We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 2

Plastic Adaptation: A Neuronal Imperative Capable of Confounding the Goals of Stem Cell Replacement Therapy for either Huntington's or Parkinson's Disease

Michael I. Sandstrom, Kevin A. Anderson, Naveen Jayaprakash, Parnit K. Bhupal and Gary L. Dunbar

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71790

Abstract

Although stem cell transplant therapy offers considerable promise for deteriorative diseases, the efficacy of its application may be mitigated by endogenous compensatory mechanisms in the host brain. Plastic compensation follows neurodegeneration, beginning at its very onset and minimizing early symptom expression. As researchers attempt to correlate symptom remission with the ability of transplanted cells to adopt specific cell phenotypes, they need to be vigilant of the possibility that competing, local compensatory effects may be altering the outcome. Clearly plastic compensatory mechanisms could confound desired transplant-derived improvements by supplanting the beneficial contributions of the transplants. As circuit-level adaptations occur, more explicit explorations of their relevance to neuronal transplantation success are needed. Conceptual models of undirected transplanted cells adopting preconceived appropriate roles require revision. The notion that newly transplanted neuronal precursors will incorporate themselves into host circuitry with mutual cooperation across both parties (i.e., transplant and host) without some symbiosis-promoting mechanism is naïve. Undirected local circuits could react to newly transplanted additions as intruders. We advocate that appropriate signaling from transplanted cells to the host environment is required to optimize the therapeutic relevance of transplantation. This review surveys critical signaling mechanisms that might promote symbiotic interdependence between the host and new transplants.

Keywords: stem cells, transplantation, Parkinson's disease, Huntington's disease, adaptive plasticity, development

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

1. Introduction

For several years now, efforts have been underway to examine and refine technology associated with promoting the incorporation of pluripotent stem cells from various origins following transplantation into the brains of patients suffering from various deteriorative diseases [1–3], or to test the viability of such treatments using experimental animal models [4–6]. The typical pattern of findings associated with clinical efforts is initial moderate symptom improvement, followed by either resumption of symptoms over time or highly variable therapeutic outcomes [7–9]. Common arguments raised for the mechanism underlying inconsistent effectiveness are that either the transplanted cells are not merging sufficiently with the host brain due to a timely competition-related synaptogenesis process, that transplanted cells are not surviving in the harsh environment of the host due to immune-system/inflammatory host responses, or both [8–11] (also see the extensive review by cell type [12]). While evidence for these arguments certainly exists, it remains unclear whether those arguments cover all the relevant possibilities that threaten the longevity of the transplanted stem cells' utility. One potential threat to the long-term efficacy of this treatment, or to stem cell transplant therapies in general, which is frequently overlooked, would be *plastic adaptation*. Briefly, plastic adaptation represents a multitude of cellular responses that occur with the apparent role of maintaining cellular homeostasis, yet within the nervous system also supports the maintenance of a sort of dynamic status quo in which compensatory changes adjust the actions or response capacities of local healthy neurons in support of a superseding circuit-associated need. Plastic adaptation also occurs within the physiologically healthy brain in order to adjust for novel needs, supporting changes such as long-term memory, habit formation, and other sorts of behavioral adaptation of organisms to new surroundings and demands (for examples see [13, 14]). A surge of inquiry into what are now known as epigenetic mechanisms supports the notion of a clear capacity of cells to respond to environmental stimuli by generating enduring changes in their genetic expression [15–18]. This, combined with numerous demonstrations of more transient receptor plasticity [19–22], defines neuronal cells as versatile in both short- and long-term periods in adjusting to their neurochemical and electrophysiological circumstances at their membranes and within their nuclei, respectively. Following transplantation, it is likely that plastic adaptation responses could occur in both populations of neuronal cells of concern, either the transplanted cells or the surrounding host cells that likely interact with the transplanted cells.

During nervous system development, the mechanisms that guide the distributions of cells and their connectivity offer a far more forgiving flexibility when compared to the harsher, more demanding adult environment we face when attempting to correct deterioration with transplants [23–26]. Growth distances for neurites are shorter given the smaller neuropil, and more overt chemical gradients support pathfinding [27–29]. A developmental neurogenesis surge supports self-repair in the event of cell destruction because phenotype commitment is guided by a progressive fulfillment of niches and feedback signals once niches are filled [30–34]. Differential neuronal responsibilities within developing circuits are coaxed into existence in the context of an enhanced adaptive plasticity on either side of synaptic clefts, where each contributes to phenotype adoption of the other while it is determined what they

might contribute to the developing circuit [35, 36]. As needs are met, postsynaptic neurons decrease their encouragement of subsequent equivalent connections by adapting their signaling [37–40]. How new afferents drive or control action potentials contributes significantly to circuit behavior, depending on factors as subtle as the proximity of synapses to a target neuron's trigger zone, while on the postsynaptic side the development of the trigger zone may modify when and how action potentials arise [41–46]. Incorporation into circuits relates to both identity and survival as neurons develop. As the neuronal phenotype is established, developing cells become increasingly dependent upon both afferent and efferent connections to other neurons. Neuronal fate seems to result from aspects of stimulation in the context of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). Excitatory postsynaptic potentials known to increase intracellular calcium seem to participate in driving developmental determinations. This was shown by a series of experiments performed on precursor cells *in vitro* where calcium chelation blocked the establishment of neuronal phenotypes normally induced by either electrical or NMDA-glutamate stimulation in conjunction with BDNF [47–49]. Thus, precursors that receive insufficient controlling input to engage their activity likely adopt non-neuronal, glial, or support cell status, modifying or diminishing their contribution to circuits. When growing neurons establish connections to other neuronal populations, this provides them with target-derived trophic support that staves off programmed cell death that is likely to occur in its absence [50–53]. Surviving long enough to establish contributions is of course also important for transplanted populations, but evidence indicates that this is easier in the more forgiving context of development. A very useful series of explorations documenting how transplantation faces diminished success as the host ages was thoroughly documented in a mini-review by Sally Temple [54]. In addition, often the younger and less "experienced" or "committed" precursor cells are shown to more easily adapt into their transplanted roles than similar, yet older, populations [55–57]. In other contexts, such as the ability to properly generate blood cells following bone marrow transplants, younger donors seem to yield more successful results than older donors, indicating this age-dependency is not limited to neuronal populations [58, 59]. The similar goals of establishing appropriate cell populations to fill various niches following transplantation suggest if the environment were less competitive or more accommodating, and cells were guided by the more overt signals available during development, the process of incorporation would be more straightforward. In the adult brain, the mechanisms of plasticity engage to maintain the continuity of established function with mechanisms to prevent deviation from working systems; otherwise all nervous systems would constantly deteriorate into chaos. Thus, while similar concerns are present with transplantation, (i.e., coaxing the new cells to make useful and appropriate contributions to established circuitry), we cannot expect that new additions will naturally get swept into correct and working interactions the way they do during development.

To tease out the contributions of plastic adaptation to the success or longevity of stem cell transplantation therapy, it seems there is a need to expand inquiry further than whether transplanted cells develop into neurons, survive, or form mutually integrative connections with endogenous neurons. It appears equally important to determine how the cell populations influence each other and how each population adapts to this influence over time. There may be important clues

to the mysteries surrounding the impermanence of replacement therapy in how these populations adjust to the presence of the *other*. This chapter was written to consider current knowledge about plastic adaptation as it pertains to the act of incorporating transplanted neuronal cells or precursors into a damaged host brain. In addition, this review represents a general call for more direct inquiry into this subject in future efforts to explore and hone such a promising therapeutic technology. If plastic changes compromise the capacity to maintain symptom-suppressing benefits of these transplants, solutions to this will likely require more than tracking the quality and longevity of behavioral benefit or the anatomical persistence of the transplants over extended periods. Success may be enhanced by recognizing the ongoing patterns of plasticity with which transplanted neuronal cells must cooperate to earn the opportunity to contribute. Given that the age of both the cells transplanted and the host into which they have been transplanted are relevant to their incorporation and therapeutic efficacy, it appears that the capacity to adapt into the new environment depends on factors or signals from both elements that need to be understood to support moving forward intelligently with this therapeutic endeavor. The remainder of this review will address concerns regarding the host adaptive responses to the transplant as well as the transplant's adaptive response to the host that ought to be considered in this regard, focusing largely on efforts with Huntington's and Parkinson's disease.

2. Achievements of "successful" transplantations

Therapeutic support derived from neural transplantation likely necessitates circuit-level reconstruction so that certain missing neurobehavioral actions are restored. However, it is important to acknowledge that circuits can be supported by either the addition of new neuronal contributions that might restore disconnected components or by bolstering the inherent capacity of compromised circuits to adjust or compensate. The brain's inherent capacity to compensate for damage/disruption or "repair itself" is considerable and likely the reason why physical or occupational therapies support function restoration. Trophic support and other general support of persisting residual circuit components, receptor sensitivity adjustments, sprouting, and several other inherent mechanisms contribute to reparation (for an extensive review of these mechanisms see [60]). These trophic or supportive contributions can be accomplished by non-neural cells or glia that likely contribute mostly indirectly to neuronal circuit actions. In fact, Blurton-Jones and colleagues [61] demonstrated that transplant-derived BDNF was eventually responsible for supporting cognitive improvements in a rodent Alzheimer's model by promoting enhanced synaptic density in the hippocampus between preexisting neurons. Thus, it appears that either the transplanted cells become active contributors to the circuit or they support the existing circuit that itself seems to engage compensatory mechanisms supporting at least partial function. Therefore, it appears beneficial to respect that plastic adaptation persists as an ongoing process, regularly promoting positive improvements in functional circuits, and that a *successful* contribution of transplanted stem cells to existing neural circuitry necessitates a recognizable supportive contribution to this endeavor. It is our overarching concern that transplant efforts do not typically respect this context, usually holding a more direct circuit reconstruction as paramount with the presumption that the host brain will somehow also recognize our clinical perspective and modify ongoing adaptive mechanisms accordingly. When

this does not happen, we appear surprised that transplantation efforts impede healthy behavior restoration over time, or show diminished effectiveness over time—but we should not be.

While beyond the scope of this chapter, we nonetheless feel it is important to also acknowledge the prominence of neuronal circuit dependence on both use and the ongoing local actions of the immune system. It is likely that a repetitive drive on the circuit due to the person or animal engaging in systemic practice to recapture the skill they once had also supports circuit-level adaptation. This is likely why Parkinson's patients who regularly move and push themselves to actively engage compromised limbs rather than remaining sedentary reap clinical benefits from those actions [62, 63]. It is intriguing to consider these effects in the context of their ultimate therapeutic mechanisms which promote restoration or strengthening of key circuits with positive contributions to adaptation efforts, as well as the fact that once a part of a circuit, transplant-derived neurons may require practice to become proficient in their established roles. Of course, immune rejection and the broader context of inflammation also enter into this equation, given the common desire to utilize transplants in the context of deteriorative diseases or trauma-related damage. While it is arguable that convincing the immune system not to overreact has been extensively studied as a factor in this context (e.g., [64]), the inflammatory response certainly has the capacity to tailor the very adaptation mechanisms we will discuss (e.g., [65]). For extensive reviews of the reciprocal interactions between neural systems and inflammatory systems relevant to plasticity see Di Filipo and colleagues [66], or Xanthos and Sandkuhler [67].

Do the new additions engage with the existing circuits in positive adaptation-enhancing ways? Along the way it appears that there are adaptations on both sides that might enhance or diminish this relationship. If the adaptations diminish this circuit-supporting relationship, then the ability of the new transplanted cells to continue their presence and effectively support positive behavioral improvements will likely be lost and the clinical efforts of transplantation will likely be considered insufficient or transient. Alternatively, if the adaptations that occur enhance the circuit-supporting relationship while avoiding interfering with ongoing adaptive efforts, the success may extend further than the initial witnessed improvements into continuous ongoing improvements, rather than plateauing at some yet incomplete recovery. In this chapter we will divide our appreciation of transplantation-related plasticity as new cells establish roles contributing to existing yet compromised circuits first into whether the endogenous circuit adopts the newcomers as team players, and second whether the transplanted cells adopt the roles required of them to contribute to the circuit or not.

3. Adaptation of endogenous host tissue to neural transplantation

Although adult neurogenesis was overlooked in the past and neuroscientists were convinced that new neurons were not produced beyond the early stages of development, it has now been demonstrated conclusively that there are select regions in the brain that regularly accept new neurons into established circuits that are derived from precursor neuroblasts that retain mitotic capacity throughout our lives, and divide asymmetrically to produce new neurons as

daughter cells (for review see [68]). Two key areas that benefit from this neurogenesis would be the hippocampus and olfactory bulb, and the degree of this natural new neuron incorporation depends on the activity levels generated in these regions. Specific functions that rely upon neurogenesis include new learning for the hippocampus [69], and rich olfactory sensory experience for the olfactory bulb [70]. As this occurs regularly in an activity-dependent manner already, it stands to reason that neuronal precursors transplanted into these regions would be more likely to receive signals encouraging their incorporation into either the hippocampus or the olfactory bulb, and in fact, this seems to be the case [71, 72]. Yet in other regions such as the striatum (the main input structure of the basal ganglia), the capacity of endogenous neuronal progenitors to become neurons seems reduced compared to exogenous transplantations [73]. The answer to why this distinction exists has been a top priority among those of us who foresee more successful replacement therapies. While the whole picture is not available, what seems clear is that there is an interactive relationship between the endogenous host cells and transplanted cells at the center. The so-called "neurogenic" regions (hippocampus and olfactory bulb) where replacement happens regularly as part of the natural progression throughout our lives would likely not be a useful target region for clinical transplantation for any key neuronal disorders, given that their potential for reconstructive replacement remains high. Yet we might initially presume that the mechanisms encouraging incorporation, such as the guidance molecules used and trophic factors encouraging survival as connections are established, or the afferent connections grown into and onto the transplant cells as the afferent component, may follow rules similar to transplant events elsewhere.

When precision is required in the placement of axon terminals, it would seem that the parameters for what might be considered functional success would be correspondingly more restrictive or demanding. Here it is appropriate to briefly describe how establishing a wide range of general chronic dopamine can provide considerable benefit in Parkinson's disease, and the distinction between "open" diffusion-capable release mechanisms versus "closed" synaptic connections circumscribed by glial borders. Due to the chronic widespread levels of dopamine persisting in extracellular space, simple diffusion-based neurotransmitter delivery is often discussed without emphasizing the more nuanced details of precise release. To illustrate, in the case of dopamine loss in Parkinson's disease, the standard drug levodopa promotes endogenous release to higher global levels without significant dependence on direct synaptic integration of the remaining endogenous dopamine neurons, as a large majority of these are gone when this treatment is prescribed (presumably after at least 70% of the endogenous innervation deteriorates). Also, dopamine-lesioned experimental model animals have been improved by treatment with synthetic slow-release nanoparticles [74] or transplantation of genetically modified fibroblasts [75] that likely neither need, nor have the capacity to respond to, afferent control. In this context these treatments, as well as the dopamine systems considered, are seen as utilizing volume transmission or "open" synapses that tend to increase release levels over larger areas, based either on simple diffusion mechanisms or low-level chronic stimulation. By contrast, there are systems that rely on comparatively local transmission or "closed" synapses that are locally circumscribed by glial cells to certain synaptic junctions, and usually depend much more heavily on the timing of inputs for their function. Systems utilizing volume transmission would, by this definition, present an ambiguity to whether they necessitate as much acceptance into the network [76, 77]. So long as they provide the requisite compound this

contribution may suffice, at least initially, in bolstering the circuit in question, though release would need to take place in key areas to be effective. Dopaminergic inputs seem to exhibit both of these characteristics ("open" and "closed"). This concept, commonly overlooked in the clinic, will be expanded upon later.

