Not(ch) just development: Notch signalling in the adult brain

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Abstract

The Notch pathway is often regarded as a developmental pathway, but components of Notch signalling are expressed and active in the adult brain. With the advent of more sophisticated genetic manipulations, evidence has emerged that suggests both conserved and novel roles for Notch signalling in the adult brain. Not surprisingly, Notch is a key regulator of adult neural stem cells, but it is increasingly clear that Notch signalling also has roles in the regulation of migration, morphology, synaptic plasticity and survival of immature and mature neurons. Understanding the many functions of Notch signalling in the adult brain, and its dysfunction in neurodegenerative disease and malignancy, is crucial to the development of new therapeutics that are centred around this pathway.

Normal brain development requires exquisite timing of differentiation programs, and Notch signalling is well known as a master regulator of neural stem cells (NSCs) and neural development[1,2]. But Notch expression persists throughout the adult brain and in mature, differentiated cells in the CNS[3,4]. The importance of Notch signalling for normal human adult brain function is demonstrated by its implication in diseases as diverse as Allagile[5,6], CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy)[7] and Hajdu–Cheney[8,9] syndromes, which have functional mutations in key Notch pathway elements, and Down's syndrome[10] and Alzheimer's disease[3,11], which have abnormal Notch expression levels. Furthermore, although they have distinct pathogeneses, each of these diseases has a cognitive impairment and/or a neurodegenerative component, which emphasizes the need to understand the functions of Notch in the adult brain.

Research into Notch function in fully differentiated cells and in the adult brain was initially hampered because of the embryonic lethality of Notch knockout mice[2]. With the development of Cre/*loxP* and viral gene transduction technologies (BOX 1), Notch expression can now be manipulated in mature animals, thus circumventing its

developmental requirement. Not surprisingly, the topic has received much attention in the past few years, and the field has seen a surge in publications on Notch signalling in the adult brain.

Box 1

Methods to manipulate Notch in the adult rodent brain

Mice with mutations in genes that encode elements of the Notch pathway frequently die before birth[2]. Thus, it has been essential to use alternative methods to analyse this signalling pathway. Owing to the exquisite dosage-dependence of Notch signalling, heterozygote mutant mice[52] probably have developmental abnormalities, as may transgenic *Notch* antisense mice[58]. Perhaps the most frequently used method of manipulating Notch signalling is by inhibiting γ-secretase. Pharmacological inhibitors block the S3 cleavage of Notch by the γ-secretase complex, are easy to use and have robust effects on Notch signalling[87]. However, Notch blockade by γ-secretase inhibitors rapidly becomes toxic to the animals, owing to effects on intestinal precursor cells[148]. As an alternative — and more specific — means to target Notch, antibodies to the receptor and its ligands have been used, but delivery to the brain parenchyma of such antibodies is challenging owing to the difficulty in administration and limited diffusion. *In vitro*, and more rarely *in vivo*, soluble ligands and ligand domain peptides have been used as both blockers and stimulators of Notch signalling[81,149]. As can be expected from such discrepant effects (both inhibitory and stimulating), the mechanism of action of soluble ligands is somewhat controversial[149]. There has been speculation that high concentrations of soluble ligands can result in multimers that can activate Notch, whereas inhibitory monomers might predominate at lower concentrations[150]. Immobilized or clustered ligands are more commonly used but require proper titration[149].

Conditional and inducible mutant mice exist for many of the key elements of the Notch pathway, including conditional Notch knockout (loss-of-function) and transgenic Notch intracellular domain (NICD) (gain-of-function) lines[2]. These lines make use of the Cre/ *loxP* system, in which the gene of interest (or a portion of the gene) is flanked by *loxP* sites ('floxed'). The floxed sequence can be removed using Cre recombinase, which recognizes the *loxP* sites. Tissue- and cell-specificity is achieved by driving *Cre* expression with an appropriate promoter, whereas inducibility is achieved by fusing Cre to a domain that sequesters it in the cytoplasm until administration of an agent, most commonly a tamoxifen-responsive mutated oestrogen receptor, promotes nuclear translocation. For neuron-specific manipulation, many Cre driver lines exist with which to conditionally manipulate the Notch pathway, though few studies have been performed using such mice[51]. The study of adult neural stem cells requires the use of inducible Cre lines, as knocking out Notch will always cause developmental abnormalities in neural stem cells when conditional drivers such as nestin–Cre and glial fibrillary acidic protein (GFAP)–Cre are employed[2]. However, inducible lines require extensive breeding, and given the need to induce nuclear translocation of inducible Cre, recombination rates are usually lower than traditional Cre lines. Frequently, recombination reporter genes, such as ROSA *yellow fluorescent protein (YFP)* or ROSA–*lacZ*, are used as a surrogate for recombination of the desired locus, but the recombination of reporter lines and floxed target genes are not linked processes, and demonstrating efficient recombination of both can be challenging[151]. Furthermore, many of the Notch receptors may have redundant roles[152], necessitating recombination of more than one Notch receptor, or alternatively, common downstream targets. Conditional transgenic dominant-negative mastermind-like (*Maml*) mice, and conditional alleles of recombining binding protein suppressor of hairless (*Rbpj*) or *Maml* — all being part of the complex that forms with NICD to

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activate transcription — offer alternatives to block the function of all Notch receptors but may also have additional, Notch-independent effects[153–155].

Notch signalling is used reiteratively in stages beyond stem and progenitor cells, including in the migratory and postmitotic stages of the neuronal lifecycle. That is to say, the core elements of the Notch pathway as used by precursors are also used to initiate several processes in neurons. Here, we review the evidence that Notch acts as master regulator of plasticity in the adult brain — from stem cells to mature neurons to degenerating neurons. We explore how Notch can affect each stage of the neuronal life cycle to generate an adaptive response, and we discuss the possible mechanisms and accessory pathways that are involved in these processes. Understanding how a master regulator like Notch functions in such diverse roles furthers our ability to develop feasible strategies to manipulate Notch in a clinically beneficial manner.

A brief history of Notch

The Notch signalling pathway is among the most well-conserved signalling pathways in animals. It arose with the evolution of multicellular organisms and the concomitant need for juxtacrine cell-to-cell communication to coordinate development[12]. As it is beyond the scope of this Review to explore the literature on Notch signalling in embryonic neurogenesis in detail[1,2,12], we briefly highlight here our current understanding of the core elements of Notch signalling (FIG. 1).

Notch functions as a receptor, and mammals have four Notch receptors (Notch1, Notch2, Notch3 and Notch4) and many ligands (TABLE 1), including jagged 1 (JAG1) and JAG2 (homologues of serrate), and delta-like proteins (reviewed in REFS 13,14). Notch and its ligands are single-pass transmembrane heterodimers (FIG. 1a,b). The extracellular domains of both Notch and its ligands consist of multiple epidermal growth factor (EGF) repeats, which can be modified by the addition of sugar moieties. Notch receptors also contain several domains that maintain the receptor in an inactivated state in the absence of a ligand[13,15]. Upon ligand binding, a series of cleavage events culminates in γ-secretase-mediated cleavage of the transmembrane domain of Notch, and release of the Notch intracellular domain (NICD) into the cytosol. The extracellular domain of Notch (NECD) is endocytosed, along with the ligand, into the 'signal-sending cell' — that is, the ligand-presenting cell (FIG. 1c–e; reviewed in REF. 13). Ligands are also subject to γ-secretase-mediated cleavage, but the function of ligand cleavage is not well understood[16].

