

# Loss of Notch Activity in the Developing Central Nervous System Leads to Increased Cell Death

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## Key Words

Notch activity · Developing central nervous system · Cell death

## Abstract

Many cells in the mammalian brain undergo apoptosis as a normal and critical part of development but the signals that regulate the survival and death of neural progenitor cells and the neurons they produce are not well understood. The Notch signaling pathway is involved in multiple decision points during development and has been proposed to regulate the survival and apoptosis of neural progenitor cells in the developing brain; however, previous experiments have not resolved whether Notch activity is pro- or anti-apoptotic. To elucidate the function of Notch signaling in the survival and death of cells in the nervous system, we have produced single and compound Notch conditional mutants in which *Notch1* and *Notch3* are removed at different times during brain development and in different populations of cells. We show here that a large number of neural progenitor cells, as well as differentiating neurons, undergo apoptosis in the absence of *Notch1* and *Notch3*, suggesting that Notch activity promotes the survival of both progenitors and newly differentiating cells in the developing nervous sys-

tem. Finally, we show that postmitotic neurons do not require Notch activity indefinitely to regulate their survival since elevated levels of cell death are observed only during embryogenesis in the Notch mutants and are not detected in neonates.

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## Introduction

The Notch pathway is used in a wide variety of cell fate decisions and has been referred to as a ‘master switch’ in directing cell fate choices such as proliferation, differentiation, and apoptosis [Artavanis-Tsakonas et al., 1999; Justice and Jan, 2002; Lai, 2004]. During development of the mammalian central nervous system, Notch activity has been shown to maintain neural progenitor cells and to inhibit neuronal differentiation [Schuermans and Guillemot, 2002]. Gain-of-function studies have revealed that constitutive Notch signaling leads to cells remaining as progenitors [Henrique et al., 1997; Ohtsuka et al., 2001; Mizutani and Saito, 2005], while decreased Notch activity is correlated with a reduction in neuronal progenitors [Hitoshi et al., 2002; Yoon et al., 2004] and increased neuronal differentiation [Ishibashi et al., 1995; de la Pompa et al., 1997]. In addition, Notch signaling is

thought to regulate glial versus neuronal identity [Wang et al., 1998; Furukawa et al., 2000; Gaiano et al., 2000; Morrison et al., 2000]. Radial glia are stem cells in the nervous system [Malatesta et al., 2000; Noctor et al., 2001, 2004; Anthony et al., 2004] and brain lipid binding protein, a marker of radial glia, has recently been shown to be a direct target of the Notch signaling pathway [Anthony et al., 2005]. While the majority of previous experiments have focused on the role of Notch activity in neurogenesis and cell fate determination, several studies have suggested that the Notch pathway may also play important roles in regulating cell survival [Miele and Osborne, 1999; Oishi et al., 2004].

In the developing nervous system, large numbers of cells are eliminated by programmed cell death [Oppenheim, 1991; de la Rosa and de Pablo, 2000]. When cell death is prevented by mutations in genes involved in apoptosis such as *apaf-1*, *caspase-3*, or *caspase-9*, major brain malformations occur [Cecconi et al., 1998; Hakem et al., 1998; Kuida et al., 1998; Yoshida et al., 1998], indicating that controlling cell survival and death may be a key mechanism by which the ultimate morphology and size of the brain is determined [Haydar et al., 1999; Kuan et al., 2000]. Thus far, the role of Notch activity in regulating cell survival and death in the developing mammalian nervous system remains ambiguous. Notch activation has recently been reported both to promote the survival of neural precursor cells [Oishi et al., 2004] and to induce programmed cell death [Yang et al., 2004]. In addition, Notch is expressed in postmitotic neurons and has been reported to regulate neurite morphology [Berezovska et al., 1999; Franklin et al., 1999; Sestan et al., 1999; Redmond et al., 2000], but little is known about whether or not Notch also functions in the survival of differentiating neurons. In this study, we examine the role of the Notch pathway in the survival and apoptosis of cells within the developing forebrain using the Cre-loxP system of conditional mutagenesis [Sauer and Henderson, 1988]. We have generated single and compound *Notch1* and *Notch3* conditional knockouts (cKOs) and double knockouts (DKOs) using two distinct Cre driver lines, one which removes Notch signaling throughout the telencephalon (*Foxg1<sup>Cre</sup>*), including all neural progenitors as well as their progeny, and one that inactivates the Notch pathway starting at a later time point in newly differentiating neurons but not in neural progenitor cells (*Dlx5/6<sup>Cre</sup>*). In this way, we can analyze the role of Notch signaling in cell survival with respect to different time points during development and in distinct populations. We show here that the Notch signaling pathway promotes cell survival in the

developing central nervous system and, in the absence of *Notch1* and *Notch3*, apoptosis is increased in neural progenitor cells as well as in differentiating neurons.

