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Brains in metamorphosis: reprogramming cell identity within the central nervous system

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Abstract

During embryonic development, uncommitted pluripotent cells undergo progressive epigenetic changes that lock them into a final differentiated state. Can mammalian cells change identity within the living organism? Direct lineage reprogramming of cells has attracted attention as a means to achieve organ regeneration. However, it is unclear whether cells in the CNS are endowed with the plasticity to reprogram. Neurons in particular are considered among the most immutable cell types, able to retain their class-specific traits for the lifespan of the organism. Here we focus on two experimental paradigms, glia-to-neuron and neuron-to-neuron conversion, to consider how lineage reprogramming has challenged the notion of CNS immutability, paving the way for the application of reprogramming strategies to reshape neurons and circuits *in vivo*.

Introduction

C. H. Waddington likened the process of cell differentiation to a marble rushing along a downward slope and ending up in one of many valleys surrounded by impassable hills. Despite long-held dogmas on the impossibility of overcoming epigenetic barriers and changing the identity of differentiated cells, it is increasingly accepted that it is possible to push the marble uphill to enter new valleys and thus convert one differentiated cell type directly into another. This process has been termed transdifferentiation or direct lineage reprogramming, and various cell types have been directly reprogrammed to acquire a new differentiated identity, across organ systems and in different species (reviewed in [1] and [2]).

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Blood, heart, and pancreas were among the first tissues where successful lineage reprogramming was described *in vivo* [3–5]. In recent years similar strategies have been used to probe the reprogramming capabilities of the mammalian CNS, exposing previously unappreciated degrees of cellular plasticity and challenging the immutability of the programs that define neuronal and glia identity.

Glia-to-Neuron Reprogramming

The idea of reprogramming endogenous glia originated from the now over a decade-old notion that radial glia during development and adulthood is at the base of neuron-producing lineages [6–9]. These findings begged the question of whether parenchymal astroglia, a very abundant cell type in the brain, could be forced to turn into neurons upon expression of transcription factors that instruct neurogenesis during development. Indeed a series of *in vitro* studies set the stage by showing that forced expression of *Pax6*, *Neurog2*, *Ascl1*, or *Dlx2* can reprogram astroglia from the early postnatal cortex into induced neurons with functional neuronal properties [10–12]. Notably, consistent with their respective roles in dorsal and ventral telencephalic development [13–16], *Neurog2* or *Ascl1*/*Dlx2* direct the conversion of astrocytes into glutamatergic and GABAergic neurons [10,11]. Thus young astroglia is capable of differentially interpreting neurogenic cues resulting in distinct neuronal terminal features.

The cellular context is a major player in determining what terminal features are acquired. *Ascl1* a central player in converting fibroblasts and other cell types into neuronal cells *in vitro* can induce the generation of different neuronal fates, from glutamatergic to dopaminergic neurons, depending on synergism with other factors, starting cell type and timing of expression. The importance of the cellular context in affecting the outcome of reprogramming transcription factors, is also exemplified by the fact that while endogenous *Ascl1* is normally required for oligodendrogliogenesis [17,18], its expression in none of the *in vitro* reprogramming paradigms resulted in the genesis of oligodendroglia. Notably, however, forced expression of *Ascl1* in adult neural stem cells of the dentate gyrus, rather than promoting neurogenesis, diverts these to oligodendrogliogenesis [19,20].

In addition to astroglia, some of the earliest studies into the reprogramming capabilities of CNS cells showed that oligodendrocyte precursor cells (OPCs, often referred to as NG2 cells) can revert back into neural stem cell-like cells following sequential exposure to growth factors [21]. NG2 cells continue to proliferate in the adult brain [22] and may thus represent an interesting target for *in vivo* direct conversion. Whether turning these cells into neurons is to be deemed *bona fide* reprogramming or rather reflects an altered programming process depends on how “terminal” one considers the differentiation of NG2 cells. In fact, while it has been reported that these cells might retain multipotency, there is also accruing evidence that they exert important glial functions in their own right [23]. Moreover, there is considerable heterogeneity in the differentiation potential observed among NG2 cells of different brain regions [24]. Notwithstanding a final verdict on these issues, NG2 cells certainly are a natural target to consider for generating neurons.