Another aspect of transplant-related plasticity is the extent to which neurons are transplanted into a typical or atypical host environment for the neuron type that is their intended end-point for the current therapy. It is clear that during early fetal periods, useful progenitor populations can naturally undergo sufficient prior developmental modification to become predisposed to becoming a certain common neural type and can be found in regionally distinct populations in the fetus. For example, cells from the lateral ganglionic eminence show a high propensity to become GABAergic striatal neurons [78], or cells from the fetal mesencephalic region show a high propensity to become dopaminergic type neurons (e.g., [79]). Specific developmental trajectory predispositions can also be coaxed from progenitor cell populations *in vitro*, where the approximate mitogens, epigenetic cues, and morphogenic signals are maintained and progressively modified to encourage specific phenotype development trajectories [6, 80, 81]. Once neural developmental predisposition is established, it is perhaps fair to suggest that there are some host regions in which predisposed neurons would thrive as they would be placed into a "familiar" environment (i.e., a *homotypic* host region; e.g., GABAergic medium spiny predisposed neurons transplanted into the striatum) and some host regions that would *not* represent environments that might foster familiarity (i.e., an *ectopic* host region; e.g., dopaminergic destined neurons transplanted into the striatum). Precedent for this homotypic versus ectopic distinction has been set [82, 83]. Keep in mind that this distinction is made to capture the regional relations of predisposed neurons for certain locations, and functional benefit concerns are secondary.

For years, neuroscientists have been studying the anatomy of neuronal populations of various types that produce different neurochemical compositions throughout the brain and the corresponding afferent connections that grow into and drive activity in these different regions. Establishing appropriate afferent drive onto the neurons that are transplanted would be a clear sign that the circuit into which the transplanted cells need to merge has accepted them as part of the equation. Clearly then, when placed into a homotypic host region this sort of acceptance would be more likely based on the proximity of the transplanted cells to appropriate afferent input that such cells need to be driven properly by the host brain architecture. The prime example of this sort of transplant that has shown considerable acceptance into the host circuit and was extensively characterized by Klas Wictorin in 1992 is the intrastriatal transplant of striatal-predisposed precursor cells obtained from the embryonic day 14–15 fetal lateral ganglionic eminence following an excitotoxic lesion of the host striatum [84]. The extensive host innervation of this transplant along with the extensive growth and integration of the transplant with the host in the context of circuit re-establishment was dramatic, long-lasting, and seemed to contribute considerable support to the lesioned circuit as seen by neurobehavioral improvement. Wictorin indicated that the initial destructive lesion to destroy local endogenous striatal neurons is crucial for enabling the sort of host integration seen, as the absence of such a lesion (i.e., transplantation into an intact striatum) yielded far less integration [85]. It stands to reason this would occur because afferent inputs would find greater ease in filling an open void or niche so long as it maintains a general presence after

the lesion. A transplant without deteriorative or destructive loss would also be unnecessary because, as stated, the transplant is meant to restore the lost contribution. Wictorin describes a considerable ingrowth of afferent inputs from the host brain into the transplant with cortical, thalamic, nigral dopaminergic, and serotonergic inputs from the raphe that showed extensive yet differential degrees of penetration into the graft [84]. Also relevant was the point made about how regions that did not appear "striatum-like" seemed far less capable of inducing dopaminergic ingrowth and that specific transplants of cerebellar or cortical tissue into the excitotoxin-lesioned striatum of adult rats yielded no such dopaminergic innervation. Electrophysiological experiments demonstrated that these innervations of the graft were synaptically functional, supporting host-originating cortical drive [86, 87], and functional dopaminergic modification of GABA release from transplanted cells into the globus pallidus and the substantia nigra reticulata [88].

Although speculative, it is possible that even if the neurons transplanted into the striatum that became GABAergic did not grow extensively into the host tissue and participate more fully in the host basal ganglia circuitry, some circuit support might be established by enhancing only local GABA release from these neurons with limited incorporation as exclusively interneurons. It has become clear that in the context of the Huntington's disease condition, even prior to the major deterioration of striatal cells, there is considerable and abnormal spontaneous activity within the dorsal striatum [89, 90], and it appears that overactive glutamate release or diminished reuptake transport of glutamate is at least partially to blame for this [91, 92]. Under these circumstances, a considerable disruption of striatal function might arise due largely to this main input region being considerably noisier than normal (electrophysiologically speaking), and under such circumstances the proper selection of outputs would necessarily become challenged. If transplanted cells were simply driven by locally increased glutamatergic inputs or the ambient glutamate levels, after which they proceeded to feed back onto local medium spiny projection neurons in a manner that minimized this noise, some presumed information processing capacity might be restored, despite the lack of full integration.

To highlight the behavioral relevance of induced electrophysiology on transplanted cells, our laboratory initiated a project that involved preliminary transduction of transplant-destined neuronal precursor cells harvested from the subventricular zone of neonatal rats (P1 to P2) with Channelrhodopsin-2 (ChR2). This receptor construct allowed for rapid and exclusive optogenetic stimulation of these cells as they became functional neurons activated by blue light. The construct also contained a transgene with a synapsin promoter as well as code for enhanced yellow fluorescent protein (EYFP) for visualization post-euthanasia. It was our interest to explore the propensity of these transplanted cells to incorporate with the circuitry of the otherwise intact dorsal striatum in a manner that would allow a movable skull-mounted iontophoresis/single-unit electrophysiology electrode with fiber optic light incorporation to locate transplanted cells by slowly moving dorsoventrally across the striatum of a freely-moving rat and searching for units that would respond to various local stimulations. There were three stimulation types that could be generated: iontophoresis of glutamate, stimulation with 473 nm blue light, and behavioral stimulation. The advantage of this strategy was that only cells transduced with ChR2 (i.e., the cells to be transplanted) would show photosensitivity to blue light. Several interesting findings arose from this work. Previous work with a similar iontophoresis electrode (modified merely to allow the inclusion of a narrow fiber optic

cable for light stimulation in the present study) had clearly shown that rats with intact striata exhibit largely low levels of spontaneous activity [93]. Thus this technique that uses pulled 4-barrel glass electrodes (recording via 3 M NaCl, and iontophoresis of 0.25 M glutamate with a 0.25 M NaCl balance barrel and a narrow fiber-optic cable delivering blue light through the final barrel, wiring and fiber-optic cable connected to a combined swivel apparatus above the chamber) took advantage of a movable electrode holder allowing multiple exploratory passes through the dorsomedial striatum while monitoring the extracellular field for potential signs of single unit activity. Iontophoresis of glutamate was our global stimulant capable of activating any striatal neuron in proximity whether it originated endogenously or from the transplant.

We went in expecting a low yield of photosensitive units given established findings that transplanted neurons tend not to incorporate well in an intact striatum (e.g., [84]). In our experiments, each animal received approximately 40,000 neuronal stem cells as 8–10 neurospheres per animal except "controls." The distribution of behaviorally responsive units was similar to previous work with similar electrodes employed without light [93] among controls. Among our main findings was that none of the units that responded to light or light/glutamate combinations also responded to the behavior of the animal. This suggests that although glutamatergic inputs from both cortex and thalamus synapsed functionally with grafts placed into the striatum following an ibotenic acid lesion [84], we saw little evidence of the corresponding freely-moving-animal incorporation that likely would have generated behavior-induced responses in our localized and verified transplanted cells. Of the 40,000 potential contributors placed directly into the pathway of our electrodes, we recorded from approximately 20 light-sensitive cells per animal at 2 weeks and then found a far smaller number (approximately 2-3) at 4 weeks. However it was not the case that the neurons derived from transplants were unable to form connections, as on multiple occasions with the subjects tested at 2 weeks post-transplantation we witnessed responses of what we predicted were spontaneously active endogenous units that were clearly inhibited during light stimulation (see **Figure 1**). None of these light-induced inhibitions were found at 4 weeks, in part because spontaneous activity was also harder to find at this later date. However, this finding and the fact that 2–3 out of the average 20 light stimulation events yielded this sort of response at 2 weeks but not at 4 weeks also suggests the possibility of temporary local synaptic connections being formed and then lost between the periods explored. While it is possible that a certain lack of drive (evidenced by the lack of behavioral drive on light-activated units) may have contributed to their demise as well as inflammatory or immune responses, another finding from this study was particularly intriguing. During the search for EYFP-expressing units at the final stages of these experiments, rats euthanized after explorations at 4 weeks contained far higher levels of fluorescent units merging into the olfactory-destined rostral migratory stream (see **Figure 2**). Newly produced neurons from the subventricular zone (where our neural stem cells were originally harvested) initially follow the edges of the lateral ventricles and then proceed ventrally into the stream headed for the olfactory bulb [94]. Fluorescent cells were consistently found to be incorporated into this system in a more rounded and presumably migratory state that, while still potentially responsive to both light and glutamate stimulation, would not be expected to have incorporated host glutamatergic drive that has been associated with driving CAM kinase II responses and the corresponding cessation of migration and synaptic arbor development [95].

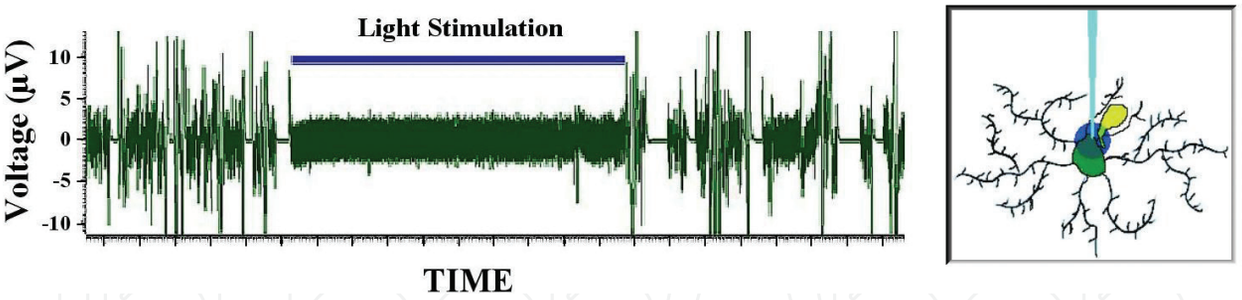

Figure 1. Spontaneous unit activity inhibited by light. Here a spontaneously active unit from within the striatum was inhibited by light. We predict that the connectivity was such that a transplanted (thus light-responsive) unit had adopted a GABAergic transmitter type and when it became activated by local light in sufficient proximity was induced to synaptically suppress the unit it had synapsed upon as depicted in the insert. *Insert Diagram*: Pulled glass electrode depicted descending from top. Darker sphere at electrode tip represents fiber-optic cable-derived blue light stimulation emanating from electrode tip. Small interneuron to electrode right depicts EYFP-expressing transplanted cell presumably sufficiently close to be excited by blue light but not to contribute to recorded *activation* response typical of "direct" stimulation. The recorded medium spiny cell, juxtaposed to the electrode tip, represents the spontaneously-active neuron providing recorded activity that was otherwise insensitive to the blue light barring GABA influence elicited from the sensitive transplanted cell connected to it.

From this study we concluded that when transplants are placed into the less-hospitable intact striatum it is possible that far fewer neurons make synapses with the local host and that when they do at earlier stages, these synaptic connections are far from permanent. It is likely that the unique proximity of the electrode, spontaneously active cell, and photosensitive cell depicted in **Figure 1**, that we hypothesize would elicit the witnessed inhibitory responses, would be serendipitous under the most opportune conditions, particularly considering the scarcity of spontaneously active striatal units within the intact brain (harder to find in general; [93]). Yet the consistency of the findings at 2 weeks and the complete lack at 4 weeks suggests that at least some transplanted units made only temporary synapses that disappeared later either due to cell death or resumption of migration. This may occur when the signals indicating the propensity for circuit interaction by the endogenous host cells are weaker, and there is subsequent reduced effort to formulate more robust and permanent interactions. Therefore, the presumption that once neurons form a synaptic relationship, the newly transplanted cells are somehow

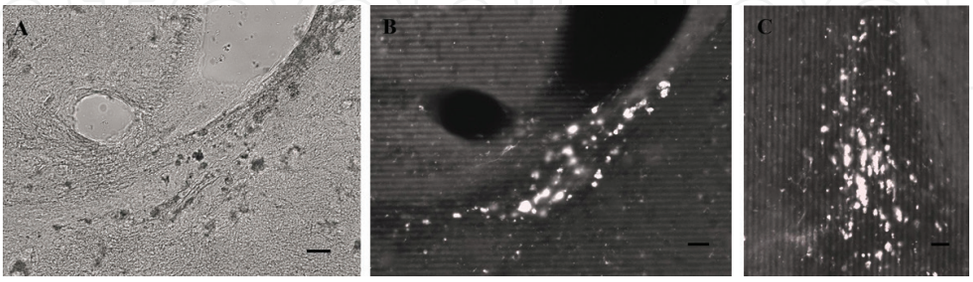

Figure 2. Fluorescent migrating cells at 4 weeks. Shown is a clear mass of cells that had migrated along the ventricles toward the bottom portion of the ventricle seen in both bright field (A) and the fluorescent images of B and C showing migrating transplant-origin cells fluorescing brightly in this location, outside of the recording area of the dorsal striatum. Line segments in each image represent 100 μm. C represents a different region of this clustering from a separate, similarly-treated animal focused at a deeper level of the rostral migratory stream. Bottom of ventricle not seen in C but is just above the upper left corner of image.

safe and likely permanently established is not supported; yet it seems to underlie the persistent sentiment that after certain periods of time, the mere existence of neuronally integrated and transplant-derived neurons in the host tissue represents a *successful* fix for the destruction or deterioration of host tissue. Such reasoning is fallacious and ignores the intricacies and constantly-shifting nature of even seemingly well-established intact neural circuitry.

Others who have transplanted into the intact striatum using lateral ganglionic eminence-derived cells witnessed a correspondingly diminished interaction with the striatal circuit. In fact, following such transplantations, Magavi and Lois [96] found a greater degree of growth into and synaptic integration with orbitofrontal cortex and the claustrum than with either striatal or nigral connections, indicating also the inability of the striatum to attract connections into the homotypic basal ganglia circuit. It is standard procedure that experiments investigating the degree of host incorporation depict dendritic arbors and other signs of likely synaptic input following transplantation, but it would be misleading to indicate that whatever snapshot taken in post-experimentation histology is a fixed and permanent condition. It would run contrary to what we know about natural endogenous synaptic plasticity to believe that any fixed depiction of synaptic status remains a permanent or "set in stone" phenomenon, as we know endogenous synapses are constantly dancing with each other, exchanging connections regularly due to competitive interactions [97]. To compete and participate in this drawn-out request for a place in the circuit, it would be important that sufficient drive is established and, after the driving elements (e.g., corticostriatal or thalamostriatal inputs) are relieved of their targets by prior lesions, there would be a likely increase in terminals seeking destinations, and this is lacking in the intact striatum. It stands to reason that neurons without such drive might continue to migrate until they can position themselves to receive it. Clearly a considerable effort is engaged by both corticostriatal and thalamostriatal afferents to synaptically integrate with striatal grafts that follow target-destructive excitotoxic lesions as well as transplanted neurons that grow far more extensive integrations into the basal ganglia circuitry [84].

