Chasing fate and function of new neurons in adult brains

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Neuron production, migration and differentiation are major developmental events that continue, on a smaller scale, into adult life in a wide range of species from insects to mammals. Recent reports of adult neurogenesis in primates, including humans, have led to explosive scientific and public attention. During the last two years, significant discoveries have revealed that the generation, recruitment and survival of new neurons in adult brains are governed by principles similar to those that shape the developing brain, such as neuronal death, sensory experience, activity levels, and learning. Similarly, many factors implicated in embryonic neurogenesis are increasingly found to regulate adult neurogenesis and survival as well. These findings now allow the first manipulations of the numbers of adult-generated neurons to address their potential behavioral function.

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Abbreviations

BDNF	brain-derived neurotrophic factor
BrdU	bromodeoxyuridine
^{3}H-thy	tritiated thymidine
HVC	song nucleus HVC, acronym used as proper name
IGF-I	insulin-like growth factor I
RA	robust nucleus of archistriatum
SVZ	subventricular zone

Introduction

In the 1960s Joseph Altman and his colleagues published a series of papers on the persistence of neurogenesis in adult animals [1,2]. Their work was met with skepticism and did not become part of canonical textbook knowledge. During the next two decades, mammalian dendrites and synapses were shown to undergo constant remodeling [3,4]. These findings indicated that adult brains were far less static than was previously assumed and the term 'adult brain plasticity' was coined. Reports of continued neurogenesis in the adult rodent olfactory system and hippocampus soon confirmed and extended Altman's original observations [5,6]. Yet, when in the early 1980s experiments documented the addition of functional new neurons to existing circuits in adult songbird brains, this phenomenon was regarded as 'a challenging biological mystery' [7] with little relevance to higher organisms [8]. The idea that the replacement of neurons could occur even in organisms with higher brain functions, such as monkeys and humans, was countered with negative data and ideological reserve [8]. The recent reports of adult neurogenesis in the brains of primates, including humans (reviewed in [9]), caused some methodological controversy [10] but, if corroborated, further establish that adult neurogenesis is a widespread phenomenon and of great clinical relevance. The peripathetic path from discovery to acceptance of adult neurogenesis is highlighted in a recent review article [11].

The focus of research has now shifted from documenting that adult neurogenesis exists to understanding the factors controlling it and its function. In this review, I summarize recent progress in elucidating the fate of adult neural precursors and their progeny, the life span of new neurons and their possible involvement in behavior. As three recent reviews focused on neurogenesis in mammalian tissues [9,11,12], I will discuss new results from the songbird system and attempt to integrate them with findings from a range of species that exhibit adult neurogenesis, including fish and insects.

Documenting new neurons

Documenting the generation of an adult-born neuron involves proof that the cell is new and that it is a neuron. To show that a neuron is new it has to be 'birthdated' by incorporation of tritiated thymidine (^{3}H-thy), bromodeoxyuridine (BrdU) or a retrovirus into its DNA during the division(s) that led to its birth. To show that it is a neuron, a number of different lines of evidence should be provided: first, its morphological and molecular characteristics must be consistent with a neuronal phenotype. Second, electrophysiological characterization must reveal neuronal activity [13•,14•,15]. However, this is experimentally cumbersome [15] and therefore rarely done. Using calcium-imaging or immediate-early gene activation to study the activity of newly generated neurons might be alternatives. Third, if new neurons are known to reach their final site of incorporation by migration, tracking the fate of ^{3}H-thy- or BrdU-birthdated neurons after different survival times should reveal cells with a morphological and molecular phenotype typical of migrating neurons, and differentiated neurons extending axons into their target regions. Some of these methods, and the situations in which they have been applied, have been summarized recently in more detail [9].

To assess the functional consequences of adult-born neurons, it is imperative to document not only the number of neural progeny generated (referred to in the text as 'neurogenesis'), but also how many neurons are functionally incorporated into existing circuitry ('neuronal incorporation'), and how many survive for some length of time ('survival'). In many systems, there is a balance between neuronal death and neurogenesis, leading to 'neuronal replacement'. It is important to remember the dynamic relationship between the birth of neurons, their incorporation, and their death when interpreting the numbers of

adult-born neurons present at different survival times after their generation.

