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Prospects for Engineering Neurons from Local Neocortical Cell Populations as Cell-Mediated Therapy for Neurological Disorders

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Abstract

There is little cell replacement following neurological injury, limiting the regenerative response of the CNS. Progress in understanding the biology of neural stem cells has raised interest in using stem cells for replacing neurons lost to injury or to disease. Stem cell therapy may also have a role in rebuilding deficient neural circuitry underlying mood disorders, epilepsy, and pain modulation among others. In vitro expansion of stem cells with directed differentiation prior to transplantation is one approach to stem cell therapy. Emerging evidence suggests that it may be possible to directly convert in vivo endogenous neural cells to a neuronal fate, providing an alternative strategy for stem cell therapy to the CNS. This review assesses the evidence for engineering subtype-specific neuronal fate of endogenous neural cells in the cerebral cortex as a function of initial cell lineage, reactive response to injury, conversion factors, and environmental context. We conclude with a discussion of some of the challenges that need to be overcome to move this alternative in vivo engineered conversion process towards becoming a viable therapeutic option.

Keywords

direct in vivo conversion; injury-induced neurogenesis; reprogramming; neural stem cell; neuronal replacement; endogenous recruitment

Introduction

Neurological disorders arising from neuronal loss or dysfunction produce a wide range of cognitive and motor deficits. Unlike other tissues, the mature central nervous system does not repair itself by replacing lost neurons. The lack of effective restorative therapies to counteract the injury or disease underlying neurological deficits results in negative consequences for the individual and for society through lost productivity. However, the discovery of adult neural stem cells (NSCs) and the continual generation of new neurons in

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specific regions of the adult mammalian brain (Gage, 2000) offer a new possibility for developing restorative therapies.

Within the adult mammalian brain, neurogenesis is confined to two discrete regions: the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricles. The SGZ produces glutamatergic granule neurons that may functionally contribute to learning and memory. Proliferation of neuronal progenitors in the SVZ is followed by migration of neuroblasts via the rostral migratory stream (RMS) to contribute primarily to new olfactory bulb interneurons (Ming and Song, 2011). However, the majority of the brain is essentially devoid of robust, constitutive addition of new neurons and is considered to be nonneurogenic, despite periodic reports of neurogenesis in different regions. For example, an initial promising report of adult cortical neurogenesis under physiological conditions (Gould et al., 1999; Gould et al., 2001) has been largely refuted by subsequent studies (Kornack and Rakic, 2001; Koketsu et al., 2003; Bhardwaj et al., 2006). If such a process does exist in the normal adult neocortex, it is extremely rare. Some studies suggest that any new cortical cells are likely small interneurons, which may have precluded their detection in other studies (Dayer et al., 2005; Feliciano and Bordey, 2013).

The existence of neurogenic centers in the adult mammalian brain suggests it may be possible to draw new neurons out to non-neurogenic regions. As a result, there has been considerable investigation into factors that could activate NSCs and direct their migration into the nonneurogenic brain. Brain injury may itself be a potent activator of endogenous neural progenitor cells, although the outcome of this response is insufficient to effect meaningful self-repair. Rodent models have demonstrated an injury-induced expansion of the SVZ progenitor population and redirection of some newly generated neuroblasts to the lesioned striatum and neocortex (Reviewed in O'hab and Carmichael, 2008; Burns et al., 2009; Kernie and Parent, 2010; Kokaia and Lindvall, 2012). Furthermore, a local response to injury has also been reported that activates resident neural progenitor cells within rodent cortex (see Table 1; Reviewed in Boda and Buffo, 2010; Ohira, 2011; Matsuyama, 2012) and potentially human cortex (Jin et al., 2006; Zheng et al., 2013). This potential capacity for induction of neurogenic fate in local neural cells suggests that endogenous progenitor cells may be an attractive target for neuronal replacement strategies.

Local recruitment of these endogenous progenitor cells may offer distinct advantages for therapeutic delivery over transplantation of cultured neural progenitor cells. Transplantation of cultured cells for cell-based therapy requires substantial infrastructure and financial resources for cell engineering, culture maintenance, and long-term storage in cell banking facilities. The surgical delivery of these cell populations to the brain is substantially invasive and grafted cells may be immunogenic. Furthermore, contamination of the transplanted cell population with a single, undifferentiated, pluripotent cell carries significant risk for tumor formation.

Manipulation of endogenous cells provides a potential means for a more rapid and minimally invasive therapy. As neurological deficits are more prevalent in an aging population, which have increased risk of surgical complication, a minimally invasive therapy is an important consideration.

This review will catalog endogenous neural cell populations with injury-induced activation capacity and summarize the current understanding of their potential for therapeutic recruitment. The potential contribution of lineage respecification tools in the recruitment of resident neural cells will also be addressed. We conclude with a perspective on the prospects and limitations for translating pre-clinical studies recruiting endogenous cells into therapeutic options for adult human cortical repair.

Potential for Recruitment of Neuroblasts from Neurogenic Zones

Neurons generated in the hippocampal dentate gyrus SGZ exhibit highly restricted migration into the adjacent granule cell layer under both naïve and injury conditions (Jin et al., 2003). The constitutive proliferation and migration of SVZ-derived neuroblasts, however, is subject to manipulation and redirection following injury and growth factor delivery, making these cells the focus of endogenous recruitment strategies for neuronal replacement. The SVZ contains a population of developmental, radial glial NSCs that persist throughout adulthood as the slowly dividing, Type B NSC. These cells generate the actively dividing transient amplifying Type C progenitor population that gives rise to the immature Type A migratory neuroblasts. Following departure from the SVZ, neuroblasts travel via the rostral migratory stream (RMS) to reach the olfactory bulb (OB), where they generate a variety of OB interneurons (Ming and Song, 2011).

The rodent SVZ is organized as a mosaic of heterogeneous spatial domains of distinct embryonic origins that produce a particular interneuron subtype (Merkle et al., 2007; Merkle et al., 2014). Neonatal isolation and heterotopic transplantation of these domains does not alter their interneuron subtype or position in the OB, implying early intrinsic fate determination (Merkle et al., 2007). Some level of specification may be actively maintained in the B cells by signaling gradients such as SHH (Ihrie et al., 2011) or Wnt (Ortega et al., 2013), but this plasticity appears to be lost by progression to the neuroblast stage (Merkle et al., 2007; Sequerra et al., 2013). This predetermination of SVZ-derived neuroblast subtype may constrain their useful contribution to cortical circuitry.

While perinatal hypoxic/ischemic injury stimulated recruitment of SVZ-derived neuroblasts to the neocortex with persistent neuronal addition (Yang et al., 2007), the response in adult brain is much more modest. Ectopic migration of SVZ-derived neuroblasts into the adult neocortex has been studied in rodent models of ischemia (Parent et al., 2002; Jin et al., 2003; Ohab et al., 2006; Shimada et al., 2010) and trauma, such as cortical aspiration (Goings et al., 2004; Sundholm-Peters et al., 2005; Saha et al., 2013) and controlled cortical impact (Kernie et al., 2001; Salman et al., 2004; Ramaswamy et al., 2005). One report demonstrated persistence of neuroblast migration for at least a year (Osman et al., 2011), similar to findings in the striatum (Kokaia et al., 2006; Thored et al., 2006). However, only a handful of studies across both ischemic (Gu et al., 2000; Jiang et al., 2001; Ohab et al., 2006; Leker et al., 2007; Li et al., 2008; Kreuzberg et al., 2010) and traumatic (Salman et al., 2004; Saha et al., 2013) injury models have reported any detectable neuronal maturation of newly generated cells in the cortex.