## Materials and Methods

### Mouse and Mouse Embryos

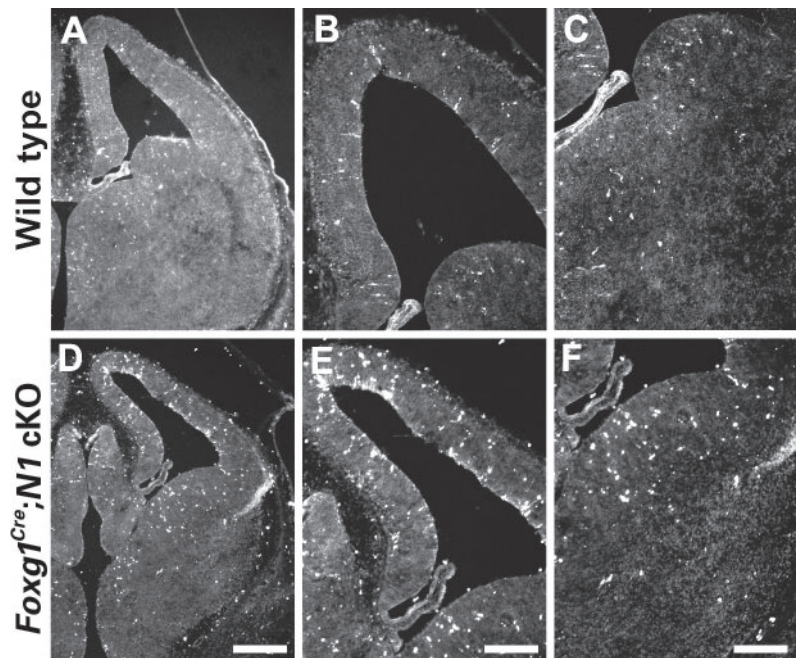
Floxed *Notch1* mice were a gift of Freddy Radtke and were genotyped as previously described [Radtke et al., 1999]. Mutant mouse embryos were obtained by crossing homozygous floxed *Notch1* mice with mice heterozygous for floxed *Notch1* and *Foxg1<sup>Cre/+</sup>*. The generation of *Foxg1<sup>Cre/+</sup>* mice was previously published [Hebert and McConnell 2000] and *Foxg1<sup>Cre/+</sup>* heterozygous mice were maintained on a Swiss Webster background. *Dlx5/6-Cre-IRES-EFEGFP* mice were generously provided by Kenny Campbell and have been previously described [Stenman et al., 2003]. *Notch3* null mutant mice are viable and fertile and were maintained as homozygous nulls [Krebs et al., 2003]. Conditional *Notch1;Notch3* double mutant mice were acquired by breeding double homozygous floxed *Notch1;Notch3* null mutant mice with mice heterozygous for floxed *Notch1* and *Dlx5/6<sup>Cre/+</sup>* on a *Notch3* null mutant background. The Z/EG Cre recombination reporter line was used to permanently label cells in which Cre-mediated recombination had occurred [Novak et al., 2000]. Plug date was defined as embryonic day E0.5.

### Tissue Preparation and Immunohistochemistry

Embryos were dissected in chilled PBS and fixed in either 2 or 4% paraformaldehyde for 4 h at 4°C, cryoprotected in 30% sucrose, embedded in Tissue-Tek® O.C.T., and sectioned at a thickness of 14–16 µm on a Leica CM3050 S cryostat. Rabbit anticleaved caspase-3 (Cell Signaling Technology, Inc., Beverly, Mass., USA) was used at 1:500, mouse antineurin (rat-401, Developmental Studies Hybridoma Bank, Iowa City, Iowa, USA) was used at 1:5, and rabbit anti-GFP (Molecular Probes, Eugene, Oreg., USA) was used at 1:1,000. Goat secondary antibodies conjugated with Cy3 or Alexa-488 were obtained from Jackson ImmunoResearch Laboratories (West Grove, Pa., USA) and Molecular Probes. The TUNEL assay was performed using the ApopTag® fluorescent kit according to the manufacturer's instructions (Intergen Company, Purchase, New York, N.Y., USA). Fluorescent images were acquired using a cooled-CCD camera (Princeton Scientific Instruments, New Jersey, N.J., USA) and Metamorph software (Universal Imaging, Downingtown, Pa., USA) and were processed in Adobe Photoshop.

## Results

To inactivate *Notch1* specifically in the embryonic mouse forebrain, we took advantage of the Cre-LoxP system of conditional mutagenesis. We used *Foxg1<sup>Cre/+</sup>* mice, which have Cre recombinase knocked into the *Foxg1* locus [Hebert and McConnell, 2000], to mediate recombination specifically in the developing telencephalon. As visualized by X-gal staining using the ROSA26



**Fig. 1.** Apoptosis is increased in the absence of *Notch1* in the forebrain at E12.5. **A–C** Coronal sections through the telencephalon of wild-type embryos at E12.5 show a low number of caspase-3-positive cells, which are present in the developing cortical plate (**B**) as well as in the ventral telencephalon (**C**). **D–F** *Foxg1<sup>Cre</sup>;N1* cKOs at E12.5 display increased immunoreactivity for caspase-3 compared to wild-type littermates. Substantially more dying cells are detected in the developing cortical plate (**E**) as well as in ventral regions (**F**) of telencephalic-specific *Notch1* mutants. Scale bar: 400  $\mu$ m in **A**, **D** and 200  $\mu$ m in **B**, **C**, **E**, and **F**.

