Neural Stem Cells, Neurogenesis, Behavior and Repair

Bi156

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Questions

• Are there stem cells in the adult brain and do they generate new neurons? How would you detect them?
• Do the new neurons integrate into the existing circuitry?
• Are they important for behavior, learning etc?
• Could adult neurogenesis be mobilized and directed to aid in recovery from injury or disease?
Detection of neurogenesis in various brain areas

Figure 1 | Changes in the view of adult neurogenesis in the mammalian brain over the past 15 years shown on a schematic diagram of the adult rat brain. In the pre-1990s, all regions were categorized as ‘non-neurogenic’ (grey). In the late 1990s, only the dentate gyrus and olfactory bulb (as well as the subventricular zone, which gives rise to the rostral migratory stream) were categorized as ‘neurogenic’ (red). Today, the two known neurogenic regions are shown in red, and areas for which there is controversial evidence for low-level adult neurogenesis are shown in pink. It should be noted that since not all of these brain regions are present on the same sagittal plane, their location is approximated on this diagram.

Gould, 2007
Summary of five developmental stages during adult SVZ neurogenesis: (1) activation of radial glia-like cells in the subventricular zone in the lateral ventricle (LV); (2) proliferation of transient amplifying cells; (3) generation of neuroblasts; (4) chain migration of neuroblasts within the rostral migratory stream (RMS) and radial migration of immature neurons in the olfactory bulb (OB); and (5) synaptic integration and maturation of granule cells (GC) and periglomerular neurons (PG) in the olfactory bulb. Also shown are expression of stage-specific markers, sequential process of synaptic integration, and critical periods regulating survival and plasticity of newborn neurons. GFAP: glial fibrillary acidic protein; DCX: doublecortin; NeuN: neuronal nuclei; LTP: long-term potentiation.

Ming & Song., 2011
Generation of new neurons in the hippocampus

Summary of five developmental stages during adult hippocampal neurogenesis: (1) activation of quiescent radial glia-like cell in the subgranular zone (SGZ); (2) proliferation of non radial precursor and intermediate progenitors; (3) generation of neuroblasts; (4) integration of immature neurons; and (5) maturation of adultborn dentate granule cells. Also shown are expression of stage-specific markers, sequential process of synaptic integration, and critical periods regulating survival and plasticity. ML: molecular layer; GCL: granule cell layer; SGZ: subgranular zone; GFAP: glial fibrillary acidic protein; BLBP: brain lipid-binding protein; DCX: doublecortin; NeuN: neuronal nuclei; LTP: long-term potentiation.
Two views of adult neurogenesis

In principle, radial glia-like “stem” cells of the hippocampus might divide symmetrically or asymmetrically, which would have different consequences on the number of new neurons generated and for the maintenance of self-renewing stem cells, from which adult neurogenesis might originate at later times. Data by Bonaguidi et al. (2011) suggest that the stem cells of the hippocampus have a range of options in terms of self-renewal versus differentiation, whereas Encinas et al. (2011), examining precursor cells at the population level, do not find evidence for such flexibility. They instead propose that stem cells terminally differentiate into astrocytes.

Kampermann., 2011
GFP-retrovirus labeled cells in adult hippocampus express neuronal and glial progenitor markers

48 hrs after virus injection:

Red = NeuN (mature neuron marker)

Tuj1 = immature neuron marker

GFAP = astrocyte markers

NG2 = neuronal progenitor marker

Van Pragg et al., ‘02
4 weeks later, GFP+ cells express markers and morphology of mature neurons

Red = NeuN

Van Pragg et al., '02
The new neurons receive synaptic inputs

EM of GFP+ cell injected with HRP (*)

Van Pragg et al., '02

4 months

4 weeks
New neurons display neuronal electrical properties

Spontaneous epsps

Van Pragg et al., '02
The new neurons are functionally similar to mature neurons.