As mentioned above, extensive dopaminergic ingrowth occurs from grafts of fetal progenitors into a lesioned striatum, indicating that not only does glutamatergic host innervation likely drive this population, but this population is also modulated by dopamine in a host-controlled manner. Despite this capacity, thus far, most experiments exploring the viability of transplanting cells as a treatment for Parkinson's disease have targeted cells predisposed to become dopaminergic ectopically into the striatum rather than the homotypic substantia nigra. The rationale behind striatal transplantation of these dopaminergic-destined cells rather than transplanting the cells into the substantia nigra region is largely because of the expectation that neurons transplanted into the substantia nigra region would not be able to grow axonal extensions sufficiently through the relatively inhospitable terrain of the adult brain to deliver the needed dopamine into the striatum. Also, striatal transplants would likely provide comparatively more dopamine in the target region. This concept was formulated by Anders Björklund and his collaborators [98, 99] as the idea of dopaminergic tissue transplants for Parkinson's disease was initially proposed. Previously described limitations to extensive axon growth through the adult CNS would clearly support this notion. Thus, the large majority of the experiments exploring replacement transplantations for Parkinsonian circumstances targets the dorsal striatum and would fall into the category of ectopic host destinations.

The question remains largely open how such cells, when they become dopaminergic neurons, will integrate into circuitry, given that they are typically stimulated at their cell body level by glutamatergic signals entering into the substantia nigra. In fact, the substantia nigra seems to receive active control inputs originating from the subthalamic nucleus and the somatosensory/motor cortex, both of which are touted as being capable of initiating rapid responses to salient events [100]. By comparison the cortical drive on striatal neurons tends to be highly converged on any individual medium spiny target from across relatively wide regions of the cortex, an organization that would require high collaboration between multiple regions to activate any single striatal neuron [101]. This common convergence is likely to be partially responsible for the relative silence observed within the striatum while otherwise intact animals are at quiet rest. The chances that cortical input into the striatum would even closely approximate that obtained from the cortical and subthalamic input to the substantia nigra is therefore low to begin with, let alone the serendipity that would be necessary to result in all such transplanted dopaminergic neurons stimulated by the same subset of glutamatergic afferents. This would severely challenge the precision of drive on these transplanted neurons, with terminations more haphazard across the transplanted population, resulting in a much more *constant* degree of afferent stimulation.

Explorations of the corticostriatal input into dopamine-predisposed grafts of fetal mesencephalic tissue have yielded mixed results from inputs that appear to take on the morphologic appearance of nigral cortical input (thick fibers giving off thin collaterals; [102]). Another point raised more recently by Braak and del Tredici [103] is that striatal medium spiny neurons tend to lose their spines over time during the ongoing pathology of Parkinson's disease and that dopaminergic inputs in the intact striatum of otherwise healthy subjects seem to interact in a complicated modulatory manner on spine shafts while the corticostriatal terminals engage their tips. As this arrangement is progressively lost, the ability of dopaminergic grafts to successfully interact with the main projection medium spiny efferents may also be jeopardized. If the drive on grafted dopaminergic neurons in the striatum is not well controlled, the ability to duplicate distinct periods of phasic release that mark events of behavioral significance may be missing from the grafted dopaminergic neurons.

Most synapses engage plastic mechanisms that adopt diminished responses to non-dynamic and unchanging levels of drive in a manner similar to the way sensory systems habituate to consistency. We know that rats given large unilateral 6-OHDA-induced lesions of dopaminergic input to the striatum tend to respond within days to apomorphine stimulation in a manner that depends upon postsynaptic modifications that establish "supersensitivity," and that there are modifications of dopamine receptors related to this occurring for extended periods following the lesion [104]. However, it has also been shown using equivalent lesions in mice that their rotation intensity diminishes over time when their lesioned hemisphere is continuously treated with apomorphine using an osmotic pump, suggesting that these modifications that support the supersensitivity *compensation* are reversible when sufficient dopaminergic stimulation remains persistent [105]. Processing the degree of postsynaptic responsivity to dopamine levels with a behavioral assay is common, since it is likely that adjustments in dopamine receptor sensitivity are continuously occurring in response to the degree of stimulation in a manner that stabilizes responses over time (e.g., [106]). It is interesting to note that, although very popular in the literature, recording diminishing rotation in response to transplantation has been deemed more distinctly inadequate

for processing transplants as only a very small boost in dopamine-producing capacity (such as 100–200 surviving transplanted neurons) seems sufficient to eliminate amphetamine-induced rotations by providing both chronic amphetamine-driven dopamine in general to the dorsal striatum [83]. Keeping this in mind, the ongoing adjustments in postsynaptic, and potentially presynaptic responses as well, are likely to reduce the dynamic responsiveness of the transplant-established dopamine system as seen by researchers, cautioning us about the inadequacy of drug-induced rotation in capturing underlying recovery dynamics [107].

The insufficiency of phasic release restoration also likely underlies the inability of Parkinson's patients on L-Dopa replacement therapy to rapidly adjust to ongoing motoric demands [108]. To accomplish a relative surge in dopamine release at a critical behavioral juncture such that the presence of dopamine provides sufficient ongoing support during more emergency situations, such as the need to escape from entrapment or predation, or falling into a body of water and needing to swim, phasic firing of nigrostriatal neurons occurs. The fact that gap junction connections have been found between nigral dopaminergic neurons and that electrophysiological behavior of the nigral population as a whole maintains consistency indicative of such electrotonic coupling [109, 110] suggests that wide ranging locations within the anterior striatal targets receive temporally consistent bursts as a result, in time with the events necessitating dopaminergic modulation. This phasic firing was recorded by Wolfram Schultz from dopamine neurons in the primate ventral tegmental area in his famous experiments that showed the cues responsible for generating increased drive on these mesolimbic neurons shifted from the initial pure reinforcement toward environmental cues *predictive* of the reinforcement and/or the risk associated with the reinforcement [111–113]. It is likely that what engages phasic drive among those neurons that support proper motivation in the arena of learning conditioning is driven in a manner somewhat distinct from the phasic drive on dopamine neurons that serve movement-related calculations within the dorsal part of the striatum. As we approximate human viability of transplantation, it is perhaps fair to mention that the borders of these two dopamine systems within the broader striatum of primates may not be as simple as their general projection parameters, and there is considerable overlap between the ascending dopamine systems (as described in [114]); there has nevertheless been a relatively consistent distinction made in the functional attributes of the projections. The apparent overlap may support the anecdotal events we have heard of where an immobile Parkinson's patient can initiate movement toward the exit of a building should this patient hear warnings of "*fire*" being exclaimed locally, though a stress-related release would be phasic.

Striatal cholinergic interneurons of the large aspiny variety are more likely to be tonically active for larger proportions of time than the main population of medium spiny GABAergic neurons, so their contributions to the ongoing processing within the region can be described as dynamic. These interneurons are controlled in a complicated way by dopamine, glutamate, and local GABA signals. Upon deeper scrutiny, the dopaminergic control of these large aspiny cholinergic neurons has been shown to involve differential employment of glutamate co-released from dopaminergic terminals along with dopamine between dorsal and ventral striata, rendering these regions distinct in how acetylcholine is driven [115]. The common understanding of the interaction between dopamine and acetylcholine in the striatum is that it is inverse, such that phasic bursts of dopamine lead to phasic pauses in ongoing activity among the large aspiny

striatal cholinergic neurons. The inverse responsivity to reward in the striatal systems driven by the dopaminergic input akin to what Schultz recorded from was clearly demonstrated by Morris and colleagues [116] in their work that looked at the dopamine responses simultaneously with the cholinergic responses. The complexities of that interaction and the manner in which it may in fact capture an extensive range of guidance information has been thoroughly described (e.g., [117]). Acetylcholine is unique compared with other transmitters in that the enzyme acetylcholinesterase that is abundantly expressed externally breaks the molecule down rapidly and restricts the domain of effectiveness to localized regions. This is relevant to our story because acetylcholine modulates dopamine release at the terminal level [118], adjusting the release that may otherwise be driven by afferent stimulation at the cell body level. In fact this level of local cholinergic control is likely to be driven differently by thalamostriatal versus corticostriatal origins of glutamatergic drive [119], providing those two differential systems unique access to this key transmitter input. On top of this, it has become clear that glutamatergic input also controls local dopamine terminal release in a receptor-dependent manner such that dopamine release may well be modified by both local striatal (reviewed in [120]) and distal nigral/VTA mechanisms. As described before, these inputs find their way in and around the spines expressed by the medium spiny neurons such that glutamatergic inputs to the spine tips get molded by dopaminergic, cholinergic, and GABAergic inputs engaging spine or dendrite shafts (see **Figure 3**) to maintain the proper final output signal that proceeds through the basal ganglia. To be effective, the timing of release would need to be carefully controlled as well as proximity. It is clear that these temporal dynamics contribute to the utility of each differential transmitter contribution, and to the overall effectiveness of the projecting efferents carrying signals to further destinations in the basal ganglia loops, as this aspect of basal ganglia function has been reviewed and explored extensively [121–123].

In the context of the present review, it is relevant to pause and ask ourselves if ectopic transplants of dopaminergic neurons into the striatum, during the course of or following extensive destruction due to Parkinson's disease, can approach the dynamic control present in the otherwise intact system. These transplanted dopaminergic neurons would likely be connected to rather haphazardly by corticostriatal or thalamostriatal glutamatergic, local cholinergic or GABAergic interneurons, and already lack the over-arching control of phasic release that is typically driven at the substantia nigra. The local control features described above have led investigators to suspect that dopamine release within the striatum might be *considerably independent* of nigral control [124], further supported by both anatomical evidence of striatal-derived fibers growing into grafts [125], and electrophysiological evidence showing approximately 50% of grafted mesencephalic cells being activated by frontal cortex stimulation [126]. However, none of these supporting findings suggest any clear resumption of the temporal dynamics of dopamine modulation in a manner that might be expected to fully restore behavioral versatility. Not only this, but such ectopic transplantations would likely lead to a dispersion of neuronal soma due to migration throughout the striatum that, while often seen as a positive attribute given the likely corresponding breadth of dopamine contribution, would also render the temporal release control much more regionally distinct. The previous point about gap junction connectivity between dopaminergic neurons suggests that at least initially, electrophysiological phasic firing is driven in a more unified manner that is more likely to be

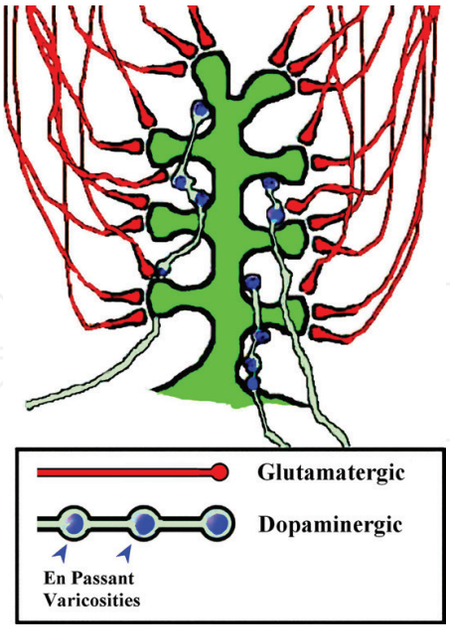

Figure 3. Simplified standard medium spiny dendrite arrangement. The prominent central aspect represents a medium spiny cell dendrite expressing standard dendritic spines. Onto this, many narrow glutamatergic inputs are depicted as synapsing onto the tips of dendritic spines, while dopaminergic input travels upward juxtaposing en-passant varicosities onto spine or dendrite shafts, modulating receptive states in a coordinated manner. Dopaminergic inputs are temporally enhanced by coincident phasic stimulation at the level of the nigra along with electrotonic coupling of nigral neurons. Proximity of cholinergic and GABAergic inputs not shown but each transmitter contribution influences the other either directly or indirectly through their converging influence on the medium spiny striatal efferent.

behavioral-event-related rather than based on local control, with local control as a secondary mechanism. As diffusion of the released dopamine occurs between such contributing neurons, this differential release control would likely establish a new consistent ambient chronic level, lacking a temporal relationship with sensorimotor events that dopamine is meant to modify, providing progressively decreasing phasic relevance to the circuit over time. At this point, if the host system has not adapted in ways that reduce dynamic sensitivities, such dopamine contributions may elicit disruptive effects, such as dyskinesias [127, 128], though other research calls this into question [80, 129]. Certainly the diminished temporal control of striatal dopamine release would be a reason for diminished support of dynamic behavioral control, and might be argued to underlie the longevity of a graft's therapeutic effectiveness.

4. Adaptation of transplanted neural cells to the endogenous host tissue

It is nearly impossible to distinguish between host-to-transplant and transplant-to-host communication because the interactions between both populations are so intimate. However, for the flow of this review, we decided to first address mechanisms of concern regarding modifications engaged by local host cells such as their growth into the transplant and efforts to exert control. Now, we turn our attention to the manner in which transplanted cells likely

recognize what they should do and how they grow into the host with an effort to exert control. As indicated previously, there are certain pro-generative regions such as the olfactory bulb and the hippocampus that are known to accommodate transplantation of new neural cells into their architecture more readily. These structures engage a system already established to continue adding new neurons into their circuits with adjustments according to demand regularly throughout adult mammalian lives, while other structures more commonly experiencing disease-driven deterioration are, unfortunately, less accommodating. Certain practical matters come into play when arranging a protocol for transplantation that limits how developmentally committed the transplant-destined population will be, deviating from the ideal circumstance of, for example, generating pure populations of dopamine and cAMP-regulated phosphoprotein (DARPP-32, marker for the classic resident medium spiny type) expressing GABAergic neurons for striatal transplant in Huntington's disease. While it is often an implicit goal to develop purified populations of only desired cell populations for transplantation, the context of "normal" developmental phenotype adoption seems to be modulated quite handily by local glial cells. If not somehow biochemically prevented from doing so, pure stem cell populations also typically develop into mixtures of neurons and glial cells in a manner approximating that expressed developmentally, with more glial cells than neurons, because the signaling molecules responsible for producing this distribution derive from the manner in which neuronal populations distinguish from epithelial populations with both distal and surface interactions [130]. By comparison, the more purified populations of specific neurons raised *in vitro* by various groups [131], would require considerable artificial manipulations compared with natural development.

As described above, previous transplant efforts have utilized populations of cells that have already developed a predisposition toward neuron types that have been responsible for some cells thriving in homotypic versus ectopic target destinations. Thus, when working *in vitro*, complete pre-differentiation and purification of specific neuronal populations requires extensive prior modification, and these efforts seem to benefit from the inclusion of developmentally-consistent proper local glial cell support [132–134]. *In vivo*, this supporting role seems largely adopted by astrocytes that enhance survival [135]. Neuronal differentiation seems to occur in conjunction with an effort to extend neurites and seek connections; a sensible concept given the previously-described importance of being connected to, and making connections in, the developing organism. After culture, the cells in question need to be lifted from their culture conditions and placed into a delivery mechanism (typically a syringe), for transplantation. It has been pointed out that primary neuron cultures might be particularly sensitive to the trypsin dissociation step and suggestions that more gentle procedures, such as papain, for dissociation of these cells have been made in recent scientific communications [136, 137], along with the notion that extensive floating cultures also face challenges. Therefore, researchers frequently opt for transplantation prior to full neurite extension and interconnectedness, even prior to full neuronal commitment, with the presumption that remaining neuronal commitment will occur *in situ*. Transplants often occur with suspensions of dissociated individual cells or of neurospheres (for review of neural sphere transplant contributions see [138]). If final neuronal commitment occurs after transplantation then, to some extent, this process will be guided by environmental cues within the host tissue.

