely disrupted, with poor lamination and multiple neuronal heterotopias (arrowheads in D, E), and invasion of the normally cell sparse layer one with neurons (arrowhead, D). The architecture is highly variable in transgenic brains, even within adjacent areas of the cortex. Areas of transgenic animal G are similarly variable (for example, see 4E versus 5H). Hippocampal architecture is variably distorted in transgenic brains. Transgenic animal K has marked thickening of the dentate gyrus (J). Sections through transgenic animal G also reveal alterations in dentate gyrus morphology (K), with poorly organized lamination, and abnormal shape (arrowhead, K). The CA fields appear less affected, but show characteristic break (arrowhead, L; see also Fig. 3G, in caudal hippocampus, and I). Bar = 200  $\mu$ m (A–E, J–L) and 1 mm (F–I, M, N).



**Figure 6.** Histologic stains of cells from transgenic brains. Bielschowsky silver staining (A), Bodian silver staining (B), neurofilament immunohistochemistry (C), immunohistochemical labeling of NSE (D), and synaptophysin staining (E) highlight large neurons in the forebrains of adult transgenic brains. (F) Inset of higher power synaptophysin staining reveals punctate accumulation characteristic of synapses. (G) Luxol Fast Blue stain for myelin highlights the presence of myelin in transgenic brains. Bar = 200  $\mu$ m (A–E, G) and 50  $\mu$ m (F).

impossible to distinguish whether the myelinated fibers arise directly from cells in these regions of the brain or are associated with fibers that merely course through the areas. Combined with the morphologic studies described above, these histological stains suggest that the excess cells in  $\beta$ -catenin transgenic brains are primarily well-differentiated, postmitotic neurons.

### Discussion

By generating transgenic animals that express relatively lowered levels of stabilized  $\Delta 90\beta$ -catenin in neural precursors, we have been able to examine the consequences of expanding the neural progenitor population in detail in two of five surviving adult animals. These adult transgenic animals have markedly enlarged forebrains characterized by a greatly expanded subventricular zone with aggregations of large and medium-sized neurons; cerebral cortices with increased surface area, variable lamination and neuronal heterotopias; distorted hippocampal and amygdala anatomy; and enlarged lateral ventricles.

### Postnatal Neurogenesis

Recent studies have demonstrated the existence of neural precursors in the brains of postnatal to adult animals (Alvarez-Buylla and Garcia-Verdugo, 2002; Gage, 2002; Gould and Gross, 2002; Rakic, 2002). These postnatal neural precursors reside in or near the subventricular zone, giving rise to neurons throughout adult life that populate the dentate gyrus of the hippocampus as well as migrate in the rostral migratory stream towards the olfactory

bulb. Adult  $\beta$ -catenin transgenic mice have dilated lateral ventricles and expanded rostral subventricular cell populations. The findings of dentate gyrus enlargement and novel aggregations of large neurons located deep to the cerebral cortex adjacent to the expanded lateral ventricles suggest that these additional neurons may derive from an expanded population of neural precursors of the subventricular zone persisting in the postnatal brain.

### ***Progenitor Cell Proliferation and Cell Cycle Exit in Cortical Development***

With the exception of cortical interneurons, which are generated from precursors in the ganglion eminences (Anderson *et al.*, 2001), the majority of the cells in the mammalian cerebral cortex arise from dividing neural precursors in the cortical ventricular zone, a pseudostratified epithelial cell layer that lines the lumen of the lateral ventricles (Caviness *et al.*, 1995a). Regulation of the decision of a progenitor to exit or re-enter the cell cycle can influence the size of the precursor pool and the number of neurons generated during cortical development. The development of the cerebral cortex is delayed relative to other rostral brain structures, and it is speculated that this delay in differentiation permits an extended period of progenitor cell divisions leading to expansion of the progenitor population prior to the onset of the production of differentiated neurons (Caviness *et al.*, 1995a). These increases in the production of progenitors could allow for dramatic increases in the total number of neurons generated during a given period of neuronal production, and thus greatly increase cortical surface area (Caviness *et al.*, 1995a; Rakic, 1995). Although the factors that regulate the onset and duration of neurogenesis, and whether a progenitor cell exits or re-enters the cell cycle, remain poorly understood, these observations have led to the proposal that subtle changes in the production of progenitor cells could underlie the expansion in surface area of the cortex (Rakic, 1995). In conjunction with the findings from embryonic transgenic mice, this current study shows that  $\beta$ -catenin expression can increase the surface area of the cerebral cortex. Although the thickness of the cortical plate in adult mice may be thinner than normal for multiple reasons (likely due to the space-occupying expansion of the novel subcortical neuronal populations), our study supports the idea that increased progenitor number can expand the surface area of the cortex without a corresponding increase in the thickness of the cortical plate.