As reports about the existence of new neurons in adult brains increase, the phenomenon is starting to be accepted *bona fide* and the burden of experimental proof seems to decrease. This development is premature, and discoveries of adult neurogenesis in new systems should receive continued scrutiny (see Box 1).

Cellular fate of neural precursors and their progeny

Spontaneous neurogenesis exists in adult insects [16], crustaceans [17], fish [18,19], reptiles [20], amphibians [21], birds [22], marsupials [23,24] and mammals [9]. A comparison across systems suggests that neural precursors in adulthood give rise to a variety of, but not all, neuronal phenotypes. Most adult-born neurons fit the characteristics of neurons generated late in central nervous system (CNS) development, which tend to be interneurons or neurons with short projections to targets within the same brain structure. Adult-born neurons that fit this description include interneurons in the olfactory bulb of arthropods [17] and mammals [25], intrinsic mushroom body neurons of insects [16], cerebellar granule neurons of fish [19], HVC neurons in songbirds [22], hippocampal granule neurons of mammals [26,27] and intracortical projection neurons in primates [28].

Are adult neural precursors restricted to become neurons of particular phenotypes?

During development of the nervous system, initially multipotent neural stem cells are thought to progressively restrict their developmental potential through the production of increasingly lineage-restricted precursor cells [29–31]. The similar fate of neurons born last in development and those born in adults raises the possibility that equivalent lineage-restricted neural progenitors are the source of both populations. This view is consistent with data from the analysis of one-year-old canaries that were injected with ^{3}H-thy during different embryonic and post-hatch ages. Neurogenesis becomes increasingly restricted to the telencephalon where it persists into adulthood [32].

Fate-restricted replacement in songbirds

Recent experiments in zebra finches also support the idea that adult neural precursors are fate-restricted, limiting the types of neurons that can be produced ([33•]; see Figure 1). Two types of new neurons are constantly added to nucleus HVC in adult songbirds: local interneurons, and neurons that extend axons towards the robust nucleus of the archistriatum (RA), thus becoming projection neurons in the motor pathway that controls production of learned song (here, designated HVC→RA). A third type of HVC neuron projects to Area X (designated HVC→X), and this type is never produced in adulthood [22].

Box 1. Nucleotide incorporation: DNA synthesis or repair – a recurrent controversy

^{3}H-thy or BrdU incorporation reflect either DNA replication or DNA repair. The fact that neurons labeled with ^{3}H-thy or BrdU can reflect newly generated neurons but also neurons that have undergone DNA repair is one of the recurrent criticisms surrounding reports of adult neurogenesis [10,86]. It is undisputed that nucleotide incorporation during DNA repair takes place on a much smaller scale than during replicative DNA synthesis, but a systematic and quantitative comparison of BrdU or ^{3}H-thy incorporation after neuronal DNA repair or after cell division has not been made in adult brain tissue. Concern about false positives resulting from DNA repair is not unwarranted: adult postmitotic neurons show high rates of spontaneous DNA mutations [87], are particulary susceptible to damage by free-radicals [88], and can repair DNA efficiently [89,90].

