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Interneurons from Embryonic Development to Cell-Based Therapy

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Abstract

Many neurologic and psychiatric disorders are marked by imbalances between neural excitation and inhibition. In the cerebral cortex, inhibition is mediated largely by GABAergic (γ-aminobutyric acid–secreting) interneurons, a cell type that originates in the embryonic ventral telencephalon and populates the cortex through long-distance tangential migration. Remarkably, when transplanted from embryos or in vitro culture preparations, immature interneurons disperse and integrate into host brain circuits, both in the cerebral cortex and in other regions of the central nervous system. These features make interneuron transplantation a powerful tool for the study of neurodevelopmental processes such as cell specification, cell death, and cortical plasticity. Moreover, interneuron transplantation provides a novel strategy for modifying neural circuits in rodent models of epilepsy, Parkinson's disease, mood disorders, and chronic pain.

New neurons are naturally added to the adult brains of many species, including humans (1–8). Adult mammalian neurogenesis, however, is largely restricted to the hippocampus and olfactory bulb, where it contributes to local neural circuit plasticity, not repair. Identification of transplantable cells that migrate and integrate into neural circuits in a manner similar to these adult-born neurons could be useful in nervous system therapy. Indeed, neuronal transplantation has a long history, but for the vast majority of cell types, the postnatal central nervous system has proven inhospitable to migration and neural circuit integration (9–11). Immature inhibitory interneurons from the embryonic ventral telencephalon, however, show a unique capacity to disperse and integrate into neural circuits of the postnatal central nervous system. In large part, this ability reflects their ontogeny: During brain development,

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ventral telencephalon-derived interneurons must migrate long distances, differentiate and survive in environments distinct from their origin, and functionally integrate into extant circuits composed of other cell types—all challenges faced by cells transplanted into the nervous system. Here, we summarize the development of telencephalic interneurons—in particular, inhibitory interneurons of the cerebral cortex—and describe their behavior after transplantation into the postnatal central nervous system. In addition, we discuss the potential of interneuron transplantation as a cell-based therapy for numerous conditions, including epilepsy, Parkinson's disease, psychiatric disorders, and chronic pain. Finally, we summarize efforts to derive forebrain interneuron precursors in vitro from pluripotent stem cells.

A Developmental Fate Realized in a Distant Time and Place

During embryonic development, molecularly, morphologically, and physiologically distinct subpopulations of interneurons originate in progenitor domains of the ventral telencephalon, including the medial and caudal ganglionic eminences (12–19). From these origins, immature interneurons undergo a remarkable process of long-distance migration to many structures of the developing telencephalon, including the cerebral cortex, where they form neural circuits with locally produced excitatory neurons (19–22). In contrast to cortical excitatory neurons, which form connections onto distant cells within and outside of the cortex, cortical interneurons form inhibitory GABAergic (γ-aminobutyric acid–secreting) connections onto local neurons and establish gap junction–mediated electrical networks with other interneurons (23). Because of this ontogeny, ventral telencephalic interneuron precursors are endowed with developmental programs that may confer an uncommon capacity to engraft into the nervous system after transplantation.

Initial studies of neural transplantation were marked by the limited dispersion of transplanted cells throughout host tissues (24–28). In contrast to cells from the embryonic neocortex, hypothalamus, thalamus, superior colliculus, rhombic lip, and spinal cord, which display minimal capacity to migrate when placed into in vitro explants, cells from the embryonic lateral ganglionic eminence [LGE; the major source of olfactory bulb interneurons (19)] and the medial ganglionic eminence (MGE) migrate substantial distances (29). Of these two populations, immature interneurons from the MGE exhibit greater migratory potential in vitro, with dispersal distances approximately two to three times those of LGE cells. When injected into the postnatal brain, MGE cells also exhibit substantial migratory capacity. Whereas embryonic cells from the LGE and dorsal forebrain remain mostly clustered at injection sites, MGE-derived interneurons disperse widely throughout developmentally distinct regions of the adult and neonatal central nervous systems, including the striatum (29, 30), hippocampus (31, 32), neocortex (29, 33), amygdala (32), thalamus (29), and spinal cord (34). Transplanted MGE interneurons migrate distances up to 2.5 mm in the adult rodent brain (30, 32) and 5 mm in the neonate (33).

Although the differentiation of cortical interneurons extends well into postnatal life (13, 35, 36), the fates of interneuron precursors are largely determined prior to their migrations out of the embryonic ganglionic eminences (37, 38). As such, when immature interneurons are heterochronically transplanted from the embryonic MGE into the postnatal cortex, they

produce the complement of interneuron subtypes normally produced by the MGE, as indicated by their expression of diverse interneuron morphologies, molecular markers, and electrophysiological phenotypes (32, 33, 39, 40). Microdissection experiments have shown that when cells from distinct subregions of the MGE are transplanted (for example, dorsal versus ventral MGE), the resultant populations vary in their compositions of neurochemically defined interneuron subtypes (38, 41). Although this strategy does not yield entirely distinct populations (for example, parvalbumin-expressing interneurons are present in both dorsal and ventral MGE transplants), it allows for the selection of populations biased toward particular types of interneurons. It remains unclear, in turn, whether various types of neurochemically defined cells target different structures and serve distinct functions after transplantation (42), yet this finding hints that distinct transplant populations could be selected for different disease pathologies.

