projection patterns to muscles[7,8]. However, essentially none of these genes are expressed in *Foxp1* mutant mice. Counter-intuitively, the projection patterns to most limb muscles and intramuscular axonal arborizations in *Foxp1* mutant mice are nevertheless similar to those of wild-type mice[3,4] (**Fig. 1c**). Notable exceptions to this rule were the axonal arborizations in muscles innervated by motor neurons pools marked by the target-induced ETS transcription factor Pea3. Dasen *et al.*[3] showed that these muscles exhibited innervation defects in *Foxp1* mutants that were comparable to those in *Pea3* mutant mice[9] (**Fig. 1c**).

How can these rather modest changes in peripheral projection pattern be reconciled with the marked alteration in molecular identity? When looked at from the perspective of one individual limb muscle, these new findings demonstrate that coordinate elimination of all molecular identity from a motor neuron pool results in less severe innervation defects than mutation of a single gene that contributes to the complex code that controls the innervation of that same muscle. These results suggest that in the complete absence of instructions in LMC motor neurons, as observed in *Foxp1* mutants, random pathfinding can nevertheless result in seemingly intact innervation patterns. Consistent with this, mice with a mutation in the homeodomain transcription factor gene *Mnx1* (also known as *Hb9*), which exhibit even earlier and more severe defects in motoneuronal specification, also manage to innervate most limb muscle targets[10,11]. Furthermore, in muscles innervated by Pea3-expressing motor neurons, *Hb9* mutant mice show projection defects similar to *Pea3* and *Foxp1* mutants (E. Vrieseling and S.A., unpublished data). Together, these findings suggest that generic axonal projections to targets can still be established in situations where many of the early cell-intrinsic genetic programs are lacking. In the context of these missing cell-autonomous programs, however, axons are no longer capable of reading target-derived cues that are essential for axonal arborization in target muscles.

Despite the rather minor alterations of overall peripheral projections to limb muscles, *Foxp1* mutants showed severe deficiencies in the topographical organization of motor neuron cell bodies in the spinal cord. These defects were revealed upon retrograde labeling of motor neurons from individual muscles. Although a motor neuron pool innervating one muscle represents a tightly clustered cell body aggregate in a defined position in the spinal cord in wild types, corresponding cell bodies in *Foxp1* mutant mice showed a randomly dispersed pattern[3,4]. These findings further underscore the arbitrary nature of the motor axonal outgrowth process in *Foxp1* mutants. As a consequence, these changes are also expected to result in notable alterations of central presynaptic connectivity onto these motor neurons. The embryonic lethality of *Foxp1* mutants has so far prevented analysis of these later phenotypes, including expected alterations in motor behavior, an interesting avenue of research to pursue in the future.

Finally, Dasen *et al.*[3] offer an intriguing evolutionary interpretation of their results. The motor neuronal spectrum generated in *Foxp1* mutant mice mirrors the composite that is present in early aquatic vertebrates that lack PGC and LMC, where simple locomotion is driven entirely by MMC neurons and a set of motor neurons innervating hypaxial muscles that could correspond to the amniote HMC set[12]. The recruitment of the Hox symphonic repertoire and its maestro FoxP1 to such an ancestral organism may thus have been instrumental in the evolutionary rise of the primitive vertebrate motor system to the one represented in mammals today, enabling them to perform refined motor behaviors. And, perhaps not by chance, FoxP1's sibling FoxP2 has been linked to human speech and language[13], which depend on highly refined motor control of larynx and mouth. Future work on when and how the Hox symphony and its conductor FoxP1 evolved and were brought together in the evolution of motor circuits may give us deep insights into the developmental and functional aspects of spinal motor system evolution.

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Regenerating your senses: multiple roles for neurogenesis in the adult brain

Paul W Frankland & Freda D Miller

The adult mouse brain continuously supplies new neurons to the olfactory bulb and hippocampus. A new study in this issue shows that ongoing neurogenesis is essential for maintenance of the olfactory bulb and for spatial memory.