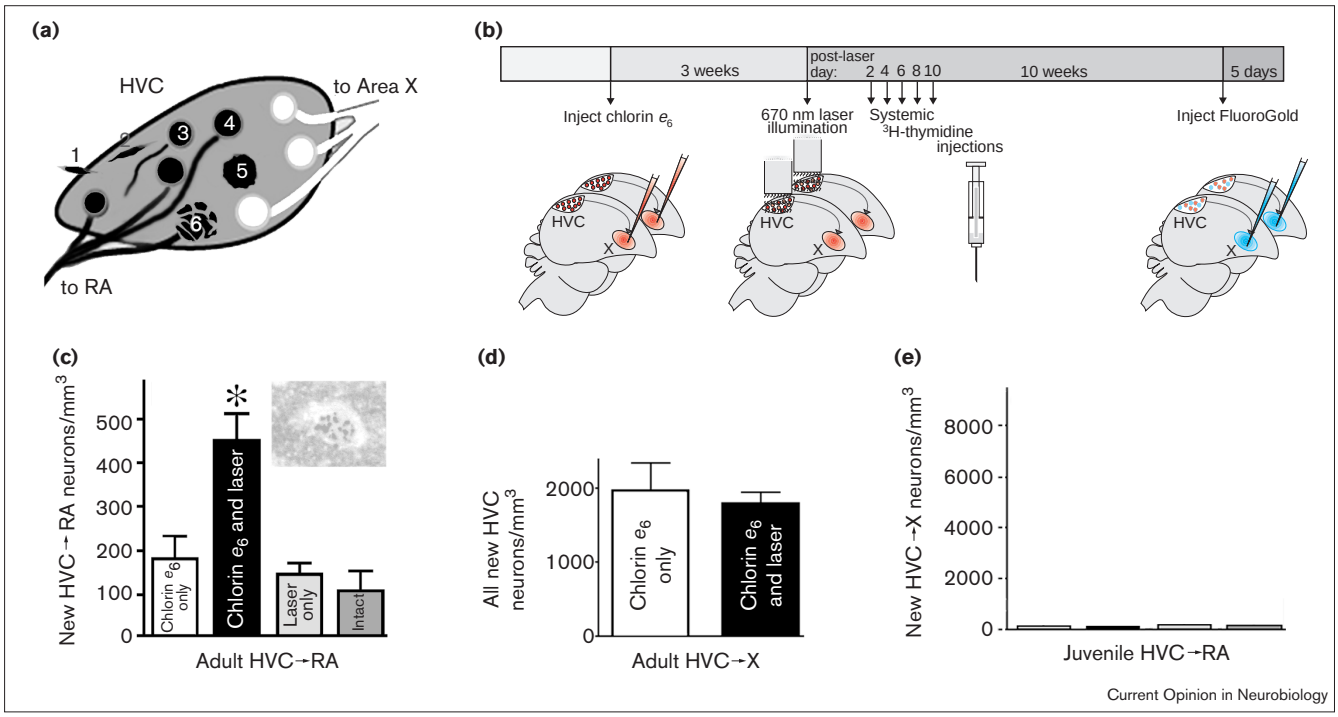
Mounting interest in the therapeutic potential of adult neurogenesis in brain repair has led to an increase in studies in which upregulation of neuronal is observed subsequent to experimentally induced neuronal injury [33•,34••,91–93]. In these experiments, it is particularly important to include additional appropriate controls for DNA repair, as many of the criteria used to prove the identity of the newly generated cells do not in fact distinguish between a newly generated neuron and a neuron that has undergone injury-induced DNA repair. Among those ambiguous criteria are the presence of synapses, connectivity to distant targets, expression of neuronal markers and expression of certain proteins involved in the cell cycle [94].

Controls to distinguish between neurogenesis and DNA repair could include:

1. retrograde labeling of projection neurons with vital dyes *before* administration of BrdU/^{3}H-thy and documentation that none of the previously labeled neurons subsequently incorporate BrdU/^{3}H-thy.
2. comparison of silver-grain counts from ^{3}H-thy-labeled cells known to divide (e.g. endothelial) and putative new neurons [95].
3. use of antibodies against proteins associated with DNA repair but not with DNA replication [96].
4. use of mice with targeted deletions of DNA repair genes [87] to demonstrate presence of adult neurogenesis.
5. comparison of ^{3}H-thy or BrdU incorporation of cells with experimentally induced DNA damage and putative new neurons [97].
6. use of antibodies associated with cell division but not with DNA repair [98].

A final cautionary note is raised by a recent publication that points out the deleterious effects BrdU incorporation can have on stem cell populations, including those in the brain [99]. Just two injections of 60 mg/kg BrdU administered at different points during rat development (from embryonic day [E]11 through postnatal day [P]10) resulted in severe morphological and behavioral abnormalities, depending on the timing of the treatment. Presumably, the permanent replacement of the thymidine by BrdU on the replicating DNA strand causes cumulative mutations in rapidly expanding cell populations. The severity of this effect on adult-born neurons depends on the number of divisions a neural precursor undergoes before terminal differentiation, which is not known. If adult-born neurons arise from a relatively small number of founder cells, BrdU-induced replication errors could be expected to affect many of the neuronal offspring. This is not an unlikely scenario, as the doses and the number of injections used to assess adult neurogenesis frequently exceed those used in this study.

