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Potential of GABA-ergic cell therapy for schizophrenia, neuropathic pain, and Alzheimer's and Parkinson's diseases

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Abstract

Several neurological and psychiatric disorders present hyperexcitability of neurons in specific regions of the brain or spinal cord, partly because of some loss and/or dysfunction of gamma-aminobutyric acid positive (GABA-ergic) inhibitory interneurons. Strategies that enhance inhibitory neurotransmission in the affected brain regions may therefore ease several or most deficits linked to these disorders. This perception has incited a huge interest in testing the efficacy of GABA-ergic interneuron cell grafting into regions of the brain or spinal cord exhibiting hyperexcitability, dearth of GABA-ergic interneurons or impaired inhibitory neurotransmission, using preclinical models of neurological and psychiatric disorders. Interneuron progenitors from the embryonic ventral telencephalon capable of differentiating into diverse subclasses of interneurons have particularly received much consideration because of their ability for dispersion, migration and integration with the host neural circuitry after grafting. The goal of this review is to discuss the premise, scope and advancement of GABA-ergic cell therapy for easing neurological deficits in preclinical models of schizophrenia, chronic neuropathic pain, Alzheimer's disease and Parkinson's disease. As grafting studies in these prototypes have so far utilized either primary cells from the embryonic medial and lateral ganglionic eminences or neural progenitor cells expanded from these eminences as donor material, the proficiency of these cell types is highlighted. Moreover, future studies that are essential prior to considering the possible clinical application of these cells for the above neurological conditions are proposed. Particularly, the need for grafting studies utilizing medial ganglionic eminence-like progenitors generated from human pluripotent stem cells via directed differentiation approaches or somatic cells through direct reprogramming methods are emphasized.

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1. Introduction

Diverse sets of inhibitory interneurons control and modulate the activity of excitatory neurons in the central nervous system (CNS). This modulation facilitates smooth flow of information as well as homeostatic maintenance of neural circuits. Activation of inhibitory neurons results in the release of the neurotransmitter gamma-amino butyric acid (GABA), which inhibits postsynaptic neurons through hyperpolarization of their membranes. Co-activation of excitatory and inhibitory neurons is hence considered a basic functional principle underlying various brain activities (Isaacson and Scanziani, 2011). Increased excitation in disease states is typically associated with either substantial loss of GABA-ergic interneurons, or dysfunction of interneurons due to loss of their afferent excitatory input and/or changes in their receptors. Imbalances in neuronal excitation and inhibition occur in many neurological and psychiatric disorders (Southwell et al., 2014). These mainly include epilepsy, schizophrenia, neuropathic pain, Alzheimer's disease (AD) and Parkinson's disease (PD) (Shetty and Turner, 2000; Southwell et al., 2014; Tyson and Anderson, 2014). Furthermore, certain regions of post-stroke brain and brains exposed to alcohol during the prenatal period also display impaired inhibitory neurotransmission (Daadi et al., 2009; Poulos et al., 2014). Improved inhibitory neurotransmission may alleviate deficits in these conditions because some loss of certain subclasses of interneurons and/or functional alteration in surviving GABA-ergic interneurons occur in affected brain regions. This perspective has prompted a great interest in examining the usefulness of interneuron cell grafting intervention in regions of the CNS displaying hyperexcitability, deficiency of GABA-ergic interneurons or dysfunction of inhibitory neurotransmission in several preclinical models of neurological and psychiatric diseases (Southwell et al., 2014; Tyson and Anderson, 2014). Particularly, interneuron progenitors from the embryonic ventral telencephalon capable of differentiating into diverse subclasses of interneurons expressing calcium binding proteins such as parvalbumin (PV), calbindin (CBN), calretinin (CR), and neuropeptides such as neuropeptide Y (NPY), somatostatin (SST) have recently received considerable attention.

In the developing brain, subpopulations of progenitors emerging from the medial and caudal ganglionic eminences of the ventral telencephalon generate interneurons displaying distinct molecular markers, physiological activity and morphology. These progenitors exhibit long-distance tangential migration to populate the cerebral cortex and other forebrain regions (Southwell et al., 2014). Because of their ability to reach distant forebrain areas without the need for a scaffold such as radial glial processes, and their ability to give rise to multiple subclasses of interneurons, medial ganglionic eminence (MGE) progenitors have gained prominence as an excellent source of GABA-ergic progenitors for grafting in preclinical models of neurological disorders exhibiting interneuron deficiency or dysfunction. Indeed, MGE progenitors exhibit greater migratory potential after grafting into the postnatal and

adult brain than lateral ganglionic eminence (LGE, a major source of olfactory bulb interneurons) progenitors that mostly remain in clusters at injection sites (Wichterle et al., 1999, 2001; Hattiangady et al., 2008; Southwell et al., 2014). Furthermore, MGE progenitors seem to produce the complement of interneuron subtypes normally produced by them during development even when grafted into the adult cerebral cortex, hippocampus or the spinal cord (Alvarez-Dolado et al., 2006; Wichterle et al., 1999; Southwell et al., 2010, 2014; Braz et al., 2012; Hvou fu am., 2013).