The ability to stimulate recruitment of neuroblasts from the SVZ is likely dependent on the extent and proximity of cortical injury to the neurogenic site. Neuroblast migration into the striatum has been shown to correlate with the extent of ischemic damage (Thored et al., 2006). Direct comparison of cortical ischemic lesions found recruitment of SVZ neuroblasts only from those that involved the striatum (Shimada et al., 2010). An important contribution of the Shimada et al. (2010) study was their internal comparison of two MCAO lesion models, as a large inter-laboratory variation in infarct size is found in the literature (Liu et al., 2009b). Most studies have not demonstrated the source of the neuroblasts found in the cortex following cortical-only injury, while other viral pre-labeling studies of the SVZ have failed to demonstrate migration to the cortex (Buffo et al., 2008; Nakagomi et al., 2009). Given the variability in observed migration following injury, an alternative explanation could be that observed newly generated neuroblasts were of a local cortical origin. Modest injury-induced migration from SVZ may have additional constraints in human patients as infarct volumes in human stroke are typically only 4–14% of cerebral hemispheric volume (Carmichael, 2005), resulting in a diluted stimulus for migratory redirection. Another consideration in regard to the potential redirection of SVZ-derived cells to the cortex is that the migratory distances from the SVZ to cortex are already significant in rodents and are far greater in humans (Figure 1).

In addition to successful migration, appropriate neuronal subtype specification will be required for therapeutic purposes. There is limited detectable neuronal fate of potential SVZ-derived neuroblasts to the neocortex and results have been mixed in regard to the neuronal subtypes generated in striatum through the recruitment of SVZ-derived neuroblasts. Initial reports found the majority of adult born neurons following an extensive ischemic lesion labeled for the medium spiny projection neuron (MSN) marker, DARPP32 (Arvidsson et al., 2002; Parent et al., 2002). As MSNs comprise 95% of the striatal neuron population, this would suggest an appropriate replacement neuron is being specified. In contrast, an identical lesion in another report produced only calretinin interneurons, which comprise the majority of newborn OB interneurons under naïve conditions (Liu et al., 2009a), suggesting a redirection of migration without modification of neuronal subtype lineage. To date, functional studies on the ischemic striatum have validated only a generic interneuron phenotype of newly generated cells (Yamashita et al., 2006; Hou et al., 2008). The apparent predetermined lineage specification of SVZ derived cells suggests that they may not acquire cortical neuronal subtype specification without additional regional cues, which may be lost in ischemic or traumatic cortical injury.

Regional cues appear to contribute to refinement of neuronal subtype diversity. For example, signaling from surrounding intact tissue, achieved only in models of discrete neuronal loss, has been successful in recruiting neuronal progenitors ((Magavi et al., 2000; Nakatomi et al., 2002; Chen et al., 2004; Tonchev et al., 2005; Brill et al., 2009; Vessal and Darian-Smith, 2010; Benraiss et al., 2013). In an elegant study, a selective cortical lesion resulted in replacement projection neurons that acquired pyramidal morphology and sent axons to layer appropriate thalamic targets (Magavi et al., 2000). Similar results were achieved with corticospinal motor neurons (Chen et al., 2004). As the evidence suggests that these newborn projection neurons originated from the SVZ, this would imply a substantial regional re-specification of neuroblasts destined to become olfactory interneurons to an

excitatory, cortical layer-specific fate. Independent replication of these findings traced at least partial origin of these cells to a novel SVZ population that contributes to juxtaglomerular glutamatergic neurons to the olfactory bulb (Brill et al., 2009). These progenitor cells expressed markers typical of cortical precursors and appeared competent to respond to the re-expression of developmental factors from the surrounding parenchyma for further layer-specific subtype specification (Sohur et al., 2012). These new corticocallosal neurons also expressed the appropriate upper cortical layer marker, Cux1 (Brill et al., 2009).

The current evidence from adult human brain indicates that recruitment of SVZ-derived neuroblasts may not be a viable therapeutic approach. Neurogenesis does exist in the adult human dentate gyrus (Eriksson et al., 1998; Spalding et al., 2013), but has been controversial in the SVZ. Although there is one report of robust, adult human, SVZ-derived OB neurogenesis (Curtis et al., 2007), the overwhelming evidence suggests a drastic decline to relatively rare proliferating cells and neuroblasts with an absence of OB neurogenesis after eighteen months of age (Sanai et al., 2004; Sanai et al., 2007; Arellano and Rakic, 2011; Sanai et al., 2011; Wang et al., 2011; Spalding et al., 2013). A recent study utilizing retrospective C14 dating does report low levels of constitutive adult human neurogenesis in the striatum that over a lifetime produces new interneurons, but not medium spiny neurons (Ernst et al., 2014). There was no direct evidence linking the adult born neurons to an SVZ origin, leaving open the possibility that they were locally derived. Despite the activation of the human SVZ following ischemia, there is no evidence for any significant injury-induced migration (Macas et al., 2006; Martí-Fàbregas et al., 2010). Given the low numbers, transient existence, and limited subtype specificity of SVZ-derived new neurons in rodent models, combined with the migratory distances needed to reach human cortex, it seems unlikely that human SVZ could provide a viable source for recruitment to cortex or other non-neurogenic brain regions. As a result, identification of candidate cells for local recruitment may be a suitable alternative strategy for neuronal replacement in the cortex.

Injury-Induced Recruitment of Resident Cortical Progenitors

The potential existence of neural progenitor cells outside of the SVZ and dentate gyrus has been demonstrated (Palmer et al., 1999). Numerous reports of cortical NPC isolation have since emerged in various injury models including laser-targeted cell death (Sirko et al., 2009), stab wound (Buffo et al., 2005; Buffo et al., 2008; Sirko et al., 2013), cortical impact (Itoh et al., 2005) and ischemic lesions (Nakagomi et al., 2009; Shimada et al., 2010; Shimada et al., 2012). NPCs have also been derived from surgically resected (Arsenijevic et al., 2001; Richardson et al., 2006a) and post-mortem adult human tissue (Palmer et al., 2001). Neuronal commitment of newly-generated cortical cells does not occur spontaneously; it appears that injury is necessary to induce multipotent progenitors in the cortex. As the isolated cells in previous studies demonstrated multipotency, but limited self-renewal, we will refer to them as neural progenitor cells (NPC).

In addition to the SVZ and dentate gyrus, there is evidence suggesting the existence of a minor neurogenic niche at the surface of the brain in cortical layer 1, where a small population of newly generated cells express the inhibitory, GABA producing enzyme, GAD67 (Ohira et al., 2010). These so called Layer 1-Inhibitory Neural Progenitor (L1-INP)

cells proliferate in response to ischemic injury, migrate into deeper cortical layers, and express multiple inhibitory neuron subtypes along with some evidence for functional activity, despite a lack of NeuN expression. Chronic treatment of the serotonin selective reuptake inhibitor (SSRI), fluoxetine, in uninjured animals also led to an increase in L1-INP proliferation and differentiation into multiple inhibitory subtypes (Ohira et al., 2013). It is possible that the L1-INPs may be the source of the rare, newborn cortical interneurons reported in the uninjured brain (Dayer et al., 2005) and of newly generated cells labeled prior to ischemic injury that co-expressed immature neuronal markers in the cortical penumbra (Jin et al., 2003). While further characterization of the L1-INP population is needed, their location would make them a valuable local source of diverse interneuron production for cortical repair as an alternative to recruiting cells from the SVZ or dentate gyrus. These findings also suggest that we may not yet have detected all potential progenitor cell populations in the CNS.

If local cortical NPCs can be recruited under injury conditions, it becomes important to define their pre-injury identity. A few candidate cell populations have emerged, including reactive astrocytes, reactive pericytes, oligodendrocyte progenitor cells, and white matter progenitor cells (Table 1). The evidence from animal models in support of an NPC role for each of these cell populations will be discussed in turn, followed by a discussion of evidence for cortical NPCs in the human brain.

