

Neurogenesis or non-neurogenesis: that is the question

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Commentary

Neural stem/precursor cells (NPCs) that reside within germinal niches of the adult CNS have more complex roles than previously expected. In addition to their well-documented neurogenic functions, emerging evidence indicates that NPCs exert non-neurogenic functions that contribute to the regulation and preservation of tissue homeostasis under both physiological and pathological conditions. In this issue of the *JCI*, Mohammad et al. found that DCs efficiently patrol the CNS only when the germinal niche of the subventricular zone functions properly. Indeed, DCs traveled from the ventricles along the rostral migratory stream to the olfactory bulb (a cervical lymph node access point) to dampen anti-CNS immune responses. The authors' findings further support a non-neurogenic role for NPCs in maintaining tissue homeostasis and promoting tissue protection in the adult brain.

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Neurogenesis or non-neurogenesis: that is the question

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Neural stem/precursor cells (NPCs) that reside within germinal niches of the adult CNS have more complex roles than previously expected. In addition to their well-documented neurogenic functions, emerging evidence indicates that NPCs exert non-neurogenic functions that contribute to the regulation and preservation of tissue homeostasis under both physiological and pathological conditions. In this issue of the JCI, Mohammad et al. found that DCs efficiently patrol the CNS only when the germinal niche of the subventricular zone functions properly. Indeed, DCs traveled from the ventricles along the rostral migratory stream to the olfactory bulb (a cervical lymph node access point) to dampen anti-CNS immune responses. The authors' findings further support a non-neurogenic role for NPCs in maintaining tissue homeostasis and promoting tissue protection in the adult brain.

Neurogenesis: A tale of two germinal niches

In the adult rodent CNS, lifelong neurogenesis – the process of neuron generation from neural stem/progenitor cells (NPCs) – primarily occurs in two distinct areas of the brain (i.e., germinal niches), the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles (1, 2). Depending on the germinal niche, NPCs have distinct fates. Adult NPCs generated in the SGZ migrate a short distance into the granule cell layer of the dentate gyrus (DG) and become indistinguishable from preexisting cells, an activity that is considered necessary for

modulating and refining the neuronal circuits involved in hippocampus-dependent memory processing and behavior (1–3). Newly formed NPCs from the SVZ migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB), where they integrate within the granule and glomerular cell layers to maintain and reorganize the OB system (1, 2). Recent compelling evidence challenges the limited view that neurogenic areas of the brain act solely as sources of newly formed neurons for replacement of neuronal cells in the hippocampus and OB (4). In fact, the exclusive neurogenic role of the SVZ has been questioned due to recent data clearly indicating that adult OB neurogenesis might not have any functional significance in humans. In adult humans, 700 new neurons are added to the hippocampus each day (corresponding to an annual turnover of 1.75% of the

neurons within the renewing fraction); however, retrospective birth dating has established that the majority of OB neurons are of the same age as the individual, and that additional neurons in the adult human OB account for less than 1% of the total neurons exchanged over a century (4).

Not only for neurogenesis: SVZ-derived NPCs exhibit non-neurogenic functions

How can we explain the apparent paradox of NPCs being produced by the SVZ, yet no evident neuron turnover in the OB? One thought-provoking explanation comes from recent studies indicating that adult NPCs residing within the SVZ exert non-neurogenic functions – such as protecting and regulating homeostasis – as alternatives to cell replacement, in both physiological and pathological conditions (Table 1 and Figure 1). For example, it has been shown that SVZ-derived NPCs have phagocytic activity toward maturing neurons, which requires the intracellular engulfment protein ELMO1 to promote Rac activation downstream of phagocytic receptors (5). Additionally, SVZ-derived NPCs have been described as having a secretory protein profile (including secretion of VEGF) distinct from other brain cells and capable of modulating activation, proliferation, and phagocytosis of microglia (6).

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Table 1
Non-neurogenic functions exerted by NPCs within the SVZ in physiological and pathological conditions