Just as MGE interneurons migrate when transplanted outside the cortex, they are also able to differentiate into mature inhibitory interneurons in foreign regions of the central nervous system. For example, when heterotopically transplanted into the spinal cord, immature interneurons from the ventral telencephalon survive and eventually express some of the molecular, morphologic, and physiologic properties of cortical interneurons (34). Thus, interneuron transplantation is a method for adding specific and, depending on the target, foreign elements to neural circuits (Fig. 1, A to C). Together, these transplantation studies highlight the notion that the behaviors and phenotypes of cortical interneurons are largely determined by intrinsic developmental programs established during the early stages of their development in the embryo.

In the host brain, transplanted MGE-derived interneurons display electrophysiologic activity patterns typical of the various subtypes of physiologically defined interneurons (32, 33). Transplanted interneurons also exhibit spontaneous and evoked synaptic currents, indicating that they receive functional synaptic inputs from neurons of the host brain (30, 33, 40) (Fig. 1D). Transplantation increases the frequency of inhibitory events in projection neurons (34, 40, 42) (Fig. 1E), but curiously, these effects are largely insensitive to the number of cells transplanted; in the cerebral cortex, transplant-mediated inhibition reaches a plateau with transplant sizes just 5 to 10% of that which the cortex can support (40). Simultaneous electrode recordings of transplanted interneurons and host projection neurons have shown that transplanted interneurons make functional inhibitory synaptic connections onto host neurons (43), consistent with the possibility that transplanted interneurons modify inhibitory circuits in the host cortex by forming new inhibitory synapses. Electron microscopy also has revealed synaptic contacts between transplanted interneurons and neurons of the host cortex (39, 40), whereas transsynaptic tracer experiments have demonstrated the synaptic connectivity of interneurons transplanted into the spinal cord (34).

Transplanted cortical interneurons have a capacity to integrate synaptically into host brain circuits, where they appear to primarily target host excitatory neurons (39). It is unknown, however, whether transplanted interneurons form inhibitory synapses onto one another, nor whether they form gap junction–mediated electrical networks. Nonetheless, these studies have provided a strong rationale for the use of interneuron transplantation to study and experimentally modify inhibition in the host nervous system.

Interneuron Transplantation as a Window into Brain Development and Function

Interneuron transplantation permits the introduction of immature cells into foreign and developmentally distinct environments, allowing researchers to study how cell-autonomous and environmental factors determine the fates of developing neurons. For example, transplantation of MGE interneurons from mutant donors into wild-type hosts has illustrated the cell-autonomous role of the Dlx1 transcription factor in controlling the branching and survival of specific subsets of cortical interneurons. Interneuron transplantation has also proven useful for studying genes that, when mutated in all tissues, cause perinatal lethality. For example, the transplantation of *Nkx2.1* (44) and *TrkB* mutant interneurons (40) has allowed an examination of the roles of these genes in interneuron fate determination and migration, respectively, neither of which could be assessed directly in mutant animals because the animals die early in postnatal life (45, 46).

Just as transplantation of mutant MGE interneurons has revealed some of the cell-autonomous determinants of interneuron development, transplantation has elucidated non–cell-autonomous determinants as well. For example, exposing wild-type MGE cells to sonic hedgehog prior to transplantation biases them to form somatostatin-expressing rather than parvalbumin-expressing populations (38, 47). Conversely, transplantation of wild-type interneurons into mutant host backgrounds may also be a valuable approach for examining how non–cell-autonomous factors contribute to neurodegeneration (48).

Interneuron transplantation has also been used to study developmental cell death, where it has challenged a long-standing hypothesis of neurodevelopment (40). Throughout nervous system development, programmed cell death eliminates large fractions of developing neurons shortly after the formation of synaptic contacts (49, 50). According to the neurotrophin hypothesis (49–52), the engraftment of transplanted interneurons should be limited by competition for survival signals derived from the host: Transplanted cells that establish sufficient connectivity receive target-derived survival signals, while those that do not receive such signals die. By contrast, transplantation studies indicate that cortical interneuron survival is determined independently of signals produced by other cell types (40). Transplanted interneurons undergo developmental cell death in the host cortex, but they die asynchronously from interneurons of the host at a time consistent with their own intrinsic developmental program (Fig. 2). Over transplant sizes that vary by a factor of 200, the extent of transplant cell death remains constant and is similar to the extent of native interneuron cell death during normal development. Additionally, transplanted interneurons that lack TrkB, the principal neurotrophin receptor in the central nervous system (53), do not demonstrate reduced survival relative to wild-type transplanted interneurons. Together, these findings suggest instead that intrinsic developmental programs regulate interneuron cell death. These programs are not governed by cellular competition for survival signals produced by other cell types, although they may involve competition for survival signals produced by other interneurons. This pattern of intrinsically determined cell death may explain why transplanted MGE interneurons can survive and augment neuronal populations both inside and outside of the cerebral cortex.