Reactive Astrocytes

The glial nature of NSCs prompted investigation of stem cell features in astrocytes within the developing and mature CNS (Gotz et al., 2002; Doetsch, 2003). For example, multipotent neurospheres can be generated from cortical astrocytes up to ten days after birth (Laywell et al., 2000). Upon injury, local astrocytes become “reactive” and undergo hypertrophy with marked up regulation of GFAP. In more severe injury, a subset of these reactive cells proliferate and express nestin, vimentin, and sox2, suggesting partial dedifferentiation toward a multipotent state, reminiscent of their radial glial origin (Sofroniew and Vinters, 2010; Robel et al., 2011). However, astrocyte proliferation is relatively modest and is often overstated due to the massive upregulation of GFAP (Sofroniew and Vinters, 2010). Furthermore, reactive astrocytes do not migrate in response to lesion (Tsai et al., 2012; Bardehle et al., 2013). In vivo imaging has also identified a subset of reactive astrocytes undergoing proliferation following a stab wound injury that is juxtaposed to vascular elements (Bardehle et al., 2013). Culture studies from lesioned cortical tissue previously labeled by genetic lineage tracing (Buffo et al., 2008) or viral vector lineage tracing (Shimada et al., 2010; Shimada et al., 2012) support a role for reactive astrocytes as potential NPCs. However, exhaustive grafting studies of the astrocyte-derived neurospheres into the neurogenic regions of the embryonic, neonatal, and adult brain resulted in no mature neurons (Shimada et al., 2012). Therefore, it appears that injury alone is insufficient to instruct reactive astrocytes into full and successful neuronal lineage commitment.

Reactive Pericytes

CNS lesions produce reactive gliosis along with a fibrotic response directly bordering the gliotic scar that walls off the CNS from further insult (Burda and Sofroniew, 2014). A subset of resident perivascular cells has recently been shown to contribute to fibrotic scar formation following both spinal cord injury (Goritz et al., 2011) and focal cortical ischemia (Fernandez-Klett et al., 2013). Resident CNS perivascular cells are vital regulators of the neurovascular niche and include the capillary ensheathing pericytes and vascular smooth muscle cells in the larger vessel walls that are identified by expression of surface markers NG2 and PDGFR-beta (Winkler et al., 2011). A subset of pericytes in peripheral organs has been shown to contribute to tissue-specific repair, exhibiting a differentiation capacity similar to that of mesenchymal stem cells (MSCs) (Crisan et al., 2008). Consistent with a role for resident tissue repair, isolation and culture of microvascular CNS pericytes demonstrated their potential to differentiate along the neural lineage (Dore-Duffy et al., 2006; Dore-Duffy, 2008). Cortical pericytes arise from a neuroectodermal lineage, as forebrain pericytes are reported to be uniquely neural crest-derived (Korn et al., 2002). Their neuroectodermal origin may explain the in vitro capacity of forebrain pericytes to differentiate into a neural lineage, while also suggesting that this capacity may not be present in mesencephalic or rhombencephalic pericytes.

Cortical ischemia produces a reactive response in pericytes in both rodents and humans. The core of an ischemic lesion displays an immediate loss of capillary pericytes and astrocytes followed by a loss of endothelial cells. These events are paralleled by massive proliferation of PDGFR-beta expressing cells originating from larger vessels and their subsequent migration and distribution throughout the ischemic core. This subpopulation of perivascular cells gradually downregulates pericyte markers NG2 and CD13 and adopts a mesodermal phenotype indicated by upregulation of the stromal markers fibronectin, alpha-SMA, and CD105 (Fernandez-Klett et al., 2013). While increased numbers of circulating bone marrow-derived cells have been identified in humans following stroke (Jung et al., 2011), a local origin for the reactive pericytes had been established using chimeric bone marrow (Fernandez-Klett et al., 2013). In a recent report, Paul et al. (2012), using both pericyte and MSC markers, isolated cells that could exhibit both neural and MSC lineage potential under specific culture conditions. These isolated human pericytes also expressed the NSC marker nestin and expressed factors typical of a glutamatergic identity upon neuronal differentiation (Paul et al., 2012). Taken together, these studies suggest that CNS pericytes may have broad lineage potential under certain circumstances.

The reported potential of brain pericytes suggests a link with similar reports identifying cells expressing nestin. Multipotent neurospheres could be derived from ischemic lesion core tissue that expressed both nestin and PDGFR-beta along with expression of multiple neural crest genes (Nakagomi et al., 2009). A number of more recent studies reported a progenitor response, mainly nestin expression, that appeared to initiate at the cortical surface and radiate into deeper cortical layers (Bifari et al., 2009; Nakagomi et al., 2009; Sirko et al., 2009; Xue et al., 2009; Ohira et al., 2010; Nakagomi et al., 2011). The initial location of the reactive perivascular cells prior to injury is in the larger vessels, which are located closer to the cortical surface and which also exist throughout pial vessels. The pia mater is also of

neural crest origin (Ilaria Decimo, 2012) and microdissection and culture of pial cells from either naïve postnatal (Bifari et al., 2009) or adult ischemic cortex (Nakagomi et al., 2011) demonstrated multipotency. These data may point to a potential progenitor niche at the cortical surface, which would have unique access for activation by therapeutic factors added to the bathing CSF.

Oligodendrocyte Progenitor Cells

Oligodendrocyte progenitor cells (OPCs) comprise 5–8% of all cells in the rodent brain and represent the majority of proliferating cells, including those in the cerebral cortex. They are routinely identified by the Neuron-Glia 2 (NG2) antigen and thus are also known as NG2 cells. OPCs are also identified by PDGFR-alpha (Nishiyama et al., 2009; Trotter et al., 2010). Cortical OPCs are spatially oriented in a non-overlapping lattice or grid-like distribution that is dynamically maintained by growth and repulsion (Hughes et al., 2013). Similar characteristics of distribution and proliferation are seen in the human cortex (Geha et al., 2010). OPCs are a primary responder to injury where they undergo rapid proliferation (Simon et al., 2011) and migration at the lesion site (Sirko et al., 2013). OPCs have been shown to receive synaptic communication from neurons (Bergles et al., 2000) that is believed to signal myelinogenesis, as synaptic activation has been shown to effect the proliferation, migration, and differentiation of OPCs (Mangin and Gallo, 2011). Identifying further functional roles for cortical OPCs remains an area of investigation.

The concept that adult OPCs could be multipotent has been highly controversial (Kondo, 2000; Richardson et al., 2011). Multipotent neurospheres were generated from early postnatal CNP-reporter mice (Belachew et al., 2003), yet a lack of neuronal potential was observed following isolation of NG2 reporter cells (Zhu et al., 2008). In general, early postnatal cells can retain substantial developmental plasticity (Laywell et al., 2000; Zhu et al., 2011), but in contrast to these reports, derivation of adult OPCs resulted in the absence of neurosphere formation from FACS-sorted cells labeling for either NG2 or PDGFR-alpha (Buffo et al., 2008). There have also been reports of neuronal potential of OPCs in vivo (Dayer et al., 2005; Rivers et al., 2008; Guo et al., 2010). However, these findings were not replicated in an independent genetic lineage study that failed to detect any reporter-labeled neurons (Kang et al., 2010), raising the possibility of leaky control of the reporter gene in the previous studies. Recent studies further support the restriction of postnatal OPCs to an oligodendrocyte lineage in vivo (Clarke et al., 2012; Zhu et al., 2012). The preponderance of evidence suggests that adult cortical OPCs are not multipotent under standard conditions, but their potential warrants further investigation.