Condition (mice)	Effect	Cell target	Activity	Mechanism	Reference
Physiology					
Adult C57BL/6; <i>Elmo1</i> ^{-/-}	Cell phagocytosis	Neuroblasts	Regulate neurogenesis	Phagocytosis via ELMO1	5
Adult C57BL/6; <i>Cx3Cr1-EGFP</i>	Bystander neuroprotection	Microglia	Regulate cell proliferation, activation, and phagocytosis	Secretion of growth factors (VEGF)	6
Adult C57BL/6; 2D2; <i>Cx3Cr1-EGFP</i>	Favoring cell migration	DCs	Regulate immune responses within the CNS	Migration from the ventricle along the RMS	7
Pathology					
EAE (adult C57BL/6; 2D2; <i>Cx3Cr1-EGFP</i>)	Favoring cell migration	DCs	Regulate CNS immunity	Migration from the ventricle along the RMS	7
Stroke and epilepsy (WT C57BL/6; NestinTK)	Bystander neuroprotection	Medium spiny neurons	Regulate afferent neuronal excitatory transmission	Secretion of endocannabinoids (AEA)	9
Brain tumors (WT C57BL/6; <i>nestin-GFP</i> ; <i>Trpv1</i> ^{-/-} ; <i>Ccnd2</i> ^{-/-} ; <i>Faah</i> ^{-/-} ; SCID)	Bystander neuroprotection	High-grade astrocytoma cells	Exert a cytotoxic effect	Secretion of endovanilloids (AEA, OEA, PEA)	10
Demyelination (adult C57BL/6; NG2-DsRed)	Oligodendrogenesis	NG2 ⁺ oligodendrocyte progenitors	Regulate formation of new oligodendrocytes	Glutamatergic synapse formation with demyelinated axons	16
Stroke (adult C57BL/6; N4; GFAP-GFP; <i>Thbs4</i> ^{Cre/+})	Astroglioneurogenesis	Peri-ischemic area	Support stabilization of the glial scar and BBB	Astroglioneurogenesis promoted by THBS4	17
SCI (adult C57BL/6; <i>Foxj1</i> -rasless)	Astroglioneurogenesis; bystander neuroprotection	Neurons; lesion site	Support neuronal survival and structural stabilization of the lesion site	Astroglioneurogenesis; secretion of growth factors (CNTF, HGF, IGF-1, TGF- β 1)	18

AEA, arachidonylethanolamide; CCND2, cyclin D2; CNTF, ciliary neurotrophic factor; ELMO1, engulfment and cell motility protein 1; FAAH, fatty acid amide hydrolase; N4, *nestin-creERTM4*; OEA, oleoyl ethanolamide; PEA, palmitoyl ethanolamide; SCI, spinal cord injury; TRPV1, transient receptor potential vanilloid; THBS, thrombospondin.

In this issue of the *JCI*, Mohammad and colleagues present evidence of an additional non-neurogenic homeostatic mechanism occurring within the SVZ niche (7). Using pharmacological and toxic methods, the authors identified and characterized the RMS as a pathway for DC trafficking through the CNS to the systemic immune compartment. Ablation of NPCs and the RMS with the antiproliferative agent cytosine- β -D-arabino-furanoside (ARA-c) led to DC retention in the murine brain. Furthermore, this pathway directly modulated Treg function in the cervical lymph nodes and reduced CNS-confined immune reactions. Finally, disruption of immune cell trafficking in the brains of 2D2 mice via localized infusion of the drug fingolimod, which inhibits T cell trafficking, resulted in reduced CNS immune tolerance, enhanced anti-CNS autoimmune responses, and CNS-associated inflammatory diseases such as EAE (7). While chemorepulsive factor gradients seem to guide NPC trafficking toward the OB (8), it remains to be determined whether and how DCs and NPCs physically interact along the RMS.

SVZ NPCs: Guardians of the brain

Emerging data certainly support the concept that SVZ NPCs act as guardians of the brain. In fact, NPCs are capable of sensing and contrasting danger signals to trigger an inflammatory process involving the innate arm of the immune system (Figure 1). Apart from the aforementioned relationship between SVZ NPCs and DCs, a recent study indicates that SVZ NPCs might also protect striatal neurons from glutamatergic excitotoxicity by releasing endogenous endocannabinoids (e.g., arachidonylethanolamide [AEA]), which are capable of binding to their respective neuronal receptors (CB1 and CB2) (9). This NPC-mediated protection is tuned up during CNS-compartmentalized inflammatory insults, such as those occurring in the early phase of ischemic stroke and epilepsy. In addition, SVZ NPCs may mediate suppression of high-grade astrocytomas (HGAs) by releasing endovanilloids that activate the transient receptor potential vanilloid subfamily member-1 (TRPV1) on HGA cells, thus triggering cell death and prolonging overall survival

(10). Together, these data help to explain previous results in support of the concept of therapeutic plasticity of transplanted SVZ-derived NPCs. Various approaches have consistently shown that transplanted SVZ-derived NPCs, while remaining undifferentiated, promote CNS tissue healing via secretion of immunomodulatory and neuroprotective molecules capable of reducing detrimental responses (the so-called bystander effect) (11–14).