Van Pragg et al., '02

Postsynaptic currents following stimulation of perforant path
Functions of adult neurogenesis

- There are discrepancies in the literature regarding the role of adult neurogenesis in various hippocampal and olfactory tests. But these experiments differ as to the type and timing of precursor ablation, type of behavioral tests used, and type of animals used.
- Nonetheless, studies have suggested significant contributions of new hippocampal neurons to spatial learning and pattern discrimination, trace and contextual fear conditioning, reorganization of memory traces.
- New olfactory bulb neurons are implicated in short-term olfactory memory, olfactory fear conditioning, long-term associative memory, and regulation of pheromone-related behaviors such as mating and social recognition.
Mice with ablated neurogenesis due to focal x-irradiation show impaired spatial memory for similar, but not distinct, spatial locations in the radial arm maze (RAM). (A) Mice were irradiated 2 months before behavioral testing. (B and C) Irradiation significantly reduced the total numbers of immature Dcx+ cells in IR mice [white arrows at right in (C)] compared with sham controls [(C), left] P < 0.001. GCL, granule cell layer; HL, hilus. (D) Pattern separation was tested using a DNMP (delayed non-matching to place) protocol in the RAM by varying the distance between sample and correct arms: S2, low; S3 and S4, high (S, start arm). (E) Each trial consisted of a sample phase (left) and a choice phase (right). The mouse had to non-match to the new location. (F) IR mice were impaired at low (S2) but not high (S3 and S4) separations in the DNMP task. The horizontal black line represents chance. Error bars indicate SEM.

Clelland et al., 2010
Neurogenesis and pattern separation

Mice with ablated neurogenesis due to focal x-irradiation show impaired spatial discrimination for similar but not distinct spatial locations, but not impaired associative object-in-place memory, in the mouse touch screen. Mice were irradiated 2 months before behavioral testing as in (A). After pretraining for 7 to 10 days in which mice learned to nose-touch stimuli on the infrared touch screen (B) to obtain a reward, mice were trained on an associative object-in-place task (PAL) (C). For example, as in the left panel of (C), mice had to choose a flower at left as a correct association over the incorrect association of a plane at right to obtain a reward. (D) IR mice learned the PAL task at the same rate as sham controls (horizontal black line represents chance). (E) Mice were then tested on a two-choice spatial discrimination task in which they had to respond to the correct location [e.g., left illuminated box of left screen in (E)] until a criterion of seven out of eight consecutive correct touches was recorded before reversing to the previously incorrect location [e.g., right illuminated box of left screen in (E)]. Mice were tested on either the low (S2, left screen) or high (S4, right screen) separation, as depicted in (E) during each testing day. (F) IR mice exhibited significantly impaired performance at low (S2) but not high (S4) separations during acquisition of this task, consistent with a pattern separation deficit similar to that observed in the first experiment (Fig. 1).

Clelland et al., 2010
Genetic decrease in neurogenesis causes deficit in pattern separation

Mice with decreased neurogenesis due to targeted Lentiviral expression of dnWnt show impaired spatial memory for similar, but not distinct, spatial locations in the radial arm maze in a similar pattern to that seen in irradiated mice. (A and B) dnWnt expression [right in (B)] significantly reduced the total numbers of immature Dcx+ cells in dnWnt mice (A) compared with GFP controls [left in (B)] [independent samples t test: t(24) = 3.47, P = 0.002]. Single channel images depicting Dcx+ cells are shown below triple channel images in (B). (C) Pattern separation was tested using a DNMP as in Fig. 1. dnWnt mice were impaired at low (S2) but not high (S3 and S4) separations in the DNMP task. The horizontal black line represents chance. Error bars indicate SEM. Scale bars, 50 mm. **P < 0.01; *P < 0.05.
Converse: Genetic *increase* in DG neurogenesis

**a**, Schematic illustrating genetic gain-of-function strategy to increase adult hippocampal neurogenesis. (1) In the adult dentate gyrus, a substantial fraction of adult-born neurons undergo BAX-dependent programmed cell death (pale red). (2) Nes-CreERT2-mediated ablation of Bax in type I and type II cells results in the generation of adult-born neurons that lack BAX, thereby preventing their death. **b**, Experimental design. IP, intraperitoneal. **c**, Representative coronal hippocampal sections immunostained for DCX from vehicle (Veh)- and TAM-treated NCff mice (top). Insets are at higher magnification; arrows in insets indicate DCX1 neurons with at least tertiary dendrites. Quantification of DCX1 population (bottom): total number of DCX1 neurons, 6,9746600 (NCff1Veh mice) and 12,63661764 (NCff1TAM) (*, P50.038, unpaired two-tailed Student’s t-test; n53 mice per group); and total number of DCX1 neurons with at least tertiary dendrites, 1,8006340 (NCff1Veh) and 4,0906285 (NCff1TAM) (**, P50.006; n53 mice per group).