Directly adjacent to the cortex, a substantial population of OPCs is found in the subcortical white matter (SCWM). Acute isolation of cells isolated by the early OPC marker A2B5 from adult human SCWM followed by immediate transplantation into fetal rat brains resulted in differentiation into all three neuronal lineages (Nunes et al., 2003). The SCWM region of the human brain is extensive (Figure 1); should multipotent progenitors be confirmed in the SCWM, they would provide a spatially advantageous source for recruitment for cortical repair.

Local Response to Human Injury

Although limited, the human evidence also supports a local progenitor response to injury. Multiple immature neuronal markers were identified in the ischemic penumbra of the adult human cortex from five to 42 days post-injury. Proliferating doublecortin-expressing cells were identified in close association with blood vessels (Jin et al., 2006). Resected cortical tissue from patients undergoing surgical intervention for traumatic brain injury (TBI) also found evidence for progenitor markers, including doublecortin (Zheng et al., 2013). Multiple markers were used in both studies to verify proliferation and co-expression of progenitor markers. The detection of these cells in the cortex by five days following ischemia and within hours of TBI likely excludes cell migration from the distant SVZ, which had not shown any neuroblast migration from the SVZ by 23 days post-ischemia (Martí-Fàbregas et al., 2010).

Engineering Cell Fate

Adult tissue stem cell populations exist throughout the body and provide for cellular turnover for organs more susceptible to environmental wear such as the skin, intestine, or liver. In contrast, neurons are generally designed to persist throughout the life of the organism with no expectation for turnover. The primary response to injury in mammals has become one of containment through scarring. The response to extensive injury in the adult CNS is also that of scar formation with the only consistent reports of ectopic neurogenesis being in models of discrete neuronal death where the surrounding tissue architecture remains intact, as discussed above. What may be perceived as a failed attempt at repair may actually be an evolved mechanism for containment of the lesion, as blocking these mechanisms leads to increased lesion size (Sofroniew and Vinters, 2010; Robel et al., 2011). In support of this view, despite the increased plasticity of reactive astrocytes, they essentially remain intrinsically predisposed to the astrocyte lineage (Shimada et al., 2012), suggesting the dedifferentiation process may solely serve to generate sufficient numbers of glial scar-forming astrocytes.

As discussed above, there may be proliferative and migratory responses to injury in the adult CNS, but a final mature neuronal fate is rarely achieved from the various progenitor cell populations. This suggests that specification of cell fate is the limiting factor in generation of new neurons in non-neurogenic regions of the brain. Recent advances in cellular reprogramming provide new tools for re-specification of cell fate. The introduction of select transcription factors can convert a post-mitotic, adult fibroblast to an embryonic-like stem cell (Takahashi and Yamanaka, 2006) or directly into a functional neuron (Vierbuchen et al., 2010). Conversion has also been achieved in adult human fibroblasts to both iPSCs (Takahashi et al., 2007) and directly to functional neurons (Ambasudhan et al., 2011; Caiazzo et al., 2011; Yoo et al., 2011). An important proof of principle came with the direct conversion of exocrine cells to insulin-producing beta cells via in vivo gene delivery to the adult pancreas (Zhou et al., 2008). A similar approach was used to target proliferating, scar-forming fibroblasts following myocardial infarction resulting in their direct conversion to functionally integrated cardiomyocytes (Qian et al., 2012; Song et al., 2012). These

advances in engineering cell fate provide an alternative avenue for brain repair through direct, in vivo, lineage specification.

Direct Reprogramming to Subtype-Specific Neurons

There is a large diversity of neuronal subtypes in the adult CNS (Molyneaux et al., 2007) and the controlled generation of subtype-specific neurons will be required for effective repair. The direct conversion of mouse fibroblasts into functional glutamatergic and inhibitory neurons, termed induced neurons, has been achieved with a combination of three factors (Brn2, Ascl2 (also known as Mash1), and Myt1l, collectively known as the BAM factors) and without requiring passage through an intermediate pluripotent state (Vierbuchen et al., 2010). In the developing CNS, expression of transcription factors defines a code that specifies neuronal subtype identity (Reviewed in Guillemot et al., 2006; Molyneaux et al., 2007; Rouaux et al., 2012; Kelsom and Lu, 2013; Kepecs and Fishell, 2014). While generation of various neuronal subtypes derived from pluripotent cells has been achieved previously through presentation of environmental cues as seen in development, the successful manipulation of intrinsic factors to direct or re-specify cell fate could bypass the need to recapitulate, in its entirety, the signaling complexity seen in development, thus providing a powerful therapeutic tool to convert resident progenitor cells into needed mature cell phenotypes (Rouaux et al., 2012; Amamoto and Arlotta, 2014). Defining the effective transcriptional code for directing neuronal subtype specification will need to be elucidated in vitro and subsequently validated in vivo to develop neuronal conversion as a therapy. Progress has been made in vitro with sets of transcription factors directing differentiation of human pluripotent cells into glutamatergic (Zhang et al., 2013) and GABAergic (Au et al., 2013) subtypes or transdifferentiation of human fibroblasts into dopaminergic (Caiazzo et al., 2011) and cholinergic motor neurons (Son et al., 2011).

Importantly, unique transcription factor sets also appear to correlate with positional identity within the layers of the neocortex (Molyneaux et al., 2007). For example, a single factor, fezf2, could re-specify committed, post-mitotic striatal projection neurons in the developing embryo into corticofugal projection neurons that sent projections into appropriate cerebral peduncle and thalamic targets (Rouaux and Arlotta, 2010). Fezf2 was also sufficient to re-specify embryonic, Layer 2/3 corticocallosal neurons to a corticofugal phenotype (Rouaux and Arlotta, 2013). Decreased conversion efficiency was exhibited at P3 and lost at P21, indicating a limited temporal window of such plasticity, which may be terminated once a stable connection has been established with targets. It remains to be determined whether the addition of fezf2 to existing glutamatergic conversion cocktails (BAM) could induce neurons of a predominant corticospinal subtype. It would also be of great interest to evaluate if the delivery of fezf2 to different functional regions of the developing cortex resulted in maintenance or alteration of positional identity and projection to the appropriate functional target for that cortical region.

Lineage Specification of Local Cortical Neural Cells

Rather than converting cells to a distant lineage, such as fibroblasts to neurons, transdifferentiation within a similar developmental lineage should provide a more efficient, robust, and reliable level of re-specification with less genetic manipulation. This is

supported by the direct conversion of glia to functional neurons with only a single transcription factor (Heinrich et al., 2010; Guo et al., 2014). The complexity of neuronal physiology may also be more susceptible to epigenetic remnants from previous cell identity, which may be more problematic in direct conversion without the epigenetic reset of a pluripotent intermediate (Kim et al., 2010; Marro et al., 2011) or cell passaging (Hack et al., 2004; Heinrich et al., 2010). Emerging evidence suggests that glial populations may retain identity linked to their developmental origin that could influence subtype specification upon conversion (Heinrich et al., 2010).

We previously identified neural cell populations that responded to injury, but did not complete neuronal differentiation. We had suggested that it would likely be necessary to direct their fate. Below we will summarize progress in conversion of astrocytes, pericytes, and oligodendrocyte progenitor cells (OPCs) into subtype-specific neurons (Table 2).