Increased cortical surface area can be generated in different ways. For example, reduction of programmed cell death by targeted mutation of Caspase 9 causes severe brain malformations characterized by cerebral enlargement with increased surface area, ectopic growth, and thickening of the ventricular zone (Hakem *et al.*, 1998; Kuida *et al.*, 1998). In contrast, our findings suggest that subtle changes in the expansion or maintenance of the neural precursor population are sufficient to cause horizontal expansion of the surface area of the developing cerebral cortex without increases in cortical thickness.

### ***Increased Neuronal Numbers in Adult Transgenic Mice***

The increased number of neurons in transgenic brains appears so extensive that in some areas of the posterior forebrain, they nearly obliterate the overlying cerebral cortex. It is likely that these aggregations of large neurons derive from an expanded population of adult neural precursors. Although neurons derived from postnatal precursors in the subventricular zone normally migrate toward the olfactory bulbs, the olfactory bulbs of adult

transgenic animals are not increased in size relative to those of normal mice, suggesting that the normal migratory behavior of these neurons may be impaired or misdirected. The dentate gyrus, the other area in the normal mouse brain with persistent adult neurogenesis, also appears to be distorted and enlarged compared with normal. Taken together, these findings are consistent with the idea that  $\beta$ -catenin regulates the decision of neural precursors to exit or re-enter the cell cycle, and adult mice expressing  $\beta$ -catenin in precursors have an expanded progenitor population that gives rise to abnormally large numbers of neurons derived from postnatal precursors

### ***Consequences of Expanded Precursor Populations***

The studies of embryonic transgenic mice with expanded cerebral cortices and adult transgenic mice with enlarged brains support the idea that  $\beta$ -catenin can regulate the size of the neural precursor population, and as a consequence, regulate neuronal production during all stages of development. The findings that embryonic transgenic brains have increased cortical surface area and adult brains have increased cortical surface area with large subcortical aggregations of neurons and hippocampal enlargement suggest that expanded neural precursor pools can give rise to neurons both embryonically and postnatally.

Recent studies also provide support that  $\beta$ -catenin is essential for development of other brain regions as well. Mice which had  $\beta$ -catenin specifically deleted in the region of Wnt-1 expression had brain malformations and failure of craniofacial development (Brault *et al.*, 2001). In particular, the entire cerebellum and part of the midbrain failed to form, similar to the phenotype observed in Wnt-1 mutant animals. The defects in craniofacial development suggested a role for  $\beta$ -catenin in neural crest cell production. These findings support the idea that  $\beta$ -catenin plays a pivotal role in a variety of neural precursor cells during development.

It is unclear whether the same precursors that give rise to cortical neurons also generate postnatal neural precursors. The lateral ventricles and adjacent subventricular zones where postnatal neural precursors reside appear expanded in our adult transgenic animals, supporting the idea that the postnatal precursor pool, like the embryonic precursor pool, is also expanded by  $\beta$ -catenin expression. Although many questions remain regarding the developmental origin of the new neurons characteristic of these enlarged transgenic brains, these results support the notion that regulation of precursor pool size will determine the size of the final neuronal population. Creation of a stable transgenic line may allow more detailed analysis of neurogenesis in response to  $\beta$ -catenin overexpression. Nonetheless, modest changes in whether precursors re-enter or exit the cell cycle appear able to ultimately result in dramatic differences in the size of the precursor pool, and consequently, the size of different parts of the brain (Caviness *et al.*, 1995c; Rakic, 1995).