Clearly, to adopt a specific neuronal fate with exclusive neurotransmitter-releasing properties, there are specific genes that must be expressed, as each phenotype would require either exclusive gene expression to produce or post-translationally select specific neuropeptides for release, or to process precursors enzymatically to generate classic neurotransmitters. These genes and immediate products have generated a new phenomenology that captures the immunohistochemical characterization of cell types in modern histology. It has, however, become abundantly clear that gene expression responsible for this is controlled by complicated internal processes that, despite early presumptions to the contrary, are not permanent or irreversible. The more prominent of these involve histone manipulations such as acetylation or methylation of DNA. This leads to the DNA source of specific genes becoming buried within inaccessible Gordian-like knots, which may be unwound to allow for DNA transcription under certain contexts. Current technology maintains the clear capacity to take committed cells and reverse this genetic commitment to generate what is now called "induced pluripotency," essentially restoring a precursor status to cells, even after they have become a more standard somatic type. In order for this induced pluripotency to be remotely possible, as well as subsequent guided induction of specific types of neurons from such cells, some genetic propensities must remain, even in cells that have outwardly adopted noticeably distinct fates. Early explorations of neuronal phenotype commitment involved explorations of the bizarre and seemingly extreme tendency of sympathetic neurons that ended up targeting the sweat glands to *convert* from a noradrenergic to a cholinergic phenotype [139]. Considerable controversy surrounded the search for the target-derived factor that was responsible for inducing this switch, which was clearly necessary given the cholinergic receptor population of the gland, and research centered on a cytokine family molecule [140]. The capacity of neurons to switch transmitter expression or take on more complicated forms of expression during development is broader than this [141], but these examples clearly demonstrate that the mechanisms underlying neuronal phenotype determination remain versatile and responsive to external signals, even after neuronal differentiation. This suggests that precursor cells would harbor an even more versatile capacity to properly respond to host signals and, as such, merge into the circuit in a regionally attentive manner.

The process of becoming a neuron is sensitive to the degree of electrical stimulation in that the corresponding calcium increases tend to facilitate neuronal differentiation [47]. In the case of the earlier stage fetal development following more complex anterior-posterior differentiation of the neural tube and the establishment of prosencephalon, mesencephalon, and rhombencephalon, more distinct neuronal populations begin to emerge due to specific morphogen combinations and temporal sequences of exposure [142, 143], with certain regions producing environmental signals conducive to specific neuronal subtypes. As glutamatergic neurons distinguish from GABAergic neurons within regionally distinct sub-areas of the subventricular source of new neurons, basic helix-loop-helix transcription factors are induced to kick in, such as neurogenins, leading to a glutamatergic fate, and Mash-1 along with the "distal less" homeodomain genes presumably induced into expression by positional signals Dlx1 and Dlx2 that seem to promote a GABAergic fate, with the degree of initial neurogenin or Mash-1 expression a seemingly deciding factor [144]. When cells that are transplanted into the early developing nervous system are evaluated for the expression of regionally specific markers, such as

these transcription factors, they seem to express themselves as if they pay little heed to their ectopic location. For example, one experiment demonstrated that only about 6% of the transplanted cells that took up residence in the striatum expressed Dlx immunoreactivity, indicating a GABAergic trajectory, while 37% of the transplanted cells residing in the tectum, a region typically devoid of this marker, expressed this marker [145]. Such data suggest that there may be a longer-term set of guidance steps that feed into promoting the regionally specific neuronal phenotypes that include an earlier need for cell juxtaposition interactions that generate homeodomain predilections prior to the final departure of neurons from the cell cycle. The circumstances seem somewhat different in vitro with populations of neural stem cells, because when growth factors are carefully removed from such populations within N2B27 media (conducive of neuronal differentiation), the large majority of neurons (over 80%) produced adopt the GABAergic phenotype [146]. Another informative embryonic-to-embryonic transplant study that broadens the developmental factors associated with phenotype decisions was performed by Magrassi and colleagues [147]. They found that when ganglionic eminence-derived neurons clustered together as aggregates, they supported each other in maintaining their GABAergic phenotype fate while, by contrast, neurons that migrate into ectopic locations as individuals may adopt alternative fates guided by local signals. The capacity of cells that are predestined to adopt alternative fates based on responding to positional signals has been demonstrated in that they are able to adopt cortical-like morphologies when they migrate into the cortex, presumably as individual neurons [148]. In fact, at this early point in transplantation evaluation, outcome assessments based largely on the morphology of neurons indicated that transplantation into any region seemed to be guided by local cue phenotype induction toward locally appropriate fates (e.g., [149]). These days such assessments are largely considered insufficient, and a more marker-specific immunocytological phenotype determination is encouraged. When these are evaluated with the subventricular-zone-derived adult neural stem cells and their common migration trajectory into the olfactory bulb, it has been suggested that differential phenotypes or phenotype-restrictions might begin to be established quite early, prior to migration to the destination, given the diversity of expressions despite common local cues within the bulb [150]. However, a more recent hypothesis-driven review compiled by Sequerra and colleagues [151] suggests that the capacity for true phenotype guidance from local cues can be quite extensive, such that environmental circumstances can differentiate between glutamatergic and GABAergic phenotypes and manipulations of morphogen expressions, such as sonic hedgehog, by blocking it in ventral locations or ectopically expressing it in dorsal locations can "dorsalize" neuronal phenotypes in ventral sectors or "ventralize" them in dorsal sectors respectively. Transplantation of small numbers of embryonic stem cells into various regions and subsequent specific tracking of resulting neurons indicates that within the intact mouse brain there is a regionally distinct capacity to promote the incorporation of new neurons that is largely progressively lost with age, but when neurons merge into the circuit during more accommodating developmental periods, they typically adopt regionally appropriate functional contributions.

What about the projection potential of transplanted neurons as they attempt to integrate with the host? As described before, Wictorin and his collaborators [84] explored the placement of presumed striatal-predestined rat embryonic ganglionic eminence-derived grafts into the striatum of adult rats following excitotoxic lesions in this same-target location. They witnessed

significant growth into the basal ganglia circuitry with the majority of the graft adopting a GABAergic phenotype and projecting *myelinated* axon growth into the host globus pallidus, with only a few projections showing retrograde transport indicating they reached the substantia nigra reticulata. Interestingly in his review, Wictorin [84] also mentions control studies performed in which cerebellar precursor tissue was transplanted into the striatum instead of the ganglionic eminence-derived cells, and that this ectopic transplant resulted in considerably diminished outgrowth and diminished integration with either glutamatergic or dopaminergic host-derived afferent ingrowth. The migration of neurons was even affected when transplants were placed into the developing neonatal striatum in a restricted manner when cerebellar precursor tissue was used instead of striatal precursor tissue, indicating the relevant guidance cues are established early [152]. As might be imagined, hindbrain (rhombencephalon) precursor tissue, transplanted into the adult cerebellum after excitotoxic lesions in that region, adopts several local phenotypes and seems to grow extensively into this region, recapitulating that circuit to an arguably regionally specific, yet similar, degree [153]. This indicates that homotypic versus ectopic concerns are more universal and relevant to multiple regions. Interestingly, human-derived precursor cells transplanted into the rat brain have also been described as growing more extensively into the rat host than do either rat- or mouse-derived precursor cells, though they seemed also to be sensitive to being placed within a homotypic domain (striatal into striatum) versus an ectopic domain (cerebellar into striatum), once again expressing significantly reduced growth into the latter host location [154]. It is intriguing to speculate about how human-derived neural precursors attain a more prominent and extensive host integration into rat host tissue when the signals presumably inspiring growth are likely distinct, though it has been speculated that human cells harbor a propensity to grow for greater distances before target-derived signals are expected while exhibiting a relative insensitivity to growth-inhibiting signals that are produced by the host. The bottom line message of this section is that cell populations seem to acquire, and become limited by, their: (1) neuronal status where they depart from the mitosis cycle, (2) neurotransmitter phenotype that limits their range of influence, and (3) regional predilection that bolsters their contribution to the circuit when they recognize "home" and diminishes contributions from cells delivered elsewhere. This regional predilection has been described above for striatal, or ganglionic eminence-derived neurons, transplanted into the striatum. Apparently, it is also relevant to dopaminergic neuron transplants of fetal ventral mesencephalon, which typically includes both nigral (A9) and ventral tegmental (A10) "type" neurons and for which the ability to successfully re-innervate the striatum is far superior among the nigral type, both anatomically and in terms of behavioral support [155–157]. It seems clear that there are niche components integrated into neuronal phenotypes that extend beyond merely the transmitter they express.

So what does this say about the ectopic dopaminergic cell transplantation into the striatum and the idea that transplant contributions will be more successful if placed within their target region? These efforts do require some background explanation. Parkinson's disease has been understood as mainly a loss of forebrain or more specifically striatal dopamine for most of its history. Although the specific temporal and spatial actions of striatal and greater basal ganglia neurons have been better understood for quite some time, there has been a corresponding lack of attention to the temporal dynamics of the dopamine provisions to that system in the clinical world, presumably because the tools available seem to work without a need for such a

concern. The virtue of the most common pharmaceutical treatment, Levodopa, seems to derive largely from ensuring a more consistent dopamine presence. Dopamine agonists, also used as a pharmacological treatment, likely linger outside any strict temporal parameters in that they are likely removed only by diffusion. Animal models benefit from rudimentary delivery mechanisms that also largely appear to maintain dopamine presence with little to no dynamic shifting according to "need," as might be expected of the phasic attributes of an intact dopamine system. Clearly the previously-mentioned movement tests that reveal the insufficient temporal precision of behavioral control with classic treatment has heightened awareness of the concern [108]. Nevertheless it is readily apparent that dopamine cell transplantation for the Parkinson's patient remains largely conceptualized as a more sophisticated delivery system for dopamine that may become increasingly necessary as the ongoing deterioration of dopaminergic neurons diminishes the patient's capacity to convert Levodopa into dopamine. The enzyme aromatic L-amino acid decarboxylase is necessary to complete this conversion step and experimental animal tests indicate that the effects of Levodopa both depend on this action and that serotonergic neurons within the brain, which also harbor this enzyme, may be capable of supporting continued benefit from the drug [158]. Dopamine neuron transplantation into the striatum likely provides improved benefit beyond this serotonergic neuron involvement in that transplanted cells would be capable of growing into greater proximity, and that their terminals would maintain an improved reuptake transport control of the corresponding dopamine released that serotonergic neurons would lack. Nonetheless, for the reasons mentioned in the previous section, the absence of controlled dynamic modification would develop into a problem over time as the greater circuit compensates, and should be attended to as clinical strategies are formulated.

Presumably behavioral support would be improved if derived from dopamine release from more fully reconstructed dopaminergic projections from a grafted nigra into the striatum as there would be improved potential for dynamic temporal control by more "appropriate" afferents. Despite the fact that most reviews of transplants for PD mention this point (e.g., [155]), to our current knowledge, despite several apparent successes in establishing nigra-to-striatum re-innervation from nigral dopaminergic grafts [159–165], there have been no systemic assessments of the afferent control of these grafts established by the host. Clearly the main interest at present with such a grafting strategy is to ensure the dopaminergic reconstruction extends across the inhospitable terrain of the adult brain from the nigra to the striatum. Likely due to the expression of considerable disruptive signals within the adult CNS and the need for more continual support during the growth process, initial efforts to coax this reconstruction from homotypic nigral-placed grafts were unsuccessful in breaching the divide, though even these relatively nigral-restricted grafts did provide some behavioral support [166], likely due to the importance of dendritic dopamine release within the nigra [167]. In fact, the neuronal populations upon which the dendritic dopamine release likely plays its role are GABAergic neurons in the reticulata, and efforts to transplant GABA-producing neurons into this region have also demonstrated some behavioral benefit, presumably by somehow expanding the repertoire of this basal ganglia output region [83, 168]. Such an effect speaks volumes, questioning the precision of the disinhibitory feedback loop formed by striatal efferents to the nigra reticulata and

on to the ventrolateral and ventromedial thalamus, understood to form the basis of motor program selection provided by the basal ganglia [169]. These GABAergic transplants into the nigra also may contribute benefit by increasing the local suppression of noise as described previously for the seemingly noisy striatum. As efforts expanded, it became clear that considerable neurotrophic support was necessary and this was either provided by "bridge" tissue grafts that might be likely to release such compounds, such as Schwann cell type cells, or the local cells were induced to release these compounds by viral transgenic expression (e.g., [82, 160, 170]). This strategy renders the CNS territory through which the new dopaminergic fibers must grow more hospitable, presumably also providing retrograde support signals, inspiring continued growth, and staving off the previously-described cell death that results from the lack of connectedness during this growth journey.

It is notable that while many of these strategies were being tested, a well-recognized age dependency was revealed in that even significant dopamine-depleting lesions performed on young and neonatal animals yielded only mild or dramatically diminished behavioral deficits [171, 172]. At the same time, these animals, when grown to adults, still depended upon dopamine for their locomotor behavior, albeit in an altered way [173], and produced sufficient but diminished levels of striatal dopamine to accomplish this [174]. Perhaps the enhanced plasticity supporting this maintenance of dopamine-dependent behavioral control was derived from the natural expression of neurotrophic factors that maintain a higher presence during early postnatal periods of development [175]. A general protection of dopaminergic neurons has been shown to derive from glial-derived neurotrophic factor (GDNF) in particular [176]. In fact, it has been determined that developing nigral dopaminergic neurons depends considerably on GDNF for their survival and maintenance by the establishment of a conditional GDNF knock-out mouse that exhibits clear dopaminergic disruption-related hypokinesia and diminished tyrosine hydroxylase among dopaminergic neurons once GDNF production is blocked during adulthood [177]. The age-dependency factor, regarding the dopamine system, has also been demonstrated in the ability to incorporate dopaminergic transplants. Efforts to unilaterally transplant dopaminergic fetal grafts into the substantia nigra on postnatal days 3, 10, and 20 into rats that had received bilateral 6-OHDA lesions on postnatal day 1 resulted in the intriguing finding that transplants given on postnatal days 3 and 10 showed evidence of nigrostriatal regrowth or fuller incorporation into that circuit, while those receiving transplants on postnatal day 20 did not [178]. It seems GDNF and BDNF may cooperate, to some extent, in supporting dopaminergic cells, as BDNF has also been used successfully to promote a sparse re-innervation of the striatum from a nigral-targeted graft [179]. The neurotrophic factors that seem to play supportive roles expand considerably when observed in the light of what supports the original production of the medial forebrain bundle during development [180].