Figure 1



Targeted neuronal death upregulates neuronal recruitment to HVC in a cell-type-specific manner. **(a)** Diagram of HVC showing schematically the two projection neuron types, HVC→RA (black) and HVC→Area X (white). Neuronal replacement occurs only in the HVC→RA projecting neurons but not the HVC→X population. Newly generated neurons migrate (1,2) into HVC where they incorporate, differentiate, and extend axons towards RA (3,4). Other HVC→RA neurons die (5) and disappear (6). **(b)** Bilateral cell-type-specific neuronal degeneration in young and adult male zebra finches was induced by photoactivation of retrogradely labeled HVC projection neurons, using a 674 nm laser. The retrograde label consisted of chlorin e_6-conjugated nanospheres that were injected either into Area X or into RA. After allowing sufficient time for retrograde transport, HVC was non-invasively illuminated with 674 nm laser light. This activates chlorin e_6 to release cytotoxic singlet oxygen, resulting in apoptosis of the targeted neurons. The procedure spares cells that are not labeled as well as those that have taken up chlorin e_6-conjugated nanospheres but lie outside of the region over which the laser is focused. To monitor cell birth, ^{3}H-thy was injected systemically every other day for 10 days, starting on the day after laser illumination. Three months later, the retrograde tracer FluoroGold (FG) was injected into the same target that had previously been injected with chlorin e_6. This FG injection allowed assessment of how many HVC neurons projected to this target at the time of perfusion, including any neurons that had escaped killing by the targeted photolysis as well as any new neurons that replaced those that were killed. The latter cell type could be positively identified by the combined FG and ^{3}H-thy label; see inset in (c). **(c)** Induced death of the HVC→RA neurons in adults upregulates incorporation of new neurons into HVC. The number of newly recruited HVC→RA projection neurons was significantly higher after targeted neuronal death of the HVC→RA neurons (black bar) than in the three control groups ($n_{hemispheres}$ for white = 6, black = 18, light gray = 4, dark gray = 4 hemispheres. ANOVA, $p = 0.012$; $F=4.3$). The control groups were not significantly different from each other ($p>0.05$). There was no significant difference in HVC volumes among groups (not shown). **(d)** Induced death of the HVC→X neurons in adults does not affect incorporation of new neurons into HVC. Neuron addition to HVC after induced death of the HVC→X neurons in adult zebra finches was equivalent in the experimental group that received chlorin e_6 injections before the laser (black bar) and the control group that received laser illumination only (white bar). $p = 0.8$ Mann-Whitney test, Z adjusted for ties −0.286, n = 3,2. **(e)** Induced death of the HVC→X neurons in juveniles does not cause replacement of HVC→X neurons. After induced death of HVC→X neurons, no new HVC→X neurons were observed in any group (n_{birds}=6,6,3,6).

It is not known why only HVC→RA neurons and HVC interneurons, but not HVC→X neurons, die and are replaced in normal adult birds. Correlative evidence suggests that recruitment of new neurons is regulated by prior neuronal death. To test whether the lack of incorporation of HVC→X neurons in adulthood is caused by the lack of instructive signals from HVC→X-neuronal death, HVC→X neurons were selectively destroyed using a photolytic lesion technique. However, no new HVC→X neurons were encountered after the induction of selective death. In contrast, targeted ablation of the HVC→RA neurons resulted in a significant increase of the number of these neurons. This indicates that death-associated signals can indeed regulate the recruitment of adult-born neurons, but only of the type that normally undergoes replacement in adulthood. To address whether age-related extrinsic factors prevented the recruitment of HVC→X neurons, HVC→X neurons were also ablated in juvenile zebra finches, in which favorable conditions for axonal outgrowth towards Area X still persist; such favorable conditions include transiently expressed molecular factors. Even under these circumstances, no new HVC→X neurons were found. These results are consistent

with the notion that neural precursor cells in the avian brain are lineage-restricted and that death-related signals cannot induce a change of fate.