In addition to these newly appreciated effector functions, it is remarkable that during CNS-confined pathological processes, SVZ NPCs might also alter their neurogenic differentiation default pattern in order to confine and limit tissue damage. In toxin-induced demyelination of the corpus callosum, less than 4% of newly formed SVZ-derived cells differentiate into myelinating oligodendrocytes (15); however, newly formed SVZ-derived glial precursors can promote remyelination by forming functional glutamatergic synapses with demyelinated axons (16). Astrocyte production from the postnatal SVZ niche in response to localized photothrombotic/

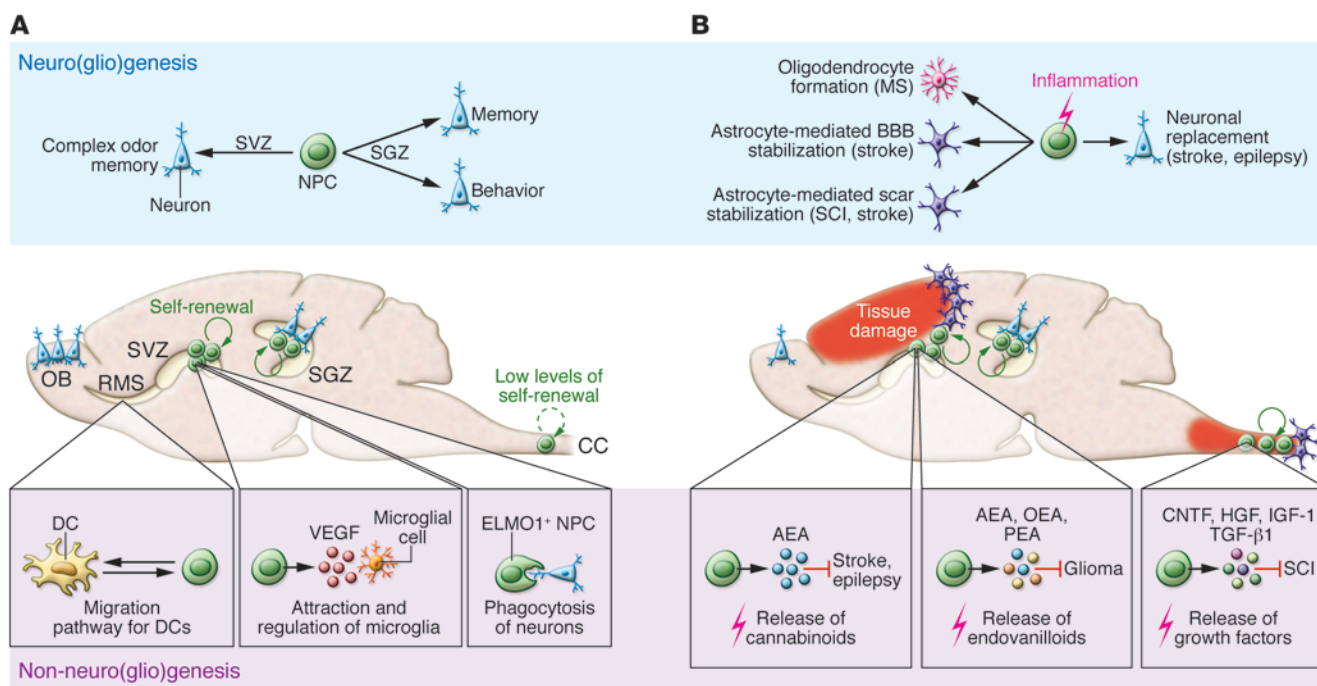


Figure 1

NPC functions during physiological and pathological conditions. **(A)** In physiological (homeostatic) conditions, NPCs reside within 2 main neurogenic niches, the SVZ and the SGZ, and undergo neurogenesis to maintain specific CNS circuits in the OB and in the DG. Concurrently, NPCs might exert non-neurogenic functions, such as controlling microglial cell behavior, through secretion of VEGF, and phagocytosis of maturing neurons. A recently reported non-neurogenic SVZ NPC function is the ability to act as scaffolding cells for the proper migration of DCs patrolling the CNS, allowing DC trafficking from the ventricles toward cervical lymph nodes. **(B)** In maladaptive (stressful) conditions, such as those occurring during pathology/inflammation, NPCs might also exert neurogenic and non-neurogenic functions aimed at maintaining or reestablishing dysfunctional CNS circuits. Depending on the pathological process, neurogenic functions are finalized to generate new neurons capable of integrating into functional circuits, whereas non-neurogenic functions are mainly aimed at limiting and/or preventing tissue damage and promoting tissue recovery. Non-neurogenic tissue restoration operates via a bimodal mechanism of action. On the one hand, SVZ NPCs do sense and contrast danger signals in order to avoid harmful inflammatory reactions through the production of soluble factors, such as cannabinoids, endovanilloids, and growth factors. On the other hand, SVZ NPCs generate new astrocytes that stabilize the glial scar and the BBB and new oligodendrocytes that promote remyelination. AEA, arachidonylethanolamide; CC, central canal; CNTF, ciliary neurotrophic factor; OEA, oleoyl ethanolamide; PEA, palmitoyl ethanolamide; SCI, spinal cord injury.