The study of transplant-induced cortical plasticity further illustrates how interneuron-intrinsic developmental programs shape cortical development and regulate the engraftment of transplanted cells (40) (Fig. 2). During a critical period that occurs in late postnatal life, rodent ocular dominance plasticity is transiently heightened, presumably because of the maturation of cortical inhibition in the visual cortex (54). Manipulations of GABAergic inhibitory signaling alter ocular dominance plasticity (35, 55–57), suggesting that transplanted interneurons could affect plasticity of visual cortex through their known effects on inhibition (33). In mice, ocular dominance plasticity reaches a maximum in the fourth postnatal week, when cortical interneurons are approximately 5 weeks old, and then declines sharply thereafter (58). Transplantation of MGE interneurons into the visual cortex induces ocular dominance plasticity well after the normal critical period (43). This plasticity is only observed approximately 5 weeks after transplantation, when the transplanted interneurons are of a cellular age equivalent to that of native interneurons at the peak of the critical period. These findings suggest that the critical period is in part governed by the execution of an interneuron-intrinsic developmental program and that transplanted interneurons induce new plasticity by retaining and executing this program when transplanted into the postnatal cortex. The mechanism of transplant-induced plasticity is unknown, but it is unlikely to result merely from increased inhibition, as the pharmacologic enhancement of inhibition does not induce plasticity after the critical period (56). Transplanted interneurons make synapses that are three times as numerous yet approximately one-third as strong as those made by native interneurons, which may destabilize the cortical network and elicit functional reorganization (43). Alternatively, or in addition, transplanted interneurons may secrete molecules that alter the extracellular matrix, allowing native neurons to form new synapses (54, 59).

The Clinical Potential of Interneuron Transplantation

A number of neurologic and psychiatric disorders are thought to result, at least in part, from the dysfunction of cortical interneurons. These conditions, recently termed “interneuronopathies” (60), include epilepsy, autism, schizophrenia, and possibly Alzheimer’s disease (61–66). Other conditions, such as Parkinson’s disease, Huntington’s disease, spasticity, and chronic pain, result from imbalances in neural excitation and inhibition secondary to the dysfunction of other neuronal populations. Interneuron precursor transplantation has thus been explored as a strategy for restoring inhibition to neural circuits affected in these conditions (Fig. 3). Moreover, because some of them manifest in the adult, yet could arise from developmental abnormalities that endow neural circuits with a “presymptomatic signature” (67), interneuron transplantation has also been studied as a means of preventing the manifestation of clinical symptomatology in disease models. Through its effects on host brain plasticity, interneuron transplantation may also be a strategy for augmenting functional recovery after neurologic injury. Finally, through their ability to disperse and intercalate throughout neural tissue, transplanted interneurons may serve as vectors for the delivery of therapeutic agents.

Epilepsy is a heterogeneous disorder of both developmental and traumatic etiologies. Together, epilepsies are marked by the hyperexcitability of neuronal networks, which, in many cases, reflects the dysfunction of inhibitory circuits (66). It follows that interneuron

transplantation, through its ability to augment local GABA-mediated synaptic inhibition, may be a means for modifying epileptogenic circuits and limiting the spread of seizure activity across neural networks. In fact, a number of animal studies have provided evidence that transplanted interneurons improve disease phenotypes in both developmental and acquired forms of epilepsy.

Interneuron transplantation was first shown to suppress spontaneous seizures in a mouse potassium channel mutant (*Kv1.1*) that simulates a neuronal ion channelopathy associated with severe tonic-clonic seizures in humans (39). MGE interneuron transplants were made into the neo-cortex at neonatal stages, before the manifestation of seizures. Thirty days after transplantation, seizure monitoring by videoelectroencephalography demonstrated a 90% reduction in seizure number during the monitoring period. Although the potassium channelopathy was present across all cell populations of the brain, seizure suppression was achieved through interneuron transplants largely restricted to the cerebral cortex. These experiments suggest that neonatal interneuron transplantation could have a prophylactic effect in a congenital seizure disorder, but they did not address whether transplantation can suppress seizures when performed after epilepsy has emerged.

Other studies provide evidence that interneuron transplantation to neonates and adults can suppress seizures initiated by acute epileptogenic stimulation of the adult brain. Two months after transplantation of MGE interneurons to the neonatal mouse cortex, some host animals exhibited increased seizure thresholds, decreased seizure severity, and decreased seizure-associated mortality in a maximum electroconvulsive shock model, a traditional assay for anti-epileptic drug screening (68). Transplantation of interneurons into the adult cortex also reduced the power in local field potential recordings used to monitor seizure-like electrical activity induced by focal administration of the convulsive drug 4-aminopyridine to the cortex (69). This effect was largely independent of the number of cells that survived in the host cortex, which may corroborate other studies indicating that small numbers and large numbers of transplanted interneurons exert equally strong effects on neuronal inhibition (40).