Sahay et al., 2011
Genetic *increase* in DG neurogenesis *enhances* pattern discrimination

Sahay et al., 2011

a, Experimental design to test rapid one-trial contextual encoding. b, On day 1, both groups showed negligible levels of freezing in context A before a single 2-s, 0.75-mA foot shock (denoted by the red lightning bolt) was delivered. Controls(NCff1Veh)(n=514) and mice with more adult-born neurons (NCff1TAM) (n=514) showed comparable levels of conditioning to training context A and negligible levels of freezing in a distinct context, C. TAM treatment alone did not affect contextual encoding, as reflected in the similar levels of freezing of ff1Veh (n=515) and ff1TAM (n=516) mice in contexts A and C. c, Experimental design to test discrimination between two similar contexts, A and B. d, Analysis of discrimination ratios. NCff1TAM mice show significantly higher levels of discrimination between the two contexts than do NCff1Veh mice. e, Freezing behaviour of mice with increased adult hippocampal neurogenesis (NCff1TAM) and controls(NCff1Veh) over the duration of the experiment. Although both groups show comparable and extensive generalization between the two contexts at the beginning of the experiment, NCff1TAMmice (n=511) distinguished between contexts A and B more rapidly than didNCff1Veh mice (n=59). f, NCff1Veh mice were able to discriminate between the two contexts by day 9 of testing; *, P<0.05; **, P<0.01. b, d–f, Results are presented as Mean+/-s.e.m.
Environments rich in odors or contexts stimulate neurogenesis in the olfactory bulb (OB) and dentate gyrus (DG), respectively. Similarly, learning stimulates neurogenesis in the OB and DG depending on whether the modalities are olfactory or contextual and spatial. Other manipulations such as exercise or antidepressants stimulate neurogenesis primarily in the DG. Stress, aging, and sensory deprivation result in a decrease in neurogenesis in both the DG and OB. We propose that an increase in neurogenesis favors pattern separation, which alters the balance that normally exists between pattern separation (taking place in DG or OB) and pattern completion (taking place in CA3 or PC). Conversely, a decrease in neurogenesis impairs pattern separation, which shifts the balance in favor of pattern completion and results in generalization. These shifts may be a part of the normal adaptive response to changing environments. **In an enriched environment, discrimination and cognitive flexibility (which result from increased pattern separation) are advantageous because they favor exploration and learning; in contrast, in a dangerous environment, generalization (which results from decreased pattern separation) may be advantageous because it favors avoidance of new and potentially dangerous situations.** However, these normal adaptive responses when exaggerated may lead to pathologies: excessive generalization may for example lead to anxiety disorders such as post-traumatic stress disorder (PTSD) or to the impairments that often accompany aging such as mild cognitive impairment (MCI). Similarly, excessive pattern separation may lead to an excessive attention to details such as seen in some psychiatric disorders such as autism and obsessive-compulsive personality disorder (OCPD).