ischemic cortical injury (controlled by the Notch modulator thrombospondin 4 [THBS4]) has been found to stabilize the blood-brain barrier (BBB) (17). In spinal cord injury (SCI), NPC-derived astrocytes stabilize the scar and are required to restrict secondary enlargement of the lesion and further axonal loss (18). Nonetheless, NPC progeny also appear to exert a neuroprotective effect required for survival of neurons adjacent to the SCI-associated lesion. In an experimental model of epilepsy, SVZ-derived cells that migrate toward the hippocampus have been described to terminally differentiate into glial cells, but not neuronal cells (19). This process might be protective, because in temporal lobe epilepsy, newly derived neurons aberrantly migrate and integrate in the dentate hilus, exacerbating hippocampal epileptic activity (20).

Conclusions

Replacement of damaged cells does not appear to be the sole operating mechanism of SVZ-derived NPCs, and it is likely that the neurogenic and non-neurogenic behaviors of SVZ NPCs are influenced by specific characteristics of the microenvironment. Strategically, cells of the SVZ are in communication with two different microenvironments, due their contact with both blood vessels and cerebrospinal fluid (CSF) by apical processes (8, 21–23). Furthermore, the SVZ is very close to crucial areas of the midbrain – including basal ganglia and striatal structures – that contain GABAergic neurons capable of efficiently regulating and modulating interconnections among several cortical and subcortical brain areas (24). Finally, prior studies have demonstrated that inflammation occurring as a consequence of auto-

immunity and/or traumatic and ischemic injuries might alter NPC proliferation and differentiation characteristics in a non-cell-autonomous fashion (25).

The study by Mohammad and colleagues describes a role for SVZ-derived NPCs in regulating immune trafficking in the CNS (7). It is tempting to speculate that endogenous SVZ NPCs maintain and/or restore CNS homeostasis through both neurogenic and non-neurogenic functions. In addition to differentiating into neuronal cells, NPCs are capable of sensing danger signals coming from the periphery and producing a response settled to restraining conditions that might prove noxious for proper neural cell function.

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Dangerous liaisons: flirtations between oncogenic BRAF and GRP78 in drug-resistant melanomas

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BRAF mutations in aggressive melanomas result in kinase activation. BRAF inhibitors reduce BRAF^{V600E} tumors, but rapid resistance follows. In this issue of the JCI, Ma and colleagues report that vemurafenib activates ER stress and autophagy in BRAF^{V600E} melanoma cells, through sequestration of the ER chaperone GRP78 by the mutant BRAF and subsequent PERK activation. In preclinical studies, treating vemurafenib-resistant melanoma with a combination of vemurafenib and an autophagy inhibitor reduced tumor load. Further work is needed to establish clinical relevance of this resistance mechanism and demonstrate efficacy of autophagy and kinase inhibitor combinations in melanoma treatment.

Protein kinases and cancer

Molecular analyses of human tumors have highlighted the numerous mutations in protein kinase genes that contribute to the development of cancers. For example, the gene encoding BRAF kinase is mutated in more than 60% of

melanomas, the most aggressive human skin cancer. Indeed, a mutation that results in a single amino acid substitution, V600E, accounts for 90% of identified BRAF mutations. It is worth noting that this “oncogenic” BRAF (BRAF^{V600E}) is present in many melanocytic nevi, which are benign neoplasias that can persist for decades without transitioning to malignancy (1). The fact that BRAF^{V600E}-containing melanocytic nevi are not intrinsically aggressive hints that additional steps — such as acquisition of

further mutations, metabolic reprogramming (2), or alterations in other cellular processes — are required for conversion of the proliferative melanocytes into full-fledged malignant melanomas. This raises the exciting possibility that understanding some of the internal brakes on malignancy will yield novel treatments for melanoma that fails to respond to most current anticancer therapies.

Cancer-promoting mutations frequently result in constitutive activation of the mutant kinase, which has prompted pharmaceutical companies to develop kinase inhibitors to slow or reverse the oncogenic process (3). However, the networks of signaling pathways that control cell growth in normal and cancer cells means that most kinase inhibitors are cytostatic, causing cell cycle arrest rather than cell death. Some kinase inhibitors, however, show spectacular results in eradicating tumor cells. For example, the BCR-ABL tyrosine kinase inhibitor ima-

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