Finally, it is critical to consider that the adult brain also contains a source of non-neural cells that may be ideal starting populations for reprogramming into neurons. In a first demonstration of this concept, Karow et al. showed that pericytes, cells normally found juxtaposed to blood vessels, can be isolated from the adult human brain and converted into functional induced neurons upon forced expression of *Sox2* and *Ascl1* [25].

Collectively, these studies set the stage for subsequent work aimed at reprogramming resident cells into neurons within the vastly more complex context of the living brain (Figure 1). First attempts at *in vivo* reprogramming of endogenous glia utilized retroviruses encoding *Pax6* to target cells that proliferate in response to brain injury [26]. Encouragingly, DCX-positive cells were produced. However, they quickly disappeared suggesting abortive neurogenesis. Almost a decade later, these attempts have now been repeated with novel factors at hand. Work from several laboratories utilized retro- or lentiviral vectors to express different neurogenic fate determinants in brain-resident cells. Torper et al conditionally expressed into striatal astrocytes the cocktail of transcription factors first described to reprogram fibroblasts into neurons [27]. Despite the caveat of potentially unwanted targeting of additional cell types due to promoter leakage, this led to the generation of morphologically and neurochemically identifiable neurons, suggesting that glia can be reprogrammed into neurons in the adult brain [27]. This conclusion is supported by studies in neocortical and striatal lesion models, where retrovirus-mediated expression of *Neurog2* was found to induce neurogenesis, albeit the identity of the starting cells remained unknown [28]. However, neither of these studies reported on the functionality of the neurons. Employing a stab wound injury model Guo et al. found that the gene encoding the basic helix loop helix transcription factor NeuroD1 can reprogram both reactive astroglia and NG2 cells into neurons with a surprising degree of efficiency [29]. Recordings from brain slices showed that these neurons were functional in that they received synaptic inputs and were capable of repetitively firing action potentials [29]. Another study succeeded in obtaining electrophysiologically functional neurons from astrocytes *in vivo* by a two-step process. Forced expression of *Sox2* in striatal astrocytes led to the generation of proliferating, DCX-positive neuroblasts, which could only be turned into functional neurons upon further treatment with brain-derived neurotrophic factor (BDNF) and the bone-morphogenetic protein (BMP) inhibitor Noggin [30]. What is particularly interesting in this second approach is that the starting number of astrocytes could be amplified by transitioning through a proliferating neuroblast stage.

Neuron-to-neuron reprogramming

As a distinct alternative to reprogramming of non-neuronal resident cells of the brain, replacement of specific classes of neurons may also be achieved via reprogramming of neurons themselves, from a class into another. Once generated during development central neurons become permanently postmitotic and do not change their identity over time. However, it is unclear whether under the influence of appropriate signals neurons could be instructed to acquire signature features of a different cell type.

It remains largely unknown how neuronal class-specific features are maintained after development, which makes it difficult to predict whether neurons can be lineage

reprogrammed. Work in invertebrates indicates that expression of key developmental transcription factors need to be maintained into adulthood in order for neurons to keep class-specific properties [31,32]. Initial evidence suggests that vertebrate neurons may have some of the same strategies in place. For example, the transcription factor *Nurr1* is necessary for midbrain dopaminergic neurons to maintain terminal features such as dopaminergic identity and expression of some lineage specific transcription factors [33]. These experiments suggest that terminal neuronal identity is at least partly maintained via “active” mechanisms of transcriptional regulation. In agreement, nuclei of some neurons when exposed to the most powerful reprogramming environment known, i.e. the cytoplasm of the egg, appear able to support the development of an entire mouse. In 2004 the nucleus of an olfactory epithelial neuron, a unique class of sensory neurons that continue to be produced in the adult, was transferred into an enucleated oocyte via somatic cell nuclear transfer, and live mice were successfully obtained [34,35]. Using a similar approach, viable mice were subsequently produced using the nuclei of postmitotic neurons from the cerebral cortex of juvenile, but not adult, mice [36–38]. This work has provided some proof-of-principle evidence that no irreversible genetic or epigenetic changes have likely taken place during neuronal development that preclude the acquisition of a new cellular identity. Intriguingly, in response to oncogenes neurons of the cerebral cortex appear able to dedifferentiate to a progenitor state and initiate glioblastoma formation [39], although rare targeting of progenitors cannot be excluded in any of these studies.