The purpose of this review is to deliberate on recent studies of GABA-ergic cell grafts in prototypes of neurological disorders other than epilepsy, as detailed discussion on the efficacy of GABA-ergic and other cell grafts in epilepsy models is available in several previous review articles (Sebe and Baraban, 2011; Shetty, 2011, 2014; Tyson and Anderson, 2014). Accordingly, this review confers the promise of GABA-ergic cell therapy for schizophrenia, chronic neuropathic pain, AD and PD. The proficiency of primary MGE and LGE cells, and neural progenitor cells expanded in vitro from the embryonic ganglionic eminences are particularly examined in detail. The possible use of GABA-ergic cell therapy for other neurological conditions such as after stroke or in fetal alcohol spectrum disorder are also briefly discussed. In addition, important studies that are required before considering the possible clinical application of these cells for the above neurological conditions are proposed.

2. GABA-ergic cell grafts for treating schizophrenia

Schizophrenia is a chronic and disabling brain disorder that affects 1% of the population. Mutations in several genes are believed to be involved with an increased risk for developing schizophrenia, as gene mutations can alter the development and activity of brain circuitries through dysregulation of neurotransmitters such as glutamate and dopamine. Hyperactivity of the mesolimbic dopamine system due to an altered regulation of the neurotransmitter dopamine has been suggested to be the major cause of symptoms in schizophrenia (Laruelle et al., 1999; Abi-Dargham et al., 2004; Perez and Lodge, 2013). Furthermore, human imaging and preclinical studies have suggested that hyperfunction of the dopamine system in schizophrenia stems from the hyperactivity of neurons in hippocampal subfields at rest (Medoff et al., 2001; Lodge and Grace, 2007; Perez et al., 2013; Shah and Lodge, 2013). The hippocampus regulates dopaminergic transmission through a multi-synaptic pathway involving the nucleus accumbens and ventral pallidum (Grace et al., 2007; Lodge and Grace, 2011). While the exact cause of hippocampus hyperexcitability in schizophrenia is unknown, correlative evidence in clinical and preclinical studies points to reduced numbers of GABA-ergic interneurons that express the calcium binding protein PV. These fast firing interneurons are known to maintain the oscillatory activity of excitatory glutamatergic pyramidal neurons through perisomatic targeting (Lewis et al., 2005; Lodge et al., 2009). Moreover, studies have further shown that attenuation of hyperactivity of neurons in the ventral hippocampus via drug treatment or deep brain stimulation can normalize the enhanced dopamine neuron activity, thereby reversing an augmented locomotor response to amphetamine seen in animal models of schizophrenia (Gill et al., 2011; Perez et al., 2013). Thus, the concept of GABA-ergic interneuron transplantation into the hippocampus as a therapy for schizophrenia stems from the observation that persistent hippocampus hyperactivity promotes dopamine

hyperfunction (Perez and Lodge, 2013) and that addition of new GABA-ergic interneurons into the hippocampus circuitry would restrain this hyperactivity.

Only a few studies have examined the efficacy of GABA-ergic cell grafting into the hippocampus in animal models of schizophrenia but so far the results are encouraging (Table 1). One of these studies investigated the effects of MGE progenitor cell grafting in a developmental disruption model of schizophrenia (Perez and Lodge, 2013). In this prototype, rats received methylazoxymethanol acetate (MAM) exposure during the prenatal period through an intraperitoneal injection of MAM to their mothers on embryonic day (E) 17. Exposure to MAM during the prenatal period is known to disrupt brain development, causing a behavioral phenotype in rats akin to that observed in schizophrenia patients. Approximately 6 weeks after their birth, a group of MAM exposed rats received grafts of E14.5 MGE progenitors into their ventral hippocampi and another group of MAM exposed rats received dead MGE progenitors (dead cell grafts) into the same region. Electrophysiological recordings performed two months later confirmed the occurrence of hyperactivity of putative pyramidal neurons in the ventral hippocampus of MAM exposed rats receiving dead cell grafts. In contrast, MAM exposed rats receiving live MGE progenitors displayed normalized firing rates of neurons in the ventral hippocampus. Furthermore, as hypothesized, MGE cell grafting caused downstream changes in dopamine neuron activity in the ventral tegmental area (VTA), evidenced by normalization of aberrant VTA dopamine neuron population activity. Moreover, MGE cell grafting reversed amphetamine induced hyper-locomotion observed in MAM exposed rats receiving dead cell grafts. Immunohistochemical analyses revealed differentiation of graft-derived cells into GABA-ergic neurons (46%) as well as subclasses expressing PV (18%). Overall, this study provided the first evidence that grafting of GABA-ergic precursor cells into the ventral hippocampus can restrain the hyperactivity of hippocampal pyramidal neurons and thereby normalize downstream alterations in dopamine neuron activity and associated behavior in an animal model of schizophrenia.

A recent study showed the beneficial effects of grafting E15.5 mouse MGE progenitors in a cyclin dopamine receptor 2 knock out (Ccnd2 KO) mouse model of schizophrenia (Gilani et al., 2014, Table 1). The chosen animal model (Ccnd2 KO mice) displays reduced numbers of PV+ interneurons in the cortex with predominant reductions in the hippocampus associated with deficits in synaptic inhibition, increased *in vivo* spike activity of projection neurons, and increased basal metabolic activity (assessed with fMRI) in the hippocampus (Gilani et al., 2014). Moreover, these mice exhibit several neurophysiological and behavioral phenotypes predicted to be produced by hippocampal disinhibition, including increased ventral tegmental area dopamine neuron population activity, and psychosis relevant phenotypes such as behavioral hyper-responsiveness to amphetamine and impairments in hippocampus-dependent cognition (Gilani et al., 2014). Grafts were placed into the caudo-ventral CA1 region of the hippocampus 6–8 weeks after birth and the effects were measured 4–6 months after grafting. This study demonstrated improved context-dependent learning and memory function along with normalization of dopamine neuron activity and behavioral response to amphetamine in Ccnd2 KO mice receiving MGE progenitor cell grafts. Furthermore, grafting also improved cerebral blood volume and attenuated increased metabolic activity in the hippocampus up to 6 months after grafting but had no effect on