Interneuron transplantation has been found to restore synaptic inhibition and rescue seizure phenotypes in mouse models of acquired epilepsy. In one study, MGE interneurons were transplanted into the adult hippocampus after the ablation of hippocampal interneurons with saporin (31). Interneuron transplantation restored local synaptic inhibition and reduced the treated animals' susceptibility to pharmacologically induced seizures. In another study designed to evaluate the clinical potential of interneuron transplantation to limit spontaneous seizures, transplants were performed into animals with acquired epilepsy secondary to drug-induced status epilepticus (32). Using a well-characterized rodent model of temporal lobe epilepsy, the most common form of epilepsy in adults, these experiments showed that interneurons transplanted into the adult hippocampus migrate and integrate into host circuits as functional, mature interneurons that comprise the subtypes of interneurons derived from the MGE. Transplantation into the hippocampus, but not the amygdala, reduced spontaneous seizure frequency by approximately 90% in animals monitored by videoelectroencephalography more than 60 days after transplantation. Because behavioral and cognitive comorbidities frequently occur in temporal lobe epilepsy patients, the effects

of interneuron transplantation on host behavior were also studied. Transplantation into the hippocampus reduced behavioral comorbidities present in the temporal lobe epilepsy model.

Together, these studies indicate that transplantation of a relatively small and locally restricted population of cortical interneurons can improve seizure phenotypes. This effect has been observed in models characterized by interneuron dysfunction (31), in genetic mutant models (39), and in models characterized by the pathology of other cell populations (32). Although the exact mechanism of seizure suppression remains unknown, transplanted interneurons likely constrain seizure activity by forming new inhibitory synapses and increasing “surround inhibition” (70). Other mechanisms may be involved, such as structural rearrangements of host circuitry, the secretion of neuropeptides or trophic factors, or the modulation of inflammation. It remains unclear, however, whether transplanted interneurons can integrate into neural circuits affected by long-standing epilepsy, or whether they exert a long-lasting effect on seizure phenotypes.

Interneuron transplantation has also been explored as a cell-based treatment for Parkinson’s disease, a neurodegenerative condition characterized by movement abnormalities, cognitive and behavioral decline, and autonomic dysregulation. The motor symptoms of Parkinson’s disease arise from the loss of dopaminergic neurons in the substantia nigra of the midbrain. This results in an imbalance of excitation and inhibition of the striatum, a basal ganglia structure that modulates motor commands. MGE interneurons were transplanted into rats that previously received 6-hydroxydopamine lesions of the substantia nigra, a manipulation that yields a model of the motor symptoms of Parkinson’s disease (30). Transplanted cells migrated, differentiated into GABAergic interneurons, and survived for more than 12 months in the damaged striatum, and they reduced Parkinsonian-like motor behaviors. Transplantation also increased motor activity in otherwise intact wild-type hosts, indicating that it also affects striatal-dependent motor behaviors in the intact brain. Although the mechanisms that underlie these effects are unknown, it is hypothesized that transplanted GABAergic interneurons introduce new inhibition to striatal circuits affected by the loss of brainstem dopaminergic inputs, thereby reducing the striatum’s inhibitory influence on motor commands. Regardless of the exact mechanism, these experiments suggest that transplanted interneurons can modify a disease phenotype secondary to the dysfunction of an entirely different cell population.

As discussed above, MGE interneurons exert disease-modifying effects when transplanted into the neocortex, hippocampus, and striatum—three structures that are normally populated by these cells. MGE interneurons have also been shown to survive, integrate, and ameliorate disease symptoms when transplanted into the spinal cord, a structure containing GABAergic interneurons that originate in the spinal cord and express morphologies different from those of cortical interneurons. In these experiments, MGE interneurons were transplanted into a model of neuropathic pain elicited by peripheral nerve injury (34). In this model, inhibitory circuits in the dorsal horn of the spinal cord were disrupted, and as a result, non-noxious stimuli became painful (allodynia) while pain behaviors provoked by normally painful stimuli were increased (hyperalgesia). When transplanted into the dorsal spinal cord 1 week after peripheral nerve injury, MGE interneurons developed molecular phenotypes characteristic of GABAergic cortical interneurons. After approximately 2 weeks, the

transplanted cells became responsive to stimuli delivered via peripheral nerves, and they made projections onto neurons that ascend to the brainstem, as indicated by activity-dependent gene expression and retrograde tracer studies. Finally, transplantation reduced behavioral measures of mechanical hypersensitivity, a hallmark of neuropathic pain. As was observed in other studies (40, 69), there was no correlation between transplanted interneuron number and functional effect. Additionally, transplantation did not affect baseline pain thresholds in the absence of nerve injury, which suggests that transplanted interneurons may not functionally alter all spinal cord circuits. These experiments indicate that interneuron transplantation can modify neuropathic pain, a sensory disorder of the spinal cord; however, it remains to be determined whether interneuron transplantation can ameliorate motor disorders of the spinal cord characterized by increased tone, such as spasticity or incontinence.

In addition to its impressive effects in animal models of neurologic disease, interneuron transplantation has been shown to affect behavioral phenotypes common to some psychiatric disorders. Transplantation of MGE interneurons to the mouse neonatal prefrontal cortex, a brain structure involved in numerous cognitive and executive behavioral functions in rodents and humans, prevents phencyclidine-induced schizophreni-form deficits in adult mice (71). By contrast, transplantation into the occipital cortex, a region involved in visual processing, does not prevent phencyclidine-induced deficits. These findings are consistent with post mortem studies of schizophrenic brains, which show altered chemical and molecular differentiation of interneurons in the prefrontal cortex (62, 64).