Should neurons retain the capability of reprogramming their identity, could neurons then be converted from one class into another within the CNS? Could this in turn result in re-wiring of circuitry and gaining of new function? Given that neurons share some pan-neuronal features, conversion of closely-related neuron subtypes may facilitate the generation of highly specialized neurons and circuits for therapeutic benefit. This field is only emerging, but some studies are exploring this possibility. Similar to the work on reprogramming non-neuronal cells, master selector genes able to drive the acquisition of class-specific neuronal identity can be powerful tools to instruct a neuronal class-switch *in vivo*. In a first application to neurons of the cerebral cortex, the transcription factor *Fezf2*, a master gene capable of instructing multiple features of identity of corticospinal motor neurons (CSMN) [40,41], was used to turn other cortical neurons into CSMN within the brain. Despite neurons being postmitotic, *Fezf2* is sufficient to directly reprogram embryonic and postnatal callosal projection neurons (CPN) into corticofugal neurons, including CSMN. Reprogrammed callosal neurons acquire molecular properties of CSMN, and change their axonal connectivity to corticofugal projections directed below the cortex towards the spinal cord [42]. Interestingly, electrophysiological properties and afferent/efferent connectivity of layer V neurons develop when *Fezf2* is expressed in layer IV stellate neurons [43]. The data indicate that neurons of the neocortex can undergo a change of identity postmitotically when exposed to cell autonomous signals instructive of a different neuronal lineage (Figure 1). In the case of callosal neurons, postmitotic neuronal identity could be changed as late as P3 and P6, approximately ten days after the neurons have become postmitotic. The data indicate that the postmitotic nature of neurons does not *per se* preclude reprogramming. This is in agreement with prior cell fusion experiments that could instruct reprogramming without cell division [44] and with direct reprogramming in the pancreas, where exocrine cells direct

conversion into beta cells does not require cell division [5]. However neuronal nuclear plasticity progressively declines over the first postnatal week and reprogramming capabilities in response to *Fezf2* have exhausted by P21 [42]. This is likely not a phenomenon unique to cortical neurons, as "complete" reprogramming of rod into cone photoreceptors quickly turns into "partial" reprogramming over a similar time window [45]. In this study, conditional removal of the fate-specifying transcription factor *Nrl* from adult rods directly reprogrammed rods into cones. Although the cells retained some rod-specific traits, the work demonstrates that partial conversion of rods into cones is feasible in adults. Notably, this study tested the potential therapeutic relevance of such conversion showing that adult rods normally sensitive to *retinitis pigmentosa* not only became disease-resistant upon conversion into cones, but that as a result, endogenous cones were spared from the secondary death that normally affects them when rods are allowed to degenerate.

Collectively, these studies begin to challenge the concept that neurons may be irreversibly differentiated cells, yet also highlight how fundamental obstacles and limitations remain. Studies are needed to extend the critical period of postmitotic neuron reprogramming to the adult brain. The mechanisms that preclude reprogramming of adult neurons are not understood and this goes hand in hand with a lack of knowledge of how neuronal class-specific identity is kept unchanged during lifetime. Some evidence exists for active maintenance of neuronal identity, but this alone could be a problem for long-lived cells as it could become deregulated with time. It is likely that epigenetic and genetic changes may also be in place to passively "lock-in" identity, even if not necessarily in an irreversible manner. In support for the existence of epigenetic blocks to neuronal reprogramming *in vivo*, failure of reprogramming of adult rods into cone photoreceptors may at least partly be due to DNA methylation at key, class-specific loci [45].