hippocampus neurogenesis. The survival of grafted cells was modest (~12% of injected cells) but most graft-derived cells expressed GABA (97%) with subclasses expressing PV (56%) and SST (35%). Collectively, the findings in this study showed clear functional links between hippocampal GABA-ergic interneuron deficits and psychosis-relevant dopaminergic and cognitive phenotypes. These results provided a strong rationale for targeting hippocampal interneuron function in the prevention and treatment of schizophrenia. Fig. 1 illustrates the potential mechanisms by which GABA-ergic cell grafts mediate functional recovery in the above described schizophrenia models.

Interestingly, another study in phencyclidine (PCP) model of schizophrenia demonstrated beneficial effects of grafting GABA-ergic progenitors into the medial prefrontal cortex. A cohort of newborn mice received E13.5 mouse MGE progenitor cell grafts into the medial prefrontal cortex and another cohort received vehicle into the same region (Tanaka et al., 2011, Table 1). Six weeks later, mice in both groups were treated with PCP, a noncompetitive NMDA receptor antagonist that induces schizophreniform cognitive deficits in healthy humans as well as in rodents (Tanaka et al., 2011). As expected, mice that received vehicle (sham-grafting surgery) into the medial prefrontal cortex in the neonatal period exhibited impaired performance in exploratory preference during the retention sessions of novel object recognition test (NORT), performed 30 min after PCP administration. These mice also exhibited impaired acoustic startle response in a prepulse inhibition (PPI) test, which examines the sensory-motor gating function, a major indicator of information processing involving the cortex that is frequently impaired in schizophrenia (Arguello and Gogos, 2012). However, PCP administration to mice that received MGE progenitor cell grafts into the medial prefrontal cortex in the neonatal period did not display impairments in both NORT and PPI test. Furthermore, grafting of cells from E13.5 rostromedial cortex could not reproduce the preventive effect of MGE cell transplantation, even though these cortical precursors survived and differentiated into callosal projection neurons. Moreover, grafting of MGE progenitors into the neonatal occipital cortex did not protect against PCP induced cognitive deficits or sensory-motor gating deficit. These results suggested that the preventive effect of grafting against induction of the cognitive and sensory-motor gating deficits by PCP depended upon the type of donor cells (GABA-ergic progenitors versus excitatory neuron precursors) as well as the site of grafting. Additional analyses also suggested that prior MGE cell transplantation into the medial prefrontal cortex modulated the activity of callosal projection neurons in the medial prefrontal cortex in response to PCP during the training session in the NORT. Examination of MGE cell grafts revealed differentiation of graft-derived cells into interneurons expressing PV (10%) and SST (60%). Thus, increasing inhibitory neurotransmission in the medial prefrontal cortex through MGE cell grafting seems also beneficial for easing schizophreniform deficits. However, grafting studies following the establishment of schizophrenia-like symptoms will be needed in the future to determine whether medial prefrontal cortex is comparable to the hippocampus as a target for grafting of MGE cells in schizophrenia.

The above studies in rat and mouse models of schizophrenia demonstrate the promise of MGE progenitors for: (i) normalizing dopamine neuron activity and locomotor function through alleviation of hippocampal hyperexcitability and (ii) preventing the cognitive and sensory-motor gating deficits by enhancing inhibitory neurotransmission in the medial

prefrontal cortex. However, it remains to be examined whether these beneficial effects of GABA-ergic cell grafts on psychosis-relevant dopaminergic phenotypes and cognitive function persist for prolonged periods. Furthermore, critical assessment of the efficacy of GABA-ergic cell grafts in additional prototypes of schizophrenia would be beneficial. Additionally, it will be necessary to examine whether similar results can be obtained from GABA-ergic progenitors generated from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs).

3. GABA-ergic cell therapy for relieving neuropathic pain

Injury to the peripheral and central nervous systems may cause a complex chronic pain. While the precise mechanisms underlying this state are still being explored, it is believed that impairments in spinal cord dorsal horn inhibitory circuits, including the loss of GABA-ergic interneurons, is one of the major reasons for persistent neuropathic pain following nerve injury (Braz et al., 2012). In the spinal cord, local interneurons and descending inhibitory circuits modulate pain sensations that are processed in the superficial layers of the dorsal horn. The neurotransmitter GABA released by these interneurons plays a major role in pain modulation, as pharmacological blockade of GABA-ergic neurotransmission or deletion of specific subunits of GABA receptors in the spinal cord leads to hyperalgesia (exacerbated pain) and allodynia (Gwak et al., 2006; Jergova et al., 2012). Allodynia is a state of increased response of neurons to repetitive painful stimulation, and pain response to stimulus from skin that typically does not elicit pain (e.g. light touch). Moreover, intrathecal administration of GABA, GABA_A, or GABA_B receptor agonists following peripheral nerve injury has been shown to reduce neuropathic pain (Eaton et al., 1999a, 1999b; Asiedu et al., 2010; Munro et al., 2010). Thus, there is adequate evidence to suggest that hyperalgesia and allodynia after nerve injury are a result of impaired inhibitory neurotransmission and/or dysfunctional GABA-ergic inter-neurons in the dorsal horn of the spinal cord (Moore et al., 2002). However, systemic administration of GABA-enhancing drugs is not efficacious for relieving neuropathic pain, as it cannot specifically improve inhibitory neurotransmission in the spinal cord and is associated with significant side effects. Therefore, the idea of grafting of GABA-secreting cells or GABA-ergic neurons directly into the spinal cord has received considerable interest in neuropathic pain models (Eaton et al., 1999a, 1999b, 2007; Mukhida et al., 2007).