Coaxing the growth-trajectory environment to also express adhesion molecules that new growth cones might grow along has also been considered (e.g., [170]). The sorts of glial cells or other tissue, which are often added to the equation of a "bridge," are generally not those known to be disruptive to axon growth such as astrocytes and oligodendrocytes. In fact when the medial forebrain bundle pathway is observed for regrowth following axotomy, sprouting of new axons is considerably enhanced by removing glial cells from the growth path by use of

a glial toxin [181]. Among the putative interfering variables to this sort of existing cell regrowth are heparan sulfate proteoglycans, chondroitin sulfate proteoglycans, and keratan sulfate proteoglycans that are derived from activated astrocytes that surround lesions [182]. Developing dopaminergic neurons of the substantia nigra must sprout axons that grow anteriorly for substantial lengths to reach their target termination zones. Also, anatomists have recognized a substantial formation of synapses *en passant* among these and other monoaminergic neuron types, suggesting multiple way stations occur within target structures prior to establishing classic terminal boutons, each subject to various degrees of local control [183]. Their extensive growth trajectory requires growth-promoting and cell-death-diminishing signal molecules during axon extension, particularly when transplants are placed during adulthood when the road is longer. Thus, regrowth from such posterior-ventral origins likely depend on the presence of cellular *guideposts* along the way that might break up the full growth required of the nigrostriatal tract into growth stints that are supported by retrograde feedback signals, as well as the removal of potentially interfering substances derived from activated glia. The involvement of glia in the diminished propensity to grow extensive connections from posterior regions may also depend on the manner in which the original lesion is created. It may be that the neurotoxins used in animal models to induce dopamine-depleting lesions (e.g., 6-OHDA, MPTP) exacerbate glia, resulting in more activation of astrocytes and thereby interfering with regrowth (see [184, 185]). However in most idiopathic cases of Parkinson's disease, there is a distinct lack of reactive astrocytes during the course of deterioration or afterward [186, 187], indicating that the contributions of chondroitin sulfate and other growth-interfering responses might be lower in this condition, despite a clear insufficiency of dopaminergic regrowth. Nevertheless, the indication that reactive astrocytes may linger for up to 90 days following 6-OHDA administration [185] is intriguing when the rat 6-OHDA treated model system is considered because usually transplantation is performed prior to that time in those animal models.

Homotypic transplant placement may also be promoted in the context of dopaminergic cells, given that their qualities may be guided more substantially by local cues, as well as gaining from local afferent control. During development, the local ventral midbrain environment seems to contribute considerable epigenetic guidance to newly generated neurons in the form of morphogens. One of these morphogens that has been classically associated with ventral development beginning at the neural tube stage is sonic hedgehog (for review see [188]). The two prominent locally secreted factors that drive dopaminergic phenotype development are fibroblast growth factor 8 (FGF-8) and sonic hedgehog [175], leading to internal genetic expression of Nurr1 and Ptx3 transcription factors that further establish phenotype delineation. This is likely why those two secreted factors are used in protocols that guide the development of dopaminergic phenotypes from more pluripotent precursors *in vitro* (e.g., [189, 190]). When ventral mesencephalic-derived embryonic stem cells are left to develop freely in culture, many of them develop as dopaminergic, but there is also a mixture of phenotypes that might be expected from the ventral midbrain or hindbrain such as serotonergic and GABAergic neurons. Efforts to improve the yield of dopaminergic phenotypes have produced multiple proposed protocols involving different steps that replicate different aspects of developmental phenotype adoption. For example, one of these uses the Wnt signaling to influence developing neurons at the location of the developing nigra. Wnt signaling seems to be established to differential degrees in the developing nervous system, in large part by

cell to cell contact information and signal gradients that get established during the course of progressive commitment in gene expression. Specifically, inducing the transcription factor known as Wnt5a via transfection, following sonic hedgehog and FGF-8 exposure, seems to generate greater dopaminergic phenotype yields than sonic hedgehog and FGF-8 alone [191]. In addition, bone marrow derived stem cells seem to require a neuronal-enhancing, region-specific environment characterized by low oxygen, retinoic acid, and continuous neurotrophin-3 stimulation, as these in combination with the aforementioned sonic hedgehog and FGF-8 stimulation seem to enhance dopaminergic phenotype expression further [192]. All this indicates that there are specific local environments that would induce phenotype commitment based on regionally-specific combinations of factors that are provided in the appropriate sequence during development, and these remain in a sort of residual form still capable of supporting, albeit at a more limited degree of commitment, in the adult structure. The capacity of dopaminergic neurons grafted into the nigra to acquire afferent control remains understudied, but this capacity would likely be higher than that of ectopic transplants into the striatum. If gap junction connections could also be established with the local endogenous dopamine neurons of the nigra, this could enhance temporal pattern production substantially. Of course, if there are ongoing deterioration-inducing challenges among the Parkinsonian endogenous dopamine neurons this could induce the closure of gap junction connections, due to sensed pH or calcium changes, as a protective response [193]. However, given the circumscribed positioning of the dopaminergic neurons within the nigra following the transplant, it would seem a far more straightforward incorporation process regarding afferent stimulation in general than what would otherwise be required within the striatum.

Multiple placement transplants have been performed using animal models that have shown more substantial support for behavior. Experiments performed by Mukhida and colleagues showed considerable improvement in behavioral control with dopaminergic-destined fetal ventral mesencephalic transplants into the striatum, substantia nigra, and subthalamic nucleus that seemed to improve behavioral recovery better than the typical single transplant alone [194]. Clearly there may be a benefit to such extended transplantation but there are two major issues drawing the practicality of such strategies into question. First, transplantation of cells into one area in human patients is already a significant procedure, fraught with considerable risk and expense. The idea of multiple sites of transplantation would need to be justified by not only significant movement restoration but also in long-term viability beyond the 5-week, post-transplantation assessments commonly used. Second, given the concerns raised in this review, each ectopic transplant performed is likely to both provide some distortion in the temporal dynamics of delivery and also would perhaps block the more successful growth and penetration of the homotypic aspect. How well would new nigrostriatal terminals grow into the striatum if there are already local striatal dopaminergic terminals competing for CNS real estate in the same region? Given the clinical limitations and the likely extended growth time that would be required for nigrostriatal restoration, it may be prudent to consider formulating temporary neurons that could be progressively eliminated as fibers reach the striatum that could maintain a "substitute" dopamine presence. The concern with dual transplants (both in the nigra and the striatum) is that striatal transplants would likely diminish the growth or synaptogenesis drive among incoming nigrostriatal growth cones in a manner similar to what seems to occur among striatal neurons transplanted into the intact

striatum (establishing limited interactions with the host as a result). At this point, developing transgenic transplantable cells with pharmacologically inducible properties may be able to accomplish this temporary substitute goal. Initial inefficient support might be maintained during the growth process and this might be progressively and selectively removed as dopaminergic growth from homotypic regions reaches the area.

5. Concluding remarks

Plastic adaptation was described above as representing a multitude of cellular responses that occur with the apparent role of maintaining cellular homeostasis, yet within the nervous system also support the maintenance of a sort of dynamic status quo in which compensatory changes adjust the actions or response capacities of local healthy neurons in support of a superseding circuit-associated need. We understand that various CNS circuits establish the capacity to process a wide range of information with various degrees of versatility that presumably evolved to provide stability in some areas of common reliance and flexibility in areas where learning functions occur regularly and synaptic adjustments are correspondingly at higher demand. Neurons appear to undergo adaptations as they attempt to enter a circuit, and the environmental guidance for the control contributed by new additions extends to various degrees backward into the history of the newly added cells in question as it signals what it can provide and encourages host connections while it negotiates for acceptance into the host circuit and the privilege of contributing. As neurons do this during development, their relative pluripotency diminishes toward the eventual niche they enter into and it is highly likely that new neuronal contributions transplanted into these circumstances go through similar steps as they adapt to the roles they play. The long-term viability of additions requires that a utility anticipated by the circuit is fulfilled or the host circuit may adapt the addition out of relevance like an efficient social system isolates and eventually eliminates an influence perceived as disruptive. As an example, a long-term neurotransmitter lingering without dynamic change could come from leaky or malfunctioning neurons, so it would benefit a circuit to recognize this and diminish postsynaptic responses until the signal once again exceeds noise. Synaptic negotiation during development of the mammalian neuromuscular system, which has been more accessible and easier to manipulate with experiments, shows a series of back and forth messages that eventually culminate in the muscle fiber accepting one motoneuron terminal and rejecting other applications for the job (see [195] for detailed discussion of this process). It is likely that whether neurons incorporate into CNS circuits depends upon their capacity to apply themselves and on whether the corresponding job has already been taken, as indicated by the diminished success of transplants into adult intact CNS structures achieving synaptic incorporation. While it is possible for neuronal precursors to be conditioned in a manner that promotes certain wanted phenotypes, the ability to properly incorporate into a workable circuit is challenged when they are placed into an ectopic environment as described above. To draw an analogy to human socialization, it's as if the cells in question either have, or are given, an agenda that may or may not merge with the agenda of the local host circuit. The mechanisms in place that promote apoptosis, in this context, are a useful and positive contribution to the overall circuit despite the fact that the death of cells seems unfortunate. Neurons in various deteriorative diseases adopt abnormal activities. In fact, the whole basis

of deep brain stimulation, as derived from earlier therapies for Parkinson's disease, was to render excessive and aberrant activity quiescent (see [196, 197]). It is important that our clinical efforts consider the adaptive nature of the host tissue, into which we desire our transplants to be incorporated as this strategy will meet with greater long-term success and fewer potentially disruptive side-effects that generate additional, unwanted measures into the equation if these concerns are not accounted for from the outset.

Acknowledgements

Support for this work was provided by the Central Michigan Neuroscience Program, the Central Michigan University Office of Research and Sponsored Programs, the John G. Kulhavi Professorship, and the Field Neurosciences Institute.

Author details

Michael I. Sandstrom[1]*, Kevin A. Anderson[1], Naveen Jayaprakash[2], Parnit K. Bhupal[1] and Gary L. Dunbar[1,3]

*Address all correspondence to: sands1m@cmich.edu

1 Neuroscience and Experimental Psychology Programs, Central Michigan University, Mount Pleasant, MI, USA

2 Biomedical Science, Marquette University, Milwaukee, WI, USA

3 Field Neurosciences Institute, Saginaw, MI, USA

References

[1] Mazzini L, Ferrero I, Luparello V, Rustichelli D, Gunetti M, Mareschi K, Testa L, Stecco A, Tarletti R, Miglioretti M, Fava E, Nasuelli N, Cisari C, Massara M, Vercelli R. Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A phase I clinical trial. Experimental Neurology. 2010;**223**(1):229-237. DOI: 10.1016/j.expneurol.2009.08.007

[2] Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. Nature. 2006; **441**:1094-1096. DOI: 10.1038/nature04960

[3] Mezey E, Key S, Vogelsang G, Szalayova I, Lange D, Crain B. Transplanted bone marrow generates new neurons in human brains. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(3):1364-1369. DOI: 10.1073/pnas.0336479100

[4] Rossignol J, Fink K, Davis K, Clerc S, Crane A, Matchynski J, Lowrance S, Bombard M, Derkorver N, Lescaudron L, Dunbar GL. Transplants of adult mesenchymal and neural stem cells provide neuroprotection and behavioral sparing in a transgenic rat model of Huntington's disease. Stem Cells. 2014;**32**(2):500-509. DOI: 10.1002/stem.1508

[5] Lescaudron L, Unni D, Dunbar GL. Autologous adult bone marrow stem cell transplantation in an animal model of Huntington's disease: Behavioral and morphological outcomes. International Journal of Neuroscience. 2003;**113**(7):945-956. DOI: 10.1080/00207450390207759

[6] Kim J-H, Auerbach JM, Rodríguez-Gómez JA, Velasco I, Gavin D, Lumelsky N, Lee S-H, Nguyen J, Sánchez-Pernaute R, Bankiewicz K, McKay R. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature. 2002; **418**:50-56. DOI: 10.1038/nature00900

[7] Li W, Englund E, Widner H, Mattsson B, van Westen D, Latt J, Rehncrona S, Brundin P, Björklund A, Lindvall O, Li JY. Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating parkinsonian brain. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**(23):6544-6549. DOI: 10.1073/pnas.1605245113

[8] Björklund A, Dunnett SB, Brundin P, Stoessl AJ, Freed CR, Breeze RE, Levivier M, Peschanski M, Studer L, Barker R. Neural transplantation for the treatment of Parkinson's disease. The Lancet Neurology. 2003;**2**:437-445. DOI: 10.1016/S1474-4422(03)00442-3

[9] Freeman TB, Olanow CW, Hauser RA, Nauert GM, Smith DA, Borlongan CV, Sanberg PR, Holt DA, Kordower JH, Vingerhoets FJ, Snow BJ, Calne D, Gauger LL. Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. Annals of Neurology. 1995;**38**(3):379-388. DOI: 10.1002/ana.410380307

[10] Napoli E, Borlongan CV. Cell therapy in Parkinson's disease: Host repair machinery gets a boost from stem cell grafts. Stem Cells. 2017;**35**(6):1443-1445. DOI: 10.1002/stem.2636

[11] Walczak P, Chen N, Eve D, Hudson J, Zigova T, Sanchez-Ramos J, Sanberg PR, Sanberg CD, Willing AE. Long-term cultured human umbilical cord neural-like cells transplanted into the striatum of NOD SCID mice. Brain Research Bulletin. 2007;**74**(1-3):155-163. DOI: 10.1016/j.brainresbull.2007.06.015

[12] Conti L, Reitano E, Cattaneo E. Neural stem cell systems: Diversities and properties after transplantation in animal models of diseases. Brain Pathology. 2006;**16**(2):143-154. DOI: 10.1111/j.1750-3639.2006.00009.x

[13] Rokni U, Richardson AG, Bizzi E, Seung HS. Motor learning with unstable neural representations. Neuron. 2007;**54**(4):653-666. DOI: 10.1016/j.neuron.2007.04.030

[14] Hyman SE. Addiction: A disease of learning and memory. American Journal of Psychiatry. 2005;**162**(8):1414-1422. DOI: 10.1176/appi.ajp.162.8.1414

[15] Baker-Andresen D, Ratnu VS, Bredy TW. Dynamic DNA methylation: A prime candidate for genomic metaplasticity and behavioral adaptation. Trends in Neurosciences. 2013;**36**(1):3-13. DOI: 10.1016/j.tins.2012.09.003

[16] Day JJ, Sweatt JD. Epigenetic mechanisms in cognition. Neuron. 2011;**70**(5):813-829. DOI: 10.1016/j.neuron.2011.05.019

[17] Tsankova N, Rentha W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. Nature Reviews Neuroscience. 2007;**8**:355-367. DOI: 10.1038/nrn2132

[18] Renthal W, Maze I, Krishnan V, Covington HE III, Xiao G, Kumar A, Russo SJ, Graham A, Tsankova N, Kippin TE, Kerstetter KA, Neve RL, Haggarty SJ, McKinsey TA, Bassel-Duby R, Olson EN, Nestler EJ. Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron. 2007;**56**(3):517-529. DOI: 10.1016/j.neuron.2007.09.032

[19] Ganguly K, Poo M. Activity-dependent neural plasticity from bench to bedside. Neuron. 2013;**80**(3):729-741. DOI: 10.1016/j.neuron.2013.10.028

[20] Abbott LF, Nelson SB. Synaptic plasticity: Taming the beast. Nature Neuroscience. 2000;**3**: 1178-1183. DOI: 10.1038/81453

