Unexpected help to repair the cerebellum

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Upon injury of the developing mouse cerebellum, endogenous repair mechanisms can heal the brain and prevent behavioral motor deficits. At the right time, with the right cues, the brain can repair itself.

Brain injuries, neurodegeneration or neuroinflammation can lead to neuronal death. With the exception of restricted areas in which neurogenesis continuously occurs, the adult brain has a very limited ability to generate new neurons. To repair the adult brain, various options have emerged. Cellular replacement by transplantation of exogenous cells has shown promising results using a variety of cell sources, including fetal brain tissue, neural stem cells, reprogrammed induced pluripotent stem cells or somatic cells directly converted into neurons *in vitro*[1,2]. Direct *in vivo* reprogramming by induced conversion of non-neuronal cell types (through electrochemical or virus-mediated gene-delivery methods) into neurons has also demonstrated the encouraging potential of this approach[3–7]. Finally, the identification of rare neural stem cells in the adult CNS has raised the possibility of directly stimulating resident stem cells for brain repair[8–11].

As reported in this issue of *Nature Neuroscience*, Wojcinski *et al.*[12] used irradiation and a diphtheria toxin receptor-based transgenic mouse model to acutely deplete granule cells in the external granule layer (EGL) of the mouse cerebellum at postnatal day (P) 1 (**Fig. 1a**). Despite severe EGL depletion observed at P3, irradiated mice displayed normal morphology, with only a small reduction in the size of the cerebellum at P30. Most strikingly, irradiated mice did not present any behavioral motor deficits 5–6 weeks after irradiation.

Because cells expressing the neural progenitor cell marker Sox2 were present in the EGL of irradiated mice at P5 but not in nonirradiated control mice, the authors hypothesized that the population of nestin-expressing progenitors (NEPs) located in the nearby Purkinje cell layer (PCL) could contribute to the replenishment of the EGL. Using multiple genetic approaches and live imaging in slices, they demonstrate that NEPs in the PCL, which normally give rise to astrocytes and Bergmann glial cells, unexpectedly switch their fate to regenerate granule neurons and to repopulate the cerebellum. Wojcinski *et al.*[12] dissected the mechanisms at the base of this formidable example of brain plasticity and shed light on certain key properties that are required for such an efficient endogenous repair response to occur.

First, the acute depletion of the EGL has to be sensed to trigger the initiation of the repair response. To investigate the pathways involved in the activation of NEPs, Wojcinski *et al.*[12] performed RNA sequencing on sorted NEPs and compared the gene expression profile between NEPs from nonirradiated mice and from irradiated mice at P5. They found a transient upregulation of genes regulated by Sonic Hedgehog (SHH) signaling at P5 in irradiated NEPs but not at P8, suggesting a role for SHH signaling in the early repair response. When they specifically depleted SHH signaling in NEPs by ablating Smoothened (*Smo*), which encodes a G-protein-coupled receptor downstream of SHH signaling, conditional-knockout mice lacking Smo in nestin-expressing cells showed impaired recovery after irradiation. Five to six weeks after irradiation, these mice had smaller cerebella due to the lack of migration of the NEPs into the EGL and showed behavioral deficits, confirming the key role of SHH signaling in cerebellar repair. The authors suggest that Purkinje cells in the PCL could be sensing the absence of excitatory input due to the depletion of excitatory granule cells in the EGL and, consequently, modulating the directionality of SHH signaling toward the NEPs in the PCL.

After the detection of the EGL depletion, a population with repair potential must be activated. An obvious target population would display features of granule cell progenitors (already expressing Pax6 and Atoh1), but Wojcinski *et al.*[12] demonstrate the unexpected activation of NEPs. As they confirm by genetically inducible fate mapping experiments, at the time of activation, NEPs are lineage-committed, express neural stem cell markers and give rise to astrocytes and Bergmann glial cells. Yet the extrinsic signals provided to these cells through SHH signaling and environmental cues are able to trigger their adaptive reprogramming toward granule cell progenitors. This *in vivo* reprogramming occurs through a two-step process by which NEPs first undergo a proliferation phase in the PCL and then migrate into the EGL, where they lose their neural stem cell markers (Sox2 and nestin) and acquire granule cell lineage-specific markers (Atoh1). The fact that granule cell progenitors derived from migrating NEPs can mature properly once they reach the depleted EGL suggests that the site of lesion provides the necessary cues for progenitor development. This is in accordance with previous studies in which *in vivo* conversion of non-neuronal cells into induced neurons could be obtained following a stab-wound cortical injury but not in the unlesioned cortex[4].