Conversion of Astrocytes

The predominance of astrocytes and their role in reactive gliosis make them an attractive target for neuronal conversion. The identification of radial astroglia as the putative NSC in both development and adulthood prompted initial investigation into the neurogenic potential of parenchymal astrocytes. Pax6 had been shown to be an important neurogenic determinant in cortical radial astroglia and forced re-expression of this factor in postnatal astrocytes was the first demonstration of transdifferentiation to a neuronal fate (Heins et al., 2002). Following delivery of single neurogenic determinants, Mash1, Pax6, or Neurogenin2 (Ngn2), cultured postnatal astrocytes acquired functional electrophysiological properties, yet failed to form a pre-synaptic compartment (Berninger et al., 2007). However, delivery of Ngn2 with an improved retroviral construct for stronger and persistent gene expression led to the induction of glutamatergic neurons with functional synapses (Heinrich et al., 2010).

The conversion efficiency of postnatal astrocytes to glutamatergic neurons with the single factor, Ngn2 was significantly higher than that of embryonic fibroblasts with the three BAM factors (Vierbuchen et al., 2010) and is likely due to an intrinsic predisposition of cortical astrocytes to the glutamatergic subtype. As another example, three factors (Mash1 with Lmx1B and Nurr1) were required to convert postnatal astrocytes into functional midbrain dopaminergic neurons (Addis et al., 2011). Regional identity and lineage relationship appear to be important for conversion efficiency. Glutamatergic neurons of the rodent cortex are produced in the embryonic dorsal telencephalon (Molyneaux et al., 2007), while inhibitory neurons are generated in the ganglionic eminences of the ventral telencephalon and migrate tangentially to the cortex (Kelsom and Lu, 2013). Pax6 and downstream target Ngn2 are restricted to dorsal progenitors and indispensable for glutamatergic identity, whereas similar specification roles are found in the ventral telencephalon for Mash1 and target Dlx2 (Guillemot et al., 2006). In regard to lineage relationship, cortical astrogliogenesis has been shown to proceed following neurogenesis from a shared dorsal precursor in a similar deep to superficial pattern during cortical development (Magavi et al., 2012; Tsai et al., 2012). Conversion of cultured astrocytes to inhibitory neurons was far less efficient with single factor delivery of Dlx2, although some induced interneurons could form autaptic synapses. Either combined delivery of Mash1 and Dlx2 or delivery of Dlx2 alone following passaging

under neurosphere conditions could overcome the developmental bias and resulted in neuronal induction in nearly all transduced cells (Heinrich et al., 2010). Despite the high induction efficiency and mature membrane properties displayed with the combination of Dlx2 and Mash1, no autaptic synapses were identified. However, neurosphere conditions prior to Dlx2 delivery resulted in a five-fold increase in functional autaptic synapses and was likely due to the fact that neurosphere conditions partially erase regional identity, as previously shown in embryonic progenitors (Hack et al., 2004). As cells in the brain will retain their regional identity, in vitro conversion studies under neurosphere conditions may not be predictive of conversion success in vivo.

Conversion of Pericytes

Evidence for multipotency in pericytes (Dore-Duffy et al., 2006; Paul et al., 2012) and recent findings of their ability to undergo proliferation, migrate, and persist in the ischemic core (Nakagomi et al., 2009; Fernandez-Klett et al., 2013) suggests that activated pericytes may be a suitable population to target for neuronal replacement (Özen et al., 2012; Karow, 2013). However, another study did not find neuronal differentiation under standard culture conditions (Karow et al., 2012), which may be explained either by the use of neurosphere conditions or the addition of SHH in previous studies (Dore-Duffy et al., 2006; Paul et al., 2012). SHH has been shown to invoke stem cell potential in reactive astrocytes (Sirko et al., 2013) and has been suggested to be necessary for neural lineage induction in pericytes (Dore-Duffy, 2008).

Despite the lack of neuronal differentiation of pericytes under standard culture conditions, combined delivery of Mash1 and Sox2 produced a GABAergic phenotype with some functional properties (see Table 1; Karow et al., 2012). Mash1 alone was able to downregulate pericytes markers and led to expression of B-III-tubulin. This underscores the powerful role of Mash1 as a principle factor in establishing general neuronal potency. Mash1 is normally associated with interneuron development in the ventral telencephalon (Roybon et al., 2010), which is congruent with the findings in this study, but was also identified as the core factor in the reprogramming of fibroblasts (Wapinski et al., 2013) to glutamatergic neurons (Vierbuchen et al., 2010), dopaminergic neurons (Caiazzo et al., 2011), and cholinergic motor neurons (Son et al., 2011).

Conversion of Oligodendrocyte Progenitor Cells (OPCs)

OPCs represent the only established actively proliferating progenitor population that is widespread throughout the non-neurogenic, uninjured adult brain and OPCs possess distinct advantages as a candidate for neuronal replacement. In vivo imaging has demonstrated that local replacement occurs once a local OPC is induced to migrate following a small, focal laser lesion, essentially maintaining a distributed homeostatic population (Hughes et al., 2013). This homeostatic regulation may be an advantage for potential therapies converting OPCs to neurons. Homeostasis would return cell numbers to normal if OPCs are induced to proliferate or replace OPCs that were recruited for neuronal replacement. OPC synapses may have yet unidentified functional roles aside from signaling a myelinogenic response. The fact that OPCs maintain their synaptic connections while undergoing proliferation

(Mangin and Gallo, 2011), suggests that these cells could be recruited with minimal disruption to normal physiology.

It is reasonable to postulate from the above conversion studies on astrocytes that a developmental bias may be found for OPCs, as well. Cortical OPCs are generated in three waves during development (Kessaris et al., 2006; Richardson et al., 2006b; Rowitch and Kriegstein, 2010). The first two waves arise ventrally from the medial (MGE) and lateral/caudal (L/CGE) ganglionic eminences, respectively, during the embryonic period and a dorsal source emerges following birth. Interestingly, the first wave originating from the MGE is virtually eradicated within the first few postnatal weeks so that by adulthood, a roughly equal mix of ventral- and dorsal-derived OPCs exist in cortex (Kessaris et al., 2006).

Direct In Vivo Conversion in the Adult Brain

A series of recent reports have demonstrated the in vivo conversion of resident glia into neurons in the adult brain (Table 2). The neuronal induction of resident striatal astrocytes through expression of the BAM factors provided proof of principle for such an approach (Torper et al., 2013). A cocktail of CRE-inducible lentiviral vectors carrying the BAM factors were delivered to the striatum of GFAP-Cre mice. Transduced cells displayed neuronal morphology and expressed NeuN six weeks following injection. Despite a lack of functional data, these findings demonstrated that intrinsic factors could overcome the non-neurogenic environment and induce neuronal fate.

In vivo conversion has also been investigated in the context of injury (Buffo et al., 2005; Grande et al., 2013; Guo et al., 2014). In what could be considered the first report of in vivo reprogramming in the adult brain, retroviral gene delivery of either Pax6 or a dominant negative form of Olig2 (Olig2VP16) following a cortical stab wound generated an immature neuronal (DCX) phenotype, but most transduced cells failed to mature and survive by one month (Buffo et al., 2005). This failure to fully mature may not be due solely to the non-neurogenic environment of the cortex, as delivery of Olig2VP16 to the neurogenic dentate gyrus resulted in the retention of transduced cells expressing DCX and the reduction of mature neurons expressing NeuN (Klempin et al., 2012). Olig2 is expressed throughout the oligodendrocyte lineage, but is also found in reactive astrocytes and multipotent progenitors. Recently, it has been demonstrated that Olig2 is expressed in an oscillatory pattern in NPCs and sustained expression results in differentiation to the oligodendrocyte lineage (Imayoshi et al., 2013). Selective knockout of Olig2 in NG2 cells following stab wound in the adult cortex using a triple transgenic mouse model failed to generate a neuronal phenotype (Komitova et al., 2011), suggesting reactive astrocytes as the source of the immature neuronal phenotype described above (Buffo et al., 2005). Olig2 is necessary to induce an OPC fate (Ligon et al., 2006), but may not be necessary to maintain it (Komitova et al., 2011).