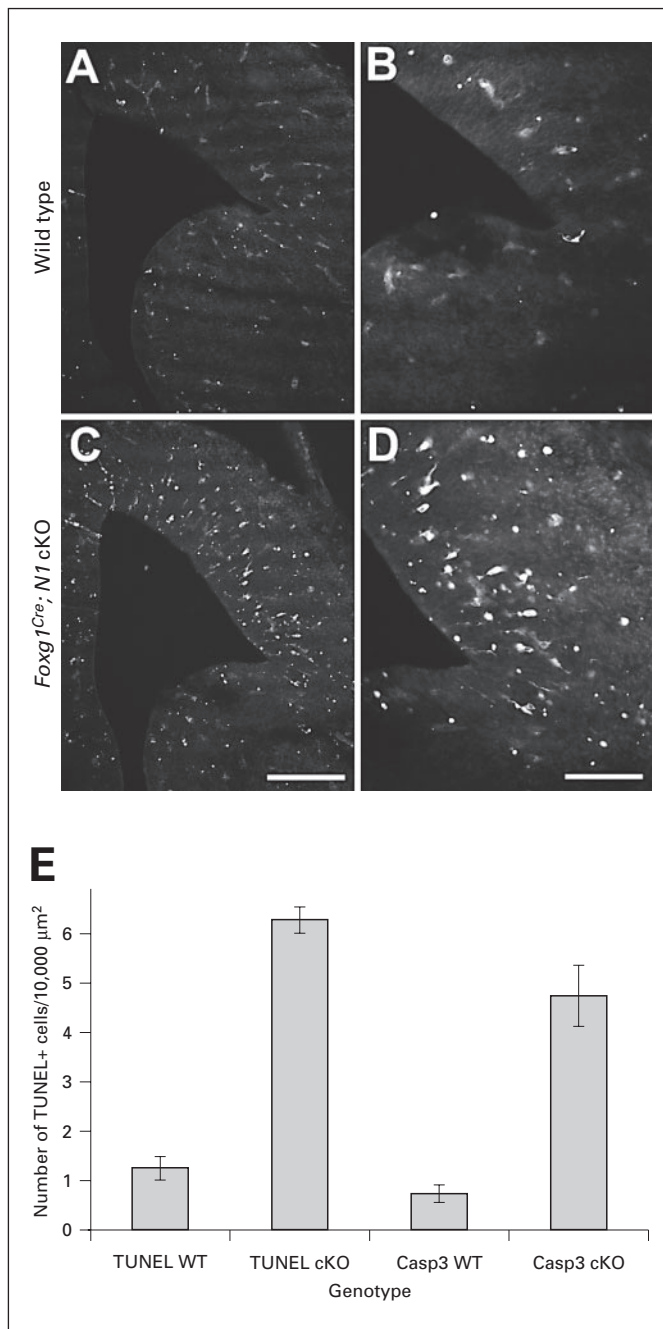
Cre reporter mouse [Soriano, 1999], *Foxg1<sup>Cre/+</sup>* induces the recombination of ‘floxed’ (flanked by loxP sites) alleles within the ventral telencephalon and anterior portion of the optic vesicles as early as E8.5 [Hebert and McConnell, 2000; Fuccillo et al., 2004] and throughout the entire telencephalon by E9.5 and E10.5 [Mason et al., 2005]. To obtain *Notch1* conditional mutants, we crossed homozygous mice in which exon 1 of the *Notch1* gene is floxed (*Notch1<sup>fl/f</sup>*) [Radtke et al., 1999] to *Notch1<sup>fl/+</sup>; Foxg1<sup>Cre/+</sup>* mice. Thus, Cre-directed recombination of the floxed *Notch1* allele is expected to occur in all telencephalic cells, including the neural progenitor cells and their newly differentiating progeny. For simplicity, we refer to *Foxg1<sup>Cre/+</sup>;Notch1<sup>fl/f</sup>* conditional knockout mice as *Foxg1<sup>Cre</sup>;N1* cKOs.