Sahay et al., 2011
Irradiation of the SVZ reduces odor-cued fear conditioning

(a) An illustration of the timeline of testing: Fear Conditioning consists of a 40-min session that includes 8 CS + shock pairings with a variable inter-trial interval of 4–6 min. At 24, 48, and 72 h after conditioning a 20-min probe session is conducted in a novel context. (B) Time course of a probe of odor-cued conditioned freezing response. Plotted is the mean amount of freezing as a percentage of each minute in the 20-min probe; all three probe sessions are included in the calculation of mean percent freezing. All mice conditioned to odor are grouped: XR n = 17 (red) and sham n = 18 (black). The increase in freezing in the minute immediately following CS (minutes 11, 13, 15, 17, and 19) is the "post-CS minute" that is used in subsequent analysis of freezing. (C) Acquisition of freezing response during conditioning. The average percent freezing is plotted for XR (n = 17) and sham (n = 18) mice; here the percent freezing is during the 2 min immediately following each CS + shock pairing. Only mice conditioned to odor are represented; mice conditioned to an audio tone displayed a similar pattern for acquisition of freezing behavior (data not shown). (D) Members of a cohort of mice tested 26-weeks post-irradiation were conditioned to a 2 kHz audio tone rather than odor. In this graph mean percent freezing from all three probe sessions for the +26-week cohort is plotted; the reduced freezing displayed by the XR group (factorial ANOVA n = 10, p < 0.05) in response to a conditioned odor was not observed for mice conditioned to an audio tone (n = 10). (E) The average percent freezing for the "post-CS minute" during each of the three probe sessions is plotted. XR mice (n = 17) freeze less than Sham mice (n = 18); repeated measures ANOVA p < 0.05. In factorial ANOVA P1 and P2 are p < 0.05, and for P3 p = 0.0627. All error bars represent SEM.

Valley et al., 2009
How can a small number of new neurons so significantly affect behavior?

- Adult-born neurons are hyper-excitable, display enhanced synaptic plasticity in response to their synaptic inputs, and are less sensitive to GABAergic inhibition.
- They are preferentially activated by specific inputs, as shown by immediate early gene responses.
- These neurons also actively inhibit the output of the local, mature circuitry in the hippocampus.
- These neurons preferentially contact distinct types of hippocampal interneurons.
- This may allow the newly integrated neurons to make distinctive contributions to information processing in their circuits.
Can endogenous neural stem cells be stimulated to produce more neurons?

Enhancers of adult neurogenesis *in vivo*:

* Neuron death (seizure, ischemia, irradiation)
* Growth factors
* Physical activity
* Enriched environment
  Caloric restriction
* Antidepressants
Enriching the olfactory experience
Enriched odor environment increases the number of new neurons in the olfactory bulb

Rochefort et al., '02
Enriched odor environment does not enhance hippocampal neurogenesis

Rochefort et al., '02
Enriched odor environment enhances olfactory memory

Rochefort et al., '02
Enriched spatial environment and exercise enhance neurogenesis in the hippocampus

Enriched environment - mouse
Enriched environment - human
Enriched cage environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis

Brown et al., '03

Red = NeurN    Green - BrdU    Blue = S100
Running and environmental enrichment are additive

**FIGURE 3** The number of BrdU-labeled cells in the dentate gyrus was determined along with the cellular phenotypes of BrdU-positive cells (NeuN indicating a neuronal, S100β an astroglial fate). We found RUNSTD to cause an increased number of BrdU-labeled cells as well as new neurons compared to STDSTD. RUNENR resulted in a further increase in the number of BrdU-positive cells and newborn neurons. Therefore, the effects of wheel running and environmental enrichment on adult hippocampal neurogenesis were additive. Fisher post hoc test after ANOVA.
Can neurogenesis be stimulated in other brain areas?

Neuron death was induced in layer VI corticothalamic neurons by retrograde labeling with a chromophore and then irradiating the head.
Targeting apoptosis in adult cortex - corticospinal motor neurons

Fig. 1. Targeting and induction of CSMN apoptosis. (A–C) Schematic of sequential targeting and photoactivation steps to induce CSMN apoptosis. (A) Green fluorescent nanospheres carrying chlorin e₈ were microinjected into the dorsal spinal cord at the cervical 5–6 level in 4-week-old mice. The nanospheres were retrogradely transported to the somata of layer V CSMN via the corticospinal tract. (B) Two weeks later, in mice at 6 weeks of age, we exposed the motor cortex through intact dura to 674-nm-wavelength light collimated at layer V. Photoactivated chlorin e₈ produced singlet oxygen within neuronal lysosomes, inducing apoptosis exclusively in nanosphere-containing motor neurons. (C) CSMN that both contain nanospheres and are exposed to light undergo selective apoptosis. Surrounding neurons and glia are undamaged. (D) Oblique coherent contrast Image of a coronal section of anterior brain (Nikon SMZ 1500), indicating location of CSMN. (E) Chlorin e₈ conjugated green fluorescent nanospheres injected into the cervical spinal cord exclusively label motor neurons in layer V. About 50–60% of the CSMN were targeted by green fluorescent nanospheres carrying chlorin e₈. (F) Enlarged view of nanosphere-labeled motor neuron in layer V with pyramidal morphology typical of CSMN. Due to the light dosimetry used, degeneration of ~10–20% of targeted projection neurons occurred. (G–I) Targeted CSMN developed pyknotic and fragmented nuclei (arrows), one indication that they were undergoing apoptotic neuronal death. Arrowheads indicate normal nuclei of surrounding healthy neurons. (G) CSMN selectively labeled by green photoactive nanospheres 8 days after photoactivation. (H) Targeted CSMN developed pyknotic and fragmented nuclei, labeled with Hoechst 33258 in blue (arrows). (I) Merged Image of G and H.