Since the initial discovery that the adult brain contains stem cells that generate new neurons[1], an important question in neurobiology has been whether or not this adult neurogenesis is functionally important. Although a substantial body of work has shown that some of these new, adult-born neurons integrate into active neural circuits, the magnitude and importance of this integration has been unclear[2]. In a definitive study, Imayoshi *et al.*[3] demonstrate that adult neurogenesis is indeed important and that, surprisingly, it may fulfill two very distinct functions (**Fig. 1**). In one brain region, the olfactory bulb, adult stem cell–generated progeny have a critical role in tissue maintenance, much as they do in tissues such as the gut and skin, whereas in a second region, the hippocampus, they serve to add new neurons that are important for adult behaviors.

Imayoshi *et al.*[3] drew these conclusions on the basis of an elegant transgenic strategy that they used to permanently label and then deplete stem cell–generated progeny

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in the adult brain. Specifically, the authors generated mice in which a tamoxifen-inducible Cre recombinase ($CreER^{T2}$) was expressed under the promoter for the neural precursor marker nestin. Using these mice, they first asked how many new stem cell–derived neurons were added to the adult brain. To do this, they crossed their mice with various reporter lines and induced recombinase activity in adults with tamoxifen, thereby efficiently (up to 87%) expressing the reporters in neural stem cells (NSCs) and their progeny. Initially, the authors followed the differentiation, migration and maturation of new neurons in the olfactory bulb. Previous work has shown that neurons are generated from NSCs in the subventricular zone of the lateral ventricles and that these neurons migrate to the olfactory bulb, where they mature and acquire features of mature granule neurons after about 1 month[4,5]. The authors' new inducible transgenic approach confirmed this earlier work and led to the conclusion that almost the entire granule cell population was replaced by new neurons over a 12-month period. The rate of neuronal replacement was nearly linear for the first 6 months, with a decrease in the pace of addition from 6–12 months, potentially reflecting a decline in olfactory neurogenesis in middle age and beyond. The exception to this almost complete replacement was in the superficial layers, where only half of the neurons were labeled, suggesting that there is at least one subpopulation of persistent granule cells in the olfactory bulb.

These data indicate that olfactory neurogenesis provides a constant supply of new neurons to replace cells in the olfactory bulb. What happens if this supply is turned off? To answer this next critical question, Imayoshi *et al.*[3] generated mice in which the majority of new adult stem cell–derived neurons were ablated. To achieve this, they mated the nestin $CreER^{T2}$ mice to mice in which a construct encompassing a floxed stop codon followed by the catalytically active fragment of diphtheria toxin was inserted into the neuron-specific enolase locus. When the resulting mice were treated with tamoxifen, diphtheria toxin expression was induced, killing newborn cells as soon as they differentiated into neuron-specific enolase–expressing neurons. The authors demonstrated the specificity of their approach, showing that it did not affect either developmentally generated or earlier (before tamoxifen) cohorts of adult-generated granule cells. Using this elegant strategy, Imayoshi *et al.*[3] found that blocking adult neurogenesis led to an extensive depletion of granule cells in the olfactory bulb. This depletion was detectable as early as 3 weeks post-tamoxifen and was even more pronounced at 12 weeks post-tamoxifen. Thus, cell death in the olfactory bulb is not a consequence of neuron addition. Instead, olfactory bulb cell death is an autonomous process that occurs in the absence of olfactory neurogenesis and that inevitably leads to substantial tissue shrinkage when stem cell function is perturbed. This situation is reminiscent of that in tissues such as the gut, skin or blood, where adult stem cells have an essential role in tissue maintenance and replacement.

