Chapter 3

Neuron Replacement and Brain Repair; Sex Does Matter

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Additional information is available at the end of the chapter

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1. Introduction

The brain is one of the main targets of gonadal steroid hormones. In addition, it contains many of the steroid metabolizing enzymes. The effect of gonadal steroids on brain development and maturation has been well documented [1,2]. The vast literature on the subject has introduced the common belief that gonadal steroids may be the only effectors of the brain sexual differentiation, overshadowing other key elements. Although there is no doubt about its importance, the dogma of the gonadal origin of somatic differentiation, including neuronal cells, usually implies that XX and XY cells, accordingly stem cells, are functionally equivalent unless gonadal secretions act on them in a sex-specific manner. The human Y chromosome encodes 27 different proteins [3] eight of which are expressed in the male brain and could have a male-specific effect on the brain, independent of any gonadal hormone influence [4,5]. Furthermore, XX cells contain an X chromosome that received a paternal genomic imprint, whereas XY cells do not, a fact that is likely to contribute to autonomous differences between male and female cells. De Vries and colleagues [6] generated mice in which the testis-determining gene *sry* was deleted from the Y chromosome and subsequently inserted onto an autosome. This experiment resulted in the generation of mice where the development of the testis occurred independently of the complement of X or Y chromosome. Although most of the sexual dimorphism correlated with the presence of testis or ovary (and therefore associated with gonadal hormones), XY mice (with testes or ovaries) were found to be more masculine than the XX mice (with testes or ovaries) in the density of vasopressin-immunoreactive fibers in the lateral septum, suggesting that sex chromosome genes contribute to the development of a sex difference in the brain. These results also suggest that one should not consider that female and male neural stem cells (NSC) are equal and react in the same manner to a specific environment or pharmacological agent. Furthermore, there are no data to support the *a priori* consideration that transplanting

young female NSC in an old female brain would result in the same neural differentiation and functional recovery as transplanting young male NSC in an old male brain. Likewise, there is no evidence to *a priori* consider that male and female NSC neurogenic properties would evolve in a sex-independent manner throughout development and aging. To support this hypothesis, sexual dimorphism has already been described in various biological aspects of several types of stem cells [7-13]. In particular, we and others recently reported a sexual dimorphism in the neurogenic capacity of rat [14,15] and primate [11] NSC. Considering the dramatic and sex-specific hormonal changes occurring throughout development and aging one might expect a sex- and sex-through-aging-specific environment to be a prerequisite for successful neurogenesis.

The chapter will first discuss the potential of stem cells for brain repair, tissue regeneration and function recovery, second the effect of sex on stem cell fate whether neural stem cells or peripheral stem cells, and third the potential translation in clinics. The goal of the present proposal is to discuss the therapeutic relevance of the largely under-explored sex- and age-based differences in the capacity of NSC to engage in neurogenesis programming. Indeed, beyond understanding the physiology and biochemistry of aging NSC, for both sexes, the overall objective is to discuss the potential foundation for future studies aimed at tailoring NSC transplantation strategies for brain repair as a function of sex/age, as well as considering sex- and age-specific pharmacological approaches towards the development of neurogenesis-inducing treatments.

2. Stem cells — Hope and hurdles

If one had to explain the excitement and exhilaration stem cell therapy triggers in the field of tissue repair and the hope it represents, a comparison that would make sense is the new horizons opened after the first man in flight or the first man in space. The hopes and hypes reflect the potential offered by stem cell therapy and in that sense, they are completely justifiable. Probing PubMed with 'stem cell' and 'therapy' as keywords in certainly the best way to understand how the interest of research community significantly evolved over 40 years. Indeed, in 1970, only 13 publications related to stem cell therapy were referenced in PubMed, compared to 7942 in 2010 (Figure 1). A slightly restricted search for 'stem cell', 'therapy' and 'brain' reveals that the number of publications related to brain repair, although increasing, is "plateauing" at 10% of the total number of publications focused on stem cell therapy for the past five years (Figure 1). This status reflects a greater difficulty to generate clear data on experimental and clinical stem cell therapy for brain repair than with any other organs. This situation is likely due to the unique architectonic of the brain, the way it interfaces with the peripheral compartment and the seemingly endless cell phenotypes that compose the brain.