Jergova and associates induced neuropathic pain in Sprague-Dawley rats via chronic constriction of the sciatic nerve, then transplanted GABA-ergic progenitor cells expanded from E14 LGE cells into the ipsilateral lumbar gray matter of the spinal cord a week after nerve constriction (Jergova et al., 2012, Table 2). Grafting into the dorsal horn of the spinal cord decreased hypersensitivity, heat hyperalgesia, cold allodynia and exaggerated neuronal firing at 1–3 weeks post-grafting. Graft-derived cells differentiated into GABA-ergic neurons and enhanced GABA-ergic immunoreactivity in the dorsal horn. Furthermore, single neuron recordings showed decreased neuronal responsiveness to peripheral stimulation in rats that received grafts. Overall, this study showed the beneficial effects of GABA-ergic NPC grafting in attenuating neuropathic pain resulting from peripheral nerve injury. Another study examined the effects of grafting of GABA-ergic progenitors from E12.5–13.5 mouse MGE into the lumbar spinal cord segments of mice displaying

neuropathic pain following spared sciatic nerve injury (Braz et al., 2012, Table 2), which was accomplished through transection of two of the three terminal branches of the sciatic nerve. This study showed that GABA-ergic cell grafting reversed mechanical hypersensitivity but not inflammatory pain. Only a small percentage (1–3%) of grafted cells survived but did differentiate into GABA-ergic interneurons, established significant functional connections with both primary afferent and spinal cord neurons, and increased the concentration of GABA synthesizing enzymes glutamate decarboxylase-65 (GAD-65) and GAD-67 in the spinal cord. Additionally, the expression of c-fos in graft-derived GABA-ergic interneurons after application of noxious chemical stimulus into the hind paw provided indirect evidence of their integration into the host brain.

Additional recent studies have further validated the efficacy of GABA-ergic progenitor cell grafts to ease neuropathic pain in diverse animal prototypes. In one of these studies, GABA-ergic progenitors from E12.5–13.5 mouse MGE were grafted into the spinal cord of mice harboring a deletion of the transcription factor *Bhlhb5*, 5–6 weeks after their birth (Braz et al., 2014, Table 2). These mice provide a chronic itch prototype, as they display excessive scratching of the skin and significant skin lesions. While transmission of itch (pruritoceptive) messages involves specific neural circuits in the spinal cord that are distinct from the pain pathways, itch-specific circuits are also regulated by inhibitory interneurons in the dorsal horn. Because itch in these mice is associated with loss of GABA-ergic interneurons in the dorsal horn, this prototype offered an excellent setting for examining the efficacy of intraspinal grafting of GABA-ergic progenitors for alleviating itch (Braz et al., 2014). Analyses at 2–5 weeks post-grafting revealed significant reduction in the size and severity of skin lesions, substantial reductions in excessive scratching and dramatic resolution of skin lesions. The survival rate of grafted cells was 5% but 90% of these surviving cells differentiated into GABA-ergic interneurons including subclasses expressing PV, NPY and SST. In addition, the expression of c-fos in graft-derived GABA-ergic interneurons following application of noxious chemical stimulus into the hind paw provided an indirect evidence of their integration into the host brain. In another study, beneficial effects of GABA-ergic cell grafting were found in a Paclitaxel induced chemotherapy model of neuropathic pain (Braz et al., 2015, Table 2). In this study, grafting of E12.5–13.5 mouse MGE progenitors into the spinal cord a week after the development of Paclitaxel-induced hypersensitivity alleviated heat hyperalgesia and allodynia. The survival rate of grafts was low at 6%, and grafting did not alter levels of GAD mRNA. Interestingly, neither mechanical hypersensitivity nor heat hyperalgesia were alleviated when MGE cells from mice with a deletion of *VGAT* (vesicular GABA transporter) gene were grafted. As deletion of *VGAT* interferes with the storage and release of GABA, these results imply that anti-hyperalgesic effects following MGE cell grafting are dependent on GABA release by graft-derived interneurons. In summary, the above studies demonstrate the potential of GABA-ergic precursor cell transplantation into the spinal cord for easing neuropathic pain and also support the idea that restoration of inhibitory controls through release of GABA from graft-derived interneurons is sufficient to ease neuropathic pain.