A more recent study co-delivered EGF/FGF with retroviral Pax6, Mash1, or Ngn2 during a cortical stab wound lesion (Grande et al., 2013). Co-administration of growth factors may have a dedifferentiating effect similar to that seen in neurosphere cultures of reactive astrocytes that renders them more susceptible to neurogenic fate determinants (Heinrich et

al., 2010). Growth factor delivery appeared to facilitate the neuronal differentiation of Ngn2 transduced cells as indicated by a large increase in the percentage of DCX expressing reporter cells, yet less than one percent of these cells expressed NeuN at later time points. The addition of growth factors with Pax6 induced a neuronal phenotype in a negligible number of cells with an absence of any such effect with Mash1.

In contrast to the cortex, delivery of Ngn2 to the striatum resulted in a lower efficiency of neuronal induction, but nearly half proceeded to express NeuN, suggesting the striatal environment is more conducive to maturation and survival (Grande et al., 2013). Delivery of a retrograde tracer to the globus pallidus co-labeled with a small number of GFP-labeled cell bodies in the striatum, suggesting appropriate axon targeting. These findings require further validation as some mature MSNs could also be found labeled with delivery of the control vector. The induced striatal neurons expressed GABA, but lacked expression of MSN marker Darp32. The conversion of resident striatal cells to mature neurons with Ngn2 is of interest, as it is not involved with normal striatal development. This may be explained by a report where exchanging the coding sequence of Mash1 with Ngn2 during development resulted in normal ventral telencephalic development (Parras et al., 2002). However, these compensatory mechanisms would be restricted in the adult brain and likely account for the lack of complete MSN reprogramming (Grande et al., 2013).

Just recently, cortical slice recordings established the direct conversion of resident glia to functional neurons in the injured, adult neocortex following retroviral delivery of NeuroD1 (Guo et al., 2014). Cell specific promoters were utilized to determine the specific effect of NeuroD1 on reactive astrocytes or OPCs. The targeting of reactive astrocytes resulted in greater than ninety percent conversion to a mature neuronal phenotype after one week. This robust efficiency of induction relative to the previous injury studies may be attributed to several factors. The previous studies used a vector construct with transgene expression driven by the weaker LTR promoter (Buffo et al., 2005; Grande et al., 2013). In vitro studies had shown that full reprogramming required the use of the persistent and higher expressing CAG vector (Heinrich et al., 2010), which was used in the current study. The cortex is particularly non-permissive to neuronal maturation as indicated by the transient existence of doublecortin cells from either SVZ migration or delivery of neurogenic determinants following injury and their general failure to persist as a mature phenotype. NeuroD1 is a downstream target of Ngn2 and is expressed during the terminal differentiation of embryonic dorsal progenitors into glutamatergic cortical neurons (Guillemot et al., 2006; Osorio et al., 2010). Ngn2 may not induce NeuroD1 expression in all transduced cells and this may partly be due to non-permissive or inhibitory factors in the cortical environment. However, once activated, NeuroD1 may effectively bypass such inhibition and autonomously carry the cell to terminal differentiation.

The competency of astrocytes to respond to the terminal differentiation effect of NeuroD1 may be explained by the predisposition of astrocytes to a glutamatergic identity. This may mean that cells with a ventral telencephalic origin may have limited competency to respond to NeuroD1, as it does not contribute to normal GABAergic differentiation. For example, selective expression of Neurod1 in NG2 cells induced only half of the reporter labeled cells, which coincides with the respective contribution of the ventral and dorsal waves of OPCs

during development discussed earlier (Kessaris et al., 2006). It is possible that a subset of these cells may also be NG2-expressing pericytes. Transduction of cultured NG2 cells with NeuroD1 resulted in a small number of interneurons (Guo et al., 2014). The presence of interneurons in these cultures may arise from SVZ-derived OPCs that migrate to cortex (Menn et al., 2006), as NeuroD1 is involved in the terminal differentiation of subsets of interneurons in the OB (Roybon et al., 2009).

An alternative approach to directing a terminal neuronal fate is to generate proliferating neuroblasts followed by maturation cues. Ectopic expression of Sox2 in striatal astrocytes resulted in induction of doublecortin expressing neuroblasts that remained proliferative for four weeks without tumor formation, but rarely became mature neurons, similar to native neuroblasts in ectopic regions following injury (Niu et al., 2013). However, co-delivery of BDNF/Noggin or valproic acid enabled the terminal maturation of neurons of a MSN phenotype. Striatal slice recordings found that the majority of these neurons exhibited electrophysiological features suggestive of local network integration. Mature cells contained dendrites with a spiny morphology, but the expression of MSN associated proteins, such as DARPP32, was not evaluated. While these results are promising, they may not predict similar success in the cortex, as the striatum has been shown more conducive to maturation and survival (Grande et al., 2013).

Outlook for Repair

The above studies demonstrate that resident neural cells can be directly converted to functional neurons in vivo (Table 2). Successful translation of this novel approach will require the specific, regional targeting of a desired cell population with highly efficient reprogramming factors for the generation of clinically relevant numbers of subtype-specific neurons. The cellular response to injury/disease and the developmental bias towards a particular neuronal subtype will determine the ideal cell population to target for neuronal conversion. The contribution of the targeted cell population to normal physiology or as a response to injury and its subsequent loss through conversion must also be considered. For example, astrocytes serve essential roles following injury, such as uptake of glutamate to prevent excitotoxicity and the gliotic scar limits the extent of damage from injury or infection (Sofroniew and Vinters, 2010). Thus, redirecting astrocytes to neuronal conversion may have implications for their contribution to normal homeostatic and injury response function. We now discuss selection of appropriate neural cells and potential repair strategies for their direct conversion in the context of neurological injury or disease. A significant advantage of manipulating endogenous cells for repair is the potential for minimally invasive intervention. We conclude with a discussion of some emerging delivery options.

Selection of Appropriate Target Cell Population for Conversion

Given the diversity of neurons, therapeutic conversion of local neural cells to neurons will require a strategy to achieve the needed neuronal subtype, for example, replacing projection neurons as opposed to interneurons. Replacement of projection neurons is an imposing challenge, as restored function requires axonal growth through the mature brain to appropriate long distance targets. There is evidence that axonal growth and targeting is intrinsically defined and that guidance cues may persist into adulthood (Gaillard et al., 2007;

Gaspard et al., 2008; Espuny-Camacho et al., 2013). There is also an emerging appreciation of regional heterogeneity in astrocytes (Emsley and Macklis, 2006), suggesting it is possible that resident cortical astrocytes retain even further specification, such as position and layer identity. If astrocytes retain this identity as they are specified into neurons, this could enhance their value for replacement of projection neurons. The regional specification of neuronal subtype during cortical development is determined by the positional identity of the ventricular radial glia stem cell, which is patterned by morphogen gradients across the ventricular axes (Kriegstein and Alvarez-Buylla, 2009). The layers of the cortex are then generated radially through an intrinsic temporal mechanism in a deep to superficial sequence (Gaspard et al., 2008; Espuny-Camacho et al., 2013). Two recent reports have established that cortical astrocytes and neurons share a common progenitor and maintain strict positional identity within a radial column (Magavi et al., 2012; Tsai et al., 2012). Even following injury, reactive astrocytes do not migrate from the radial column into adjacent damaged areas (Tsai et al., 2012; Bardehle et al., 2013).