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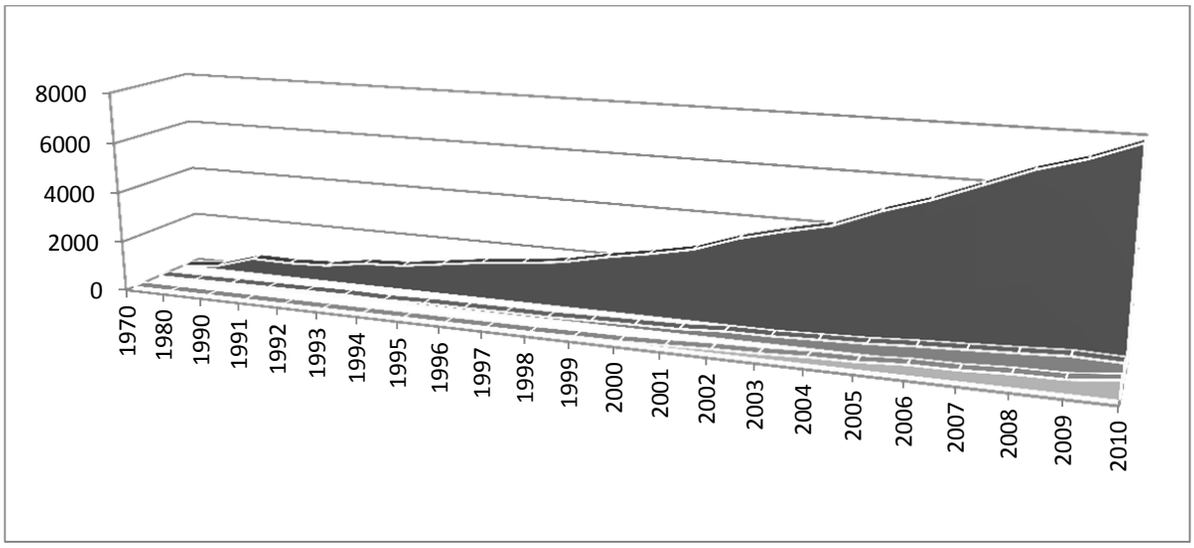

Figure 1. Number of publications per keyword and per year. Light grey "neural stem cells"; medium grey "stem cells AND therapy AND brain"; dark grey "stem cells AND therapy".

It is remarkable that the first report using the words 'neural stem cells' was published not that long ago in 1992, reaching the hundred only in 2001, catching up in 2010 with 714 references with the number of publications that include 'stem cell', 'therapy' and 'brain' (Figure 1). Although one cannot deny that the amount of data degenerated nowadays on NSC and brain repair is getting more important, the rather slow pace at which progresses are still reported does not prefigure any substantial impact in clinics to be made in the near future. Many hurdles lie on the road of brain stem cell therapy. The first one, and not necessarily by order of importance, is directly related to the various types of stem cells considered as a potential source for brain transplantation. Human embryonic stem cells (hESC) [16,17], fetal NSC [18,19], post-natal or adult NSC [20-25], induced pluripotent stem cells (iPS) [26-29], bone marrow stem cells (BMSC) [30-36], mesenchymal stem cells (MSC) [37,38], umbilical cord blood-derived stem cells [39-41] and adipose tissue-derived stem cells [42,43]. This luxury of choice turned out to become an increasingly difficult situation to handle, generating at best a non-consensual agreement as to which stem cell type should be used for what type of neurological disorder. Overall, it seems that potential stem cell sources have been insufficiently characterized, impeding clinical translation. Although it sounds like a very easy statement to make, we have to admit that our knowledge in long-term outcome of stem cell transplantation in the brain is extremely limited. In example, grafting stem cells not from neurodermal origin in an attempt to replace degenerated neurons supposes a transdifferentiation mechanism of the transplanted stem cells prior to undergo successful differentiation in the appropriate neuronal phenotype [44-49]. Very little literature is currently available in transdifferentiation mechanisms and even more so, recent data reported that transdifferentiation is reversible and may be restricted to certain type of stem cells [48,49], raising the question of what would happen in vivo. A major concern is the development of gliomas or other brain cancer types secondary to stem cell transplantation. Indeed, the property of stem cells to self-renew is exactly what confer them the capacity of being highly tumorigenic [50-53]. It is particularly true for the hESC which