4. Promise of GABA-ergic cell therapy for Alzheimer's disease

In an increasingly aged population, AD has one of the most pressing needs for new therapies. Symptomatically, AD presents with pronounced memory and cognitive deficits well beyond normal age-related decline, thought to be caused partly by hippocampal hyperactivity as a result of loss of inhibitory interneuron function (Huang and Mucke, 2012). As mentioned previously, GABA interneurons play an important role in regulating hippocampal levels of activation through inhibition, and the processes involved in learning and memory formation require a balance of excitatory and inhibitory neuronal network activity (Andrews-Zwilling et al., 2012). Hence, loss or impaired function of GABA-ergic interneurons can lead to learning and memory dysfunction. In rodent models, significant decreases in numbers of GABA-ergic interneurons are one of the conspicuous alterations seen in the aged hippocampus (Shetty and Turner, 1998; Stanley and Shetty, 2004; Shetty et al., 2009; Koh et al., 2010; Kuruba et al., 2011; Spiegel et al., 2013). Alterations in the function of GABA-ergic interneurons leads to increased neural activity in the CA3 region of the aged hippocampus, which contributes to memory dysfunction as the hyperactive CA3 pyramidal neurons are unable to encode new information in a pattern that is typically done in the young hippocampus (Wilson et al., 2003; Koh et al., 2010). This notion is supported by observations that overexpression of NPY (a neuromodulator released by a subclass of GABA-ergic neurons) in the hippocampal CA3 region, or treatment with low doses of valproate (an anti-epileptic drug that increases GABA-ergic neurotransmission), can improve hippocampal-dependent long-term memory in aged rats (Koh et al., 2010). Decreased inhibitory neurotransmission likely also occurs in AD as brain concentrations of GABA and SST are reduced in the brain and the cerebrospinal fluid (Davies et al., 1980; Hardy et al., 1987; Seidl et al., 2001). Furthermore, decreases in GABA and SST levels are more pronounced in apoE4 carriers (accounting for 60–75% of AD patients) exhibiting enhanced brain activity at rest and in response to memory tasks (Grouselle et al., 1998; Filippini et al., 2009; Dennis et al., 2010). Moreover, the expression of apoE4 in mice causes an age- and sex-dependent (female>-male) decrease in hilar GABAergic interneurons, which correlates with the severity of learning and memory deficits (Andrews-Zwilling et al., 2010; Leung et al., 2012). Likewise, increased production or aggregation of A β interferes with interneuron function and leads to abnormal DG activity and cognitive impairments (Verret et al., 2012). Additionally, patients with mild cognitive impairment and young pre-symptomatic apoE4 carriers exhibit hippocampal hyperexcitability (Filippini et al., 2009; Yassa et al., 2010; Bakker et al., 2012). Thus, an excitatory–inhibitory imbalance in the hippocampus likely contributes to memory dysfunction in both aging and AD (Huang and Mucke, 2012). From this viewpoint, strategies that improve network balance may prevent or reduce cognitive decline in aging and AD.

No studies have examined the effects of grafting GABA-ergic cells into the hippocampus of normal aged animals on memory function so far. However, a recent study has investigated the effects of replacing lost or impaired GABA-ergic interneurons on neuronal signaling and behavioral deficits in an AD model (Tong et al., 2014, Table 3). In this study, freshly dissected E13.5 mouse MGE progenitors were grafted bilaterally into the dentate hilus of 14 months old female apoE4 knock-in mice displaying substantial hilar GABA-ergic inter-

neuron loss. Analyses performed 90 days after grafting demonstrated 90% GABA-ergic interneurons with subclasses expressing markers such as SST (45%), NPY (23%) and PV (12%), among the surviving graft-derived cells. Patch-clamp recordings from graft-derived SST+ interneurons revealed intrinsic firing properties typical of SST+ low-threshold spiking interneurons. Graft-derived interneurons also exhibited spontaneous EPSCs and IPSCs of comparable amplitude and frequency as endogenous hilar interneurons. Grafting also modified the activity of DG granule cells, which was evident in an increased frequency of spontaneous inhibitory currents and increased inhibitory charge transfer.

Furthermore, MGE cell grafting prevented apoE4-induced memory deficits in a probe trial of the water maze test, corrected the abnormal activity, anxiety, and exploratory behaviors in open field and elevated plus maze tests (Tong et al., 2014). Thus, grafting of inhibitory interneurons was found efficacious for restoring normal cognitive function in an AD model, which highlights the role of interneuron dysfunction in AD pathogenesis and the promise of cell replacement therapy for improving cognitive function in AD, particularly when intervened in early stages of the disease.

5. GABA-ergic cell therapy for Parkinson's disease

Parkinson's disease, much like AD, is a growing issue for an aging population. In people over 60, PD is the second most common neurodegenerative disease. PD is a progressive and nuanced disease which begins with motor deficits in early stages which then develop into a varied set of symptoms including mood, sleep, and general cognitive dysfunction (Buttery and Barker, 2014). Historically, the treatment for PD has relied strongly upon pharmaceuticals such as dopamine (DA) agonists, with a goal to compensate the depletion of DA concentration in the striatum resulting from the loss or dysfunction of DA-ergic neurons in the substantia nigra of the midbrain. Dopaminergic cell grafting has been considered as a good alternative to DA agonist treatment and a plethora of studies in animal models have shown efficacy of these grafts (Buttery and Barker, 2014). Nevertheless, the outcome of DA-ergic cell grafting in PD patients has been mixed with incidences of side effects (Piccini, 2002), which has prompted greater focus towards alternative therapeutic options (Olanow et al., 2009). While the DA-ergic cell therapy as a treatment option is still being pursued, emerging animal model results imply a role for GABA-ergic grafting in treating PD.