Conversely, the glutamatergic bias of cortical astrocytes may not make them the ideal cell source for the generation of interneurons (Heinrich et al., 2010). Cortical OPCs contain a mixed population of dorsal- and ventral-derived precursors (Kessaris et al., 2006). Ventral-derived OPCs may exhibit high conversion efficiencies if introduced to the appropriate interneuron fate determinants. As an active cycling progenitor population capable of migration, OPCs may retain less developmental memory than resident astrocytes and be a better choice for conversion to cell types of non-telencephalic origin, such as serotonergic or dopaminergic neurons. OPCs are also the primary proliferatively active progenitors in the uninjured brain, as astrocytes require injury to invoke their reactive progenitor potential, making OPCs better suited for targeting neurological disorders other than traumatic or ischemic injuries that may lack a substantial reactive component.

Considerations for Therapeutic Use of Direct In Vivo Neuronal Conversion

Severe traumatic or ischemic brain injury results in a necrotic cavity void of neural tissue and any behavioral improvements are attributed to preservation and synaptic remodeling of the uncompromised surrounding tissue. A subset of pericytes has recently been shown to preferentially migrate, proliferate, and survive in the ischemic core where they contribute to the fibrotic portion of the lesion scar (Paul et al., 2012; Fernandez-Klett et al., 2013) and provide a potential target to extend regeneration into the ischemic core. This response of reactive pericytes may provide an opportunity to engineer a reconstruction of the tissue lost in the ischemic core. For example, targeting reactive pericytes to express exogenous growth factors may facilitate cellular growth into the core. Rebuilding post-injury circuitry in a lesion cavity may also benefit from a bioengineering approach using biomaterial gel. These gels can be coated with substrates for cell adhesion, such as laminin, or with nanospheres filled with various factors for sustained or temporally-defined release (Tam et al., 2014). Following ischemic injury, the blood vessels realign toward the ischemic lesion (Ohab et al., 2006) and the delivery of a silicone gel with VEGF nanospheres into the ischemic core resulted in infiltration of these vessels into the engineered matrix (Zhang et al., 2007). Therapeutic bioengineering offers numerous opportunities for controlled manipulation of the

environment and will likely benefit from a combination of both cell transplantation and endogenous recruitment approaches for such severe injuries.

Direct neuronal conversion of local neural cells may have a therapeutic advantage over ex vivo cell transplantation in cases of diffuse injury, such as mild TBI or neurodegenerative disease, where conversion factors could be delivered over large areas. Mild TBI has been gaining significant attention recently for its increasing role in neurological impairments among soldiers and athletes in contact sports. The pathological mechanisms generating downstream neurological impairment are not well defined, but diffuse TBI may produce scattered micro-islands of gliotic lesions throughout the zone of impact (Burda and Sofroniew, 2014). The utility of neuronal replacement in neurodegenerative disease is dependent on the diversity of neuronal subtypes affected and the extent to which disease progression is an intrinsic property of the afflicted neurons. Although endogenous cells share identical genetics, a newborn neuron may effectively reset the disease pathology progression clock. This concept is supported by decreased levels of mutant protein detected in newly generated neurons compared to existing striatal neurons in a model of Huntington's disease (Benraiss et al., 2013). Environmental toxicity should also be considered in cell replacement therapy for neurodegenerative diseases. Although neuronal loss in amyotrophic lateral sclerosis (ALS) is selective for motor neurons, there is accumulating evidence that astrocytes contribute to neuronal toxicity, thus replacement neurons will be susceptible to similar insult (Haidet-Phillips et al., 2011; Meyer et al., 2014; Re et al., 2014). Cell therapy for Alzheimer's disease will require the replacement of multiple subtypes. In this case, the retention of positional identity in resident astrocytes may allow diffuse delivery of a generic glutamatergic factor, such as NeuroD1, to direct subtype-specific conversion. Retroviral delivery of NeuroD1 was also shown to generate functional neurons in an aged model of Alzheimer's disease, demonstrating feasibility of this approach in a hostile diseased environment (Guo et al., 2014).

In addition to replacing lost neurons, in vivo neuronal conversion may have a role for adding new neurons to therapeutically modulate neural circuitry. Transplantation studies have demonstrated that the addition of inhibitory neurons to various CNS regions (reviewed by (Tyson and Anderson, 2014) can alleviate symptoms in disorders such as epilepsy (Hunt et al., 2013), neuropathic pain (Braz et al., 2012), and schizophrenia (Tanaka et al., 2011). A current limitation for direct conversion in vivo is whether a clinically relevant number of neurons can be produced. The generation of GABAergic neurons may be particularly suited for this approach, as minimal numbers may exert a powerful inhibitory tone. For example, activation of the parvalbumin interneuron population, that comprises less than five percent of hippocampal neurons, alleviated epileptiform activity (Krook-Magnuson et al., 2013). Serotonergic neurons have been directly differentiated from mouse ESCs with transcription factors (Nefzger et al., 2011) and achieving direct conversion of local neural cells into serotonergic neurons may provide improvement of the non-motor symptoms of Parkinson's disease (Politis et al., 2012). Replication of these GABAergic transplant studies may serve as an ideal starting point to evaluate the clinical potential of in vivo conversion.

Targeted delivery of reprogramming factors

The population chosen for recruitment requires cell-specific and regional targeting for efficiency and safety. Delivery of conversion factors should ideally be non-invasive. However, the CNS is well protected, making delivery a challenge. The tightly adhered endothelial cells of the blood brain barrier (BBB) present a well-known hurdle to vascular drug delivery. The superficial aspect of the cortex helps make parenchymal delivery of conversion factors less invasive. However, intracranial access is still required, presenting an undesired level of invasiveness, particularly when widespread regions must be targeted.

Current experimental approaches using viral delivery offer the best starting approach for developing therapeutic delivery. Adeno-associated viral (AAV) vectors have been established as a safe means of gene delivery in numerous clinical trials. AAV vectors display low immunogenicity. In addition, AAV vectors do not integrate into the host genome and thus avoid the risk of insertional mutagenesis. Numerous serotypes have been developed that offer the ability to preferentially target specific cell populations with a defined distribution (Ojala et al., 2014). Further specification can be achieved with the use of a cell-specific promoter for gene expression. AAV is limited by the size of gene inserts, but neuronal conversion may be achieved with only a few factors. The use of a polycistronic vector, such as 2a peptide linkers, can increase efficiency if multiple genes are required (Szymczak et al., 2004). Importantly, systemic delivery of AAV9 to the adult mouse tail vein led to transduction of astrocytes throughout brain and spinal cord (Foust et al., 2009), suggesting that viral vectors may be able to circumvent the requirement for intracerebral injection.

Minimally invasive delivery of conversion factors may also be achieved utilizing recent advances in nanodrug delivery systems (Wong et al., 2012; Masserini, 2013). As stable cellular reprogramming can be achieved with only a transient introduction of the appropriate transcription factors, sustained transgene expression could be replaced by peptide (Kim et al., 2009) or small molecule (Hou et al., 2013) delivery. Systemic delivery of nanoparticles in combination with focused ultrasound could create a safe and transient opening in the BBB for localized drug delivery (O'Reilly and Hynynen, 2012; Aryal et al., 2014). Nanoparticles could be used to transport therapeutics to the leaky BBB at the injury site (Lin et al., 2012). All of these drug carriers can be functionalized with specific antibodies or receptor ligands for cell specific targeting (Alexander-Bryant et al., 2013; Sanna et al., 2014). The PDGFR-beta receptor has already been used in the clinic to target pericytes in non-neural tissue and deliver cancer therapeutics (Bansal et al., 2012).

Conclusions

Direct in vivo neuronal conversion offers a new avenue for restorative therapies to treat neurological injury and disease. Recent progress has identified potential source cells for conversion and proof of principle that this can be achieved in vivo. Investigation now needs to center on the specificity of neuronal subtypes, the efficiency of their generation, the expansion of the population to clinically relevant numbers, and demonstration of the functional integration and contribution of new neurons generated through direct in vivo

neuronal conversion. Advances in delivery systems will also be needed to enable minimally invasive therapeutic delivery of conversion factors.