Notably, the authors found that this adaptive reprogramming did not occur during adulthood. After depleting the EGL in adult mice, they could not detect any proliferation of NEPs or production of granule cells, suggesting that a critical time window exists during which NEPs can replenish the cerebellum. It will be interesting to investigate whether NEPs from the PCL do not proliferate because of the absence of SHH signaling from the Purkinje cells or whether Purkinje cells sense and signal the depleted EGL but NEPs lose their ability to switch fate during adulthood. Do intrinsic

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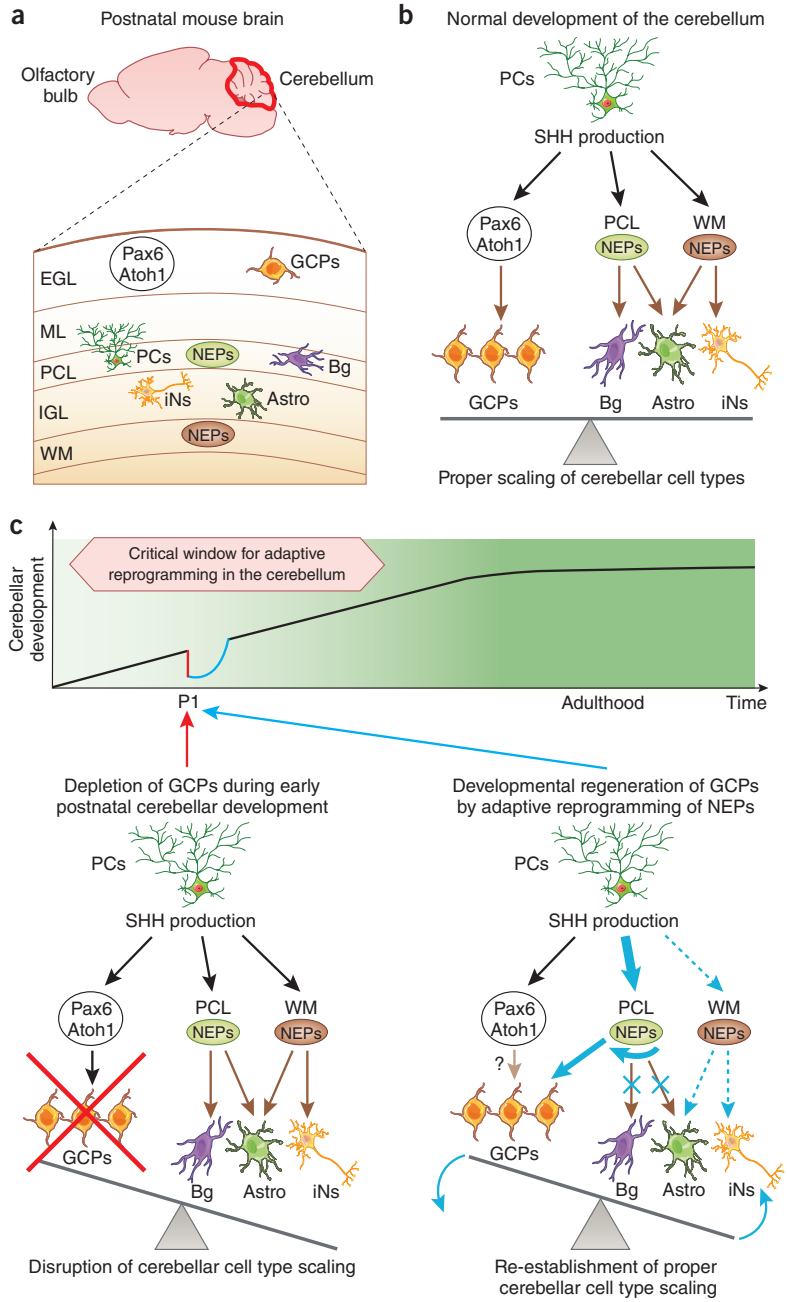