Chen et al., ’04
New neurons differentiate specifically in injured area of cortex

Chen et al., '04
Some of the new neurons send axons to the appropriate target area

Chen et al., '04
Various methods of neural induction of pluripotent stem cells have been described such as embryoid body formation, retinoic acid treatment, stromal feeder cocultures, or defined media containing mitogens FGF2 and EGF. Inhibition of TGFβ signaling using various molecules enhances neuralization. Defined combinations of morphogens, small molecules, growth factors, or transcription factors have been shown to specify neural progenitors to certain subtypes of neurons as well as glial cells. Some of the best-characterized pathways of clinically important cell types are illustrated above. Efficient methods to produce astrocytes have yet to be determined.

Han et al., 2011
Molecular mechanisms regulating adult neurogenesis

- Morphogens: Notch, Shh, Wnts, BMPs
- Growth factors, cytokines, hormones: BDNF, FGF1, LIF, IL-6
- Neurotransmitters: GABA, glutamate, acetylcholine, serotonin, norepinephrine
- Adhesion molecules: β1-integrin, PSA-NCAM, tenascin-R, reelin
- Cell cycle regulators, transcription factors: P16, p21, p53, Sox2, TLX, Id, FoxOs, Prox1, NeuroD, Olig2, Pax6, Dlx-2, Mash-1, CREB
- Epigenetic regulators: DNA methylation, histone modifications, non-coding RNAs
Adult neurogenesis and mental disorders

- Mutations in presenillin linked to early onset familial Alzheimer’s disease affect self-renewal and differentiation of adult SVZ precursors.
- KO of Fmrp, the gene responsible for fragile X syndrome, alters proliferation and differentiation in the SVZ.
- MeCP2, the gene responsible for Rett syndrome, regulates maturation and spine formation of new neurons in the adult hippocampus.
- DISC1, a gene implicated in schizophrenia, bipolar and major depression, affects proliferation, dendrite growth and synapse formation in the adult hippocampus.
- Thus, aberrant postnatal neurogenesis may contribute to juvenile and adult onset of many mental disorders.
FMRP KO in adult NSCs causes a deficit in neurogenesis

Fmrp deletion in Nestin-expressing cells resulted in fewer YFP+ cells in the dentate gyrus. (d) 56 d after tamoxifen injection. Red, Fmrp; green, YFP; white, NeuN; blue, DAPI. Left scale bar, 20 μm; right scale bar, 10 μm. (e) Sample images of YFP+ cells in the dentate gyrus 56 d after tamoxifen injection. Green, YFP; blue, DAPI. Scale bar, 50 μm. (f) Quantification of the number of YFP+ cells in cKO;Cre;YFP and Cre;YFP control mice. GCL, granule cell layer; ML, molecular layer; TAM, tamoxifen. Error bars indicate means ± s.e.m. n = 5 per genotype per time point.

Guo et al., 2011
FMRP is critical for hippocampal learning

(c,d) Context (c) and tone (d) trace learning analyses of Cre;YFP mice (which express Fmrp), cON;Cre;YFP mice (with Fmrp restored in nestin-expressing cells) and cON;YFP (no Fmrp) littermates as determined by the percentage time of freezing to training context (c) or tone (d). (e) DNMP-RAM analyses of Cre;YFP (which express Fmrp), cON;Cre;YFP mice (with Fmrp restored in nestin-expressing cells) and cON;YFP (no Fmrp) littermates as determined by the percentage of correct entry in separation 2 tests and separation 4 tests. Error bars indicate means ± s.e.m. cON;Cre;YFP, n = 6; Cre;YFP control, n = 8; cON;YFP control, n = 6. H, hilar region of the hippocampus.