[21] Ziles K. Neuronal plasticity as an adaptive property of the central nervous system. Annals of Anatomy - Anatomischer Anzeiger. 1992;**174**(5):383-391. DOI: 10.1016/S0940-9602(11)80255-4

[22] Klein WL, Sullivan J, Skorupa A, Aguilar JS. Plasticity of neuronal receptors. The FASEB Journal. 1989;**3**(10):2132-2140. DOI: 10.1096/fj.1530-6860

[23] Hou ST, Jiang SX, Smith RA. Permissive and repulsive cues and signaling pathways of axonal outgrowth and regeneration. International Review of Cell and Molecular Biology. 2008;**267**:125-181. DOI: 10.1016/S1937-6448(08)00603-5

[24] Yu LMY, Leipzig ND, Shoichet MS. Promoting neuron adhesion and growth. Materials Today. 2008;**11**(5):36-43. DOI: 10.1016/S1369-7021(08)70088-9

[25] Chen DF, Tonegawa S. Why do mature CNS neurons of mammals fail to re-establish connections following injury—functions of Bcl-2. Cell Death and Differentiation. 1998; **5**(10):816-822. DOI: 10.1038/sj.cdd.4400431

[26] Silver J. Inhibitory molecules in development and regeneration. Journal of Neurology. 1994;**242**(Suppl.1):S22-S24. DOI: 10.1007/BF00939236

[27] Hidalgo A. Neuron-glia interactions during axon guidance in Drosophila. Biochemical Society Transactions. 2003;**31**(1):50-55. DOI: 10.1042/bst0310050

[28] Bovolenta P, Mason C. Growth cone morphology varies with position in the developing mouse visual pathway from retina to first targets. Journal of Neuroscience. 1987; **7**(5):1447-1460

[29] Li GN, Liu J, Hoffman-Kim D. Multi-molecular gradients of permissive and inhibitory cues direct neurite outgrowth. Annals of Biomedical Engineering. 2008;**36**(6):889-904. DOI: 10.1007/s10439-008-9486-z

[30] Panicker MM, Rao M. Stem cells and neurogenesis. Chapter 18 In: Marshak DR, Gardner RL, Gottlieb D, editors. Stem Cell Biology. Cold Spring Harbor Monograph Series. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. Vol. 40. 2001. pp. 399-438. DOI: 10.1101/087969673.40.399

[31] Scherson T, Serbedzija G, Fraser S, Bronner-Fraser M. Regulative capacity of the cranial neural tube to form neural crest. Development. 1993;**118**:1049-1062

[32] Uylings HBM. Development of the human cortex and the concept of "critical" or "sensitive" periods. Language Learning. 2006;**56**:59-90. DOI: 10.1111/j.1467-9922.2006.00355.x

[33] Henderson C. Role of neurotrophic factors in neuronal development. Current Opinion in Neurobiology. 1996;**6**:64-70. DOI: 10.1016/s0959-4388(96)80010-9

[34] Rabinowicz T, de Courten-Myers GM, Petetot JM-C, Xi G, de los Reys E. Human cortex development: Estimates of neuronal numbers indicate major loss late during gestation. Journal of Neuropathology and Experimental Neurology. 1996;**55**(3):320-328. DOI: 10.1097/00005072-199603000-00007

[35] Lardi-Studler B, Fritschy J-M. Matching of pre- and postsynaptic specializations during synaptogenesis. The Neuroscientist. 2007;**13**(2):115-126. DOI: 10.1177/1073858406296803

[36] Garner CC, Zhai RG, Gundelfinger ED, Ziv NE. Molecular mechanisms of CNS synaptogenesis. Trends in Neurosciences. 2002;**25**(5):243-250. DOI: 10.1016/S0166-2236(02)02152-5

[37] Kwon H-B, Kozorovitskiy Y, Oh W-J, Peixto RT, Akhtar N, Saulnier JL, Gu C, Sabatini BL. Neuroligin-1-dependent competition regulates cortical synaptogenesis and synapse number. Nature Neuroscience. 2012;**15**:1667-1674. DOI: 10.1038/nn.3256

[38] Peng HB, Yang J-F, Dai Z, Lee CW, Hung HW, Feng ZH, Ko C-P. Differential effects of neurotrophins and Schwann cell-derived signals on neuronal survival/growth and synaptogenesis. Journal of Neuroscience. 2003;**23**(12):5050-5060

[39] van Ooyen A, Willshaw DJ. Competition for neurotrophic factor in the development of nerve connections. Proceedings of the Royal Society B - Biological Sciences. 1999;**266**(1422): 883-892. DOI: 10.1098/rspb.1999.0719

[40] Kaiser M, Hilgetag CC, van Ooyen A. A simple rule for axon outgrowth and synaptic competition generates realistic connection lengths and filling fractions. Cerebral Cortex. 2009; **19**(12):3001-3010. DOI: 10.1093/cercor/bhp071

[41] Gulledge AT, Kampa BM, Stuart GJ. Synaptic integration in dendritic trees. Journal of Neurobiology. 2005;**64**(1):75-90. DOI: 10.1002/neu.20144

[42] Lippman J, Dunaevsky A. Dendritic spine morphogenesis and plasticity. Journal of Neurobiology. 2005;**64**(1):47-57. DOI: 10.1002/neu.20149

[43] Spruston N, Stuart G, Hausser M. Dendritic integration. In: Stuart G, Spruston N, Hausser M, editors. Dendrites. New York: Oxford University Press; 1999. pp. 231-270. Ch10

[44] Wefelmeyer W, Puhl CJ, Burrone J. Homeostatic plasticity of subcellular neuronal structures: From inputs to outputs. Trends in Neurosciences. 2016;**39**(10):656-667. DOI: 10.1016/j.tins.2016.08.004

[45] Katsuki T, Joshi R, Ailani D, Hiromi Y. Compartmentalization within neurites: Its mechanisms and implications. Developmental Neurobiology. 2011;**71**(6):458-473. DOI: 10.1002/dneu.20859

[46] Kuba H, Oichi Y, Ohmori H. Presynaptic activity regulates Na^+ channel distribution at the axon initial segment. Nature. 2010;**465**:1075-1078. DOI: 10.1038/nature09087

[47] Yamada M, Tanemura K, Okada S, Iwanami A, Nakamura M, Mizuno H, Ozawa M, Ohyama-Goto R, Kitamura N, Kawano M, Tan-Takeuchi K, Ohtsuka C, Miyawaki A, Takashima A, Ogawa M, Toyama Y, Okano H, Kondo T. Electrical stimulation modulates fate determination of differentiating embryonic stem cells. Stem Cells. 2007;**25**(3):562-570. DOI: 10.1634/stemcells.2006-0011

[48] Babu H, Ramirez-Rodriguez G, Fabel K, Bischofberger J, Kemperman G. Synaptic network activity induces neuronal differentiation of adult hippocampal precursor cells through BDNF signaling. Frontiers in Neuroscience. 2009;**30**:49. DOI: 10.3389/neuro.22.001.2009

[49] Cho T, Ryu JK, Taghibiglou C, Ge Y, Chan AW, Liu L, Lu J, McLarnon JG, Wang YT. Long-term potentiation promotes proliferation/survival and neuronal differentiation of neural stem/progenitor cells. PLoS One. 2013;**8**(10):e76860. DOI: 10.1371/journal.pone.0076860

[50] Jontes JD, Smith SJ. Filopodia, spines, and the generation of synaptic diversity. Neuron. 2000;**27**(1):11-14. DOI: 10.1016/S0896-6273(00)00003-9

[51] Personius KE, Balice-Gordon RJ. Activity-dependent editing of neuromuscular synaptic connections. Brain Research Bulletin. 2000;**53**(5):513-522. DOI: 10.1016/S0361-9230(00)00384-1

[52] Bibel M, Barde Y-A. Neurotrophins: Key regulators of cell fate and cell shape in the vertebrate nervous system. Genes & Development. 2000;**14**:2919-2937. DOI: 10.1101/gad.841400

[53] Gordon N. Apoptosis (programmed cell death) and other reasons for elimination of neurons and axons. Brain and Development. 1995;**17**(1):73-77. DOI: 10.1016/0387-7604(94)00109-B

[54] Temple S. The development of neural stem cells. Nature. 2001;**414**:112-117. DOI: 10.1038/35102174

[55] Soares S, Sotelo C. Adult neural stem cells from the mouse subventricular zone are limited in migratory ability compared to progenitor cells of similar origin. Neuroscience. 2004;**128**(4):807-817. DOI: 10.1016/j.neuroscience.2004.07.031

[56] Zhang Y, Klassen HJ, Tucker BA, Perez M-TR, Young MJ. CNS progenitor cells promote a permissive environment for neurite outgrowth via a matrix metalloproteinase-2-dependent mechanism. Journal of Neuroscience. 2007;**27**(17):4499-4506. DOI: 10.1523/JNEUROSCI.0200-07.2007

[57] Shetty AK, Hattiangady B. Postnatal age governs the extent of differentiation of hippocampal CA1 and CA3 subfield neural stem/progenitor cells into neurons and oligodendrocytes. International Journal of Developmental Neuroscience. 2013;**31**(7):646-656. DOI: 10.1016/j.ijdevneu.2013.05.006

[58] Van Zant G, Liang Y. Concise review: Hematopoietic stem cell aging, life span, and transplantation. STEM CELLS Translational Medicine. 2012;**1**:651-657. DOI: 10.5966/sctm.2012-0033

[59] Akiyama M, Hoshi Y, Sakurai S. Changes of telomere length in children after hematopoietic stem cell transplantation. Bone Marrow Transplantation. 1998;**21**:167-171. DOI: 10.1038/sj.bmt.1701060

[60] Stein DG, Brailowsky S, Will B. Brain Repair. New York: Oxford University Press; 1995. 147 p

[61] Blurton-Jones M, Kitazawa M, Martinez-Coria H, Castello NA, Muller F-J, Loring JF, Yamasaki TR, Poon WW, Green KN, LeFeria FM. Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. Proceedings of the National Academy of Sciences. 2009;**106**(32):13594-13599. DOI: 10.1073/pnas.0901402106

[62] Alberts JL, Linder SM, Penko AL, Lowe MJ, Phillips M. It is not about the bike, it is about the pedaling: Forced exercise and Parkinson's disease. Exercise and Sport Sciences Reviews. 2011;**39**(4):177-188. DOI: 10.1097/JES.0b013e31822cc71a

[63] Mayer E, Brown VJ, Dunnett SB, Robbins TW. Striatal graft-associated recovery of a lesion-induced performance deficit in the rat requires learning to use the transplant. European Journal of Neuroscience. 1992;**4**:119-126 https://doi.org/10.1111/j.1460-9568.1992.tb00858.x

[64] Barker RA, Widner H. Immune problems in central nervous system cell therapy. NeuroRX. 2004;**1**(4):472-481. DOI: 10.1602/neurorx.1.4.472

[65] Ideguchi M, Shinoyama M, Gomi M, Hayashi H, Hashimoto N, Takahashi J. Immune or inflammatory response by the host brain suppresses neuronal differentiation of transplanted ES cell-derived neural precursor cells. Journal of Neuroscience Research. 2008;**86**(9):1936-1943. DOI: 10.1002/jnr.21652

[66] Di Filipo M, Sarchielli P, Picconi B, Calabresi P. Neuroinflammation and synaptic plasticity: Theoretical basis for a novel, immune-centred, therapeutic approach to neurological disorders. Trends in Pharmacological Sciences. 2008;**29**(8):402-412. DOI: 10.1016/j.tips.2008.06.005

[67] Xanthos DN, Sandkuhler J. Neurogenic neuroinflammation: Inflammatory CNS reactions in response to neuronal activity. Nature Reviews Neuroscience. 2014;**15**:43-53. DOI: 10.1038/nrn3617

[68] Alvarez-Buylla A, Garcia-Verdugo JM. Neurogenesis in adult subventricular zone. Journal of Neuroscience. 2002;**22**(3):629-634

[69] Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. Nature Neuroscience. 1999;**2**:260-265. DOI: 10.1038/6365

[70] Lledo P-M, Saghatelyan A. Integrating new neurons into the adult olfactory bulb: Joining the network, life-death decisions, and the effects of sensory experience. Trends in Neurosciences. 2005;**28**(5):248-254. DOI: 10.1016/j.tins.2005.03.005

[71] Christie KJ, Turnley AM. Regulation of endogenous neural stem/progenitor cells for neural repair-factors that promote neurogenesis and gliogenesis in the normal and damaged brain. Frontiers in Cellular Neuroscience. 2013;**6**:70. DOI: 10.3389/fncel.2012.00070

[72] Johnson MA, Ables JL, Eisch AJ. Cell-intrinsic signals that regulate adult neurogenesis in vivo: Insights from inducible approaches. BMB Reports. 2009;**42**(5):245-259. DOI: 10.5483/bmbrep.2009.42.5.245

[73] Luzzati F, DeMarchis S, Parlato R, Gribaudo S, Schutz G, Fasolo A, Peretto P. New striatal neurons in a mouse model of progressive striatal degeneration are generated in both the subventricular zone and the striatal perenchyma. PLoS One. 2011;**6**(9):e25088. DOI: 10.1371/journal.pone.0025088

[74] Pahuja R, Seth K, Shukla A, Shukla RK, Bhatnagar P, Chauhan LKS, Saxena PN, Arun J, Chaudhari BP, Patel DK, Singh SP, Shukla R, Khanna VK, Kumar P, Chaturvedi RK, Gupta KC. Trans-blood brain barrier delivery of dopamine-loaded nanoparticles reverses functional deficits in Parkinsonian rats. ACS Nano. 2015;**9**(5):4850-4871. DOI: 10.1021/nn506408v

[75] Chen S, Xianwen C, Dehua X, Zhenguo L, Lingfei X, Smith SW, Zhongcheng Z. Behavioral correction of Parkinsonian rats following the transplantation of immortalized fibroblasts genetically modified with TH and GCH genes. Parkinsonism & Related Disorders. 2003; **9**(2):91-97. DOI: 10.1016/S1353-8020(03)00020-8

[76] Zoli M, Agnati LF. Wiring and volume transmission in the central nervous system: The concept of closed and open synapses. Progress in Neurobiology. 1996;**49**(4):363-380. DOI: 10.1016/0301-0082(96)00020-2

[77] Zolia M, Torria C, Farraria R, Janssonb A, Zinia I, Fuxeb K, Agnatia LF. The emergence of the volume transmission concept. Brain Research Reviews. 1998;**26**(2-3):136-147. DOI: 10.1016/S0165-0173(97)00048-9

[78] Pritzel M, Isacson O, Brundin P, Wiklund L, Björklund A. Afferent and efferent connections of striatal grafts implanted into the ibotenic acid lesioned neostriatum in adult rats. Experimental Brain Research. 1986;**65**:112-126. DOI: 10.1007/bf00243834

[79] Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. New England Journal of Medicine. 2001;**344**(10):710-719. DOI: 10.1056/NEJM200103083441002

[80] Lee CS, Cenci MA, Schulzer M, Björklund A. Embryonic ventral mesencephalic grafts improve levodopa-induced dyskinesia in a rat model of Parkinson's disease. Brain. 2000; **123**(Pt. 7):1365-1379 https://doi.org/10.1093/brain/123.7.1365