Figure 1 Model of cerebellar repair by adaptive reprogramming of nestin-expressing progenitors. (**a**) Postnatal cerebellar organization (ML, molecular layer; IGL, internal granule cell layer; WM, white matter) and the location of the different cell types: progenitors expressing Pax6 and Atoh1, NEPs, granule cell progenitors (GCPs), Purkinje cells (PCs), Bergmann glia (Bg), interneurons (iNs) and astrocytes (Astro). (**b**) During normal development of the cerebellum, Purkinje cells produce SHH and activate progenitors expressing Pax6 and Atoh1, NEPs from the PCL and NEPs from the WM. PCL NEPs give rise to GCPs, Bergmann glia and astrocytes; WM NEPs give rise to astrocytes and interneurons. (**c**) Top: cerebellar development over time, highlighting the critical window for adaptive reprogramming in the postnatal cerebellum. White-to-green shading represents the assumed progressive loss over time of the cerebellum's adaptive reprogramming potential. Bottom left: upon depletion of the EGL at P1, GCPs are destroyed, and tissue homeostasis is impaired. Remaining production of Bergmann glia, astrocytes and interneurons would disrupt the scaling of cerebellar cell type. Bottom right: during the repair response, Purkinje cells may redirect SHH signaling toward NEPs from the PCL, leading to their proliferation and migration to the EGL. In the EGL, NEPs lose their neural stem cell phenotype, acquire granule cell lineage-specific factors and produce GCPs to repopulate the EGL. Decreased SHH input to WM NEPs transiently slows the generation of astrocytes and interneurons, restoring proper cerebellar cell type scaling and ensuring normal cerebellar function.

signals (for example, specific chromatin state, enhancers or transcriptional factors) maintain NEP plasticity during the early phase of cerebellar development, or are extrinsic signals particularly potent during this period to override the lineage commitment of NEPs? The answers to these questions remain to be uncovered.

Another broad consequence of EGL lesion is a sudden loss of tissue homeostasis at a time when multiple populations that contribute to the structure of the cerebellum are expanding. To investigate the influence of the depletion of granule neurons, the most numerous cell type in the cerebellum, on the generation of other cerebellar cell populations, Wojcinski *et al.*[12] analyzed the behavior of NEPs located in the white matter and in the internal granule cell layer. They found that white matter and internal granule cell layer NEPs transiently decreased their expansion right after irradiation (P3), resulting in a delay in the production of interneurons and astrocytes. This delay could help reset the postnatal development clock of the cerebellum to maintain proper scaling of cerebellar cell types. It is thus possible that Purkinje cells behave as main regulators of the cerebellum repair response by redirecting SHH signaling toward NEPs from the PCL to induce adaptive reprogramming and boost the generation of granule cells, while limiting SHH input to white matter and internal granule cell layer NEPs to slow the production of other cell types and to eventually ensure normal cerebellar circuit formation (**Fig. 1**).

Overall, it is tempting to speculate that, if given the right cues, the rare neural stem cells present in the adult cerebellum[9] could contribute to brain repair. But considering the complexity of the repair response as a whole—the various cellular players and the combination of extrinsic and intrinsic signals involved—it is likely that early postnatal days represent a critical window of plasticity (**Fig. 1c**) that will be difficult to recreate in the adult brain. However, recent work shows that Müller glia, which lose their neurogenic potential in the retina by P16 in the mouse, can be reprogrammed in the adult mouse by combining the forced overexpression of a transcription factor with treatment with a histone deacetylase inhibitor[13]. Simultaneously targeting multiple pathways might be a way to exploit in the adult brain the intrinsic regenerative potential of the cerebellum revealed by Wojcinski *et al.*[12].

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Yummy or yucky? Ask your central amygdala

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Central amygdala directs behavioral responses to emotionally salient stimuli. While most studies have focused on aversive responses, some central amygdala neurons promote feeding and are positively reinforcing.

You take a first bite of an unfamiliar food, and then… do you spit it out or swallow? If you are an infant not yet versed in manners, the answer will purely reflect whether this food is tasty or disgusting. This basic decision lies at the heart of ingestive behavior. Proper identification of the tastes of harmful substances (whether innately recognized or previously learned) and of palatable food are vital for animal health. More generally, avoidance of harmful stimuli and approach toward appetitive stimuli are essential for an organism's survival. The central amygdaloid nucleus (CeA) promotes avoidance of threats, leading to the view that threat avoidance is the main function of CeA[1]. Indeed, work in the last few years has identified genetically distinct CeA subpopulations regulating avoidance of a variety of noxious stimuli, ranging from bitter tastes to pain[2–6]. However, CeA has also been implicated in appetitive behaviors[5], and bulk activation of most CeA neurons induces complex (appetitive) hunting behavior in mice[7]. Nevertheless, the specific CeA populations involved in appetitive behaviors and the circuit mechanisms by which they act remain elusive. In this issue of *Nature Neuroscience,* Douglass *et al.*[8] help fill this gap by identifying a new population of CeA neurons involved in appetitive behaviors in mice. By combining state-of-the-art tools, they demonstrate that these neurons promote feeding behavior by enhancing the rewarding properties of food following initial consumption.