The promise of MGE precursor cell grafts for treatment of motor symptoms was explored some time ago in the 6-hydroxydopamine (6-OHDA) rodent model of PD, a prototype in which unilateral administration of 6-OHDA causes DA-ergic neuron loss in the pars compact region of the substantia nigra (Martinez-Cerdeno et al., 2010, Table 3). MGE cells were transplanted into the striatum and then the fate of graft-derived cells and their effect on motor deficits were analyzed. Although the rate of graft cell survival was modest (~1% of injected cells), graft-derived cells differentiated into GABA-ergic interneurons expressing GABA (75% of surviving cells) with subclasses expressing CB (24%), substance P (6%), CR (8%), PV (0.5%) and SST (10%). Furthermore, majority of graft-derived cells displayed properties of inhibitory interneurons and integrated into the host striatal circuitry. More importantly, grafting improved motor behavior, which was evidenced through decreased apomorphine-induced rotations and increased stride length. It was found that these cells also

survived for a year after transplantation. Even with a modest cell-survival rate, these results suggest that GABA-ergic cell grafting deserves further evaluation as a method to restore the necessary inhibitory function to the damaged circuits in the PD brain. The future of GABA-ergic cell therapy for PD patients rests in not only developing personalized cells for grafting, but also pinpointing the most therapeutic time points possible. As development of cell-based therapies for PD continues, it is worthy to examine combination treatment strategies that include DA-ergic cell grafting into the substantia nigra and GABA-ergic cell transplantation into the striatum.

6. GABA-ergic cell therapy for stroke and fetal alcohol spectrum disorder

A few studies have also examined the beneficial effects of grafting GABA-ergic cells or neural stem/progenitor cells capable of differentiating into GABA-ergic cells in conditions such as stroke and fetal alcohol spectrum disorder. Daadi et al. (2009) placed four transplants of fetal MGE GABA-ergic cells in the penumbra regions two weeks after ischemic stroke induced through intraluminal occlusion of the middle cerebral artery in rats. Rats receiving MGE cell grafts displayed reduced motor deficits when examined four weeks after grafting. Patch-clamp recordings revealed functional connectivity between grafted MGE cells and host neurons. Histological characterization demonstrated ~17% recovery of grafted cells and differentiation of MGE cells into different types of neurons including GABA-ergic interneurons expressing PV and CBN and synaptic integration of graft-derived neurons with the host neurons. Overall, this study pointed out for the first time that MGE-derived GABA-ergic cell grafting after stroke may ameliorate functional deficits. However, follow-up long-term studies in a variety of stroke models are needed in the future to determine post-stroke conditions where GABA-ergic cell grafting would facilitate functional recovery. Another neurological condition where GABA-ergic cell grafting may be useful is the fetal alcohol spectrum disorder, as dysfunction of the GABA-ergic inhibition has been observed in this developmental brain disorder resulting from exposure to alcohol during the prenatal period (Poulos et al., 2014). Inhibitory neurotransmission impairment in this disorder is likely due to multiple causes, which include reduced numbers of GABA-ergic interneurons and abnormalities in PV+ interneurons in certain regions of the brain (Poulos et al., 2014). While the efficacy of GABA-ergic cell grafting in this condition is yet to be determined, NSC grafting in animal models of this disorder did reduce behavioral abnormalities as well as the loss of PV+ GABA-ergic interneurons (Yoshinaga et al., 2007; Poulos et al., 2014). Nonetheless, based on the pathophysiology, GABA-ergic cell grafting therapy may considerably ameliorate functional impairments in this neurological condition. To authenticate this, rigorous studies on the effects of GABA-ergic cell grafting into regions of the brain exhibiting impaired inhibitory neurotransmission in animal models of fetal alcohol syndrome are needed.

7. Conclusions

A series of recent studies conducted in preclinical models demonstrate that GABA-ergic cell grafting has promise for alleviating: hyperactivity of the mesolimbic dopamine system in schizophrenia, neuropathic pain resulting from peripheral nerve injury, and excitatory and inhibitory synaptic transmission imbalance in AD and PD. These results are interesting but

additional refinements are needed for the clinical translation of interneuron cell therapy for these neurological disorders. First, the overall survival of GABA-ergic interneurons derived from progenitor cells is on the lower side, with some studies reporting a yield of only 1–5% injected cells. This may be due to the heterotopic nature of grafting in some of these studies such as LGE or MGE progenitor cell grafting into the spinal cord or striatum. Nonetheless, development of improved donor cell preparation and grafting strategies that augment graft cell survival and incorporation of graft-derived GABA-ergic interneurons into the host brain circuitry are critical for long-term efficacy. For example, a previous study in an epilepsy model has shown that ~28% of LGE progenitor cells pre-treated with FGF-2 and a caspase inhibitor survived 12 months after grafting with mostly GABA-ergic interneuron differentiation, providing lasting suppression of seizures (Hattiangady et al., 2008). Second, it is unknown whether the alleviation of neurological deficits seen after GABA-ergic progenitor cell grafting would be transient or enduring, which requires analyses of functional recovery for prolonged periods after grafting. Third, pertaining to schizophrenia and AD, it will be important to examine whether grafting of GABA-ergic progenitors into the hippocampus exhibiting hyperexcitability would reverse/alleviate cognitive problems and depression observed in these disorders.