*Foxg1<sup>Cre</sup>;N1* cKOs survive until birth but die within several hours. Conditional mutants have a smaller forebrain than wild-type littermates. One possible explanation that may account for the decrease in overall brain size, at least in part, is an increase in programmed cell death of neural progenitor cells, their postmitotic progeny, or both. To test whether apoptosis is increased in the absence of *Notch1*, we immunostained brain sections from *Foxg1<sup>Cre</sup>;N1* cKOs at E12.5 with antibodies to cleaved caspase-3, which is upregulated in cells undergoing apoptosis [Nicholson et al., 1995]. While sparse caspase-3-positive cells can be seen in sections from wild-type brains, the number of caspase-3-positive cells is sub-

stantially increased in the absence of *Notch1* at E12.5 (fig. 1). Dying cells are observed in both the developing cortical plate and basal telencephalon. Cells undergoing programmed cell death can also be visualized using the TUNEL assay. When we examine the forebrain of *Foxg1<sup>Cre</sup>;N1* cKOs at E14.5 using the TUNEL assay, we found that apoptosis remains elevated (fig. 2). In addition, we compared the number of dying cells using both activated caspase-3 immunostaining and TUNEL labeling and found that similar numbers of dying cells are identified with each method, although slightly more apoptotic cells are detected using TUNEL staining (fig. 2E). There is an approximately 6-fold increase in the number of dying cells in *Foxg1<sup>Cre</sup>;N1* cKOs at E14.5 compared to wild-type littermates (fig. 2E).

Apoptosis serves multiple functions in brain development, from adjusting the pool of neural progenitor cells required for proper morphogenesis to regulating the size of neuronal populations to match the size of their target fields, and it is likely that apoptosis in progenitors and postmitotic neurons has distinct roles and mechanisms [Kuan et al., 2000]. In *Foxg1<sup>Cre</sup>;N1* cKOs, dying cells can be identified within the ventricular zone (VZ), which is where the neural progenitors reside, as well as within regions of differentiating neurons, such as the mantle, at E12.5 and E14.5 (fig. 1, 2). While previous studies have investigated roles for the Notch signaling pathway in regulating cell survival and apoptosis in neural progenitor