Mice cut loose from fate restriction

One must be cautious not to overinterpret these results in favor of lineage restriction of adult neural precursors in general. The identical approach used in mice suggests the opposite: that adult neural precursors can be induced to change fate [34••]. In rodents, spontaneous neurogenesis persists in the olfactory system and the hippocampus, but not in the cortex. Magavi *et al*. [34••] report that photolytic neuronal death can induce layer VI cortical neurons to be regenerated in adulthood. This is remarkable because during development, layer VI neurons are derived from early precursors, and late progenitors are no longer competent to respond to environmental cues that specify layer VI identity [30]. In fact, the precursors that persist into adulthood in the neurogenic subventricular zone (SVZ) of rodents are normally destined to become glia and olfactory bulb neurons (reviewed in [35]). Nevertheless, Magavi *et al*. suspect that precursors residing in the SVZ, which is close to layer VI, are recruited by death-induced signals to give rise to the regenerated layer VI neurons.

Interestingly, previous attempts to respecify adult mammalian SVZ cells into cortical neurons were not successful. Even when postnatal SVZ precursors were transplanted into embryos, where environmental cues for region-specific differentiation are presumably more appropriate, SVZ precursors never colonized the cortex, though they did incorporate into many other levels of the developing neuraxis (reviewed in [35]). If confirmed, the new findings in mouse cortex [34••] imply that adult SVZ precursors are not irreversibly committed but can alter their fate in response to death-associated factors. This is consistent with the striking finding that adult *in-vitro*-expanded SVZ precursors (neurospheres) can integrate into chick and mouse embryos and their progeny can differentiate into cells of ectodermal, and even mesodermal and endodermal lineage [36••]. In view of this unexpected plasticity of SVZ cells in mice, it is curious that in songbirds replacement of neurons after targeted photolytic death was limited to the type of neuron that is replaceable in intact birds. Because adult neurons are incorporated into more brain regions in non-mammalian brains (fish, lizards, birds) than in mammals, it would seem more likely that neural precursors in non-mammalian vertebrates are actually less lineage-restricted than mammalian precursors, or that non-mammalian brains are more permissive. Taken together, these data suggest that adult neural precursors are normally lineage-restricted but, with the appropriate signals, have the capacity to become de-differentiated into a less-specified precursor.

Elimination of neural precursors by programmed cell death

Recent insights concerning adult neurogenesis in insects point towards another variable affecting adult neurogenesis. In insects, new neurons are added to adult mushroom bodies in some species (e.g. mealworms, ladybirds, rove beetles) but not in others (e.g. flies, cockroaches, honeybees) [16,37]. Even within the same order, adult neurogenesis can be present (crickets) or absent (locusts). At least in adult flies and bees, the reason for this is that the neuroblasts in the mushroom bodies undergo programmed cell death during late pupal stages [38,39]. It is not known whether, in other adult brains, certain neural precursors might undergo programmed cell death after they have generated a certain number of progeny. This, however, does not seem to be the case in the mammalian hippocampus, where the dramatic decrease of neuronal production that accompanies aging is due to age-dependent increases in corticosteroid levels rather than to programmed cell death of the progenitor pool [40].

Longevity of new neurons and factors affecting birth and survival

The life span of new neurons is probably highly relevant to the task they perform. In many species, at least some adult-born neurons survive for weeks or months after they have been born, suggesting that they are functional. However, much evidence points towards an initial overproduction and subsequent pruning of adult-born neurons analogous to processes during development. For example, Kirn *et al*. [41] recently reported that neurons start arriving in HVC about one week after they are born in the ventricular zone (VZ). Half of the newly arriving neurons apparently die within the next two weeks. What determines their death is not yet understood. But other experiments suggest that the time span of survival can be affected by environmental factors. Now, work by Wang *et al*. [42••] raises the possibility that survival of new neurons is dependent on sensory experience. Within one month after being deafened, male zebra finches had only one quarter as many new HVC neurons as did intact males, either because of a decrease in neurogenesis or because of increased death of the newly born neurons during the first month of their existence. Moreover, deafening also seemed to attenuate neuronal death at later stages, so that four months after deafening the number of new neurons was the same as it had been at one month. In contrast, in normal males, more than two thirds of the neurons present at one month were no longer present at four months. As deafening also causes song deterioration, these new data raise the question of whether changes in neuronal turnover are the cause of behavioral change (see section entitled 'Functional significance of new neurons').