The behavioral consequences of having abnormally large numbers of neurons in distorted forebrains can only be described anecdotally from this current study. Our informal observations suggest that the adult transgenic animals behaved differently compared with their wild-type littermates. Although formal behavioral testing was not performed, we observed that transgenic animals were easily startled. Furthermore, upon handling, we noted that transgenic animals were more aggressive than their wild-type littermates and would attempt to bite the investigator much more frequently than normal mice.

### ***Architectural Abnormalities in Transgenic Mice***

Analysis of these adult mice in the setting of  $\beta$ -catenin over-

expression strongly implicate a role for  $\beta$ -catenin in neuronal migration and laminar architecture in the forebrain. The adult transgenic mice show many areas of abnormal cerebral cortical architecture, ranging from normal numbers of layers that are poorly formed to gross loss of cortical architecture and massive ectopia. Large numbers of cadherin superfamily members are expressed in postmigratory neurons in the cortical plate (Hamada and Yagi, 2001) and have been suggested to be required for normal lamination. While our results cannot implicate one or another cadherin family member, they are consistent with the possibility that cadherin/catenin-regulated adhesion may be important for normal lamination.

In addition to abnormal patterns of cell lamination, many forebrain neurons appear to arrest in the subventricular zone rather than migrating out of the subventricular zone to other, more distant sites (presumably including cortex and/or striatum and amygdala). Whereas many cadherins are expressed in the cortical and subcortical ventricular zone, cadherin expression is generally down-regulated in migratory neurons of the forebrain (Kwon *et al.*, 2000). The abnormalities of migration in  $\beta$ -catenin transgenic mice suggests the possibility that down-regulation of  $\beta$ -catenin signaling might be important for proper neuronal migration.

## Notes

A.C. was a Howard Hughes Medical Institute Physician Postdoctoral Fellow. This work was also supported by RO1 NS32457 from the NINDS to C.A.W. C.A.W. is an Investigator of the Howard Hughes Medical Institute. We thank A. Barth and W.J. Nelson (Stanford) for the  $\Delta 90\beta$ -catenin-kt3 DNA construct. We thank U. Berger for *in situ*, and L. Du, T. Thompson and S. White for technical assistance.

Address correspondence to Anjen Chenn, Department of Pathology, Northwestern University, Feinberg School of Medicine, 303 East Chicago Avenue, Chicago, IL 60611-3008, USA. Email: achenn@northwestern.edu.