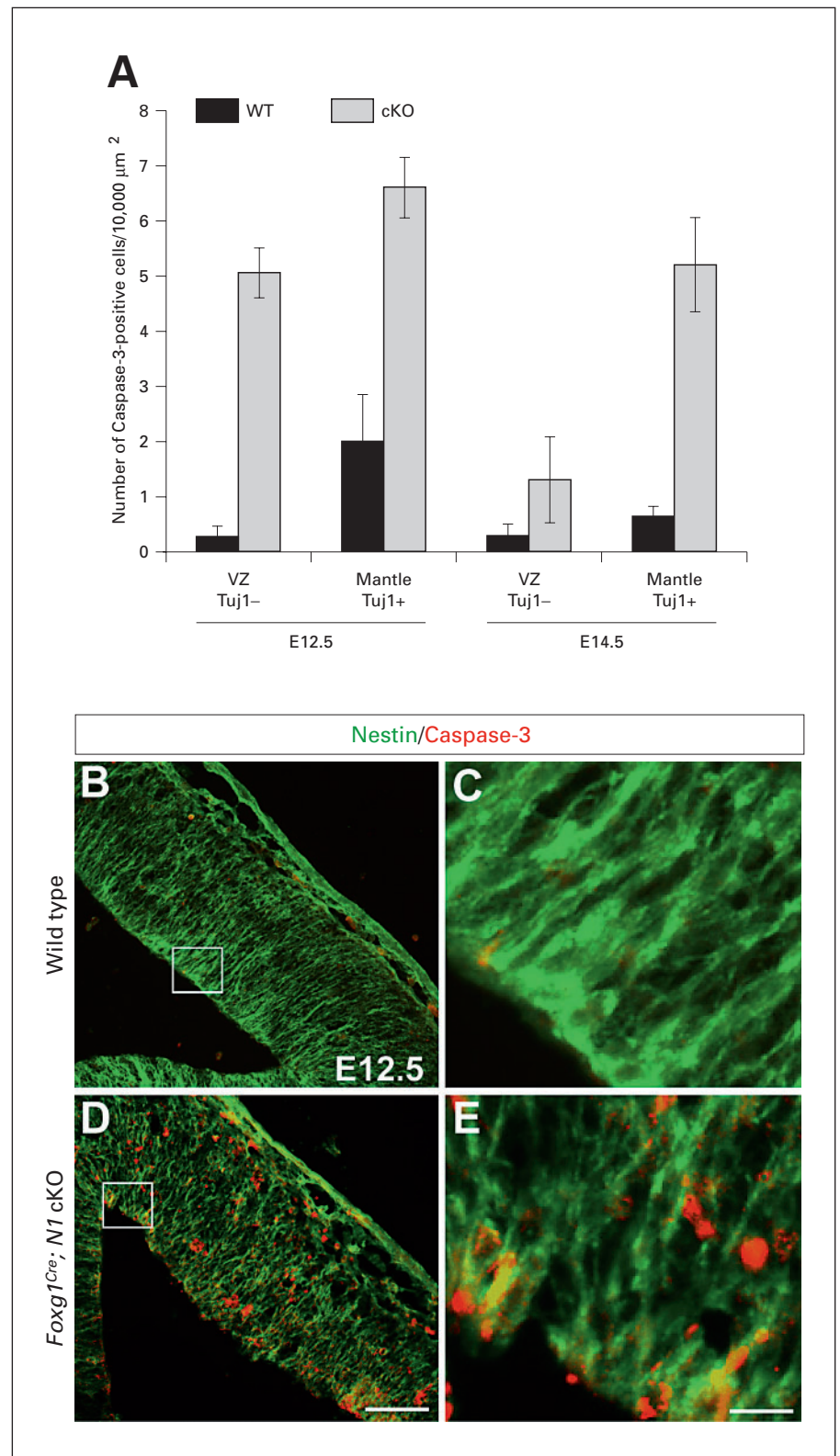


**Fig. 2.** Increased apoptosis is observed at E14.5 in telencephalic-specific *Notch1* conditional mutants. **A–D** Mutant forebrains display more TUNEL-positive cells than wild-type embryos at E14.5. **B, D** A higher magnification view of the cortical-striatal boundary region. Numerous TUNEL-positive cells are observed in *Foxg1<sup>Cre</sup>;N1* cKOs, whereas very few TUNEL-positive cells are found in wild-type littermates. Scale bar: 200  $\mu\text{m}$  in **A**, and **C** and 100  $\mu\text{m}$  in **B**, and **D**. **E** There are significantly more dying cells at E14.5 in the telencephalic-specific *Notch1* conditional mutants compared to wild-type (WT) embryos. Similar levels of cell death are observed using caspase-3 antibodies or TUNEL labeling to measure apoptosis.

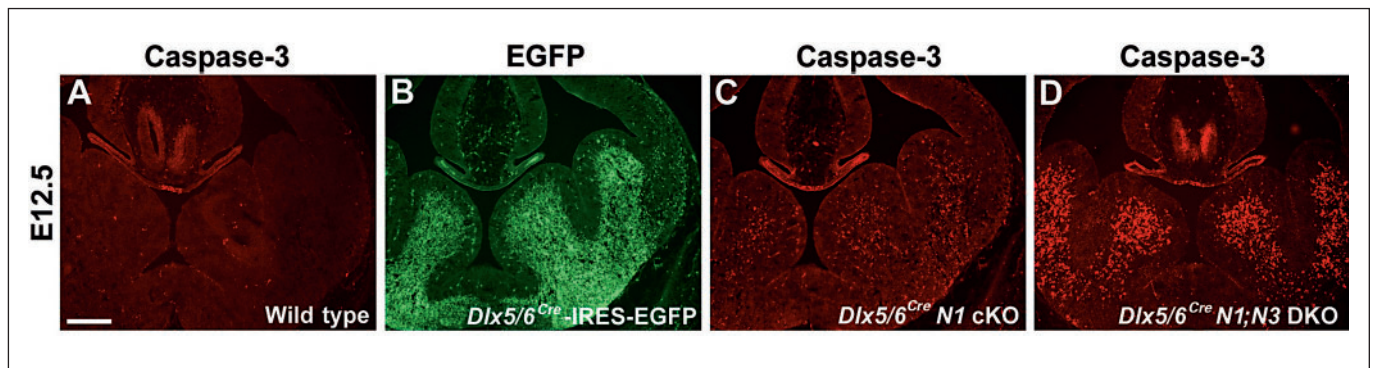
cells, little is known about the potential function of Notch signaling in the survival of more mature neurons undergoing differentiation. To distinguish between the effects of Notch on neural progenitors and differentiating neurons, we immunostained mutant and wild-type forebrains with the Tuj1 antibody, which labels newly differentiating neurons but not progenitor cells residing in the VZ. We find that apoptosis is increased both in the VZ and in differentiating neurons in the absence of *Notch1* at E12.5 and E14.5 (fig. 3A). In addition, we directly examined programmed cell death in neural progenitors by double immunostaining mutant and wild-type forebrains at E12.5 with antibodies to nestin, which labels neural progenitor cells, and activated caspase-3 (fig. 3B–E). We observe that the population of caspase-3-positive dying cells in *Foxg1<sup>Cre</sup>;N1* cKOs is mixed. There are caspase-3/nestin double-positive cells as well as caspase-3-positive/nestin-negative cells. However, there appear to be more differentiating neurons dying than neural progenitors since the majority of apoptotic cells are located within the field of Tuj1-positive neurons rather than within the VZ (fig. 3A). These results suggest that postmitotic neurons also require Notch activity to regulate their survival.