Different species use similar molecular factors to regulate both developmental and adult neurogenesis

Cyclic changes in hormone levels are correlated with changes in neuronal incorporation and survival in mice (estrous cycle [43]) and canaries (seasons [22]). One candidate factor that mediates the seasonal changes in songbirds is testosterone, which affects the longevity of HVC→RA neurons [22]. Recent evidence suggests that testosterone's

effect on neuronal survival in canaries is mediated by brain-derived neurotrophic factor (BDNF) [44]. Interestingly, both BDNF mRNA levels and neuron incorporation are linearly related to the amount of song produced, raising the intriguing possibility that neurogenesis and survival are activity-dependent [45]. In mice, a role for activity has been postulated because closure of the nostril affects the dynamics of neuronal birth and death in the olfactory bulb (reviewed in [25]) and because running increases the number of new granule neurons added to the hippocampus [46]. Two new papers are consistent with the idea that running mediates neurogenesis via insulin-like growth factor I (IGF-I) action. Peripherally injected IGF-I is taken up by the brain where it increases the generation and survival of new neurons in the hippocampus [47]. Peripheral infusions of IGF-I also mimic the effects of exercise on brain c-fos and BDNF expression [48]. IGF-II might fulfill a parallel function in songbirds, where the non-renewable HVC→X neurons strongly express IGF-II mRNA and only the renewable HVC→RA neurons are immunopositive for the IGF-II protein, suggesting a paracrine mechanism of action [49]. Retinoic acid may be another common factor that regulates neurogenesis in songbirds and the mammalian hippocampus [50,51]. A retinoic-acid-producing enzyme is highly enriched in HVC, and HVC can induce retinoic acid production in an *in vitro* assay. Pharmacological blockage of the enzymatic production of retinoic acid in HVC during development interferes with the normal development of song, suggesting that the cellular machinery needed to acquire song cannot develop in the absence of retinoic acid [50]. The effects of the blockage on adult neurogenesis in birds is still unknown, but in mammals, retinoic acid and BDNF collaborate *in vitro* to induce neuronal differentiation of adult hippocampus-derived stem cells [51].

Functional significance of new neurons

When first discovered, adult neurogenesis appeared maladaptive. Why maintain a metabolically expensive developmental program into adulthood to selectively replace or add certain cell types and send neural precursors through a dense parenchyma to sometimes distant targets? Why throw away differentiated neurons, when modification of synapses is sufficient to adjust to environmental change? Besides, how can a brain that regularly changes its components provide sufficient stability to maintain what it has learned [8]? Possible answers to the last argument come from recent research into the dynamic relationship between mechanisms that promote network plasticity and mechanisms that maintain network stability [52]. How this type of 'homeostatic plasticity' — in other words, a balance between plasticity and stability — is achieved in circuits that undergo neuronal replacement in adulthood has yet to be formally addressed. The frequent assumption is that a net addition of new neurons to an existing network facilitates plasticity, including learning. In contrast, a steady-state, or a decrease in, neuron number is associated with stability and decreased ability to learn. There is no theoretical reason why this should be so, as either incorporation or elimination of neurons from a steady-state network could induce plasticity.

Behavior influences neurogenesis?

During the past few years, results have accumulated that are consistent with the possibility that learning about the environment contributes to the generation or maintenance of adult-born neurons in the hippocampus of birds and mice (reviewed in [9]). However, as already mentioned, many other variables including motor behaviors, such as running (in mice) or singing (in canaries), also influence how many new neurons are incorporated into adult brains [45,46]. Further investigation into the nature of the link between metabolic activity, neuronal addition/survival and learning will be necessary to determine whether learning causes neuronal addition, or whether both neuronal addition and enhanced learning are consequences of metabolic activity, yet not causal to each other. Similar follow-up studies are necessary to resolve whether the above-mentioned changes of neuronal incorporation/survival in deafened zebra finches [42••] actually cause the resulting degradation of song. Alternatively, other effects of deafening, such as potentially different levels of singing activity, could play a role. A potential problem with the idea that deafening-induced changes in neuron addition/survival contribute to song deterioration is that deafening does not *always* cause song to deteriorate. When, in addition to deafening, a basal ganglia-like pathway is surgically interrupted, song remains unchanged [53•]. If new neurons were to be involved with the song deterioration normally seen after deafening, one would have to argue that these changes in neuronal turnover do not happen when deafening is combined with an interruption of the basal ganglia pathway. Also conceivable is that deafening always results in changes in neuronal turnover but that this would lead to song changes only in animals with an otherwise intact song system. In either case, one should bear in mind that manipulations of auditory feedback in songbirds are sensory in nature but could directly affect the motor pathway through changes in HVC→RA neuronal turnover.