Guo et al., 2011
Stimulating neurogenesis in a model of human neurodegenerative disease

- Neurogenesis is increased in human Huntington’s disease (HD).
- Neurogenesis in the SVZ can be stimulated by viral delivery of the neurotrophic factor, brain-derived neurotrophic factor (BDNF).
- Gliogenesis can be stimulated by the cytokine, bone morphogenetic protein (BMP). The BMP inhibitor, noggin, blocks gliogenesis in the SVZ, and potentiates BDNF-induced neurogenesis.
- Thus, it was of interest to test whether BDNF + noggin delivery could enhance neurogenesis in a transgenic mouse model of HD (R6/2) in which the human disease gene is over-expressed, causing the mice to exhibit motor symptoms and die prematurely.
Viral delivery of BDNF + Noggin stimulates neuron addition

AdNoggin potentiated AdBDNF-induced neuronal addition and integration. (A) Density of BrdU+βIII-tubulin+ cells in the striatum of R6/2 and WT mice injected once at 4 wk of age with AdBDNF/AdNoggin (AdB/N), AdBDNF, AdNoggin, AdNull, or saline. (B–E) Newly generated neurons were recognized in both WT (B) and R6/2 (C–E) mice by confocal imaging of BrdU (green) colabeling with βIII-tubulin (red, B and C), DARPP-32 (red, D), or GAD67 (red, E). (F) Density of BrdU+ cells (green) coexpressing either DARPP-32, GAD67, enkephalin (Enk), or SP in the striatum of AdBDNF/AdNoggin-, AdBDNF-, or AdNull-injected WT and R6/2 mice. (G and H) BrdU-tagged (green) enkephalergic (red, G) and SP (red, H) neurons in R6/2 mice. (I and J) FG injection of the globus pallidus revealed BrdU-tagged striatal projection neurons in AdBDNF/AdNoggin-injected 11-wk-old WT (I) and R6/2 (J) mice. *P < 0.05, **P < 0.01, ***P < 0.001, 1-way ANOVA followed by post-hoc Bonferroni t tests. Arrows denote double-labeled cells. Scale bars: 10 μm.

Cho et al., 2007
Induced neurogenesis was associated with delayed disease progression and prolonged survival. (A and B) AdBDNF/AdNoggin-cotreated R6/2 mice exhibited slower deterioration of motor performance than did AdBDNF-treated or untreated control R6/2 mice. (A) Time of sustained rotarod performance as a function of time point and treatment, using a 300-s rotarod challenge. (B) Results of the same challenge as in A presented as the percent rotarod impairment (see Methods). (C) Open-field testing revealed that AdBDNF/AdNoggin-treated R6/2 mice sustained volontary exploratory behavior, as manifested in spontaneous horizontal locomotion, longer than did mice treated with AdBDNF only or AdNull or left untreated. (D) Kaplan-Meier analysis revealed that survival was significantly extended in R6/2 mice injected with AdBDNF/AdNoggin compared with R6/2 mice injected with AdBDNF only or AdNull or left untreated. Pre, preoperative. *P < 0.05, **P < 0.01 versus AdNull and untreated; †P < 0.01 versus AdBDNF.

Cho et al., 2007
LIF stimulates recovery in a chemical model of demyelination

• The cytokine leukemia inhibitor factor (LIF) is transiently up-regulated in a variety of disease and injury states (multiple sclerosis (MS), Alzheimer’s disease, stroke, stab wound, nerve crush), and exogenous LIF can be neuroprotective.
• Demyelination like that seen in MS can be partially mimicked in the autoimmune rodent model, experimental autoimmune encephalitis (EAE).
• Exogenous LIF can protect oligodendrocytes in EAE, and also in a model of spinal cord injury.
• Demyelination can also be induced chemically by adding cuprizone, a copper chelator, to the food.
• What is the effect of viral delivery of LIF following demyelination in the cuprizone model?
LIF enhances oligodendrocyte generation after acute demyelination in the hippocampus