In the canonical Notch signalling pathway, NICD translocates to the nucleus and, with mastermind-like protein 1 (MAML1), MAML2 or MAML3, converts the recombining binding protein suppressor of hairless (RBPJ) complex from a transcriptional inhibitor to an activator (FIG. 1f,g). The most studied Notch targets are the hairy and enhancer of split-related (*HESR*) genes, with many additional genes recently having been identified as Notch targets[17]. NICD can also signal through a non-canonical pathway, presumably through protein–protein interactions and RBPJ-independent gene activation[18] (FIG. 1h).

Ligand binding permits disintegrin and metalloproteinase domain-containing protein (ADAM)-mediated S2 cleavage of the extracellular juxtamembrane region[13]. The γ-secretase cleavage of Notch is a proteolytic event that then occurs rapidly after ligand-mediated removal of the extracellular domain of Notch[13]. Cleavage by γ-secretase is a common target of pharmacological inhibition by molecules such as DAPT and DBZ (BOX 1). Such γ-secretase inhibitors are powerful blockers of Notch activity, with the important caveat that the γ-secretase complex cleaves at least 60 different proteins (and counting)[19]. Indeed, as shown in a recent study, γ-secretase-mediated effects frequently ascribed to

Notch (for example, neural stem cell renewal) can be, and often may be, in fact Notch-independent[20]. Specifically, this study showed that Notch activation was sufficient to cause astrogliogenesis from neural stem cells but that the decreased stem cell self-renewal following γ-secretase inhibition was independent of Notch signalling, as NICD could not rescue these deficits[20]. Thus, although γ-secretase inhibitors may be a useful tool to screen for Notch pathway involvement, genetic approaches are necessary for more definitive conclusions regarding such involvement. Indeed, the lack of substrate specificity of γ-secretase inhibitors and the concomitant challenges in interpreting clinical data have hampered progress in the field of Alzheimer's disease therapeutics[21].

Notch in the adult brain

The neurogenic niches of the adult brain — the subventricular zone (SVZ) and the subgranular zone (SGZ) — represent a preservation of the embryonic germinal zones, and continue to generate cells throughout life to varying extents, depending on the species[22]. These niches are only a fraction of total adult brain tissue, yet Notch pathway components are expressed throughout the adult brain[23–26]. This suggests that Notch might have roles beyond regulating stem cell maintenance and differentiation. Here we review recent evidence for Notch signalling in the adult CNS, and explore its role in regulating both progenitors and mature cell types in the adult brain (FIG. 2).

To cycle or not to cycle?

In stem and progenitor cells, proliferation is central to cell fate decision making because differentiation programs are tightly coupled to the process of cell cycle exit[27,28]. It is not surprising then, that Notch regulates the cell cycle to balance stem cell maintenance with daughter cell production, thereby preventing premature depletion of the niche. In zebrafish, which have a prominent adult telencephalic germinal zone, high levels of Notch activation in radial glia maintain the cells in a quiescent state, whereas a decrease in Notch activity leads to NSC proliferation and an increased net number of adult-born neurons[29]. Moreover, increased cell cycle exit of early precursor types[24] and impaired expansion of the progenitor pool[30] has been found in the SGZ of adult inducible Notch knockout mice, with eventual depletion of the stem population[31,32]. Consistent with the idea that Notch levels regulate the cell cycle, treating NSCs derived from human embryonic stem cells with the Notch inhibitor DAPT decreases levels of cyclin-dependent kinase 4 (CDK4) and S-phase kinase-associated protein 2 (SKP2), and increases levels of the cell cycle inhibitor cyclin-dependent kinase inhibitor 1B (p27KIP1). This prolongs the G1–S transition, and if levels of Notch signalling remain low and growth factors are withdrawn, the cell exits the cycle and differentiates into a neuron[33]. Interestingly, the relative levels of Notch signalling in neural stem cells (NSCs) can regulate the opposing states of quiescence versus proliferation in NSCs — with low levels leading to proliferation and high levels causing growth arrest[34]. However, neuronally committed, doublecortin-positive (DCX-positive) progenitors are less dependent on Notch and more responsive to environmental cues for the regulation of proliferation[30], suggesting that the capacity of Notch to regulate proliferation in the adult brain is cell type-dependent, as discussed below.

Migration and early differentiation

Although the role of Notch in NSC maintenance is well described, Notch signalling in migrating and maturing cells in the perinatal and adult brain is less well characterized. For example, Notch signalling seems to perturb neuronal migration in the cerebral cortex by altering the morphology of migrating neurons. Increased Notch signalling leads to a bipolar morphology that favours migration, whereas decreased Notch signalling leads to a more multipolar morphology that stalls migration[35]. The mechanism by which Notch regulates

migration involves reelin–DAB1 (disabled homolog 1) signalling, which inhibits NICD degradation[35]. Notably, the phenotype of reelin-lacking reeler mice — which are characterized by abnormal lamination of cortical neurons due to failed migration — could be rescued by overexpression of an NICD transgene[35]. Although these results were cell-autonomous to neurons (established by using neuron-specific promoters to drive transgene expression), it is notable that in the postnatal dentate gyrus a similar cooperative mechanism between NICD and reelin is essential for the formation and maintenance of the radial glial scaffold[36]. In DCX-positive immature neurons of the adult SGZ, NICD accumulates in the nucleus as the morphology changes to that of a migrating neuron[24,37,38]. More ramified cells show higher, more intense immunostaining for NICD, with mature cells exhibiting the highest levels. Interestingly, this pattern is consistent with the changes seen in the localization of neurogenin 3 (NGN3), as NGN3 becomes increasingly localized to the nucleus during neuronal differentiation[39].

Another possible mechanism by which Notch can modulate neuronal migration and early differentiation came from studies in primary cortical neurons. Here, treatment with the Notch ligand JAG1 downregulated spastin, a protein involved in severing microtubules, and this resulted in increased microtubule stability. Immunostaining for acetylated α-tubulin revealed notable increases in microtuble branching and neurite thickness in these cortical neurons. This effect on microtubule stability could be blocked by γ-secretase inhibition[40], indicating that it depended on NICD. Taken together, these findings demonstrate a role for Notch in regulating microtubule dynamics. Future studies will be needed to determine how direct this regulation is.

There is also evidence from studies in *Drosophila* that Notch may act non-canonically on axon guidance during development through tyrosine-protein kinase ABL signalling[41–43]. Specifically, it seems that Notch interacts with DAB and triple functional domain protein (TRIO)–ABL accessory proteins and directs axon guidance and dynamic patterning through modulation of the growth cone[43]. This is consistent with the interaction between DAB1 and Notch1 that occurs during neocortical neuron migration in cortical development[35]. An interaction between Notch and atonal has also been shown to cause dramatic alterations in axonal arborization in *Drosophila*[44]. In this study, high Notch activity diminished axonal branching, whereas low Notch activity had the opposite effect[44]. It remains to be seen whether Notch regulates axon dynamics in vertebrate neuronal development.