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A population of CeA neurons expressing protein kinase C-δ ($CeA^{PKC\delta}$ neurons) was previously implicated in aversive behavioral responses to different noxious stimuli[2,4,5]. Douglass *et al.*[8] first asked whether CeA might contain a separate population that mediates reward and does not overlap with $CeA^{PKC\delta}$ neurons. A candidate CeA population was indeed found to exist, marked by expression of the serotonin receptor Htr2a (CeA^{Htr2a} neurons). Consistent with the idea that CeA^{Htr2a} and $CeA^{PKC\delta}$ neurons exert opposing behavioral effects, direct suppression of CeA^{Htr2a} neurons and pharmacological blockade of Htr2a signaling in CeA upregulate innate fear responses[9].

Douglass *et al.*[8] used cell-type-specific optogenetics and chemogenetics to show that activation of CeA^{Htr2a} neurons increases food consumption. Using optogenetic inhibition and genetic ablation techniques, they then went on to demonstrate that CeA^{Htr2a} neuron activity is actually necessary for normal food consumption.

The authors hypothesized that CeA^{Htr2a} neurons may mediate these effects by increasing the rewarding sensations experienced during feeding. To test this idea, they performed a series of behavioral experiments while optogenetically manipulating the activity of CeA^{Htr2a} neurons. First, they demonstrated that activation of CeA^{Htr2a} neurons is itself rewarding by using place preference, in which activation of CeA^{Htr2a} neurons on one side of an arena resulted in preference for that side, and intracranial self-stimulation, in which mice performed nose pokes to receive CeA^{Htr2a} neuron stimulation. The authors then elegantly demonstrated that CeA^{Htr2a} neuron activity can make a given food item more rewarding. Specifically, by pairing CeA^{Htr2a} neuron activation with food consumption, they could condition flavor preference for a previously nonpreferred food. Furthermore, inhibition of CeA^{Htr2a} neurons reduced consumption of palatable food in mice that had *ad libitum* access to food and thus were not hungry.

In addition to insights from activating or inhibiting a neuronal population, one may obtain a more complete understanding of its function by (i) recording its activity during a relevant behavior, (ii) mapping the outputs by which it exerts its effects and (iii) mapping its sources of input. Impressively, Douglass *et al.*[8] wove together lessons learned from experiments implementing all of the above methods.

First, Douglass *et al.*[8] recorded the activity of CeA^{Htr2a} neurons in behaving mice by using microendoscopic Ca^{2+} imaging as a

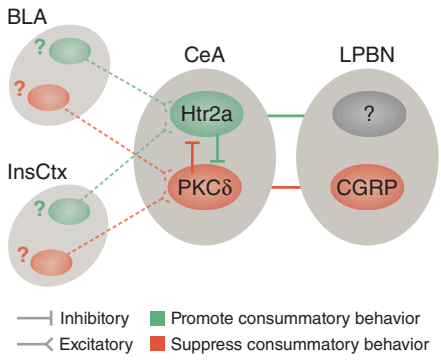

Figure 1 Neural circuits promoting consummatory behavior and suppressing consummatory behavior. CeA^{Htr2a} and $CeA^{PKC\delta}$ populations likely act, at least in part, by reciprocally inhibiting each other. $CeA^{PKC\delta}$ neurons receive information regarding noxious stimuli from LPBN $CGRP^{+}$ neurons, while CeA^{Htr2a} neurons act, at least in part, by inhibiting other ($CGRP^{-}$) LPBN neurons. Both CeA^{Htr2a} and $CeA^{PKC\delta}$ neurons receive inputs from basolateral amygdala (BLA) and insular cortex (InsCtx), among other regions. This raises the possibility that BLA and InsCtx inputs to CeA may each be composed of distinct CeA^{Htr2a}- and $CeA^{PKC\delta}$-projecting subpopulations, mediating appetitive and avoidance behaviors, respectively.