Since Notch has been shown to regulate neurogenesis, removing *Notch1* using *Foxg1<sup>Cre</sup>* may result in precocious neuronal differentiation which then leads to apoptosis as seen by Lutolf et al. [2002]. In this case, neurons that are forced to differentiate prematurely in the absence of Notch signaling may subsequently lack adequate trophic support and undergo programmed cell death due to the absence of the appropriate survival cues rather than the lack of Notch activity. To separate the potential effects of Notch signaling on neurogenesis from those on cell survival, we used *Dlx5/6-Cre-IRES-EGFP* transgenic mice (*Dlx5/6<sup>Cre</sup>*) to remove *Notch1* selectively from cells after they exit the VZ. In *Dlx5/6<sup>Cre</sup>* mice, Cre recombinase is not expressed in the VZ or in the neural progenitor cells that reside there and only becomes expressed when cells begin to differentiate and transit to the ventral subventricular zone and underlying mantle [Stenman et al., 2003]. *Dlx5/6*-expressing cells ultimately produce medium spiny projection neurons in the striatum, striatal interneurons, and GABAergic cortical interneurons [Stuhmer et al., 2002]. We found that a large number of differentiating neurons undergo programmed cell death at E12.5 when *Notch1* is removed from differentiating neurons in the ventral mantle of *Dlx5/6<sup>Cre</sup> N1* cKOs (fig. 4). We have shown that *Notch3* can functionally compensate for the loss of *Notch1* [Mason et al., 2005] and it is therefore possible that *Notch3*, which alone may not be able to promote the survival of as many

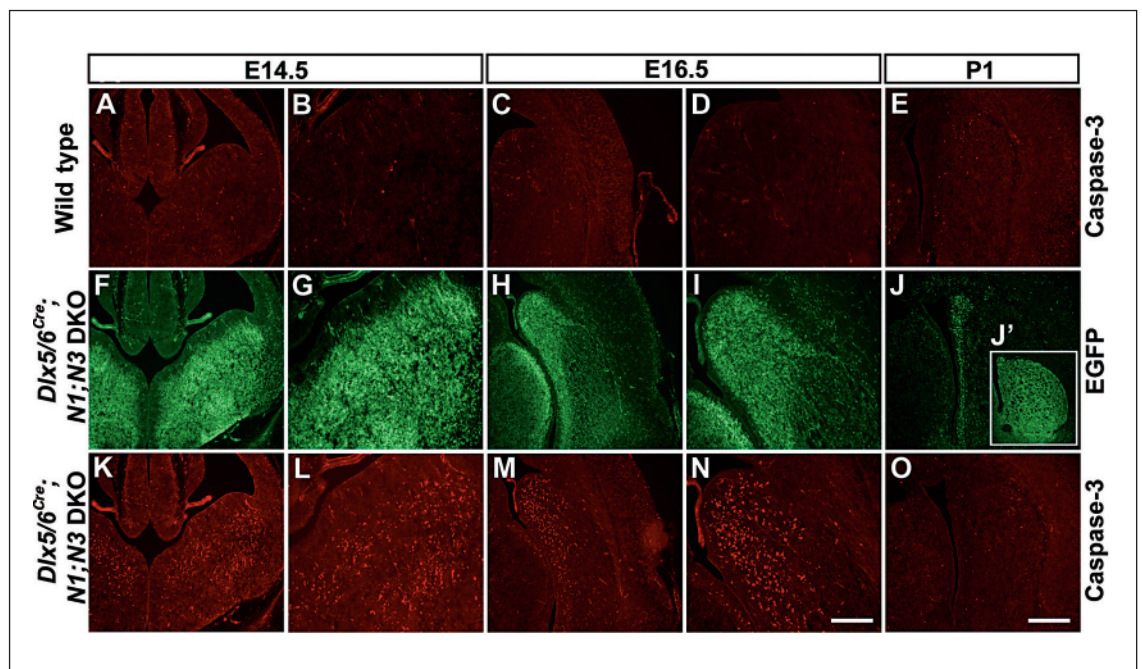
**Fig. 3.** Cell death is increased in neural progenitor cells as well as in newly differentiating neurons in the absence of *Notch1*. **A** Tuj1 immunostaining was used to distinguish neural progenitors from differentiating neurons. The number of dying cells located in the VZ (Tuj1<sup>−</sup> domain) and in the differentiating mantle (Tuj1<sup>+</sup> domain) was quantified in *Foxg1<sup>Cre</sup>;N1* cKOs and wild-type (WT) littermates. More caspase-3-positive cells are detected both in the VZ and regions of differentiating neurons (mantle) in *Foxg1<sup>Cre</sup>;N1* cKOs compared to wild-type embryos at E12.5 and E14.5. However, more dying cells are observed within the population of differentiating neurons (Tuj1<sup>+</sup> domain) than in neural progenitor cells in the VZ of telencephalic-specific *Notch1* conditional mutants. **B–E** Coronal sections of the developing cortical plate were double immunostained with antibodies to nestin (green) to identify neural progenitor cells and caspase-3 (red) to visualize dying cells at E12.5. A single dying cell expresses both nestin and caspase-3 in the wild-type forebrain (**B, C**), indicating that this neural progenitor cell is undergoing programmed cell death. Many more caspase-3-positive dying cells are observed in the forebrain of *Foxg1<sup>Cre</sup>;N1* cKOs compared to wild-type littermates but only a subset of these dying cells are neural progenitor cells (nestin and caspase-3 double positive) (**D, E**). Scale bar: 125  $\mu$ m in **B, D** and 25  $\mu$ m in **C, E**.