Male C57BL/6 mice were fed cuprizone for 5 weeks, returned to a standard diet, and given injections of Ad-LIF or Ad-LacZ. Three days after virus injection, BrdU was supplied in the drinking water for 7 d, and newly generated OLs were assessed 4 weeks after virus injection. A, A schematic provides an overview of the experimental design. i.c.v. intracerebroventricular injection. B, LIF delivery increases the number of newly generated BrdU/CC1OLs in the hippocampus compared with Ad-LacZ controls. Representative images are shown of immunostaining of the hippocampi (CA3 region) of mice treated as indicated. BrdU(green)/CC1(purple) OLs are highlighted by arrows. Quantification of BrdU/CC1cells is provided in the text. C, Quantification of the total number of CC1cells in the CA3 region of the hippocampus. ***p<0.001. The difference between the 5+4wAd-LacZ group and the 5 week group is not significant (p>0.05). D, An example of immunostaining for BrdU (green), the mature OL protein RIP (red), and axons (NF; blue) is shown for the hippocampus of an Ad-LIF-treated mouse. Scale bars, 20μm.

Deverman & Patterson, 2012
A–D, I, Ad-LIF restores expression of the compact myelin protein PLP after chronic demyelination. A–D, Confocal projection images of immunostaining for PLP (purple) and NF (green) are shown together and as individual monochrome images to the right of each dual color image. Compared with mice maintained on a standard diet (A), PLP expression is reduced after 12 weeks of cuprizone feeding (B). Recovery of PLP expression is limited 6 weeks after injection with Ad-LacZ (C) compared with mice given injections of Ad-LIF (D). I, Quantification of the ratio of the area of PLP signal above threshold over the area of NF signal above threshold.
E–H, Confocal projection images show immunostaining for Nav1.6 (red), Caspr (green), and Kv1.2 (blue). Expression/clustering of node-associated proteins is disrupted by 12 week cuprizone exposure. E, F, Compare 12 week cuprizone (F) to untreated (E). G, H, Ad-LIF treatment enhances the restoration of Nav1.6 clustering surrounded by Caspr clustering at paranodes (arrowheads) compared with Ad-LacZ treatment. J, Quantification of the number of Nav1.6 nodes flanked on both sides by Caspr paranodes. ***p<0.001 between Ad-LIF- and Ad-LacZ-treated groups. Scale bars, 20m.

Deverman & Patterson, 2012
What inhibits endogenous neural stem cells from producing more neurons?

Inhibitors of adult neurogenesis *in vivo:*

* Aging
  Stress
* Inflammation
  5-HT depletion
  Opiates
  Methamphetamines
How does aging alter neurogenesis and cognitive abilities?

• There is a very significant decline in both the number of new neurons produced and in cognitive abilities with age.
• Interestingly, both of these deficits can be ameliorated by exercise, a systemic perturbation.
• NSCs and precursors tend to be clustered very near or in contact with blood vessels – suggesting that blood-borne factors may influence neurogenesis.
• In one experiment, young mice were joined to old mice via cutaneous parabiosis so that they shared circulation.
  • Result: the young DG had fewer BrdU+ neurons than normal while the old DG had more than normal.
• Second experiment – transfusion of plasma from old mice into young mice decreased the number of neurons in the DG.
• Proteomic analysis of chemokines and cytokines enriched in old mouse serum indentified the chemokine CC11, which increases with age in mouse and human serum.
• Injection of CC11 i.v. causes and deficit in DG neurons and learning, and injection of anti-CC11 into the hippocampus blocks this effect.
Effect of CC11 on neurogenesis an learning

a, Schematic of young (3–4 months) mice injected intraperitoneally with CCL11 or vehicle, and in combination with anti-CCL11 neutralizing or isotype control antibody (Ab). Mice were treated four times over 10 days. i.p., intraperitoneal. Scale bar, 100 mm. c, Quantification of neurogenesis in the dentate gyrus after treatment. d, Schematic of young adult mice given unilateral stereotaxic injections of anti-CCL11 neutralizing or isotype control antibody followed by systemic injections with either recombinantCCL11 or PBS (vehicle). f, Quantification of neurogenesis in the dentate gyrus after systemic and stereotaxic treatment. Bars represent mean number of cells in each section. g. Learning and memory assessed by contextual fear conditioning in young adult mice injected withCCL11 or vehicle every 3 days for 5 weeks (n=12–16 mice per group). All data are represented as mean +/- s.e.m.; *P<0.05; **P<0.01; ANOVA, Dunnet’s or Tukey’s post-hoc test (c, f).