Structural plasticity

In developing cortical cells, Notch seems to modulate the complexity of the dendritic arbour. Transgenic NICD expression, ligand presentation and a high cell density — all leading to canonical Notch activation — cause a more complex, but often stunted, dendritic tree[4,45,46]. Conversely, loss-of-function experiments demonstrated that lack of Notch signalling reduced neurite numbers[4,45,46]. The modulation of dendritic arbour complexity by Notch seems to be dependent on canonical Notch signalling, as nuclear translocation of NICD is prominent and RBPJ-dependent Notch activity has been reported to occur in this process[4,46]. Similarly, levels of Notch signalling correlate with the complexity of the dendritic arbour of neurons in the postnatal and adult SGZ[24]. More recently, it was noted that adult inducible *Notch1* knockout mice have granule cell neurons with smaller dendritic trees in the dentate gyrus[30]. NGN3 has been implicated as a possible player in the regulation of structural plasticity[39,47], as have NUMB and numb-like (NUMBL) in neocortical cells[4] and in sensory neurons[48]. In the case of NGN3, which promotes neurite outgrowth, Notch activation leads to expression of *Hes* genes that inhibit *Ngn3* expression and neurite outgrowth[47]. Thus, a balance of NGN3 and Notch activity regulates dendritic complexity. NUMB and NUMBL, however, directly antagonize Notch signalling through endocytic control[49], preventing surface localization and, presumably, activation of Notch.

Strikingly, in contrast to the effect of genetic manipulations of immature neurons, rodents with transgenic manipulations of Notch in mature, postmitotic cortical neurons seem to have normal gross dendritic morphology, suggesting that there may be a critical period for Notch signalling in the regulation of neuronal aborization, possibly coinciding with the critical period for integration of new neurons[50]. An elegant Cre/*loxP* system was used to express active Notch1 selectively in cortical pyramidal cells in adult mice[51], and this manipulation resulted in reduced spine and filopodia densities without any gross changes in the dendritic arbour. These findings show that although Notch may not be able to reorganize a mature dendritic arbour during early adulthood, it can continue to fine-tune the dendritic spines.

Synaptic plasticity

A few studies have suggested that genetic manipulation of Notch influences plasticity and behaviour. An initial report described specific deficits in spatial learning and memory in adult *Notch1* heterozygote mutant mice[52]. Adult *Rbpj* heterozygote mutant mice had similar but somewhat more severe disruptions in this form of memory[52]. However, the exquisite dosage-dependence of Notch makes it difficult to interpret these findings as these mice probably had developmental defects such as premature myelination[53] and, potentially, changes in total neuron numbers[54]. Sidestepping such developmental confounds, a temperature-sensitive allele was used to examine the effect of Notch dysfunction in memory tasks in *Drosophila*. This study found that short-term memory was spared, whereas long-term memory was impaired[55]. Another study in *Drosophila*, using conditional *Notch* mutants or an induced dominant-negative *Notch*, found a similar deficit in long-term memory that could be rescued by overexpression of a wild-type *Notch* transgene[56]. Neuralized (NEUR), another molecule that modulates the Notch signalling pathway (among others) has also been implicated in long-term memory in *Drosophila*[57]. However, whether this role is dependent on Notch remains to be determined.

In the hippocampus of adult mice, reduced Notch levels (by expression of a transgenic antisense construct) led to impaired long-term potentiation (LTP) and enhanced long-term depression (LTD)[58]. Furthermore, treating hippocampal slices from these mice with an activating jagged peptide facilitated LTP[58]. However, much like Notch is transiently attenuated in the differentiation process of neurons, there is evidence that Notch signalling is interrupted — and that mRNA levels of the Notch target gene *Hes1* are reduced — during the consolidation of long-term memory 10–12 hours after passive avoidance conditioning[59]. Indeed, administration of a Notch-specific antibody, which stimulates receptor activation, blocked consolidation. Perhaps surprisingly, this study found an NICD fragment of 66 kDa (visualized using two separate antibodies) in the nuclear fraction of lysed dentate gyrus neurons — a fragment well below the usual size[59]. Further study is needed to confirm these findings and to determine whether this is a breakdown product of NICD, a novel functional fragment or perhaps an imprecise cleavage event, as has been described *in vitro*[60].

The role of Notch in synaptic plasticity is likely to be complex, as has been shown by studies in the *Drosophila* neuromuscular junction (NMJ). Here, Notch is necessary for appropriate NMJ complexity (measured in terms of axonal branches and presynaptic boutons), which can be blocked by expressing a dominant-negative Notch receptor[61]. However, the same study found that expression of higher levels of transgenic Notch or of constitutively active Notch blocks plasticity in the NMJ, suggesting that a moderate level of Notch might be ideal[61] — and again demonstrating the dose-dependent nature of Notch signalling. Interestingly, expression of Notch in the NMJ correlates with neuronal activity[61]. The finding in this study[61] that Notch can have opposing roles depending on the level of receptor activation could explain discrepant findings in different studies. For example, one study found that Notch activation decreases LTP[51], whereas another demonstrated that it enhances LTP[58]. This paradoxical pattern is also seen in studies of Notch during

neurodevelopment and — as discussed below — is probably due to the exquisite context- and dosage-dependence of Notch signalling.

Our understanding of the role of Notch in neuronal morphology and synaptic plasticity is still in its infancy and awaits further investigation. For example, the signal that stimulates Notch cleavage in neurons *in vivo* was unclear until recently published work greatly extended our understanding of the role of Notch signalling in neuronal plasticity in the mammalian[62] and *Drosophila*[63] brain. Gaiano and colleagues used transgenic *Notch* reporter mice and antibodies to Notch1 to confirm the expression and activity of Notch1 in neurons[62], and demonstrated the localization of JAG1 to synapses primarily along the axon. They noted that Notch1 expression and cleavage increased in response to synaptic activity, with Notch1 cleavage being dependent on the neuronal activity-related gene *Arc* (activity-regulated cytoskeleton-associated protein; also known as *Arg3.1*). Fascinatingly, this dependence on *Arc* was only present in neurons and not during embryonic neural stem cell maintenance, when NICD is present in abundance. *Notch1* knockout in mature neurons did not alter gross morphology but caused alterations in dendritic spines and disruption of LTP and LTD, leading to marked deficits in performance in several memory tasks[62]. Struhl and colleagues showed that in *Drosophila*, Notch was activated specifically in brain regions that are responsive to distinct odours[63]. Notably, Notch activity peaked at 24 hours after odour exposure and decreased in the following days. The authors speculated that this long decay period may be crucial for pairing of stimuli over extended periods of time[63]. Although Gaiano and colleagues noted that JAG1 was the likely ligand for Notch at the synapse in the rodent brain[62], Struhl and colleagues showed that neuronal Notch cleavage in *Drosophila* was due to Delta stimulation[63]. As is evident from these studies, increasingly precise temporal and spatial manipulations of Notch will no doubt be invaluable in the elucidation of the precise function (or functions) of Notch in neuronal plasticity.