Exploiting the power of using invertebrate systems, Oliver Hobert's group identified *C. elegans lin-53*, a chromatin remodeler, as a major inhibitor of the reprogramming of germ cells into gustatory neurons by the selector gene, CHE-1 [46]. This work put forward the guiding principle that combining chromatin modification with overexpression of fate specifying transcription factors may be a common way to facilitate reprogramming across species. It will be interesting to determine whether this molecular strategy will be successful to change the identity of cells refractory to reprogramming, like adult neurons (Figure 2).

Outlook

While the prospect of reprogramming the CNS is exciting, there remain conceptual and practical barriers for *in vivo* reprogramming to result in function-promoting outcomes or circuit modification for therapeutic benefit. Most of the direct reprogramming scenarios entail the loss of a cell of a specific identity and function. While a minor loss of cells could be tolerated, ideal strategies should allow for the replacement of the reprogrammed cell or for a local expansion of the newly generated neurons. One possibility would be the generation of an expandable intermediate cell. Should "reprogramming" of the postmitotic state of adult neurons be possible, as it has been shown for postmitotic cardiomyocytes [47], cell division may provide the additional benefit of facilitating the reprogramming process by erasing some of its epigenetic constrains.

Another crucial aspect concerns the correct incorporation of newly generated neurons into the pre-existing circuit. This is of utmost significance not only for the functional replacement of degenerated neurons, but also to avoid aberrant connections that may be detrimental to normal circuit function. The highly regulated step-by-step integration of new neurons in the adult neurogenic niches [48] can provide important lessons on the challenges a neuron derived through reprogramming must surmount in order to functionally integrate.

Finally, the greatest of all challenges would entail the regeneration of an entire circuitry consisting of distinct types of neurons and glia. Would reprogramming the identity of one neuron type result in the reorganization of the local circuitry? It is clear that in order to establish a complex nervous tissue a 1-to-1 reprogramming strategy is bound to fail and that sufficient capacity of self-organization will be required. While there is currently no concrete strategy on how to achieve this, the fact that pluripotent stem cells can generate organoids containing neurons of different regional identities in a seemingly orderly arrangement akin to real brains [49], gives hope that there will eventually be a way through this bottleneck.

A long way lays ahead for this exciting new field and it is likely that a concrete functional application of reprogramming strategies in the brain will require addressing fundamental questions regarding the way the CNS maintains its identity and the mechanisms that preclude cell conversion *in vivo*. Investigation of the mechanisms that control integration of new neurons into functional circuitry will be necessary to truly unfold the therapeutic value of these studies.

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Highlights

1. We review experimental evidence for the feasibility of direct reprogramming within the brain.
2. First we discuss achievements in the attempt of glia-to-neuron reprogramming in vivo.
3. This is followed by reviewing the state of the art of neuron-to-neuron reprogramming.
4. The challenges ahead of in vivo lineage reprogramming are discussed.