Villeda et al., 2011
Inflammation inhibits hippocampal neurogenesis

B = control
C = LPS
Green = BrdU
Red = activated microglia
Blue = vasculature

D = control
E = LPS
Red = Dcx
Green = BrdU

ED1 = activated microglia in GCL & SGZ
Indo = indomethacin (anti-Inflammatory drug)

Monje et al., 2003
Microglia inhibit neurogenesis via IL-6 in culture

Neural progenitors co-cultured with MG (microglia); CM (conditioned media)

Cultures exposed to IL-6 (dark bars)

Monje et al., 2003
Anti-inflammatory therapy restores neurogenesis following X-irradiation

Monje et al., 2003
Neurogenesis and major depression

• Hippocampal volume is reduced in major depression, and depressed subjects perform poorly on a spatial recognition task.
• Structures that show increased metabolism to anti-depression drugs (SSRIs) include hippocampus, prefrontal cortex and subgenual cingulate.
• Most anti-depressants and environmental interventions that confer benefit also stimulate adult hippocampal neurogenesis.
• The time course of maturation of the new neurons matches that of the behavioral recovery.
• The effects of fluoxetine and imipramine on depressive behavior in mice are inhibited by blocking hippocampal neurogenesis with spatially focused irradiation. These effects are strain-dependent, however.
• Perforant path LTP is enhanced by chronic fluoxetine, but not in irradiated hippocampus.
• Enhanced LTP is recovered 6 weeks after irradiation, but DCX/BrdU+ neurons have not recovered by then!
• Similar findings using nestin-driven herpes simplex thymidine kinase and gangcyclovir i.c.v. injection: new neuron production (Dcx/BrdU+) and DG LTP are down. 6 weeks later, LTP recovers but not Dcx neuron number.
• Compensation among remaining neurons? Due to an inflammation effect? Bystander toxicity?
References

Background

Student papers
CNTF administration reduces body weight and induces proliferation in hypothalamus

Kokoeva et al., 2005
Newborn cells exhibit neuronal (43%) and glial (oligodendrocyte) (23%) phenotypes.
Newborn hypothalamic cells respond to leptin

Fig. 3. (A) Newborn hypothalamic cells respond to leptin. Many BrdU^+ (red) cells of CNTF-treated mice were also positive for pSTAT3 (green) after ip leptin injection. (B) 3D confocal reconstruction of area boxed in (A). (C) Groups of DIO or ob/ob mice (n = 5) were icv infused for 7 days with CNTF (0.75 μg/day) or leptin (0.60 μg/day). For all animals, BrdU (12 μg/day) was coadministered. To induce DIO, mice were placed on a high-fat diet for 5 months. Body weight is shown as percentage difference from initial body weight. All data are mean ± SEM. Scale bars in (A), 50 μm;

DIO = diet induced obesity;  ob mice lack leptin

Kokoeva et al., 2005
Blocking CNTF-induced cell proliferation abrogates sustained weight loss

Fig. 5. Blocking CNTF-induced cell proliferation abrogates sustained weight loss. (A) BrdU+ cells (red) are virtually absent in brains treated with Ara-C or CNTF+Ara-C. Shown are fluorescence images of sections at the level of the arcuate nucleus from mice used in (B). Brains were removed 42 days after surgery. (B) Groups of mice (n = 5) were icv infused for 7 days with CNTF (0.75 μg/day) and/or Ara-C (40 μg/day). For all animals, BrdU (12 μg/day) was coadministered. Body weight is shown as percentage difference from initial body weight. All data are mean ± SEM. Scale bar, 100 μm.

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