Ageing neurons

Some of the initial studies of Notch expression were performed in the context of age-related human disease, most notably Alzheimer's disease, owing to the fact that -secretase cleavage is common to both Notch signalling and amyloid precursor protein (APP) processing[3,64]. *In situ* hybridization studies showed that mRNAs encoding Notch and presenilin — a protein that is part of the γ-secretase complex — are strongly expressed in normal brains in hippocampal and entorhinal neurons, with additional expression in cortical neurons and Purkinje cells[3]. Notably, Notch cleavage was dramatically increased in brains of patients with Alzheimer's disease compared with controls[3]. In further support of the hypothesis that Notch processing may be abnormally high in animal models of Alzheimer's disease, several studies have shown an age-dependent decrease of γ-secretase activity on Notch, as well as APP, in the normal brain[65,66]. Additionally, neuron cultures with presenilin mutations that are associated with familial forms of Alzheimer's disease frequently display altered Notch signalling[11,67], and several studies have reported interactions between Notch and APP[10,68–72], and APP and NUMB[73]. More recent studies suggest that, although not causative, aberrant Notch signalling as a result of NUMB dysregulation, and alterations in presenilin and APP, may contribute to the progressive neurodegeneration characteristic of Alzheimer's disease[74]. Unfortunately, thus far, γ-secretase inhibitors have not been very successful as potential treatments for Alzheimer's disease.

An association between Notch and neuronal disease is also seen in prion disorders. NICD protein is elevated in the cortex of mice with prion disease, and this increases with rising levels of PrP[Sc] (REF. 75). Notably, *Notch1* mRNA abundance and NICD translocation correlate with dendritic atrophy of neurons[75]. Using small interfering RNA (siRNA) to knock down *Notch1* in neuroblastoma (N2a) cells (an *in vitro* model for neurons), it was determined that activated Notch1 was primarily responsible for the decrease in neurites[75].

Moreover, treatment of mice with γ-secretase inhibitors (plus quinacrine to reduce PrP[Sc] levels) was sufficient to prevent dendritic atrophy[76]. It is interesting to contrast these findings with those of Dahlhaus *et al.*[51] and Alberi *et al.*[62], who found no change in dendritic arborization in mature, postmitotic, cortical neurons following transgenic expression of NICD or conditional *Notch1* ablation in much younger, disease-free rodents. This suggests that NICD can modulate dendritic complexity in an age-dependent manner during disease.

Ageing and pathology are often linked to shortened telomeres. A novel, mechanistic link between p53, Notch and telomere shortening in the process of neuritogenesis has recently been demonstrated[77]. Telomerase-deficient mice displayed reduced neurogenesis and had less complex neurite arbours, and this effect was dependent on p53. γ-secretase inhibition phenocopied p53 knockouts in terms of neuritic complexity, and this effect could be reversed by NICD overexpression. However, p53 did not directly regulate Notch1 or Notch3 levels[77], indicating that p53 and Notch signalling act in parallel or cooperatively. Notch and p53 could converge on RHO-associated protein kinase 1 (ROCK1) and ROCK2 (REF. 77) — downstream effectors of RHOA — to regulate actin dynamics and thus, neurite extension. It will be interesting to explore these interactions *in vivo* and to ascertain whether neurons born from aged stem cells display intrinsic differences in Notch-dependent arborization — a fact that might impact the development of strategies for endogenous repair of the aged brain.

Notch in response to neurotrauma

Several studies in rodents have reported conflicting results regarding Notch signalling in ischaemic brain injury, with some studies reporting beneficial effects of Notch signalling on progenitor proliferation and survival[78–81], and others reporting detrimental effects of Notch activation[82,83]. The caveat of many of these studies is that they examined gross changes in Notch signalling within a brain region. As we have discussed, changes in Notch signalling may differ between cell types. In addition to its roles in neurons and astroglia, Notch has profound roles in other cell types in the brain, including endothelial cells[84] and oligodendrocytes[85,86]. It may therefore be inappropriate to draw conclusions regarding the potential effect of changes in whole-tissue expression levels of Notch protein or mRNA as there are probably pleiotropic roles for Notch in these different cell types. For example, Notch blockade improves functional outcome in a mouse model of stroke through diverse means[83]. First, Notch2 expression in neurons correlates with levels of post-stroke apoptosis[82], and Notch activation in neurons predisposes them to apoptosis[83], suggesting that Notch inactivation may protect against apoptosis cell-autonomously in neurons. Second, Notch increases inflammation through increased infiltration of immune cells into the infarct[83], and Notch blockade may therefore decrease neuronal cell death in a non-cell-autonomous manner, by dampening the microglial (immune) response. Third, Notch may be involved in reactive gliosis following neurotrauma or disease[25,87,88]. This is consistent with findings seen following NICD expression in the intact brain, notably proliferation and maintenance of the glial fibrillary acidic protein (GFAP)-positive cell type in daughter cells, which are hallmarks of reactive gliosis[24,89].

Notch signalling has also been implicated in the inability to recover from demyelinating injuries and diseases. Following a compression injury of the spinal cord in adult mice, Notch pathway components were upregulated in neurons, NUMB was upregulated in GFAP-positive astrocytes and sonic hedgehog (SHH) was expressed by myelin basic protein (MBP)-positive oligodendrocytes[90]. Although differentiation programs are activated in response to injury, integration of new neurons and remyelination of existing axons does not occur, perhaps owing to a gliogenic switch in adult NSCs. Notch activation in NSCs can inhibit the generation of neuronal progenitors and promote the generation of a gliogenic

progenitor that can give rise to both oligodendrocytes and astrocytes. Further activation of Notch in the gliogenic progenitor inhibits oligodendrocyte differentiation and promotes astrocytic differentiation[91]. For example, in a mouse model of the demyelinating disease multiple sclerosis, Notch activation prevented oligodendrocyte differentiation and subsequent remyelination, leading to the generation of a glial scar[92]. However, another study found that Notch was needed to promote remyelination and differentiation of oligodendrocytes[93], again highlighting the importance of the level of Notch activation. Future studies will need to use cell type-specific means of Notch manipulation (BOX 1) to sort out the role of Notch signalling during pathologies and subsequent recovery.

It's all about the context

One of the greatest challenges in studying Notch signalling is the inability to predict the outcome of Notch activation, owing to its multiple roles (FIG. 3). The canonical Notch pathway is strikingly simple; there are no second messengers. How is it, then, that Notch signalling can have such pleiotropic functions? One mechanism is through extensive crosstalk with other pathways. It is generally accepted that Notch signalling integrates multiple pathways, thereby tuning downstream expression to fit the cell's current needs and situation, that is, the cell's context (reviewed in references REFS 94,95). This context is composed of several parts, such as the capacity to receive Notch activation, cell type, age of the animal, stimulus type and brain area. In addition, epigenetic factors[96] and tissue-specific Notch cofactors[97] can modulate the downstream targets receptive to the Notch ternary complex though changes in chromatin conformation, binding specificity or other nuclear modifications. The multiple functions of Notch signalling could be considered complementary rather than contradictory. In the following sections we will explore the factors that may determine which function of Notch signalling is relevant in a particular context. We propose that the varied and complex outcomes of Notch signalling (FIG. 3) are manifestations of a Notch signalling 'network' that controls the crucial choices that cells must make at particular stages in life[12,94,98]. When cells lose the ability to recognize their context and modulate Notch signalling accordingly, they can no longer make appropriate choices, as has been suggested in some types of brain tumour (BOX 2).