Interneuron transplantation can also reduce anxiety-like behaviors in wild-type rodents. At 30 and 60 days after transplantation, but not 15 days, transplant recipients were less likely to avoid exposed areas of an elevated plus maze, a behavior indicative of diminished anxiety (72). Likewise, aggressive behaviors were diminished in epileptic mice that received interneuron transplants to the adult hippocampus, but not the amygdala (32). Moreover, while transplantation to both structures reduced hyperactivity in adult epileptic animals, it did not produce some of the anxiolytic effects observed after transplantation to wild-type neonatal animals (72). This finding could be attributed to differences between the transplant recipients (epileptic versus wild-type), time of transplantation (adult versus neonate), and structures targeted (hippocampus and amygdala versus neocortex). Further experiments may better define the behavioral effects of transplantation to different brain regions and may determine whether transplantation can reduce abnormal behaviors when performed after their onset.

Finally, it has yet to be determined whether interneuron transplantation, through its effects on host brain plasticity (43), can enhance functional recovery after injury to the central nervous system. A number of insults, such as stroke, traumatic brain injury, viral encephalitis, and anoxic brain injury, elicit focal neuronal cell death and produce secondary motor, sensory, and behavioral impairments. By inducing temporally restricted windows of brain plasticity (43), interneuron transplantation may enhance the brain’s capacity to undergo functional recovery after such injuries. In these scenarios, interneuron transplantation would not be used to replace damaged neurons, but instead would be used to facilitate the reorganization of normal, spared neural circuits to compensate for ones

damaged by injury. Although this application of interneuron transplantation has yet to be tested, there are a number of intuitive disease contexts that await study, including amblyopia (a form of blindness secondary to impaired eye movements in early life) and neonatal hypoxia-ischemia. In both conditions, ocular dominance plasticity is disrupted and vision is impaired (54, 73). By inducing new ocular dominance plasticity in the visual cortex, transplanted interneurons may enable normal vision to develop in these conditions.

The Production of Cortical Interneurons in Vitro

The clinical potential of interneuron transplantation has thus far been investigated using cells from the rodent embryonic ventral forebrain. Ultimately, though, human applications will likely require transplantation of human cells. Although it has been suggested that substantial numbers of human cortical interneurons originate outside the ventral telencephalon (74), recent analyses of fetal brains provide evidence that the vast majority of primate cortical interneurons are in fact produced in the ventral telencephalon, at least through the second trimester of gestation (75, 76). As in the developing rodent, the human ventral telencephalon contains distinct progenitor domains (medial, caudal, and lateral ganglionic eminences) marked by similar region-specific transcription factor expression (75, 76). It remains to be seen, however, whether the morphogenetic factors that specify these progenitor domains are conserved between rodents and primates (77–79).

In vitro, cortical interneuron precursors have typically been produced from mouse and human embryonic stem cells (ESCs) through a two-stage process. First, ESCs are grown and differentiated on flat surfaces coated with cell matrix or fibro-blasts (80–82) or as spherical aggregates [embryoid bodies (83, 84)]. In this stage, undifferentiated ESCs are directed toward central nervous system progenitor identities through the use of serum-free media and mesendodermal signaling antagonists (83, 84). Second, through the timed and scaled application of morphogens that confer a ventral identity, forebrain progenitor-like cells are directed toward ventral and then MGE- and CGE-like precursor phenotypes (81, 83–90). To improve the efficiency and fidelity of ventral telencephalon-like precursor derivation, fluorescence-activated cell sorting (FACS) has been used to select for cells engineered to express fluorescent proteins under the control of forebrain-specific (83, 84, 86) and MGE-specific genetic promoters (81, 88, 89, 91, 92). Additionally, there has been progress toward the generation of specific cortical interneuron subtypes through the introduction of specific transcription factors into ESC-derived progenitors in vitro (93). Through these methods, researchers can now produce mouse and human interneurons that, after transplantation into the rodent brain, behave in a manner similar to interneurons transplanted from the mouse embryonic MGE.

The engraftment in vivo of ESC-derived interneurons was first demonstrated by the transplantation of fluorescently sorted mouse cells into the wild-type mouse brain (81). After differentiation in vitro, interneurons were purified by FACS to isolate those that expressed green fluorescent protein under the control of *Lhx6*, a promoter active in postmitotic interneurons of the MGE. This method of purification has overcome two key obstacles to the application of ESC-derived cell therapies: (i) the difficulty of selecting a specific and highly enriched donor population, and (ii) the need to exclude mitotically active cells, which could

be tumorigenic. After FACS, the purified population constituted just 2% of the initial population grown in vitro—a finding that highlights the challenge of directing cultured cells to an interneuron precursor-like identity. The transplanted cells migrated more than 2 mm, developed morphologies and electrophysiological properties of interneurons, and expressed some molecular markers characteristic of interneurons (GABA, somatostatin, parvalbumin, and neuropeptide Y, but not calretinin). The survival of the transplanted population at 1 month, however, was considerably lower than that observed in corresponding experiments using primary mouse MGE cells (approximately 2% versus 20%, respectively) (33, 40, 81). This reduced viability has been observed in other studies as well (87, 89), which suggests that in vitro systems could select for and yield cells highly dependent on a specific set of conditions not present in vivo.