[81] Fraichard A, Chassande O, Bilbaut G, Dehay C, Savatier P, Samarut J. In vitro differentiation of embryonic stem cells into glial cells and functional neurons. Journal of Cell Science. 1995;**108**:3181-3188

[82] Kauhausen J, Thompson LH, Parish CL. Cell intrinsic and extrinsic factors contribute to enhance neural circuit reconstruction following transplantation in parkinsonian mice. The Journal of Physiology. 2013;**591**(1):77-91. DOI: 10.1113/jphysiol.2012.243063

[83] Winkler JD, Kirik D, Björklund A, Dunnett SB. Transplantation in the rat model of Parkinson's disease: Ectopic versus homotopic graft placement. In: Functional Neural

Transplantation II. Novel Cell Therapies for CNS Disorders. Progress in Brain Research. 2000;**127**:233-265. DOI: 10.1016/s0079-6123(00)27012-x Ch11

[84] Wictorin K. Anatomy and connectivity of intrastriatal striatal transplants. Progress in Neurobiology. 1992;**38**:611-639. DOI: 10.1016/0301-0082(92)90044-f

[85] Labandeira-Garcia JL, Wictorin K, Cunningham ET Jr, Björklund A. Development of intrastriatal striatal grafts and their afferent innervation from the host. Neuroscience. 1991; **42**:407-426. DOI: 10.1016/0306-4522(91)90385-2

[86] Xu ZC, Wilson CJ, Emson PC. Synaptic potentials evoked in spiny neurons in rat neostriatal grafts by cortical and thalamic stimulation. Journal of Neurophysiology. 1991;**65**: 477-493

[87] Rutherford A, Garcia-Munoz M, Dunnett SB, Arbuthnott GW. Electrophysiological demonstration of host cortical inputs to striatal grafts. Neuroscience Letters. 1987;**83**:275-281

[88] Sirinathsinghji DJS, Dunnett SB, Isacson O, Clark DJ, Kendrick K, Björklund A. Striatal grafts in rats with unilateral neostriatal lesions II. In vivo monitoring of GABA release in globus pallidus and substantia nigra. Neuroscience. 1988;**24**:803-811. DOI: 10.1016/0306-4522(88)90068-1

[89] Miller BR, Walker AG, Shah AS, Barton SJ, Rebec GV. Dysregulated information processing by medium-spiny neurons in striatum of freely behaving mouse models of Huntington's disease. Journal of Neurophysiology. 2008;**100**(4):2205-2216. DOI: 10.1152/jn.90606.2008

[90] Plotkin JL, Surmeier DJ. Corticostriatal synaptic adaptations in Huntington's disease. Current Opinion in Neurobiology. 2015;**33**:53-62. DOI: 10.1016/j.conb.2015.01.020

[91] Estrada-Sanchez AM, Rebec GV. Corticostriatal dysfunction and glutamate transporter 1 (GLT1) in Huntington's disease: Interactions between neurons and astrocytes. Basal Ganglia. 2012;**2**(2):57-66. DOI: 10.1016/j.baga.2012.04.029

[92] Miller BR, Dorner J, Brunner KD, Gaither TW, Klein EL, Barton SJ, Rebec GV. Up-regulation of GLT1 reverses the deficit in cortically evoked striatal ascorbate efflux in the R6/2 mouse model of Huntington's disease. Journal of Neurochemistry. 2012;**121**(4):629-638. DOI: 10.1111/j.1471-4159.2012.07691.x

[93] Sandstrom M, Rebec GV. Characterization of striatal activity in conscious rats: Contribution of NMDA and AMPA/kainate receptors to both spontaneous and glutamate-driven firing. Synapse. 2003;**47**:91-100

[94] Liu G, Rao Y. Neuronal migration from the forebrain to the olfactory bulb requires a new attractant persistent in the olfactory bulb. Journal of Neuroscience. 2003;**23**(16):6651-6659

[95] Wu GY, Cline HT. Stabilization of dendritic arbor structure in vivo by CaMKII. Science. 1998;**279**(5348):222-226. DOI: 10.1126/science.279.5348.222

[96] Magavi SSP, Lois C. Transplanted neurons form both normal and ectopic projections in the adult brain. Developmental Neurobiology. 2008;**68**(14):1527-1537. DOI: 10.1002/dneu.20677

[97] Craig AM, Lichtman JW. Synapse formation and maturation. In: Cowan WM, Sudhof TC, Stevens CF, editors. Synapses. Baltimore: Johns Hopkins University Press; 2001. pp. 571-612. Ch12

[98] Gage FH, Dunnett SB, Stenevi U, Björklund A. Aged rats: Recovery of motor impairments by intrastriatal nigral grafts. Science. 1983;**221**(4614):966-969. DOI: 10.1126/science.6879196

[99] Björklund A, Dunnett SB, Stenevi U, Lewis ME, Iverson SD. Reinnervation of the denervated striatum by substantia nigra transplants: Functional consequences as revealed by pharmacological and sensorimotor testing. Brain Research. 1980;**199**(2):307-333. DOI: 10.1016/0006-8993(80)90692-7

[100] Watabe-Uchida M, Zhu L, Ogawa S, Vamanrao A, Uchida N. Whole brain mapping of direct inputs to midbrain dopamine neurons. Neuron. 2012;**74**(5):858-873. DOI: 10.1016/j.neuron.2012.03.017

[101] Parent A, Hazrati L-N. Functional anatomy of the basal ganglia I. The cortico-basal ganglia-thalamo-cortical loop. Brain Research Reviews. 1995;**20**:91-127. DOI: 10.1016/0165-0173(94)00007-C

[102] Doucet G, Murata Y, Brundin P, Bosler O, Mons N, Geffard M, Ouimet CC, Björklund A. Host afferents into intrastiatal transplants of fetal ventral mesencephalon. Experimental Neurology. 1989;**106**(1):1-19. DOI: 10.1016/0014-4886(89)90139-8

[103] Braak H, Del Tredici K. Assessing fetal nerve cell grafts in Parkinson's disease. Nature Medicine. 2008;**14**(5):483-485. DOI: 10.1038/nm1768

[104] Neve KA, Kozlowski MR, Marshall JF. Plasticity of neostriatal dopamine receptors after nigrostriatal injury: Relationship to recovery of sensorimotor functions and behavioral supersensitivity. Brain Research. 1982;**244**(1):33-44. DOI: 10.1016/0006-8993(82)90901-5

[105] Winkler JD, Weiss B. Reversal of supersensitive apomorphine-induced rotational behavior in mice by continuous exposure to apomorphine. Journal of Pharmacology and Experimental Therapeutics. 1986;**238**(1):242-247

[106] Turrigiano G. Homeostatic synaptic plasticity: Local and global mechanisms for stabilizing neuronal function. Cold Spring Harbor Perspectives in Biology. 2017;**4**. Article a005736. DOI: 10.1101/cshperspect.a005736

[107] Castañeda E, Fleming S, Paquette MA, Boat K, Moffett J, Stachowiak EK, Bloom DC, Stachowiak MK. Assessment of recovery in the hemiparkinson rat: Drug-induced rotation is inadequate. Physiology and Behavior. 2005;**84**(4):525-535. DOI: 10.1016/j.physbeh.2005.01.019

[108] Tunik E, Feldman AG, Poizner H. Dopamine replacement therapy does not restore the ability of Parkinsonian patients to make rapid adjustments in motor strategies according to changing sensorimotor contexts. Parkinsonism & Related Disorders. 2007;**13**(7):425-433. DOI: 10.1016/j.parkreldis.2007.02.003

[109] Vandecasteele M, Glowinski J, Venance L. Electrical synapses between dopaminergic neurons of the substantia nigra pars compacta. Journal of Neuroscience. 2005;**25**(2):291-298. DOI: 10.1523/jneurosci.4167-04.2005

[110] Grace AA, Bunney BS. Intracellular and extracellular electrophysiology of nigral dopaminergic neurons - 3. Evidence of electrotonic coupling. Neuroscience. 1983;**10**(2):333-336. DOI: 10.1016/0306-4522(83)90137-9

[111] Schultz W. Dopamine signals for reward value and risk: Basic and recent data. Behavioral and Brain Functions. 2010;**6**:24. DOI: 10.1186/1744-9081-6-24

[112] Hillerman JR, Schultz W. Dopamine neurons report an error in the temporal prediction of reward during learning. Nature Neuroscience. 1998;**1**:304-309. DOI: 10.1038/1124

[113] Schultz W, Dayan P, Montague RR. A neural substrate of prediction and reward. Science. 1997;**275**:1593-1599. DOI: 10.1126/science.275.5306.1593

[114] Haber SN, Fudge JL, McFarland NR. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. Journal of Neuroscience. 2000;**20**(6):2369-2382

[115] Chuhma N, Mingote S, Moore H, Rayport S. Dopamine neurons control striatal cholinergic neurons via regionally heterogeneous dopamine and glutamate signaling. Neuron. 2014;**81**:901-912. DOI: 10.1016/j.neuron.2013.12.027

[116] Morris G, Arkadir D, Nevet A, Vaadia E, Bergman H. Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. Neuron. 2004;**43**:133-143. DOI: 10.1016/j.neuron.2004.06.012

[117] Cragg SJ. Meaningful silences: How dopamine listens to the ACh pause. Trends in Neurosciences. 2006;**29**(3):125-131. DOI: 10.1016/j.tins.2006.01.003

[118] Threlfell A, Cragg SJ. Dopamine signaling in dorsal versus ventral striatum: The dynamic role of cholinergic interneurons. Frontiers in Systems Neuroscience. 2011;**5**. Article 11. DOI: 10.3389/fnsys.2011.00011

[119] Threlfell S, Lalic T, Platt NJ, Jennings KA, Deisseroth K, Cragg SJ. Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. Neuron. 2012;**75**(1):58-64. DOI: 10.1016/j.neuron.2012.04.038

[120] Cachope R, Cheer JF. Local control of striatal dopamine release. Frontiers in Behavioral Neuroscience. 2014;**8**. Article 188. DOI: 10.3389/fnbeh.2014.00188

[121] Cui G, Jun SB, Jin X, Pham MD, Vogel SS, Lovinger DM, Costa RM. Concurrent activation of striatal direct and indirect pathways during action initiation. Nature. 2013;**494**:238-242. DOI: 10.1038/nature11846

[122] Plamen G, Darbin O, Wichmann T. Oscillations in the basal ganglia under normal conditions and in movement disorders. Movement Disorders. 2006;**21**(10):1566-1577. DOI: 10.1002/mds.21033

[123] Grillner S, Hellgren J, Menard A, Saitoh K, Wikstrom MA. Mechanisms for selection of basic motor programs - Roles for the striatum and pallidum. Trends in Neurosciences. 2005;**28**(7):364-370. DOI: 10.1016/j.tins.2005.05.004

[124] Björklund A, Lindvall O. Cell replacement therapies for central nervous system disorders. Nature Neuroscience. 2000;**3**(6):537-544. DOI: 10.1038/75705

[125] Chkirate M, Vallee A, Doucet G. Host striatal projections into fetal ventral mesencephalic tissue grafted to the striatum of immature or adult rat. Experimental Brain Research. 1993;**94**(2):357-362. DOI: 10.1007/BF00230306

[126] Fisher LJ, Young SJ, Tepper JM, Groves PM, Gage FH. Electrophysiological characteristics of cells within mesencephalon suspension grafts. Neuroscience. 1991;**40**(1):109-122. DOI: 10.1016/0306-4522(91)90178-q

[127] Hagell P, Cenci MA. Dyskinesias and dopamine cell replacement in Parkinson's disease: A clinical perspective. Brain Research Bulletin. 2005;**68**(1-2):4-15. DOI: 10.1016/j.brainresbull.2004.10.013

[128] Ma Y, Feigin A, Dhawan V, Fukuda M, Shi Q, Greene P, Breeze R, Fahn S, Freed C, Eidelberg D. Dyskinesia after fetal cell transplantation for parkinsonism: A PET study. Annals of Neurology. 2002;**52**(5):628-634. DOI: 10.1002/ana.10359

[129] Carta M, Carlsson T, Munoz A, Kirik D, Björklund A. Role of serotonin neurons in the induction of levodopa- and graft-induced dyskinesias in Parkinson's disease. Movement Disorders. 2010;**25**(Supplementary 1):S174-S179. DOI: 10.1002/mds.22792

[130] Gotz M, Huttner WB. The cell biology of neurogenesis. Nature Reviews Molecular Cell Biology. 2005;**6**(10):777-788. DOI: 10.1038/nrm1739

[131] Lee S-H, Lumelsky N, Studer L, Auerbach JM, McKay RD. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. Nature Biotechnology. 2000;**18**:675-679. DOI: 10.1038/76536

[132] Song H, Stevens CF, Gage FH. Astroglia induce neurogenesis from adult neural stem cells. Nature. 2002;**417**:39-44. DOI: 10.1038/417039a

[133] Pfreiger FW, Barres BA. Synaptic efficacy enhanced by glial cells in vitro. Science. 1997; **277**(12):1684-1687. DOI: 10.1126/science.277.5332.1684

[134] O'Malley EK, Seiber B-A, Black IB, Dreyfus CF. Mesencephalic type I astrocytes mediate the survival of substantia nigra dopaminergic neurons in culture. Brain Research. 1992; **582**(1):65-70. DOI: 10.1016/0006-8993(92)90317-3

[135] Barres BA. The mystery and magic of glia: A perspective on their roles in health and disease. Neuron. 2008;**60**(3):430-440. DOI: 10.1016/j.neuron.2008.10.013

[136] Belland ER. Researchgate.net Blog Response to "Can anyone help me with pure primary neuronal culture?". 2014. Available from: https://www.researchgate.net/post/Can_anyone_help_me_with_pure_primary_neuronal_culture [Accessed: 31-07-2017]

[137] Wu H-Y, Geddes J, Opperman K, Kaboord B. Thermofisher Scientific Protein Biology Application Notes. An Efficient Method for the Isolation of Highly Functional Primary Neurons. 2014. Available from: https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/protein-biology-application-notes/efficient-method-isolation-highly-functional-primary-neurons.html [Accessed: 31-07-2017]

[138] Reynolds BA, Rietze RL. Neural stem cells and neurospheres—Re-evaluating the relationship. Nature Methods. 2005;**2**:333-336. DOI: 10.1038/nmeth758

[139] Landis SC, Keefe D. Evidence for neurotransmitter plasticity in vivo: Developmental changes in properties of cholinergic sympathetic neurons. Developmental Biology. 1983; **98**(2):349-372. DOI: 10.1016/0012-1606(83)90365-2

[140] Habecker BA, Symes AJ, Stahl N, Francis NJ, Economides A, Fink JS, Yancopaulos GD, Landis SC. A sweat-gland differentiation activity acts through known cytokine signaling pathways. Journal of Biological Chemistry. 1997;**272**(48):30421-30428. DOI: 10.1074/jbc.272.48.30421

[141] Spitzer NC. Neurotransmitter switching? No surprise. Neuron. 2015;**86**(5):1131-1144. DOI: 10.1016/j.neuron.2015.05.028

[142] Borodinsky LN, Belgacem YH. Crosstalk among electrical activity, trophic factors, and morphogenetic proteins in the regulation of neurotransmitter phenotype specification. Journal of Chemical Neuroanatomy. 2016;**73**:3-8. DOI: 10.1016/j.jchemneu.2015.12.001