## References

- Alvarez-Buylla A, Garcia-Verdugo JM (2002) Neurogenesis in adult subventricular zone. *J Neurosci* 22:629–634.
- Anderson SA, Marin O, Horn C, Jennings K, Rubenstein JL (2001) Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development* 128:353–363.
- Barth AI, Pollack AL, Altschuler Y, Mostov KE, Nelson WJ (1997) NH<sub>2</sub>-terminal deletion of beta-catenin results in stable colocalization of mutant beta-catenin with adenomatous polyposis coli protein and altered MDCK cell adhesion. *J Cell Biol* 136:693–706.
- Barton RA, Harvey PH (2000) Mosaic evolution of brain structure in mammals. *Nature* 405:1055–1058.
- Berger UV, Hediger MA (2001) Differential distribution of the glutamate transporters GLT-1 and GLAST in tanyocytes of the third ventricle. *J Comp Neurol* 433:101–114.
- Braut V, Moore R, Kutsch S, Ishibashi M, Rowitch DH, McMahon AP, Sommer L, Boussadia O, Kemler R (2001) Inactivation of the beta-catenin gene by Wnt1-Cre-mediated deletion results in dramatic brain malformation and failure of craniofacial development. *Development* 128:1253–1264.
- Bulfone A, Smiga SM, Shimamura K, Peterson A, Puelles L, Rubenstein JL (1995) T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. *Neuron* 15:63–78.
- Caviness V, Takahashi T, Nowakowski RS (1995a) Numbers, time and neocortical neurogenesis: a general developmental and evolutionary model. *Trends Neurosci* 18:379–383.
- Caviness VS Jr, Takahashi T, Nowakowski RS (1995b) Numbers, time and neocortical neurogenesis: a general developmental and evolutionary model. *Trends Neurosci* 18:379–383.
- Chenn A, Walsh CA (2002) Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* 297:365–369.
- Cho EA, Dressler GR (1998) TCF-4 binds beta-catenin and is expressed in distinct regions of the embryonic brain and limbs. *Mech Dev* 77:9–18.
- Clark DA, Mitra PP, Wang SS (2001) Scalable architecture in mammalian brains. *Nature* 411:189–193.
- Clevers H, van de Wetering M (1997) TCF/LEF factor earn their wings. *Trends Genet* 13:485–489.
- Cox RT, Kirkpatrick C, Leifer M (1996) Armadillo is required for adherens junction assembly, cell polarity, and morphogenesis during *Drosophila* embryogenesis. *J Cell Biol* 134:133–148.
- Elias H, Schwartz D (1969) Surface areas of the cerebral cortex of mammals determined by stereological methods. *Science* 166:111–113.
- Finlay BL, Darlington RB (1995) Linked regularities in the development and evolution of mammalian brains. *Science* 268:1578–1584.
- Gage FH (2002) Neurogenesis in the adult brain. *J Neurosci* 22:612–613.
- Galceran J, Miyashita-Lin EM, Devaney E, Rubenstein JL, Grosschedl R (2000) Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. *Development* 127:469–482.
- Gould E, Gross CG (2002) Neurogenesis in adult mammals: some progress and problems. *J Neurosci* 22:619–623.
- Hakem R, Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W *et al.* (1998) Differential requirement for caspase 9 in apoptotic pathways *in vivo*. *Cell* 94:339–352.
- Hamada S, Yagi T (2001) The cadherin-related neuronal receptor family: a novel diversified cadherin family at the synapse. *Neurosci Res* 41:207–215.
- Haug H (1987) Brain sizes, surfaces, and neuronal sizes of the cortex cerebri: a stereological investigation of man and his variability and a comparison with some mammals (primates, whales, marsupials, insectivores, and one elephant). *Am J Anat* 180:126–142.
- Huang H, Mahler-Araujo BM, Sankila A, Chimelli L, Yonekawa Y, Kleihues P, Ohgaki H (2000) APC mutations in sporadic medulloblastomas. *Am J Pathol* 156:433–437.
- Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P, Flavell RA (1998) Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* 94:325–337.
- Kwon YT, Gupta A, Zhou Y, Nikolic M, Tsai LH (2000) Regulation of N-cadherin-mediated adhesion by the p35-Cdk5 kinase. *Curr Biol* 10:363–372.
- Lee SM, Tole S, Grove E, McMahon AP (2000) A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* 127:457–467.
- McMahon AP, Bradley A (1990) The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. *Cell* 62:1073–1085.
- Moon RT, Brown JD, Torres M (1997) WNTs modulate cell fate and behavior during vertebrate development. *Trends Genet* 13:157–162.
- Oosterwegel M, van de Wetering M, Timmerman J, Kruisbeek A, Destree O, Meijlink F, Clevers H (1993) Differential expression of the HMG box factors TCF-1 and LEF-1 during murine embryogenesis. *Development* 118:439–448.
- Parr BA, Shea MJ, Vassileva G, McMahon AP (1993) Mouse Wnt genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. *Development* 119:247–261.
- Peifer M, Polakis P (2000) Wnt signaling in oncogenesis and embryogenesis – a look outside the nucleus. *Science* 287:1606–1609.
- Rakic P (1995) A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci* 18:383–388.
- Rakic P (2002) Adult neurogenesis in mammals: an identity crisis. *J Neurosci* 22:614–618.
- Yaworsky PJ, Kappen C (1999) Heterogeneity of neural progenitor cells revealed by enhancers in the nestin gene. *Dev Biol* 205:309–321.