Mouse ESC-derived interneurons have been studied in a disease model of temporal lobe epilepsy (87). Unlike other studies (81, 91), these experiments did not use FACS to enrich for interneuron precursor-like cells prior to transplantation. Nonetheless, the ESC-derived cells migrated throughout the host hippocampus, and after 2 to 3 months approximately 90% of them expressed neuronal markers. One host animal, however, developed a transplant-derived teratoma, indicating that extended differentiation protocols and selection of postmitotic neurons may be required to avoid the transplantation of tumorigenic cell types (86, 89). Approximately half of the transplanted cells developed a GABAergic phenotype, yet relatively few of them (≤10%) expressed interneuron subtype-specific markers (somatostatin, parvalbumin, and calretinin). Although a moderate fraction of the transplanted cells had electrophysiological properties of mature cortical interneurons, these cells demonstrated reduced frequencies and amplitudes of excitatory synaptic events relative to interneurons of the host brain, which suggests that their integration into host circuits was limited. Further studies are needed to determine whether these results reflect a limitation of cells produced in vitro, or whether their integration was affected by the altered physiology of the epileptic host. Transplanted ESC-derived interneurons nearly doubled the GABAergic population of the hippocampus, which was partially depleted by pilocarpine-induced seizures. Transplantation did not, however, reduce mossy fiber sprouting (a correlate of neuronal reorganization in the epileptic hippocampus). Unfortunately, the seizure phenotype of these transplant recipients was not assessed.

Finally, experiments using human ESCs and induced pluripotent stem cells have provided proof of concept that human cortical interneurons can be produced in vitro, while also elucidating challenges specific to the use of human cells (88, 89). In these studies, the development of ESC-derived human MGE-like interneurons was tracked for up to 7 months, both in vitro, on mouse feeder layers and brain slices, and in vivo, after transplantation to the postnatal mouse cortex. In one study, transplanted human ESC-derived interneurons exhibited immature phenotypes (unipolar or bipolar morphologies and expression of Dcx and Nkx2.1) 2 months after transplantation (88). When examined 7 months after transplantation (89), large fractions (as many as 50 to 75%) of the transplanted cells expressed one or more molecular markers characteristic of interneuron subtype maturation (somatostatin, calretinin, and calbindin); developed highly branched morphologies; and down-regulated expression of Dcx and Nkx2.1, two markers of immature interneurons. Essentially all cells expressed electrophysiological properties of mature interneurons and

demonstrated excitatory synaptic currents, which suggests that they integrated into the host mouse cortex (89). Tumor formation was not noted (89), and transplanted interneurons dispersed 1 to 2 mm from the site of injection. Transplanted human ESC-derived interneurons (88, 89) survived and expressed interneuron markers in greater numbers than did cells derived from mouse ESCs (87). For reasons that are unclear, however, very few of the transplanted human cells developed into mature fast-spiking, parvalbumin-expressing interneurons, despite the use of culture conditions and fluorescence purification methods expected to favor the production and selection of this cell type (88, 89).

When transplanted into the postnatal mouse brain, human ESC-derived interneurons exhibit a protracted maturation similar to that of endogenous interneurons in the neonatal human cortex; this result suggests that pluripotent stem cell–derived interneuron development parallels endogenous human interneuron development (88, 89). Thus, the time course of cellular maturation was not altered by transplantation into the neonatal mouse cortex, in which endogenous interneurons mature within 6 weeks of their production (36). These findings support the notion that interneuron maturation is more likely defined by intrinsic, organism-specific developmental programs, which, in human cells, play out over an extended time course. When cocultured on mouse cortical feeder layers, human ESC–derived interneurons may mature more quickly than cells cocultured on human cortical feeder layers (88), which suggests that environmental factors can influence their rate of maturation in vitro. Ultimately, the clinical application of human interneuron transplantation will likely require the development of cell culture systems that preserve, or possibly accelerate, the cellular mechanisms governing interneuron maturation.

The modification of neural circuits, for either therapeutic or experimental purposes, is possible through the transplantation of ventral telencephalon interneurons, a cell population that has a unique capacity to navigate and integrate into the post-natal nervous system. Given the central role of inhibition in neural circuit assembly and function, these studies of interneuron transplantation may be a prelude to the precise and directed adjustment of dysfunctional circuits in a number of disease settings. Ultimately, the generation of interneuron cell populations for transplantation, as well as the rational application of interneuron transplantation strategies, will benefit from a better understanding of this unique cell type’s development.