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Figure 1. Calibrated comparison of brain size from mouse to human

(A) Coronal cross sections through mouse, rat, rhesus monkey, and human illustrate their significant scaling differences and underscores the differences in migratory distance that SVZ-derived neural progenitors would be required to travel to replace lost cortical cells between animal models and human subjects. (B) The relative ratio of white matter to gray matter is very low in the mouse brain, whereas the abundance of white matter is greater than grey matter in the human brain (Panel A). The expansion of white matter in the human brain is primarily responsible for the increased challenge of migratory replacement for human subjects. Images of rhesus and human brain are from the MSU Brain Biodiversity Bank (<https://www.msu.edu/~brains/copyright.html>; National Science Foundation) and images for mouse and rat brain are from [Brain Maps.org](http://BrainMaps.org) (Mikula et al., 2007).

Table 1

In Vitro Neuronal Induction of Neocortical-Derived Cell

Cell Population	Reference	Cell Origin	Pre-Derivation Injury	Factors Delivered	Phenotypic Outcome	Comments
Astrocytes	Laywell et al., 2000	Embryonic to P10	Naïve		B-III-tubulin/MAP2 expression	Culture initiated under neurosphere (NS) conditions
	Buffo et al., 2008	Adult	Stab Wound		B-III-tubulin expression	Culture initiated under NS conditions
	Shimada et al., 2012	Adult	Focal Ischemia		B-III-tubulin expression	Culture initiated under NS conditions; Engraftment resulted in no neuronal differentiation
	Heins et al., 2002	Postnatal	Naïve	Pax6	B-III-tubulin expression	
	Berninger et al., 2007	Postnatal	Naïve	Ngn2 or Mash1 or Pax6	B-III-tubulin expression; spontaneous action potentials (AP) with embryonic neuron co-culture	
	Heinrich et al., 2010	Postnatal	Naïve	Ngn2	B-III-tubulin (70% induction), MAP2; glutamatergic markers: Tbr1, vGLUT1, CaMKIIa; functional autaptic glutamatergic synapses (58% induction)	Study utilized the stronger pCAG promoter
	Heinrich et al., 2010	Postnatal	Naïve	Dlx2	B-III-tubulin/MAP2 (36% induction); GABAergic Markers: GAD67, vGAT, calretinin; majority displayed immature firing patterns, few functional autaptic synapses (6% induction)	NS conditions prior to transduction (40% autaptic synapses)
	Heinrich et al., 2010	Postnatal	Naïve	Co-delivery: Dlx2/Mash1	B-III-tubulin (93% induction); generally more mature interneuron firing patterns than Dlx2, but no autaptic synapses	
	Addis et al., 2011	Postnatal	Naïve	Co-delivery: Mash1/Lmx1B/Nurr1	B-III-tubulin (35% induction), synaptophysin dopaminergic Markers: TH (50% of B-III-tubulin), Grk2; functional dopaminergic phenotype (18%); rhythmic firing pattern, calcium oscillations	Performed transcriptional profile versus embryonic substantia nigra neurons
	Guo et al., 2014	Postnatal -Human	Naïve	NeuroD1	doublecortin (90% induction), MAP2, NeuN, SV2; glutamatergic markers: tbr1, vGluT1; functional autaptic synapses,	
Pericytes	Dore-Duffy, 2006	Adult	Naïve		Neurofilament expression	Initially cultured under NS conditions
	Nakagomi et al., 2009	Adult	Focal Ischemia		B-III-tubulin, MAP2, neurofilament expression; AP generation	Ischemic Core; Initially cultured under NS conditions;

Cell Population	Reference	Cell Origin	Pre-Derivation Injury	Factors Delivered	Phenotypic Outcome	Comments
						Differentiated with retinoic acid Differentiated with retinoic acid
	Nakagomi et al., 2011	Adult	Focal Ischemia		B-III-tubulin, MAP2, neurofilament expression	Ischemic pia mater; Initially cultured under NS conditions; Differentiated with retinoic acid
	Paul et al., 2012	Adult-Human	Naïve		B-III-tubulin, DCX, HUC/D, NSE, MAP2; glutamatergic gene expression: Pax6, NeuroD1, Tbr2, Tbr1; AP generation	CD13 ⁺ CD105 ⁺ CD45 ⁻ CD31 ⁻ populations; Differentiated with SHH & cAMP
	Karow et al., 2013	Adult-Human	Naïve	Co-delivery: Mash1/Sox2	B-III-tubulin (48% induction), MAP2, NeuN; immature firing patterns; embryonic mouse neuron co-culture: repetitive APs, GABA expression, post-synaptic compartment	19% overall B-III-tubulin induction (including 36% cell death)
OPCs	Nunes et al., 2003	Adult-Human SCWM	Naïve		B-III-tubulin, GAD67 expression; AP generation	Generated neurons and glia following fetal xenograft into rodent
	Guo et al., 2014	Postnatal	Naïve	NeuroD1	B-III-tubulin, NeuN (98%) expression; vGluT1 (90%); GAD65/67 (10%); repetitive AP, both GABA and glutamate evoked currents	

Table 2

Direct In Vivo Conversion of Local Neocortical Cells

Reference	Model-Injury	Vector-Factor Delivery	Recruited Cell Type	Phenotype Outcome	Comments
Buffo et al., 2005	Cortex-Stab Wound	RV-LTR-Olig2VP16	Undetermined Reactive Cells	Immature (DCX expression); Failure to mature/survive at one month	Weak LTR vector
	Cortex-Stab Wound	RV-LTR-Pax6	Undetermined Reactive Cells	Higher DCX induction than Olig2VP16	
Torper et al., 2013	Striatum-Injection	Lenti-BAM cocktail	Astrocytes	NeuN expression persists at 6 weeks	
Grande et al., 2013	Cortex – Stab Wound	RV-LTR-Ngn2 & EGF/FGF	Undetermined Reactive Cells	DCX (20%) at 7 days post injection (dpi), NeuN (<0.5%) at 14 dpi	Weak LTR vector; No functional studies
	Cortex – Stab Wound	RV-LTR-Mash1 or Pax6 & EGF/FGF	Undetermined Reactive Cells	Negligible neuronal conversion for Pax6; No detectable conversion for Mash1	
	Striatum-Stab Wound	RV-LTR-Ngn2 & EGF/FGF	Undetermined Reactive Cells	DCX (7%) at 7dpi, NeuN (4%) at 14dpi	Note: Control vector labeled neurons
Niu et al., 2013	Striatum-Injection	Lenti-hGFAP-Sox2	Astrocytes	Proliferating, doublecortin-expressing neuroblasts (DCX); rare NeuN expression	
	Striatum-Injection	Lenti-hGFAP-Sox2 & BDNF/Noggin	Astrocytes	Medium Spiny Neuron phenotype, Spontaneous APs in striatal slice recordings	Valproic acid also promotes neuronal differentiation
Guo et al., 2014	Cortex-Injection	RV-CAG-NeuroD1	Undetermined Reactive Cells	DCX, NeuN (~100% induction at 3 week post injection); Tbr1 expression; Spontaneous and evoked synaptic responses in cortical slice recordings	
	Cortex-Injection	RV-GFAP-NeuroD1	Astrocytes	B-III-tubulin, NeuN expression (93% induction at 8 dpi)	
	Cortex-Injection	RV-NG2-NeuroD1	OPCs (pericytes?)	B-III-tubulin, NeuN expression (42.5% induction at 8 dpi)	
	Cortex-Aged AD Mouse	RV-CAG-NeuroD1	Undetermined Reactive Cells	Spontaneous APs in cortical slice recordings	