[143] Borodinsky LN, Belgacem YH, Swapna I, Sequerra EB. Dynamic regulation of neurotransmitter specification: Relevance to nervous system homeostasis. Neuropharmacology. 2014;**78**:75-80. DOI: 10.1016/j.neuropharm.2012.12.005

[144] Roybon L, Mastracci TL, Ribeiro D, Sussel L, Brundin P, Li J-Y. GABAergic differentiation induced by Mash1 is compromised by bHLH proteins neurogenin2, NeuroD1, and NeuroD2. Cerebral Cortex. 2009;**20**(5):1234-1244. DOI: 10.1093/cercor/bhp187

[145] Wernig M, Benninger F, Schmandt T, Rade M, Tucker KI, Bussow H, Beck H, Brustle O. Functional integration of embryonic stem cell-derived neurons in vivo. Journal of Neuroscience. 2004;**24**(22):5258-5268. DOI: 10.1523/jneurosci.0428-04.200

[146] Burney MJ, Johnston C, Wong K-Y, Teng S-W, Beglopoulos V, Stanton LW, Williams BP, Bithell A, Buckley NJ. An epigenetic signature of developmental potential in neural stem cells and early neurons. Stem Cells. 2013;**31**(9):1868-1880. DOI: 10.1002/stem.1431

[147] Magrassi L, Ehrlich ME, Butti G, Pezzotta S, Govoni S, Cattaneo E. Basal ganglia precursors found in aggregates following embryonic transplantation adopt a striatal phenotype in hetrotopic locations. Development. 1998;**125**:2847-2855

[148] Fishell G. Striatal precursors adopt cortical identities in response to local cues. Development. 1995;**121**:803-812

[149] Shihabuddin LS, Hertz JA, Holets VR, Whittemore SR. The adult CNS retains the potential to direct region-specific differentiation of a transplanted neuronal precursor cell line. Journal of Neuroscience. 1995;**15**(10):6666-6678

[150] Merkle FT, Mirzadeh Z, Alvarez-Buylla A. Mosaic organization of neural stem cells in the adult brain. Science. 2007;**317**:381-384. DOI: 10.1126/science.1144914

[151] Sequerra EB, Costa MR, Menezes JRL, Hedin-Periera C. Adult neural stem cells: Plastic or restricted neuronal fates? Development. 2013;**140**:3303-3309. DOI: 10.1242/dev.093096

[152] Wictorin K, Lagenaur CF, Lund RD, Björklund A. Efferent projections to the host brain from intrastriatal striatal mouse-to-rat grafts: Timecourse and tissue-type specificity as revealed by a mouse specific neuronal marker. European Journal of Neuroscience. 1991;**3**(1):86-101. DOI: 10.1111/j.1460-9568.1991.tb00814.x

[153] Kritsanasrinuan N, Kotchabhakdi N, Therapanchareon V. Development of embryonic rhombencephalon transplanted into adult rat cerebellum. Journal of the Science Society of Thailand. 1993;**19**:129-142

[154] Wictorin K, Brundin P, Gustavii B, Lindvall O, Björklund A. Reformation of long axon pathways in adult rat CNS by human forebrain neuroblasts. Nature. 1990;**347**:556-558. DOI: 10.1038/347556a0

[155] Gaillard A, Jaber M. Rewiring the brain with cell transplantation in Parkinson's disease. Trends in Neurosciences. 2011;**34**(3):124-133. DOI: 10.1016/j.tins.2011.01.003

[156] Grealish S, Jonsson ME, Li M, Kirik D, Björklund A, Thompson LH. The A9 dopamine neuron component in grafts of ventral mesencephalon is an important determinant for recovery of motor function in a rat model of Parkinson's disease. Brain. 2010;**133**(Pt2): 482-495. DOI: 10.1093/brain/awp328

[157] Björklund A, Dunnett SB. Dopamine neuron systems in the brain: An update. Trends in Neurosciences. 2007;**30**:194-202. DOI: 10.1016/j.tins.2007.03.006

[158] Lopez A, Munoz A, Guerra MJ, Labandeira-Garcia JL. Mechanisms of the effects of exogenous levodopa on the dopamine-denervated striatum. Neuroscience. 2001;**103**(3):639-651. DOI: 10.1016/s0306-4522(00)00588-1

[159] Gaillard A, Decressac M, Frappe I, Fernagut PO, Prestoz L, Besnard S, Jaber M. Anatomical and functional reconstruction of the nigrostriatal pathway by intranigral transplants. Neurobiology of Disease. 2009;**35**:477-488. DOI: 10.1016/j.nbd.2009.07.003

[160] Wilby MJ, Sinclair SR, Muir EM, Zietlow R, Adcock KH, Horellou P, Rogers JH, Dunnett SB, Fawcett JW. A glial cell line-derived neurotrophic factor-secreting clone of the Schwann cell line SCTM41 enhances survival and fiber outgrowth from embryonic nigral neurons grafted to the striatum and to the lesioned substantia nigra. Journal of Neuroscience. 1999;**19**(6):2301-2312

[161] Brecknell JE, Du JS, Muir E, Fidler PS, Hlavin ML, Dunnett SB, Fawcett JW. Bridge grafts of fibroblast growth factor-4-secreting schwannoma cells promote functional axonal regeneration in the nigrostriatal pathway of the adult rat. Neuroscience. 1996;**74**(3):775-784. DOI: 10.1016/0306-4522(96)00167-4

[162] Isacson O, Deacon TW. Specific guidance factors persist in the adult brain as demonstrated by pig neuroblasts transplanted to the rat. Neuroscience. 1996;**74**:827-837. DOI: 10.1016/0306-4522(96)00305-3

[163] Wang Y, Tien LT, Lapchack PA, Hoffer BJ. GDNF triggers fiber outgrowth of fetal ventral mesencephalic grafts from nigra to striatum in 6-OHDA-lesioned rats. Cell Tissue Research. 1996;**286**(2):225-233

[164] Wictorin K, Brundin P, Sauer H, Lindvall O, Björklund A. Long distance directed axonal growth from human dopaminergic mesencephalic neuroblasts implanted along the nigrostriatal pathway in 6-hydroxydopamine lesioned adult rats. Journal of Comparative Neurology. 1992;**323**(4):475-494. DOI: 10.1002/cne.903230403

[165] Dunnett SB, Rogers DC, Richards SJ. Nigrostriatal reconstruction after 6-OHDA lesions in rats: Combination of dopamine-rich nigral grafts and nigrostriatal "bridge" grafts. Experimental Brain Research. 1989;**75**(3):523-535. DOI: 10.1007/bf00249903

[166] Nikkhah G, Bentlage C, Cunningham MG, Björklund A. Intranigral fetal dopamine grafts induce behavioral compensation in the rat Parkinson model. Journal of Neuroscience. 1994;**14**(6):3449-3461

[167] Cheramy A, Leviel V, Glowinski J. Dendritic release of dopamine in the substantia nigra. Nature. 1981;**289**(5798):537-542. DOI: 10.1038/289537a0

[168] Winkler JD, Bentlage C, Nikkhah G, Samii M, Björklund A. Intra-nigral transplants of GABA-rich striatal tissue induce behavioral recovery in the rat Parkinson model and promote the effects obtained by intrastriatal dopaminergic transplants. Experimental Neurology. 1999;**155**(2):165-186. DOI: 10.1006/exnr.1998.6916

[169] Chevalier G, Deniau JM. Disinhibition as a basic process in the expression of striatal functions. Trends in Neurosciences. 1990;**13**(7):277-280. DOI: 10.1016/0166-2236(90)90109-n

[170] Zhang C, Jin Y, Ziemba KS, Fletcher AM, Ghosh B, Truit E, Yurek DM, Smith GM. Long distance directional growth of dopaminergic axons along pathways of netrin-1 and GDNF. Experimental Neurology. 2013;**250**:156-164. DOI: 10.1016/j.expneurol.2013.09.022

[171] Bruno JP, Sandstrom MI, Arnold HM, Nelson CL. Age-dependent neurobehavioral plasticity following forebrain dopamine depletions. Developmental Neuroscience. 1998;**20**(2-3): 164-179. DOI: 10.1159/000017311

[172] Erinoff L, Macphail RC, Heller A, Seiden LS. Age-dependent effects of 6-hydroxydopamine on locomotor activity in the rat. Brain Research. 1979;**164**(1-2):195-205. DOI: 10.1016/0006-8993(79)90015-5

[173] Johnson BJ, Bruno JP. D1 and D2 receptor contributions to ingestive and locomotor behavior are altered after dopamine depletions in neonatal rats. Neuroscience Letters. 1990; **118**(1):120-123. DOI: 10.1016/0304-3940(90)90263-9

[174] Castañeda E, Wishaw IQ, Lermer L, Robinson TE. Dopamine depletion in neonatal rats: Effects on behavior and striatal dopamine release assessed by intracerebral microdialysis during adulthood. Brain Research. 1990;**508**(1):30-39. DOI: 10.1016/0006-8993(90)91113-U

[175] Abe M, Kimoto H, Eto R, Sasaki T, Kato H, Kasahara J, Araki T. Postnatal development of neurons, interneurons, and glial cells in the substantia nigra of mice. Cellular and Molecular Neurobiology. 2010;**30**(6):917-928. DOI: 10.1007/s10571-010-9521-0

[176] Tomac A, Lindqvist E, Lin LF, Ogren SO, Young D, Hoffer BJ, Olson L. Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. Nature. 1995;**373**(6512):335-339. DOI: 10.1038/373335a0

[177] Pascual A, Hildago-Figueroa M, Biruat JI, Pintado CO, Gomez-Diaz R, Lopez-Barneo J. Absolute requirement of GDNF for adult catecholaminergic neuron survival. Nature Neuroscience. 2008;**11**:755-761. DOI: 10.1038/nn.2136

[178] Bentlage C, Nikkhah G, Cunningham MG, Björklund A. Reformation of the nigrostriatal pathway by fetal dopaminergic micrografts into the substantia nigra is critically dependent on the age of the host. Experimental Neurology. 1999;**159**(1):177-190. DOI: 10.1006/exnr.1999.7110

[179] Somoza R, Juri C, Baes M, Wyneken U, Rubio FJ. Intranigral transplantation of epigenetically induced BDNF-secreting human mesenchymal stem cells: Implications for cell-based therapies in Parkinson's disease. Biology of Blood and Marrow Transplantation. 2010;**16**(11):1530-1540. DOI: 10.1016/j.bbmt.2010.06.006

[180] Aubert I, Ridet J-L, Gage FH. Regeneration in the adult mammalian CNS: Guided by development. Current Opinion in Neurobiology. 1995;**5**(5):625-635. DOI: 10.1016/0959-4388(95)80068-9

[181] Moon LDF, Brecknell JE, Franklin RJM, Dunnett SB, Fawcett JW. Robust regeneration of CNS axons through a track depleted of CNS glia. Experimental Neurology. 2000;**161**(1):49-66. DOI: 10.1006/exnr.1999.7230

[182] Moon LDF, Asher RA, Rhodes KE, Fawcett JW. Relationship between sprouting axons, proteoglycans, and glial cells following unilateral nigrostriatal axotomy in the adult rat. Neuroscience. 2002;**109**(1):101-117. DOI: 10.1016/S0306-4522(01)00457-2

[183] Prensa L, Parent A. The nigrostriatal pathway in the rat: A single-axon study of the relationship between dorsal and ventral tier nigral neurons and the striosome/matrix striatal compartments. Journal of Neuroscience. 2001;**21**(18):7247-7260

[184] McNaught KSP, Jenner P. Altered glial function causes neuronal death and increases neuronal susceptibility to 1-methyl-4-phenylpiridinium- and 6-hydroxydopamine-induced

toxicity in astrocytic/ventral mesencephalic co-cultures. Journal of Neurochemistry. 1999;**73**(6):2469-2476. DOI: 10.1046/j.1471-4159.1999.0732469.x

[185] Haruhiko A, McGeer PL. Microglial response to 6-hydroxydopamine-induced substantia nigra lesions. Brain Research. 1989;**489**(2):247-253. DOI: 10.1016/0006-8993(89)90857-3

[186] Mirza B, Hadberg H, Thomsen P, Moos T. The absence of reactive astrocytosis is indicative of a unique inflammatory process in Parkinson's disease. Neuroscience. 1999;**95**(2):425-432. DOI: 10.1016/S0306-4522(99)00455-8

[187] Forno LS, DeLanney LE, Irwin I, Di Monte D, Langston JW. Astrocytes and Parkinson's disease. Progress in Brain Research. 1992;**94**:429-436. DOI: 10.1016/s0079-6123(08)61770-7

[188] Dessaud E, McMahon AP, Briscoe J. Pattern formation in the vertebrate neural tube: A sonic hedgehog morphogen-regulated transcriptional network. Development. 2008;**135**:2489-2503. DOI: 10.1242/dev.009324

[189] Perrone-Capano C, Di Porzio U. Genetic and epigenetic control of midbrain dopaminergic neuron development. International Journal of Developmental Biology. 2000;**44**:679-687

[190] Trzaska KA, King CC, Li K-Y, Kuzhikandathil EV, Nowycky MC, Ye J-H, Rameshwar P. Brain-derived neurotrophic factor facilitates maturation of mesenchymal stem cell-derived dopamine progenitors to functional neurons. Journal of Neurochemistry. 2009; **110**:1058-1069. DOI: 10.1111/j.1471-4159.2009.06201.x

[191] Parish CL, Castelo-Branco G, Rawal N, Tonnesen J, Sorensen AT, Salto C, Kokaia M, Lindvall O, Arenas E. Wnt5a-treated midbrain neural stem cells improve dopamine cell replacement therapy in parkinsonian mice. Journal of Clinical Investigation. 2008;**118**(1): 149-160 https://doi.org/10.1172/jci32273

[192] Tatard VM, D'lppolito G, Diabira S, Valeyev A, Hackman J, McCarthy M, Bouckenooghe T, Menei P, Montero-Menei CN, Schiller PC. Neurotrophin-directed differentiation of human adult marrow stromal cells to dopaminergic-like neurons. Bone. 2007;**40**(2):360-373. DOI: 10.1016/j.bone.2006.09.013

[193] Paracchia C. Chemical gating of gap junction channels: Roles of calcium, pH and calmodulin. Biochimica et Biophysica Acta (BBA – Biomembranes). 2004;**1662**:61-80. DOI: 10.1016/j.bbamem.2003.10.020

[194] Mukhida K, Baker KA, Sadi D, Mendez I. Enhancement of sensorimotor behavioral recovery in hemiparkinsonian rats with intrastriatal, intranigral, and intrasubthalamic nucleus dopaminergic transplants. Journal of Neuroscience. 2001;**21**(10):3521-3530

[195] Narayanan K, Lichtman JW. The role of neuronal identity in synaptic competition. Nature. 2003;**424**:426-430. DOI: 10.1038/nature01836

[196] Lozano AM, Snyder BJ, Hamani C, Huchison WD, Dostrovsky JO. Basal ganglia physiology and deep brain stimulation. Movement Disorders. 2010;**25**(51):571-575. DOI: 10.1002/mds.22714

[197] Benabid AL. Deep brain stimulation for Parkinson's disease. Current Opinion in Neurobiology. 2003;**13**(6):696-706. DOI: 10.1016/j.conb.2003.11.001