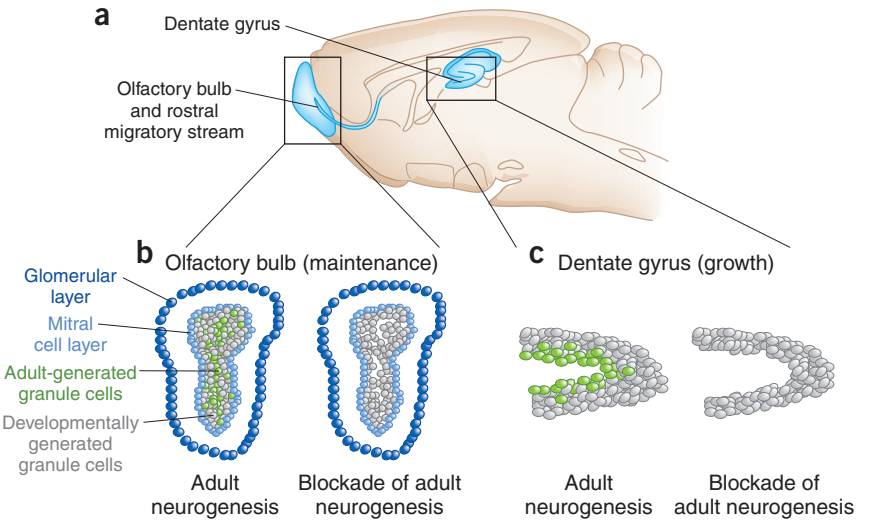

Figure 1 Two functions for adult neurogenesis in the olfactory bulb and hippocampus. (**a**) In the adult brain, neurogenesis persists in two regions: the subventricular zone of the lateral ventricle (new neurons migrate from here along the rostral migratory stream to populate the olfactory bulb) and the subgranular zone of the hippocampus (new neurons migrate the short distance from the subgranular zone to the inner granule cell layer of the dentate gyrus). (**b**) Adult-generated neurons are essential for maintaining the olfactory bulb. When olfactory neurogenesis is blocked, there is extensive depletion of granule cells in the olfactory bulb. (**c**) In contrast, in the dentate gyrus, adult-generated neurons contribute to tissue growth. When hippocampal neurogenesis is blocked, dentate gyrus growth is attenuated.

The second place in the adult brain where neurogenesis clearly persists is the subgranular zone of the hippocampus, a brain region that is important in learning and memory[6]. Does neurogenesis serve a similar maintenance/replacement function in the hippocampus? Imayoshi *et al.*[3] took advantage of a second nestin-$CreER^{T2}$ line that had substantially higher recombination efficiency in the hippocampus to ask this question. Consistent with previous observations[2,4,5], they found that these adult-generated granule cells populated the innermost layers of the dentate gyrus, where they comprised 10% of the cells after 6 months, a number that remained constant for up to 12 months. Surprisingly, however, ablating hippocampal neurogenesis produced a very different pattern of results to the olfactory bulb. Here, blocking neurogenesis didn't lead to shrinkage. Instead, the number and density of granule cells remained constant in the dentate gyrus for up to 24 weeks following neurogenesis blockade. This contrasted with control animals, in which there was an increase in cell number and density over the same period of time, as previously reported[7]. Thus, adult neurogenesis has a quite different role in the hippocampus, adding (rather than replacing) neurons and contributing to hippocampal growth (rather than maintenance) throughout the animal's lifespan. Why is there this profound difference? One possible explanation has to do with differences in circuitry. The afferent input to the olfactory bulb comes from peripheral olfactory receptor neurons, a population that is itself constantly dying and being replenished. Perhaps the ongoing deafferentation of the olfactory bulb affects neuronal survival, much as it does for developing neurons, leading to an increased rate of neuronal death and necessitating a steady supply of replacement neurons. In contrast, although structural remodeling undoubtedly occurs in the hippocampus, steady deafferentation does not.

How then do these profound changes in structure affect neural function? The mice in which adult-born neurons were depleted provided Imayoshi *et al.*[3] a definitive means

of addressing the functional role of adult neurogenesis. However, their studies revealed surprising differences when they examined the impact of blocking hippocampal and olfactory neurogenesis on behavior. To assess hippocampal learning, they trained mice in spatial (Barnes maze) and fear learning tasks following ablation of hippocampal neurogenesis. In both tasks, mice showed learning and memory deficits, demonstrating that the addition of new neurons to the dentate gyrus is essential for normal functioning of hippocampal memory. Although this conclusion is consistent with a number of recent studies[8–10], the precise manner in which these new neurons contribute to hippocampal memory is still unclear.