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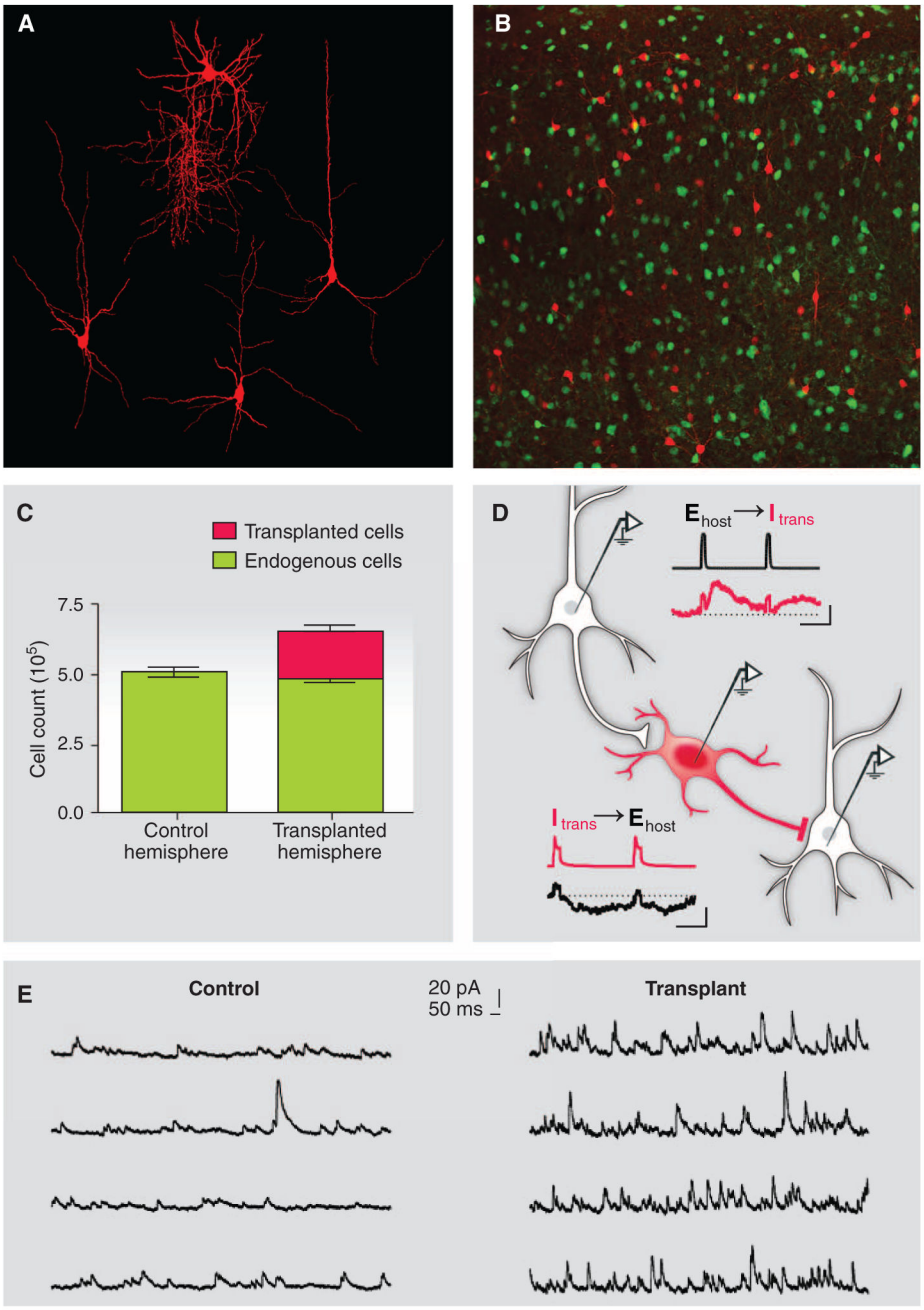

Fig. 1. Interneuron transplantation augments interneuron population size and increases inhibition in the host nervous system

(**A**) Section from the mouse cerebral cortex depicting transplanted interneurons, labeled by a red fluorescent protein. Transplanted immature interneurons disperse, survive, and develop extensive arborizations throughout the parenchyma of the host neocortex. Image width, ~400 μm. (**B**) Section from the mouse cerebral cortex depicting host interneurons (labeled by green fluorescent protein) and interneurons transplanted from the embryonic ventral telencephalon (red). Image width, ~800 μm. (**C**) Immature interneurons (red) disperse, survive, and integrate into the postnatal nervous system, where they increase the host

cortical interneuron population (green) by up to 35% (40). (**D**) Transplanted interneurons receive excitatory synapses from host pyramidal neurons and make inhibitory synapses onto host pyramidal neurons. Simultaneous electrode recordings from a transplanted inhibitory neuron (red) and host pyramidal neurons (white). Stimulation of a host pyramidal neuron elicits excitatory postsynaptic potentials in the transplanted interneuron (red). Depolarization of the transplanted interneuron evokes inhibitory postsynaptic potentials in a postsynaptic host pyramidal neuron. Scale bars, 25 ms and 90 mV (presynaptic), 25 ms and 0.125 mV (postsynaptic). (**E**) Interneuron transplantation increases the frequency of inhibitory signaling events in host pyramidal neurons. Representative traces of inhibitory postsynaptic currents were recorded from host pyramidal neurons in vitro (left, control; right, transplant recipient). [(B) and (C) from (40), (D) from (43), (E) from (33)]