Box 2

Pathological stem cell maintenance: Notch and glioma

Gliomas are brain tumours that arise from glia cells, including radial glia. These are often aggressive, rapidly growing tumours with poor prognosis, mainly because of their resistance to chemotherapy and radiation, and their high incidence of recurrence. Gliomas are thought to arise from aberrant neural stem cells — in cancer contexts they are often referred to as cancer stem cells — and not surprisingly, Notch signalling is involved in the generation and/or progression of gliomas. For example, overexpression of the Notch intracellular domain (NICD) in a neural stem cell line is sufficient to transform it into a tumorigenic cell line[156]. Aberrant Notch signalling leads to aberrant epidermal growth factor receptor (EGFR) signalling[157], a feature of the most malignant glioma, glioblastoma[158]. Likewise, disrupted regulation of Notch owing to mutations in *NUMB* or neuralized (*NEUR*) leads to tumour formation[159,160].

Although not all gliomas arise due to disrupted Notch signalling, Notch signalling is involved in maintaining the cancer stem cells and in promoting their proliferation. It is activated in response to hypoxia and angiogenesis, two processes characteristic of gliomas, especially the aggressive glioblastoma[161,162]. Notch may be moderately expressed and inactive in low-grade tumours, but it is highly expressed and active in glioblastoma[163]. By maintaining the relatively quiescent cancer stem cells, Notch promotes radioresistance of gliomas, as these quiescent cells are not sensitive to the

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therapy and survive to repopulate the tumour[164]. However, Notch signalling can also limit the growth and severity of gliomas. In some medulloblastomas, Notch1 inhibits growth, whereas Notch2 promotes tumorigenesis[165]. Although current understanding of how Notch signalling contributes to the formation and progression of gliomas is limited, it has led to the development of more effective therapies. For example, inhibition of Notch using γ-secretase inhibitors decreases proliferation and increases radiosensitivity of glioblastoma cell lines[164,166]. Studies are underway to determine if treating patients with γ-secretase inhibitors may make gliomas more sensitive to current interventions (see the ClinicalTrials.gov website. Identifiers: NCT00572182, NCT01119599 and NCT01122901).

Directional signalling: sender or receiver?

If a cell is to use Notch signalling to make a choice, it must possess functional Notch receptors and be in contact with cells that express the ligand. It is generally thought that activation of the Notch pathway results in a mix of non-cell-autonomous (ligand-mediated) and cell-autonomous (receptor-mediated) effects. Indeed, disruption of Notch ligands seemingly leads to non-cell-autonomous impairment of neurogenesis[99], whereas disruption of Notch receptors or RBPJ has been reported to lead to cell-autonomous effects[31,32]. This suggests that Notch signalling occurs in one direction, from the signal- sending (that is, ligand-presenting) cell to the signal-receiving (that is, Notch-presenting) cell[12] (FIG. 1). The mechanisms that determine whether a cell will be a signal sender or a signal receiver remain largely unknown. Some studies indicate that this process may be stochastic[100], whereas studies by Kageyama and colleagues, using elegant bioluminescence studies, attribute it to oscillation of the levels of pathway components, especially HES1 (REF. 101), or to post-translational modifications that affect receptor and ligand affinity and availability[102]. Intrinsic and extrinsic factors, as well as transcriptional feedback, regulate the levels of ligand and receptor, such that subtle initial differences in the ligand-to-receptor ratio are amplified and a signalling or receiving mode is established[13,103]. *Cis* ligand–receptor interactions inhibit Notch activation[104], proteins such as deltex, NUMB and NEUR regulate the cellular distribution and surface availability of Notch, and non-canonical ligands modulate signalling strength — that is, the probability that a sufficient number of Notch receptors is activated to elicit a response[14]. The level of functional receptor that is available dictates the ability of the cell to receive a signal, especially given that each receptor can only be activated once owing to the irreversible cleavage events that are necessary for signalling[12,13,34].

Molecular context

Many factors converge to determine the outcome of Notch activation, but perhaps the single most important factor is the type of cell that is receiving activation. This is because the repertoire of genes available for modulation (that is, the 'molecular context') varies greatly between cell types. There is extensive crosstalk between Notch and pathways implicated in different phases of adult SGZ and SVZ neurogenesis, and it is this crosstalk that allows signals to be integrated and consolidated (FIG. 3). Conditional knockout mice of the SHH pathway component protein patched homologue 1 (PTCH1)[105] and of cilia proteins[106] (which seem to mediate SHH signalling) display similar deficiencies as *Notch1*-inducible mutant mice — notably the depletion of primary, radial glial-like SGZ precursors[81,107–109] — suggesting that both pathways are necessary for precursor maintenance. Likewise, WNT–β-catenin[110] and Notch signalling seem to interact on several levels. WNT stimulates the expression of Notch ligands in progenitors[111], thus provoking Notch signalling in adjacent NSCs, whereas Notch activation modulates the threshold of WNT signalling[112]. The two pathways also interact through glycogen synthase kinase-3β (GSK3β)[113] and fibroblast

growth factor (FGF)[114] to link inhibition of differentiation with proliferation, and to cooperatively regulate the number of new neurons generated. Notch can also integrate signals from multiple growth factors in adult neural stem and progenitor cells. Pigment epithelium-derived factor (PEDF) increases the self-renewal capability of stem cells in the adult SVZ by upregulating the Notch target gene *Egfr* (epidermal growth factor receptor)[23]. However, another report indicates that in the adult SVZ, EGFR-expressing cells limit Notch signalling through a NUMB-dependent mechanism[115] and suggests that Notch and EGFR cooperate to balance stem and progenitor cell numbers.

In contrast to its role in promoting stem cell maintenance and survival, Notch activation can also be pro-apoptotic in cycling cells. Putative disinhibition of Notch signalling using inducible *Numb* and *Numbl* knockout mice compromises progenitor survival[116]. However, the relationship between Notch and the cargo-specific adaptor protein NUMB and its closely related and partially- redundant NUMBL protein is quite complex. In *Drosophila*, NUMB acts as a cell-fate determinant by asymmetrically localizing during mitosis and by inhibiting Notch in one of the daughter cells[117]. However, although the role of Notch in maintaining neural stem in vertebrate cells exquisitely mirrors its role in the fruitfly, the role of NUMB is apparently not as well conserved[117]. For example, although *Notch* manipulations that result in gain-of-function consistently maintain the neural stem cell fate, manipulations of NUMB and NUMBL that result in loss of function, and that would be expected to result in an upregulation of Notch, do not show the anticipated outcome of massively decreased neurogene-sis[118]. However, morphogenesis is grossly disrupted. This is probably due to the role of NUMB in cadherin recycling[116,118] and its interactions with other pathways and molecules such as p53 (REF. 119), SHH[120], integrins and partitioning defective (PAR)-family proteins[121]. Indeed, overexpression of NUMB promoted the abnormal perdurance of the radial glial morphology — a phenotype that would be more commonly expected after Notch activation — owing to cadherin-based maintenance of the adherens junctions[118]. In the postnatal brain, a similar cadherin-based phenotype (that is, loss of ventricular zone integrity owing to loss of adherens junctions) was seen upon *Numb* and *Numbl* ablation[116]. However, Notch can be inhibited by overexpressing *Numb* in postmitotic neurons[4]. Futhermore, multiple groups have found interactions between Notch, NUMB and APP[69,72,73], allowing for the possibility that changes in the dynamics[122] of these interactions may be involved in the pathogenesis of Alzheimer's disease. Taken together, owing to its effects on many diverse signalling pathways, the role of NUMB defies a simple categorization as a Notch inhibitor[117].