**Fig. 4.** *Notch1* and *Notch3* regulate the survival of nascent neurons in the embryonic forebrain. **A** Very few dying cells are detected with caspase-3 antibodies at E12.5 in the wild-type telencephalon. **B** *Dlx5/6*<sup>Cre</sup>-IRES-EGFP transgenic mice express Cre recombinase only in differentiating neurons in the ventral telencephalon, which is visualized here at E12.5 with EGFP immunostaining. **C** In *Dlx5/6*<sup>Cre</sup> *N1* cKOs, apoptosis is increased and many caspase-3-positive cells are observed within the ventral mantle at E12.5. **D** Removing both *Notch1* and *Notch3* results in substantially more cell death than *Notch1* alone. More caspase-3-immunopositive cells are observed in *Dlx5/6*<sup>Cre</sup> *N1*;N3 DKO than in *Dlx5/6*<sup>Cre</sup> *N1* cKOs or in wild-type littermates. Scale bar represents 200 μm.



**Fig. 5.** Increased apoptosis occurs during a restricted temporal window during embryogenesis in *Dlx5/6*<sup>Cre</sup> *N1*;N3 DKO. **A–E** Few casapase-3-postive cells are observed in wild-type mice at E14.5 (**A, B**), E16.5 (**C, D**), and P1 (**E**). **F–J** Cre recombinase is expressed in the ventral regions of the telencephalon as visualized here with EGFP immunostaining in *Dlx5/6*<sup>Cre</sup>-IRES-EGFP; *N1*;N3 double mutants at E14.5 (**F, G**), E16.5 (**H, I**), and P1 (**J**). Although EGFP expression driven by the *Dlx5/6* enhancer decreases by P1 (**J**), all neuronal cell bodies located in the striatum have undergone Cre-mediated recombination at some point during their development as shown here in the inset using the Z/EG Cre recombination reporter mouse (**J'**). **K–O** Increased numbers of caspase-3-positive cells are observed in *Dlx5/6*<sup>Cre</sup> *N1*;N3 DKO at E14.5 (**K, L**), E16.5 (**M, N**) but not at P1 (**O**). Scale bar: 200 μm in **A, C, E, F, H, J, K, M, O** and 400 μm in **B, D, G, I, L, N**.

cells as *Notch1*, may mediate the survival of some cells in the *Dlx5/6<sup>Cre</sup> N1* cKOs. To test this possibility, we examined levels of cell death in double mutants (*Dlx5/6<sup>Cre</sup> N1;N3* DKO). Substantially more dying cells are observed in *Dlx5/6<sup>Cre</sup> N1;N3* DKOs at E12.5 compared with the *Notch1* single conditional mutant (fig. 4). These results suggest that, like *Notch1*, *Notch3* functions to promote the survival of postmitotic neurons.

The observation that removing *Notch1* and *Notch3* in newly differentiating neurons dramatically increases apoptosis suggests that cells continue to be dependent on Notch signaling as they mature, raising the question of whether neurons require Notch activity continuously, even as fully mature neurons, or whether their dependence on the Notch pathway is a transient developmental phenomenon. To test the temporal requirement of Notch activity in neuronal survival, we examined cell death at different developmental time points in *Dlx5/6<sup>Cre</sup> N1;N3* DKOs, from embryogenesis to postnatal day 1 (P1). While we observe a robust increase in the number of dying cells in double mutants compared to wild-type littermates from E12.5 to E16.5 (fig. 4, 5), cell death decreases and by P1 the number of dying cells is equivalent in mutants and wild-type littermates (fig. 5). It is worth noting that *Dlx5/6<sup>Cre</sup>* expression also decreases as embryonic development proceeds and can be visualized with the progressive decrease in EGFP expression at P1 (fig. 5J). However, we performed fate-mapping experiments to identify the cells that had been exposed to Cre activity during their development by crossing *Dlx5/6<sup>Cre</sup>* transgenic mice with the Z/EG Cre recombination reporter mouse [Novak et al., 2000], which turns on EGFP in response to Cre-mediated recombination events. In this way, cells that have been exposed to Cre activity will be permanently marked by EGFP. In *Dlx5/6<sup>Cre</sup>;Z/EG* mice, all neuronal cell bodies in the striatum express EGFP at P1 (fig. 5J') [Mason et al., 2005], indicating that all neurons in this region have undergone recombination and therefore should lack Notch activity. Taken together, these data suggest that there is a limited window during development when neurons require Notch signaling to promote their survival. Once this period is over, neurons appear to be able to survive independent of Notch signals.