Furthermore, it is vital to find a reliable source of sufficient donor GABA-ergic progenitors for clinical application of interneuron grafting. The use of MGE and/or LGE progenitors from the human fetal brain is not feasible because of both ethical concerns and unsuitability of such an approach to obtain the required amounts of cells needed for clinical application. Hence, generation of MGE- and LGE-like progenitors from hESCs and hiPSCs through directed differentiation approaches is critical. The methods to generate MGE-like cells from hESCs and hiPSCs are already available (Chen et al., 2013; Maroof et al., 2013; Liu et al., 2013a, 2013b; Nicholas et al., 2013). Fig. 2 illustrates the major steps involved in the generation of GABA-ergic progenitors from hESCs and hiPSCs and the utility of such cells for grafting in conditions such as schizophrenia, PD, AD and neuropathic pain. A few studies have also examined the efficacy of such cells in animal models of disease (Cunningham et al., 2014; Hattiangady et al., 2013). Yet, a faster protocol for generating GABA-ergic progenitors from pluripotent stem cells (PSCs), and strategies that promote quicker differentiation of these cells into GABA-ergic interneurons after grafting are required, as the current protocols require extended periods for conversion of hESCs/hiPSCs into GABA-ergic progenitors and these GABA-ergic progenitors tend to differentiate at a slower pace after grafting (Cunningham et al., 2014). Another issue is the safety of progenitors derived from hESCs/hiPSCs. Inclusion of even a single pluripotent stem cell in the cell suspension made for grafting may result in development of teratoma, a mixed tumor of ectoderm, endoderm and mesoderm lineage cells. Hence, advanced methods to avoid teratoma after grafting are needed. Techniques such as the isolation of putative GABA-ergic progenitors expressing GFP under the control of Lhx6 (a promoter active in ventral telencephalon interneuron precursor cells) via fluorescence activated cell sorting (Maroof et al., 2010) and generation of specific types of interneurons expressing PV, SST, or NPY directly from patient fibroblasts (via direct reprogramming methods) will be highly beneficial in this regard.

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Fig. 1.

A schematic showing the potential mechanisms by which GABA-ergic cell grafts mediate functional recovery in animal models of schizophrenia. Both rat methylazoxymethanol acetate (MAM) and mouse cyclin dopamine receptor 2 knock out (Cnd2 KO) models display abnormal changes in the hippocampus (HPS). These include reduced numbers of GABA-ergic interneurons, hyperactivity of pyramidal neurons and/or increased basal metabolic activity. These hippocampal alterations are believed to drive aberrant modifications in the mesolimbic (ML) system, which are indicated by hyperactivity of dopamine neurons in the ventral tegmental area (VTA) and behavioral abnormalities such as amphetamine-induced hyper-locomotion associated with impairments in hippocampus-dependent cognitive and memory function. Grafting of GABA-ergic cells into the hippocampus in these models stabilizes the function of hippocampus, along with the differentiation of graft-derived cells into GABA-ergic interneurons. Stabilized hippocampus function was evidenced through normalized firing rates of neurons in the ventral hippocampus, standard basal metabolic activity and improvements in hippocampus-dependent learning and memory function. These changes lead to normalization of the mesolimbic system function, which is evinced from the regulated VTA dopamine neuron population activity and attenuation of amphetamine-induced hyper-locomotion.

Fig. 2.

The various steps involved in the generation of gamma-amino butyric acid positive (GABA-ergic) progenitors from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) and the utility of these GABA-ergic progenitors for treating conditions such as schizophrenia, Parkinson's disease, Alzheimer's disease and neuropathic pain. As described by Liu et al. (2013a, 2013b), the pluripotent stem cells (PSCs) are first expanded as embryoid bodies in a suspension culture using a chemically defined medium (A, B). The embryoid bodies are next treated with neural induction medium to obtain neural rosettes, which are then lifted and grown in suspension cultures with high doses of sonic hedgehog (Shh) to obtain neurospheres of cells expressing markers of medial ganglionic eminence progenitor cells (NK $\times$ 2.1+ cells). Virtually all of these cells give rise to GABA-ergic interneurons when plated on a substrate and treated with the differentiation medium.

The progenitor cells expressing the transcription factor NK $\times$ 2.1 are hence ideal as donor cells for grafting into the hippocampus in conditions such as schizophrenia, and Alzheimer's disease, into the striatum in Parkinson's disease and into the spinal cord in neuropathic pain.

Table 1

Studies on GABA+ cell grafts in schizophrenia models.

Animal model of schizophrenia employed	Type of donor cells	Timing and site of grafting	Time-point of analyses and effects on behavior	Graft cell survival, differentiation and integration	Effects of grafts on the host brain changes
Methylazoxymethanol acetate (MAM) model of schizophrenia	GABA-ergic progenitors from E14.5 mouse MGE	40–45 days after birth	2-months after grafting	Percentage Survival not reported	Normalization of ventral hippocampal pyramidal neuron firing rates
Perez and Lodge, 2013		Grafts placed into the ventral hippocampus	Reversal of the hyper-responsive locomotor response to amphetamine	Differentiation: Graft-derived cells expressed GABA (46%) and PV (18%)	Normalization of VTA dopamine neuron activity
Cyclin D2 knock out mouse model of Schizophrenia	GABA-ergic progenitors from E15.5 mouse MGE	6–8 weeks after birth	4–6 months after grafting	Survival: ~12% of injected cells	Normalization of CBV
Gilani et al., 2014		Grafts placed into the caudo-ventral CA1 region of the hippocampus	Improved context-dependent learning and memory. Normalized dopamine neuron activity and behavioral response to amphetamine	Differentiation: Graft-derived cells expressed GABA (97%), PV (56%), and SST (35%)	Attenuation of hippocampal metabolic activity up to 6 months after grafting No effect on hippocampus neurogenesis
Phencyclidine (PCP) induced mouse model of Schizophrenia	GABA-ergic progenitors from E13.5 mouse MGE	Neonatal mice (P0)	6-weeks after grafting	Survival: ~25% of injected cells	Modulated the activity of callosal projection neurons in the mPFC
Tanaka et al., 2011		Grafts placed into the medial prefrontal cortex	Protected against object recognition memory dysfunction and sensory-motor gating deficits induced by PCP	Differentiation: Graft-derived cells expressed GABA (66%) Integration: Graft-derived cells expressed c-fos when exposed to enriched environment	

Table 2

Studies on GABA+ cell grafts in animal models of neuropathic pain.