Several Notch-interacting pathways that are beyond the scope of this Review have been identified in adult NSCs[123,124]. However, little is known about interacting pathways in postmitotic neurons. Notch signalling can promote the survival of postmitotic newborn neurons through α-synuclein in the adult hippocampus[38]. As discussed above, Notch signalling in neurons is dependent on activity-induced genes[62] and cooperates with nerve growth factor (NGF) and its receptor p75 to regulate synaptic plasticity[47]. Hopefully, the growing body of knowledge about these signalling pathways and their context-dependent, cell type-specific interactions may one day allow us to fit together the molecular hierarchy to create a mechanistic model of the neurobiology of Notch signalling in the adult brain.

Physical context

The neurogenic niches are discrete areas of the brain, and it is only in these niches that neurogenesis normally occurs in adults[22]. It is well established that the niche itself provides extrinsic cues that Notch integrates to modulate cellular output accordingly[125]. It is not surprising, therefore, to find that neural stem cells isolated from the SVZ are distinct from stem cells in the SGZ. For example, SVZ stem cells generate inhibitory interneurons, whereas SGZ stem cells generate excitatory granule cell neurons[123]. Thus, caution should be

taken when extrapolating findings from one niche to the other. Nevertheless, results from conditional and inducible *Notch* knockout mice suggest that Notch signalling maintains astroglial stem cells in both the SVZ and SGZ by preventing differentiation[32].

Although much work is needed to fully understand the role of the niche environment in modulating the identity, potency and proliferation of neural stem cells, we have learned that both extracellular signals and neighbouring cells contribute to this modulation. Both vascular endothelial cells and astrocytes (which wrap the cerebral vasculature) stimulate neurogenesis from adult NSCs[126,127], and this has led to the hypothesis that neurogenesis occurs preferentially in a 'vascular niche' created by this network of vessels and astrocytes in the SVZ and SGZ[128,129]. An intruiging study showed that endothelial cells in the adult SVZ induce Notch activation in NSCs indirectly through the candidate factor PEDF, thus biasing cell fate towards radial glia[23]. PEDF activates NF-κB in a non-canonical manner, and this induced nuclear export of both the p65 subunit of NF-κB and a nuclear receptor co-repressor (NCOR) protein from the Notch-responsive promoters of *Hes1* and *Egfr*. By removing NCOR — and thereby disinhibiting the Notch target genes — stem cells are more likely to activate the Notch target genes responsible for self-renewing, asymmetric divisions.

Contrary to expectation, the vascular niche does not depend on high levels of oxygenation to support NSCs in the adult brain. Unlike mature neurons, NSCs are resistant to hypoxia, and even display more rapid expansion and enhanced self-renewal in 1% O_2 compared with 20% O_2 (REF. 130). In fact, evidence suggests that hypoxia — which is a physiological condition during embryonic development — contributes to a niche that supports stem cells and that is does so through Notch signalling[131]. The hypoxia-inducible factor 1α (HIF1α) is stabilized under hypoxic conditions and cooperates with Notch signalling to promote expression of Notch target genes and proliferation of NSCs and progenitors in the adult brain (such as in some tumours[130,132] and perhaps after ischaemia), although no studies to date have examined the interaction of Notch and HIF1α after ischaemia in the adult brain. Intriguingly, under grossly normoxic conditions HIF1α is expressed and stabilized specifically in NSCs and progenitors in the adult brain[133], suggesting that a physiological role for hypoxia may persist in the adult stem cell niche.

Neighbouring progenitor cells constitute a second important constituent of the neurogenic niche, providing both direct Notch ligands and a myriad of other cell–cell interactions. In the SVZ and SGZ, stem cells are surrounded by progenitors and mature cells[134,135], and cell–cell interactions between NSC and progenitors, which provide the major Notch ligand input[136], are required for neurogenesis — although few studies have formally examined the ability of adult progenitors to send a signal. Despite evidence that mature cells in these niches (for example, neurons or ependymal cells) express ligands, it has not been shown that either of these cell types can directly activate Notch signalling in adult brain. Further studies are needed to understand the implication of Notch signalling in different brain regions beyond the neurogenic niches and beyond stem and progenitor cells. For example, does Notch activation in neurons of the cortex have the same effect as in neurons of the cerebellum?

Neurogenic demand

Adult neurogenesis in the SVZ and SGZ is activity dependent[137–139], but the mechanisms that underlie this responsiveness remain unknown. Given the integral part that Notch plays in regulating proliferation and quiescence of NSCs, it seems likely that Notch would be involved in activating stem cells in response to exercise, learning or injury (BOX 3). In this section we discuss the current evidence for this and speculate about the pathways that might interact with Notch to regulate neurogenesis in response to stimuli.

Box 3

A role for Notch in response to neurogenic demand?

Neural stem cells (NSCs) are quiescent but can be recruited in response to demand ranging from physiologic, such as learning or exercise, to pathologic, such as injury[167]. Notch has been implicated in regulating the transition from quiescence to activation, and levels of Notch signalling have been shown to grossly change in each of these situations. NSCs are not the only cells that can respond to stimuli. The effect of various stimuli on each stage of neurogenesis are shown in the table. Although Notch signalling has been implicated in regulating some or all of these processes in other contexts, we have limited data on its functional role in the adult brain (see the table, bottom row). However, it is clear that Notch remains a key player in cellular plasticity at all stages, and we must understand how it works to fine-tune potential Notch-based therapies.

Cell type	Exercise	Learning	Stroke and injury
Radial glial-like NSC	• Activated • Increased proliferation but no increase in cell number[140,141] • Increased asymmetric divisions?	?	• Activated • Increased proliferation[158]
Horizontal NSC	• Quiescence • No change in proliferation or cell number[140]	• Activated? • Delayed proliferation?[37]	?
Progenitors	• Increased proliferation and cell number[137]	• Delayed proliferation?[37]	• Increased proliferation and cell number[79,80]
Neuroblasts	• Increased proliferation and cell number[137] • Increased cell cycle exit[37]	• Delayed increase in apoptosis[147]	• Increased apoptosis[158]
Newborn neurons	• Increased cell number, owing to increased survival and more progenitors[142] • Increased structural complexity[143]	• Increased survival • Increased structural complexity • Increased synaptic plasticity[147]	• Increased apoptosis[83]
Ependymal cells	–	–	• Conversion to neuroblast?
Associated changes in Notch signalling	• NICD increased in neuroblasts but not in NSC or progenitors[37] • Inducible Notch ablation in NSC and progenitors blocks running-induced proliferation[30]	• Activated during learning but generates a novel 66-kDa NICD fragment[59] • Sharply attenuated during consolidation[59].	• Increased NICD and *Hes1* in progenitors[79] • Notch inhibition blocks ischaemia-induced progenitor proliferation[80] and neuronal death[83] but promotes neuroblast differentiation[78,79]

NICD, Notch intracellular domain.