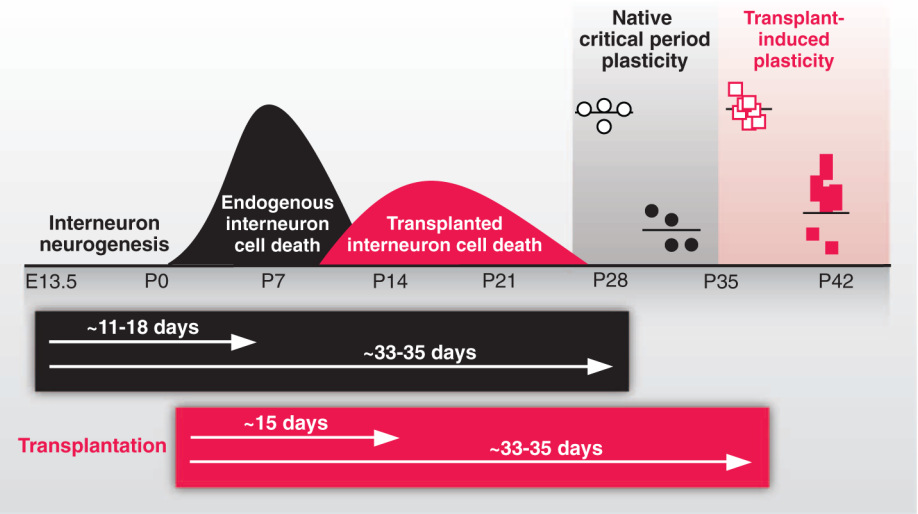

Fig. 2. Transplanted embryonic interneurons retain and execute intrinsic developmental programs when grafted into the postnatal brain

During mouse cortical development, inhibitory neurons undergo a pattern of cell death that peaks around postnatal days 7 to 11, when they reach an intrinsic cellular age of approximately 11 to 18 days (black curve). Later, around postnatal day 28, when surviving inhibitory neurons are approximately 33 to 35 days of age, a critical period for ocular dominance plasticity occurs in the visual cortex (black circles). When newborn inhibitory neurons are transplanted from the embryo into the postnatal brain (red), they undergo a similar pattern of cell death, which reaches a maximum when the transplanted cells are likewise approximately 15 days old (40). Moreover, when transplanted interneurons reach a cellular age of approximately 33 to 35 days, they induce ocular dominance plasticity in the host visual cortex (43). These findings suggest that interneuron development is governed by developmental programs that are established in the embryo, and that when transplanted, embryonic interneurons retain and carry out these developmental programs in the postnatal host nervous system.

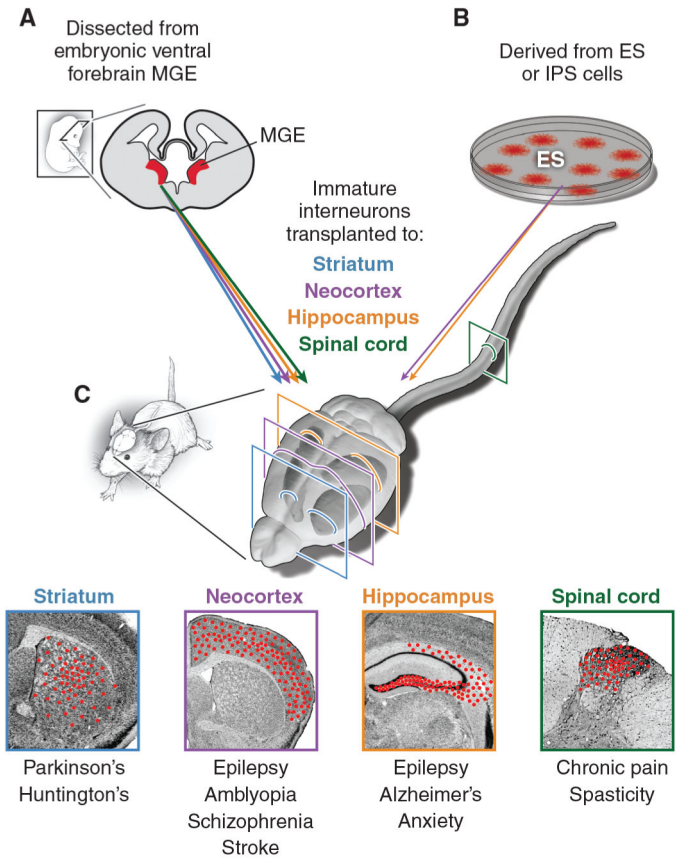

Fig. 3. Sources of immature interneurons for transplantation and therapeutic targets

(**A** and **B**) Immature interneurons can be transplanted (A) directly from the medial ganglionic eminence (MGE) of the embryonic ventral forebrain, or (B) from in vitro systems in which immature interneurons are generated from embryonic stem (ES) or induced pluripotent stem (IPS) cells. (**C**) Interneuron transplantation has been studied in diverse regions of the central nervous system, including the striatum, neocortex, hippocampus, and spinal cord. Depending on the site of transplantation, interneurons have been shown to modify disease phenotypes in animal models of Parkinson's disease, epilepsy, schizophrenia, anxiety disorder, and chronic pain. Interneuron transplantation may also be a method for therapeutically modifying neural circuits in conditions such as Huntington's disease, amblyopia, stroke, Alzheimer's disease, and spasticity.