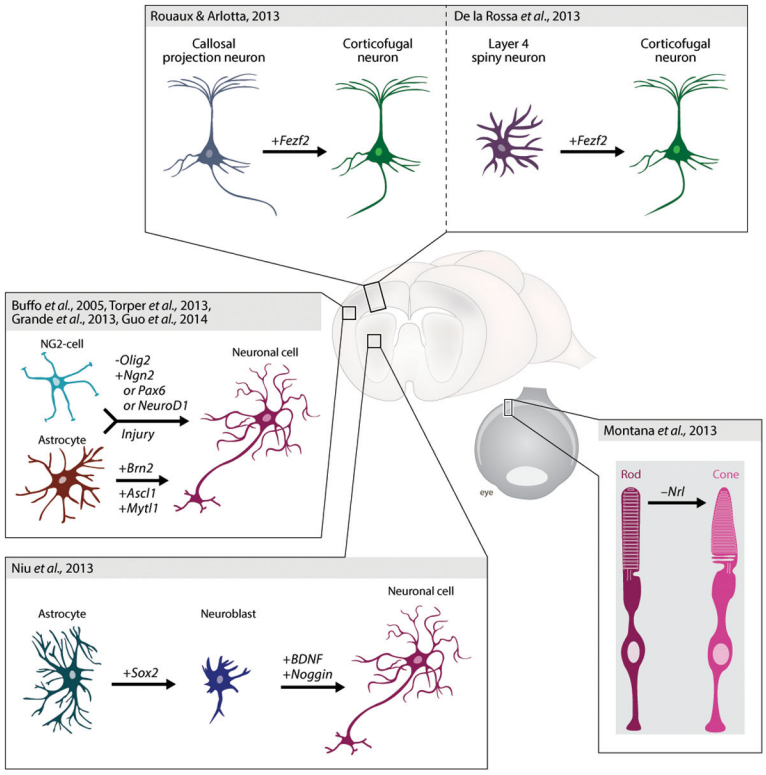

Figure 1. Direct lineage reprogramming of glia and neurons *in vivo*

Astrocytes and other glial cells have been converted into neuron-like cells within the parenchyma of the brain. Similarly, young postmitotic neurons in the neocortex and the retina have been directed to acquire new neuronal class-specific identities.

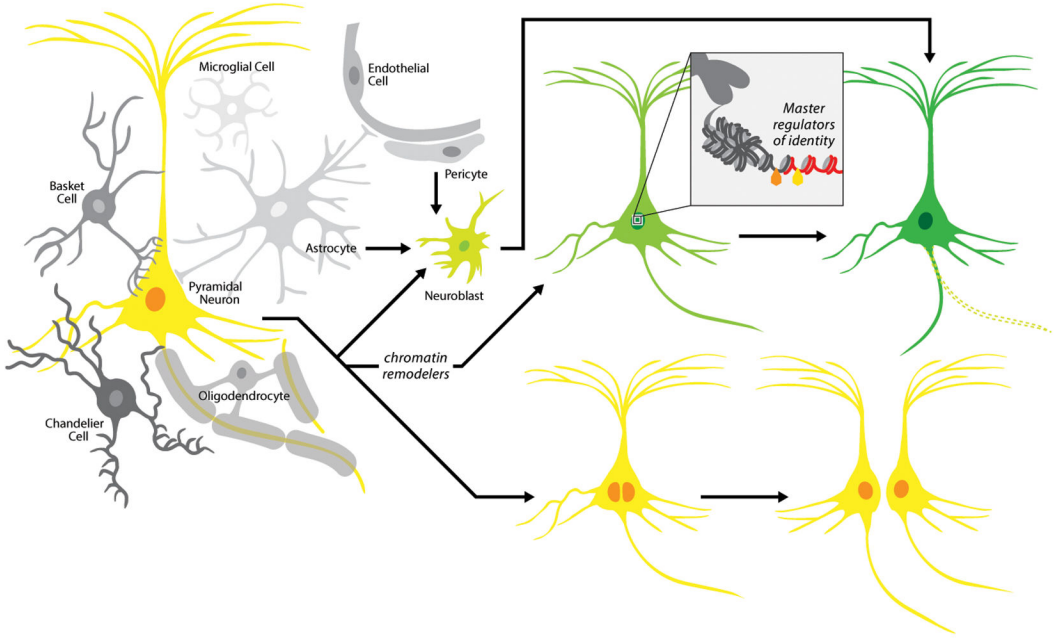

Figure 2. Theoretical ways of generating new neurons *via* reprogramming *in vivo*

Within the multicellular, complex environment of the adult CNS, astrocytes and other resident cell types, like pericytes, may be instructed to convert into plastic cellular intermediates (e.g. neuroblasts), which in turn may be receptive to instructive signals and become neurons. Neurons may transition to a more plastic cell state by undergoing epigenetic remodeling, which in turn could make them more permissive to fate specifying transcription factors able to induce the acquisition of a new neuronal class-specific identity. They also could revert to a more plastic cellular intermediate (e.g. neural stem cells, neuroblasts etc.). An alternative route to neuronal replacement could include the scenario of neuronal cell division. This process may also contribute to reset some of the epigenetic barriers normally blocking reprogramming.