Exercise is one of the most potent stimulators of adult neurogenesis, particularly in the SGZ[137], and several recent studies have shown that in mice, NSCs in the SGZ proliferate in response to running[140,141]. A study using *Hes5*–green fluorescence protein (GFP) reporter mice found that proliferation of Notch-responsive SGZ stem cells increased after running[140], but it is unknown whether Notch is required for this response. In inducible *Notch1* knockout mice, running was not sufficient to rescue the number of SGZ stem cells to wild-type levels[30], but this study did not explore the proliferative status of NSCs. However, uncommitted SGZ progenitors did not proliferate in response to running, whereas the number of DCX-positive neuroblasts was increased, suggesting that there may be a stage-specific role for Notch in activity-dependent neurogenesis. Exercise increases survival of adult-born neurons in the murine SGZ through serine/threonine-protein kinase AKT signalling[142,143] with a concomitant rise in levels of nuclear NICD in these newborn DCX-positive cells after running[37], suggesting that Notch may have a role in promoting activity-dependent survival. However, survival of new neurons in the SGZ was not affected in inducible *Notch1* knockout mice after running[30]. Thus, it remains to be determined if there is a causal link between Notch signalling and enhanced SGZ neurogenesis after exercise. It is likely that Notch plays an accessory part in other pathways, possibly through crosstalk with growth factor signalling and neurotrophic signalling, to permit responsiveness to neurogenic stimuli.

In a similar way to exercise, ischaemia induces proliferation of adult NSCs. However, Notch activation in differentiated ependymal cells after ischaemia has the opposite effect. Ependymal cells, which line the ventricles in the adult brain, do not proliferate under normal conditions and cannot self-renew. Yet, ependymal cells in forkhead box protein J1 (FOXJ1)-specific *RBPJ*-inducible knockout mice generate proliferating neuroblasts in response to ischaemic injury, unlike their wild-type littermates[144]. These results highlight a role for Notch signalling in postmitotic cells, preventing de-differentiation and cell-cycle entry. However, Kageyama and colleagues could not replicate these findings using similar nestin-specific inducible *RBPJ* knockout mice[32]. It was later found that the purportedly ependymal cell-specific FOXJ1 promoter employed in REF. 144 is also present in neurogenic astroglia and many other cells in that lineage[145], indicating that there may be methodological confounds in the interpretation that FOXJ1-derived neurons exclusively descend from ependymal cells. Nevertheless, a recent study showed that there is an astonishing degree of plasticity of cell types in the SVZ, with ependymal cells and astrocytes transitioning back and forth between phenotypes[146]. This study also showed that Notch is upstream of ephrin-type B receptor 2 (EPHB2) forward signalling in maintaining ependymal cell differentiation. This raises the intriguing question of how Notch mechanistically mediates the maintenance of astrocytic neural stem cells[23,115] and the differentiation of ependymal cells simultaneously when both have the potential to transform phenotypically into each other[146]. For example, do the same ligand(s) regulate both processes? And how is the equilibrium between cell fates regulated? Thus, further studies are needed to determine the precise role of Notch in regulating ependymal cell differentiation from the astroglial lineage and vice versa.

Although still incompletely understood, a role for Notch in modulating neurogenesis during memory and learning is emerging. Studies in adult rats and mice suggest that spatial learning correlates with increased neurogenesis in the SGZ — specifically, the survival and integration of newborn neurons[147], indicating that the addition of new neurons is important for the formation of new memories. It is plausible that Notch may be involved in recruiting quiescent NSCs to generate the needed neurons, although no studies have examined this so far. As we have discussed above, Notch can promote survival of new postmitotic neurons[38],

and is specifically required for spatial memory[52], suggesting that Notch signalling may modulate neurogenic demand at the level of the newborn neuron. It is possible that Notch could regulate the survival of newborn neurons directly or indirectly by modulating integration and synaptogenesis during learning — processes that are known to be crucial for the survival of new neurons. However, no studies have yet determined a causal or mechanistic link between Notch signalling and integration of adult-born neurons that are recruited during learning and memory formation.

Conclusions

The studies reviewed here suggest that Notch signalling in the adult brain displays a wealth of contextual diversity that we are only beginning to understand. Clearly, Notch signalling plays a prominent part in balancing stem cell maintenance with progenitor differentiation, but we do not understand how Notch signalling can fine-tune neurogenesis (from NSC proliferation to neuron integration) to suit the demand, under various conditions, in the life of an animal. In this Review we have suggested that this fine-tuning occurs in cooperation with crosstalking pathways that themselves might be regulated by the particular context or situation, whether it is running or recovering from ischaemia, learning or ageing. However, much of this is speculation and future studies will have to determine whether there is indeed any role for Notch in regulating neuronal output under demand conditions. It will not be enough to simply show that Notch is involved; rather, future studies must also focus on the underlying molecular mechanisms, so that we may gain a clearer understanding of the 'Notch signalling network'. Notch may be a master regulator of cell choices, but it does not do this in isolation. Finally, as we have emphasized in this Review, the outcome of Notch activation is to a large extent dependent on the type of cell that receives the signal. We now have some of the tools that we need (BOX 1) to parse out the complex nature of Notch signalling in a cell-specific manner. For example, knowing that Notch regulates the structure and function of newly born neurons and that it potentially has crucial roles in synaptic plasticity and in the response to disease and trauma, using conditional and inducible mutant mice we can begin to isolate and study Notch signalling specifically in new neurons of the adult brain, both under normal conditions, in response to activity, and in disease models.

Understanding how Notch can modulate stem cell fate and the addition of neurons to the adult brain, as well as the survival and function of mature neurons, has broad implications for the development of therapies aimed at combating brain injury and neurodegeneration. First and foremost, in many cases of diffuse, widespread neurodegeneration, modulation of Notch may be sufficient to increase neuron survival, such as in the case of prion-related disorders — or, more speculatively, in Alzheimer's disease. Alternative stem cell therapies are often proposed as potential treatments for neurodegenerative disorders, but we do not know the critical pathways involved in adding new neurons to existing circuitry. Notch signalling might be used to differentiate stem cells into a particular neuron type, or it might be important in regulating the survival of the new neurons once they have been implanted. Crosstalk with other pathways will need to be considered in addition to manipulating Notch itself. It is clear, however, that Notch is a key player in the adult brain and it will be the focus of many future studies towards understanding function and dysfunction.