To assess olfactory bulb function, the authors gave the same mice a battery of smell tests. Most surprisingly, blocking olfactory neurogenesis appeared to have little effect on olfactory-mediated behaviors. Mice could still readily discriminate between odors and learn to associate specific odors with a rewarding stimulus even at 6 months post-tamoxifen, when neuronal depletion in the olfactory bulb was very pronounced. These results suggest that, although the continuous replacement of dying olfactory bulb neurons is essential for maintaining olfactory bulb structure, it is not necessary for the maintenance of olfactory-mediated learning or behaviors. This conclusion is at odds with those of some previous studies[11], and, as the authors acknowledge, it is impossible to rule out the possibility that the behavioral analyses were insufficiently comprehensive to detect impairments. Indeed, evidence indicates that olfactory neurogenesis is important in maternal behaviors[12] and in mate selection[13] in female mice.

Together with previous work, this study provides us with clear evidence for the importance of adult neurogenesis in the normal adult brain. Adult stem cell–derived neurons are critical for the maintenance of the olfactory bulb, much as adult stem cell–derived progeny are essential for cell replacement in other parts of the body. When this process is perturbed, the tissue itself degenerates. In contrast, adult neurogenesis in the hippocampus is not required for maintenance, but is instead required for neuronal addition and hippocampal growth, thereby potentially contributing to the ability to accumulate new memories throughout life. Whether or not these, or other potential populations of nestin-positive adult NSCs, can be recruited for neural repair[14] can now also be addressed using the same elegant approaches.

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Pavlov's moth: olfactory learning and spike timing–dependent plasticity

Julian P Meeks & Timothy E Holy

Spike-timing dependent plasticity is a favored synaptic mechanism for learning. However, a surprising new study by Ito and colleagues in the insect mushroom body suggests that it cannot account for a paradigmatic form of learning.

Pavlov and many others have noticed that timing is important in learning; for example, any action (such as ringing a bell) that typically precedes feeding tends to be quickly associated with an impending meal. Neuroscientists have long sought the circuit and cellular mechanisms that underlie such learning. Much attention has been focused on so-called Hebbian mechanisms[1], particularly a form known as spike timing–dependent plasticity (STDP) (reviewed in ref. 2), as these mechanisms mimic, at a cellular level, the requirement for a predictive association between stimulus (ringing the bell/presynaptic activity) and reward (food/postsynaptic spike). In this issue, Ito *et al.*[3] report their studies of this question in a setting, olfactory conditioning of moths, that bears a notable similarity to Pavlov's original experiment. Surprisingly, they found that the timing of physiological activity in the mushroom body, a center for olfactory learning[4], is all wrong: odor-induced activity largely disappeared well before the optimum time for delivery of the reward. This violates the central prediction of STDP and might indicate that, at least for this archetypal example of learning, key pieces of the overall puzzle of memory remain to be elucidated.

STDP typically (but not always[5]) manifests as a strengthening of a synapse when presynaptic activity precedes postsynaptic depolarization and an attendant weakening of the synapse when presynaptic activity follows postsynaptic depolarization[2,6]. In modeling studies, this simple rule endows synapses, cells and circuits with a number of very attractive properties for both development and learning, allowing plasticity and circuit optimization without 'runaway' positive feedback[7]. Several experiments have also shown that STDP can be induced *in vivo* and correlative evidence for STDP-induced alterations in sensory processing has been obtained using natural stimuli (reviewed in ref. 8).

The timing requirements for STDP are fairly tight, typically a few tens of milliseconds[2,7]. Although the evidence for neuronal activity with this degree of timing precision is widespread, the temporal gap between 'stimulus' and 'reward' in classical conditioning can be many seconds, causing some doubts about the ubiquity of STDP for tasks on behavioral time scales[9]. One attractive proposal, therefore, is the notion that a short-term memory of the stimulus,

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