## Discussion

Our data indicate that Notch functions to promote the survival of neural progenitors and differentiating neurons in the developing forebrain. Oishi et al. [2004] reported

a similar prosurvival function of *Notch1* based on in vitro studies of neural progenitor cells. Our data build on those findings in several important ways. First, in addition to promoting the survival of neural progenitor cells, we show that Notch signaling is also important in newly differentiating neurons. We identified a number of differentiating neurons in our *Foxg1<sup>Cre</sup>;N1* cKOs that were apoptotic but, in this case, could not rule out the possibility that cells died because they were forced to differentiate prematurely since *Foxg1<sup>Cre</sup>* leads to the removal of *Notch1* in both the neural progenitor cells and their differentiating progeny. However, when Notch activity is removed from cells after they start to differentiate using *Dlx5/6<sup>Cre</sup>*, we also observed elevated levels of programmed cell death. We do not believe that the excessive apoptosis observed in these conditional mutants is a secondary consequence of abnormal neurogenesis since *Dlx5/6<sup>Cre</sup>* is active in cells only after they exit the VZ and begin to differentiate as neurons. Since the Notch pathway is a method of cell-cell communication, it is possible that neighboring neurons may use Notch to communicate with one another to reinforce their correct positioning, and hence survival, while mislocalized neurons may not be near enough to other neurons to receive Notch activation and thus initiate programmed cell death.

We also show that *Notch3* can functionally compensate for the survival-promoting effects of *Notch1*, although not entirely since elevated apoptosis is also observed in the *Notch1* single cKOs. This may be a consequence of decreased overall levels of Notch signaling in the *Notch1* single cKOs because we detect lower levels of *Notch3* mRNA than *Notch1* in the developing telencephalon of wild-type mice and see no obvious upregulation of *Notch3* mRNA in *Notch1* single cKOs [Mason et al., 2005]. Oishi et al. [2004] show that the RAM domain of the Notch intracellular domain is required for the survival-promoting effects of *Notch1*. Although it has been reported that *Notch3* is a poor activator of Notch target genes and can even repress some *Notch1* target genes [Apelqvist et al., 1999; Beatus et al., 1999, 2001], our data indicate that, with respect to cell survival, *Notch3* functions redundantly with *Notch1*. This may occur independent of *Hes* genes, canonical downstream targets of Notch, since Oishi et al. [2004] revealed that Notch's prosurvival function in neural progenitor cells involves a *Hes*-independent mechanism and *Notch3* has been reported to be a poor activator of *Hes1* and *Hes5* [Beatus et al., 1999].

In contrast to our findings, Yang et al. [2004] have recently reported that constitutive activation of *Notch1* in-

duces programmed cell death in neural progenitor cells while removing *Notch1* blocks apoptosis early in forebrain development (E10–E11.5), suggesting that Notch activity has a proapoptotic role. Several possibilities may account for this apparent inconsistency. First, the transgenic mice used contained numerous (10–20 copies) of activated *Notch1*, which is substantially higher than levels of endogenous activated *Notch1*. Oishi et al. [2004] reported that relatively high levels of Notch intracellular domain (1.2 times greater than endogenous levels) resulted in the apoptosis of neural progenitor cells whereas lower levels efficiently promoted their survival. Moreover, we did not observe elevated cell death in response to early embryonic infections of retroviruses expressing activated *Notch1* in the telencephalon [Gaiano and Fishell, unpubl. obs.]. Second, *Notch1* is constitutively activated in the transgenic mice generated by Yang et al. [2004] and, consequently, cells are exposed to continuously elevated levels of Notch activity, which is much longer than cells in vivo. Third, the authors assessed apoptosis in their *Notch1* cKOs during a very restricted early temporal window (E10–E11.5) and it is possible that they would have observed an increase in death at later stages in their mutants.

While it remains possible that the precise actions of Notch activity in the nervous system may be context-dependent, as it appears to be in the hematopoietic system [Deftos et al., 1998; Jehn et al., 1999; Miele and Osborne, 1999; Morimura et al., 2000; Tan-Pertel et al., 2000], our

data suggest that Notch signaling plays a prosurvival role in the embryonic forebrain. We carried out a series of loss of function experiments in which we used conditional mutagenesis to remove *Notch1* and *Notch3* in intact mice and then examined apoptosis at several different time points of forebrain development. We show here that Notch signaling promotes the survival of neural progenitors and differentiating neurons in the developing forebrain. In *Notch1* cKOs and in *Notch1* and *Notch3* double mutants, massive numbers of dying cells are observed from E12.5 to E16.5. At P1, cell death is no longer elevated in *Dlx5/6<sup>Cre</sup>;N1;N3* DKO mice, suggesting that there is a critical period during which neurons are dependent on Notch signaling to regulate their survival and, after that period is over, they ultimately are able to survive independently of Notch activity.

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