Acknowledgments

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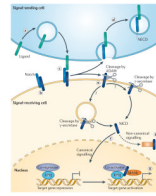
Figure 1. Notch signal transduction

A model of canonical Notch signalling. Notch signalling is unidirectional, with a 'signal-sending cell' that presents the Notch ligand (the 'signal') to the 'signal-receiving cell', which expresses the Notch receptor. **a** | Notch ligands, such as Delta-like protein 1 (DLL1; shown in green), are presented on the neuronal membrane and subsequently endocytosed. These ligands can be 'activated' by an as-yet-unknown mechanism and re-presented to the membrane. **b** | Notch is synthesized as a single peptide and then cleaved in the golgi compartment (not shown) to form a heterodimer (shown in dark and light blue) that is presented on the cell membrane. **c** | Upon being re-established on the membrane, the ligand can bind Notch. **d** | According to one model, the Notch heterodimer is pulled apart through the force of endocytosis in the signal-sending cell, thereby transendocytosing the Notch extracellular domain (NECD). **e** | The Notch domain that remains on the signal-receiving cell is cleaved by disintegrin and metalloproteinase domain-containing protein (ADAM) and subsequently by γ-secretase. The precise location of the γ-secretase cleavage is controversial, with some data indicating that it occurs in the endosome and other data indicating that it can happen both on the membrane and in the endosome, leading to different Notch intracellular domain (NICD) molecules. **f** | In either case, after cleavage NICD translocates to the nucleus. **g** | In the nucleus it dislodges the repressor complex from recombining binding protein suppressor of hairless (RBPJ), forming a complex that is stabilized by mastermind-like protein (MAMLs). NICD also recruits co-activators to initiate transcription of Notch target genes. **h** | More recently, it has been determined that Notch signalling can occur in the absence of transcriptional activation, through protein–protein interactions, or that it can activate non-RBPJ-dependent transcription (not shown), collectively referred to as 'non-canonical' signalling.
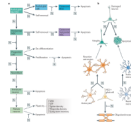
Figure 2. Pleiotropic roles for Notch signalling in the adult brain

a | The generation of neurons in the adult brain is thought to proceed through several stages, beginning with radial glial-like neural stem cells (top left part) and ending in a mature neuron (bottom part). Early in the development of adult-born neurons, cells proceed through several divisions (shown by solid black arrows). Notch activation (N) occurs during, or immediately after, these divisions. Notch activation can also occur in postmitotic or quiescent cells in the absence of division (shown by dashed arrows). In radial glial-like stem cells (RGCs) Notch promotes a radial glial fate. In both RGCs and horizontal neural stem cells (NSCs), Notch activation promotes self-renewing divisions. Notch has also been implicated in regulating quiescence of NSCs. In progenitors, Notch activation is important for proliferation. Interestingly, in the subventricular zone, pigment epithelium-derived factor (PEDF) can sensitize progenitors such that low-level activation of Notch immediately following division can promote de-differentiation (that is, induce 'stemness'). In its most well-known role, Notch inhibits neuronal differentiation of NSCs and progenitors. More recently, it has been appreciated that Notch regulates migration of neuroblasts and arborization of postmitotic newborn neurons. Notch has also been implicated in regulating synaptic plasticity of mature neurons (bottom right part) in the adult brain, regulating features of synaptic strengthening such as long-term potentiation (LTP) and long-term depression (LTD), and increases in the density of filopodia and spines. Finally, Notch is crucial for the survival of all stages of neurogenesis in both the developing and adult brain, either promoting or inhibiting apoptosis, depending on the stage. **b** | Following brain injury or degeneration, the developmental programs of Notch are reactivated to control a diverse set of responses to the insult, including promoting neuronal atrophy and death, increasing microglial activation, and potentially regulating reactive gliosis by initiating proliferation and astrogliogenesis from resident progenitors. However, the response is counter-productive and Notch activation limits the ability of oligodendrocytes to differentiate and remyelinate damaged neurons.
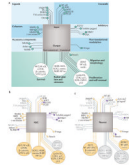
Figure 3. Notch integrates signals to coordinate a context-specific response

It is generally accepted that Notch signalling integrates multiple pathways, thereby tuning downstream expression to match the cell's current needs and situation. This could be considered to resemble a processor on a circuit board, integrating inputs from various computing processes and directing output to appropriate hardware to generate a coordinated response. **a** | A representative sample of the various 'inputs', which include Notch ligands and input through crosstalking pathways, is shown (top part). The ability of a cell to receive input is regulated by the availability of the Notch receptor (not shown) and ligands, and by post-translational modification of both the receptor and ligands by fringe family proteins — such modification results in some Notch ligands being activating and others inhibiting. Input capacity is further modulated by intracellular 'accessory pathway' components and cofactors, such as deltex, neuralized (NEUR) and mastermind-like proteins (MAMLs), and extracellular inhibitors, all of which can bias Notch activation in one cell of the signalling pair. The output of Notch signalling (a representative sample of the currently identified 'outputs' is shown; bottom part) results from both canonical Notch signalling (activation of genes whose expression is regulated by recombining binding protein suppressor of hairless (RBPJ)-dependent Notch signalling) and presumably non-canonical Notch signalling mechanisms. However, data on non-canonical signalling are limited. The output depends in part on the Notch pathways that are available in a given cell type and in part on the combination of inputs. **b** | In neural stem cells (NSCs), for example, Notch integrates growth factor (epidermal growth factor receptor (EGFR) and pigment epithelium-derived factor (PEDF)) signalling with input from key stem pathways, such as sonic hedgehog (SHH) and WNT, to promote expression of genes that maintain stemness and self-renewal. **c** | In mature neurons, in which SHH has a less prominent role, Notch may integrate WNT, neurotrophin and NF-κB signalling to modulate synaptic complexity and survival. Most of the input and key pathways that interact with Notch in mature neurons are unknown (shown by dashed lines), as are the downstream effectors. Probable candidates include neurotrophins (for example, brain-derived neurotrophic factor (BDNF)), activity-dependent modulators of synaptic and structural plasticity (for example, NF-κB), as well as effectors of cytoskeletal rearrangement (for example, RAS-related C3 botulinum toxin (RAC) proteins and β-catenin). Inputs that inhibit Notch signalling are shown in purple, and inputs that potentiate Notch signalling are shown in yellow. Inputs and outputs that play a less prominent part in a given stage are shown in grey with open circles. ADAM, a disintegrin and metalloproteinase domain-containing protein; AKT, serine/threonine-protein kinase AKT; BLBP, fatty acid-binding protein, brain; CDK2, cyclin-dependent kinase 2; DLL, Delta-like protein; DNER, Delta and Notch-like epidermal growth factor-related receptor; DLK, protein delta homologue 1; EGFL7, epidermal growth factor-like protein 7; FBW7, F-box/WD repeat-containing protein 7; HESR, hairy and enhancer of split-related genes; HIF1α, hypoxia-inducible factor 1A; IGFR, insulin-like growth factor receptor; mTOR, mammalian target of rapamycin; STAT3, signal transducer and activator of transcription 3.

Table 1

Notch receptor and selected ligand expression

Notch pathway protein	Expression pattern in forebrain	Refs
Notch1	Neuron, astrocyte, precursor, ependymal cell, endothelium	4,24,26,144
Notch2	Precursor? Neuron?	4,168
Notch3	Precursor?	168
Notch4	Endothelium	169
DLL1	Intermediate neural progenitor, postmitotic neuron?	26,170,171
DLL3	Intermediate neural progenitor?	170
DLL4	Endothelium	172
JAG1	Precursor, intermediate neural progenitor, neuron	24,26,62,170,173
JAG2	Neuron	26,169
DNER	Neuron	174

DLL1, Delta-like protein 1; DNER, Delta and Notch-like epidermal growth factor-related receptor; JAG1, jagged 1.