Neurogenesis influences behavior?

A second related, but distinct, question is whether the incorporation of new neurons in adult brains mediates behavior. Again, no conclusive evidence is available, but some recent data are compatible with the hypothesis. Song recovery after targeted ablation of HVC→RA neurons in zebra finches coincides with the replacement of the ablated neurons [33•]. Whether song recovery after other manipulations [54,55] correlates with changes in neuronal incorporation has not been tested yet, but recent data from Scott *et al.* [56•] address an interesting corollary to the idea that neuronal replacement drives behavioral change. These authors reasoned that in the absence of appropriate auditory instruction new HVC neurons might compromise established neural function, resulting in song

deterioration. To test this, the authors checked whether the rate of neuronal addition after deafening co-varied with song deterioration in two species of songbirds whose song either degrades rapidly after deafening (bengalese finches), or slowly (zebra finches). Indeed, a high percentage of new neurons added to HVC after deafening in bengalese finches correlated with fast song deterioration, whereas a lower percentage of new HVC neurons in zebra finches corresponded to a slower process of song changes. This could be due to a constitutively higher rate of neuronal addition or to species differences in neuronal turnover in response to deafening. Attenuated addition or turnover could also account for the remarkable finding that songs of older adult zebra finches take significantly longer to deteriorate after deafening than songs of younger adult birds [57•]. As rates of spontaneous neurogenesis decline with age [32], it is possible that the age-related resistance to deafening is mediated by slower incorporation or slower turnover rates in old animals.

These findings highlight the need for studies on the interaction between neuronal replacement and auditory processing in HVC. Most HVC neurons respond preferentially to the bird's own song (BOS; reviewed in [58]), and recent *in vivo* intracellular recordings show that all HVC neuron types (HVC→RA, HVC→X and interneurons) respond in a BOS-specific manner, albeit with distinct spiking and subthreshold characteristics [59••]. The opportunity now beckons to record intracellularly from newly generated HVC→RA neurons of different maturation stages and thus establish how they become functionally incorporated into existing circuits. This should reveal whether new HVC→RA neurons are 'entrained' by adjacent neurons, as has been proposed [56•,60].

Experimental manipulations of the number of new neurons

As in the experiments discussed above, most studies correlate changes in rates of neuron addition with behavioral changes. One primary example is the correlation between seasonal changes in canary song plasticity and HVC neuron incorporation [61]. Similar correlations have been reported in various other systems [43,62–64,65•]. What cannot be ruled out in any of the studies that report changes in behavior and neuron number is that these changes are either coincidental or co-regulated, but not causally related. To address this problem, it is necessary to manipulate the birth and/or survival of adult-born neurons directly, and methods to do so are now becoming available. Baseline levels of the generation of new neurons or their survival can be experimentally up- or down-regulated (Table 1).

Unfortunately, so far all of these approaches also affect additional variables besides the desired increase/decrease of neuron incorporation or survival, making it difficult to assess whether a behavioral effect is specific to the changed levels of neuron incorporation (Table 1). Future attempts to ablate identified populations of adult-born neurons in a temporally and spatially precise manner will doubtlessly become more feasible as more information becomes available about the molecular and cellular steps that characterize the differentiation of neural precursors [12,66,67].

A system that might be well suited to targeted ablation of adult-born neurons is that of insects. New neurons in insects are added mainly to the mushroom bodies, a brain structure implicated in the learning and memory of odors [68]. Using the elegant molecular genetic approach of clonal analysis to trace the lineage of mushroom body neuroblasts in *Drosophila*, the generation and projection

Table 1

Some manipulations of the number of new neurons in adult brains: effects on behavior?