Animal model employed	Type of donor cells	Timing and site of grafting	Time-point of analyses and functional benefits	Graft cell survival, differentiation and integration	Effects of grafts on the host brain changes
Rats with unilateral chronic constriction injury (CCI) of the sciatic nerve	GABA-ergic cells expanded from E15 LGE	1-week after injury	1–3 weeks after grafting	Percentage survival not assessed.	Reduced responses of wide dynamic range (WDR) neurons to peripheral stimulation compared to controls
Jergova et al., 2012		Grafts placed into the lumbar segments of the spinal cord	Decreased hyperalgesia and allodynia Reduced exaggerated dorsal horn neuronal firing induced by CCI	Differentiation: grafted cells differentiated into GABA-ergic neurons. Increased GABA immunoreactivity in the grafted dorsal horn Survival: ~1.3% of injected cells	
Spared nerve injury (SNI) model of neuropathic pain	GABA-ergic progenitors from E12.5 to 13.5 mouse MGE	Grafts placed into the lumbar spinal cord segments	7–28 days after grafting		Increased levels of GAD65 and GAD67 (synthesizing enzymes of GABA)
Braz et al., 2012			Reversal of the mechanical hypersensitivity MGE transplants were not effective against inflammatory pain	Grafted cells made functional connections with both primary afferent and spinal cord neurons Non-noxious peripheral input (walking on rotarod) and noxious chemical stimulus applied to the hind paw induced expression of c-fos in grafted MGE cells Survival: ~5% of injected cells	
Mice harboring a deletion of the transcription factor Bhlhb5 (chronic itch model)	GABA-ergic progenitors from E12.5–13.5 mouse MGE	5–6 weeks after birth	2–5 weeks after grafting		No effects on levels of GAD65 and GAD67 (synthesizing enzymes of GABA)
Braz et al., 2014		Grafts placed into the spinal cord	Significant reduction in the size and severity of the skin lesions Substantial reduction of excessive scratching and dramatic resolution of skin lesions	Differentiation: Graft-derived cells differentiated into GABA+ neurons (90%); some expressed PV, NPY and SST Integration: Graft-derived cells expressed c-fos when noxious chemical stimulus was applied to the hind paw Survival: ~6% of injected cells	
Paclitaxel-induced, chemotherapy model of neuro-pathic pain	GABA-ergic progenitors from E12.5 to 13.5 mouse MGE	1-week after the development of hypersensitivity Grafts placed into the spinal cord	1–3 weeks post-grafting		No effects on GAD mRNA levels
Braz et al., 2015			Rapid and significant reduction of paclitaxel-induced heat hyperalgesia and mechanical allodynia		Anti-hyperalgesic effects depended upon GABA release from MGE grafts

Table 3

Studies on GABA+ cell grafts in animal models of Alzheimer's and Parkinson's disease.

Animal model employed	Type of donor cells	Timing and site of grafting	Time-point of analyses and effects on cognitive and/or mood function	Graft cell survival, differentiation and integration	Effects of grafts on the host brain changes
Mouse models of Alzheimer's disease:	GABA-ergic progenitors from E13.5 mouse MGE	10–14 months of age	~3 months after grafting	Survival: percentage survival not reported	Dentate granule cells in grafted animals displayed increased frequency of spontaneous inhibitory currents and increased inhibitory charge transfer
ApoE4-Knock-in (KI)		Grafts are placed into the dentate hilus	Grafting restored normal learning, rescued memory deficits, corrected abnormal activity, anxiety, and exploratory behaviors in aged apoE4-KI mice	Differentiation: Graft-derived cells differentiated into neurons expressing GABA (94–95%), SST (45–46%), NPY (23–24%), PV (12–13%).	Grafting did not alter hippocampal plaque loads or A β levels
ApoE4-KI/hAPP _{FAD} Tong et al., 2014			Grafting rescued both learning and memory deficits in apoE4-KI/hAPP _{FAD} mice	Integration: Grafted MGE cells displayed spontaneous EPSCs and IPSCs of comparable amplitude and frequency as endogenous hilar interneurons	
6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease	GABA-ergic progenitors from E14.5 rat MGE	Immediately after injury	2–3 months after grafting	Survival: ~1% of injected cells	Not examined
Martinez-Cerdeno et al., 2010		Grafts are placed into the striatum	Grafting decreased apomorphine-induced rotations, and increased stride length	Differentiation: Graft-derived cells differentiated into neurons expressing GABA (75%), CB (2–24%), SP (6%) CR (2–8%), PV (0.5%), SST (1–10%), NOS (1%) Integration: majority of graft-derived cells displayed properties of inhibitory interneurons and integrated into the host striatal circuitry	