Manipulation	Reference	Number of new neurons	Where	Behavior	Alternate mechanism
Infusions					
bFGF (peripheral)	[80]	+	SVZ and OB	Not tested	Identity of new cells unknown, affects other brain areas
IGF-1 (peripheral)	[47] [48]	+	Hippocampus	Not tested	Affects other brain areas
BDNF (local)	[44]	+	HVC	Not tested	Affects HVC in multiple ways
BDNF-blocking antibodies (local)	[44]	–	HVC	Not tested	Affects HVC in multiple ways
Opiates (local)	[81]	+	Hippocampus	Not tested	Affects other brain areas
Ara-C (local)	[67]	–	SVZ	Not tested	Affects all dividing cells
Methamphetamine (peripheral)	[82]	+	Hippocampus	Not tested	Affects other brain areas
Genetic manipulations					
DNA fragmentation factor 45	[83]	+	Hippocampus	Better water-maze performance	Affects other brain areas, developmental effects
Hu-Bcl-2	[84]	+	Hippocampus	Fine motor problem	Affects other brain areas, developmental effects
NCAM	[85]	–	OB and hippocampus	Problem with odor novelty detection	Developmental effects, hippocampus also has fewer new neurons

Ara-C, cytosine-β-D-arabinofuranoside; bFGF, basic fibroblast growth factor; NCAM, neural cell adhesion molecule; OB, olfactory bulb.

pattern of the three neuron types that constitute this brain structure have been recently elucidated [69]. If a similar approach can be modified to include an inducible cell-death signal and be adapted to insects with persistent neurogenesis in adulthood, it would hold great promise for a clean analysis of the behavioral relevance of adult neurogenesis in olfaction. Analysis of the functional role of new neurons in insect, arthropod, marsupial and mammalian olfaction could provide insights into the way in which convergent evolution has not only resulted in designs with similar neuroanatomical and physiological characteristics [70], but also the potential functional need for continuous turnover of new neurons.

Are adult-born neurons involved in fish behavior?

In fish, new neurons are added to many parts of the brain, including the visual and auditory system as well as the cerebellum [18,19]. A frequently cited and parsimonious explanation for this is the continued growth of many adult fish [71] which necessitates the addition of new neurons to sensory structures and their connected brain areas. However, like songbirds, fish are an ideal model for pursuing the question of whether neurogenesis is relevant to behavior because of their variety of reproductive, vocal, and sexually dimorphic behaviors with identified brain substrates [72,73]. A relationship between adult neurogenesis and behavior has been hypothesized for the pacemaker nucleus of the weakly electric gymnotiform fish, *Apteronotus*, but has so far not been explicitly tested [19]. It would be particularly interesting to pursue the way in which the addition of new neurons can be compatible with the precise temporal firing pattern that is characteristic for both the pacemaker nucleus in fish and for HVC of songbirds.

Conclusions and future directions

Emerging evidence about the regulation of neural precursors in adult animals indicates that their fate is normally quite restricted. However, under special circumstances, neural precursors can apparently differentiate into a variety of cell types. Elucidating the signals that induce this flexibility will be a major task in the future. Broadening the study of adult neurogenesis to a greater variety of species has already yielded interesting parallels between brain regions that share structural and functional homologies (hippocampus in birds and mammals [74], and the olfactory system in most species studied). Similarities among species are now also evident in the use of certain molecular factors involved in the regulation of adult neurogenesis. Establishing a definite role for new neurons in adult behavior will require the development of genetic mutants in which adult neurogenesis is regulated in a temporally and spatially specific way. In addition, approaches that use a variety of species to correlate changes in specific behaviors with changes in the rate of neuronal turnover will continue to play an important role in identifying what aspects of behavior are affected by, or are affecting, adult neurogenesis and survival [46,75,76]. Awareness of the fact that all behaviors are an assortment of distinct but interrelated components is of key importance in accomplishing this task. For example, memory, motor ability, motivation, perception, arousal and attention all contribute to the execution of the seemingly simple 'cognitive' Morris water-maze task. The extensive knowledge about different aspects of behavior gathered by experimental psychologists and ethologists is a resource whose power for studying the function of adult-born neurons is only starting to be recognized [77]. This emphasizes the need for a diversification of behavioral assays, including those that tap the natural behavior of the species under investigation [78,79].

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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