tumorigenic properties have been extensively studied [52]. In addition, iPS have been developed to exactly mimic embryonic stem cells neurogenic capacity without having the capacity to induce brain tumor formation. However, the iPS safety is still being debated [52]. A clinical case has been reported very recently regarding a young boy who developed glioneuronal neoplasm four years after having received allogenic hESC transplantation in an attempt to improve his ataxia telangiectasia symptomatology [50]. It is remarkable that the tumor cells were identified as deriving from at least two of the donors composing the hESC pool from which the young boy received multiple transplantations. This specific data points out the possibility that using pool of donors may increase the risk of developing tumor or even host-versus-graft reaction [54-57]. Beyond the trivial concern of stem cells tumorigenesis remains the question of inducing the proper neural phenotype needed to replace a specific type of neuron. Parkinson's disease is without any doubt the neurodegenerative disease for which brain transplant has been studied the most extensively. A consensual strategy seems to pre-differentiate the stem cells toward the dopaminergic fate prior to conduct the transplantation [58,59] and as a consequence, many studies have been conducted to develop suitable protocols [58-62]. The same question arises to ensure that stem would undergo GABAergic differentiation prior to be used in patients suffering from Huntington's disease [63]. However, despite many efforts to establish validated differentiation protocols, the phenotypic fate followed by transplanted stem cells still remain largely uncontrolled [64-66] and likely involves stem cells intrinsic properties, i.e. region or context dependency, sex or age [14,67-70]. In a medical context of growing interest for global personalized medicine in general [71] and focused on regenerative medicine in particular [27,72-74], answering the question "What stem cell for whom?" never appeared as critical.

3. Stem cells transplantation in clinics — From hype to disillusion, keeping the faith alive

The main reason of the arisen interest for stem cell therapy in central and peripheral nervous system lies in the fact that it addresses neuropathologies and conditions for which neurological damages are extensive, socially debilitating and irreversible and for which there is no 'magic pill'. Stem cell therapy for tissue repair is generating very high expectation to treat neurodegenerative diseases like Parkinson's disease [66,75], amyotrophic lateral sclerosis [76-81], Huntington's disease [18,19,82-84], Alzheimer's disease [85-87], multiple sclerosis [88-90], spinal cord injury [91,92] and retina degeneration [26,31,93]. Clinical translation has been, up to now and by far, most exclusively conducted in patients suffering from Parkinson's, Huntington's disease or amyotrophic lateral sclerosis and, to our knowledge, there is no data reported clinical evaluation of stem cell transplantation in Alzheimer's disease or retina degeneration.

3.1. Parkinson's disease

Two groups actively involved in conducting clinical studies assessing the therapeutic effectiveness of stem cell transplantation to treat Parkinson's disease reported conflicting results

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[94-97]. In a first study, six Parkinson patients were to received bilateral transplantation of fetal nigral tissue the post-commissural putamen [95]. The fetal material was obtained from the mesencephalon of legally aborted fetus and used as solid grafts. Each patient received tissue pooled from 3 to 4 fetuses. Two years outcome measurement showed an improvement of the Unified Parkinson's Disease Rating Scale (UPDRS), and an increase of [^{18}F]-fluorodopa uptake in the putamen. On the downside, all patients started experience dyskinesia few weeks after the transplantation. When the same group performed the same type of study on a much larger cohort, the outcome was much more deceiving [97]. Out of the 34 patients followed up to 2 years, none of them displayed clinically relevant improvement although post-mortem analysis showed a robust survival of grafted dopaminergic neurons. These results led the authors to conclude that fetal nigral transplantation could not constitute a therapy for Parkinson's disease. One can argue that using solid grafts rather than well characterized stem cell/ neuroblast primary culture might have impaired the capacity of newly formed neurons to establish connections with the pre-existing network. This point is extensively discussed in a very recent review [66]. A more striking issue to us is the lack of information related to the sex of the donor suggesting that the tissue samples were pooled and grafted regardless of a potential sex-based difference in stem cell biology which could have an impact on the clinical outcome. In an other study, 40 patients were bilaterally grafted in the putamen with a mixture of stem cells/dopaminergic neuroblasts obtained from cultured mesencephalic tissue from 4 embryos [94]. Follow-up at one year post-transplantation showed some benefit but only for the patients 60 years of age or younger. In addition, the benefit was clinically significant only in some areas of the UPDRS and 15 percent of the patients became dyskinetic. A post-analysis of the one-year follow-up data revealed a sex-based difference in the graft outcome [96]. Indeed, the male patients displayed more clinical benefit than the female patients after the first year but the progress rate increased in the female to catch up with the male at the end of the second year of follow-up [96]. In addition, a follow-up performed over 4 years on 33 patients out of the 40 initially included showed that the age-based difference observed earlier [94] disappeared as the oldest patients' overall UPDRS was catching-up over the 4-years period of time on the youngest' one [96]. The authors concluded that some clinical benefit was still clearly present 4 years after transplantation with however no correlation with the [^{18}F]-fluorodopa uptake. Although the measured outcome seemed to be more encouraging than in the first series of experiments, the cell preparation may account for it, the results remain rather inconsistent and deceiving. On the same line, earlier clinical studies reported a somewhat beneficial effect of fetal mesencephalic neurons in patients affected by Parkinson's disease [75,98,99]. Several reasons have been proposed such inconsistency, among which the number of cells, the grafting site, the preparation of the cells, the use of immunosuppressant or the lack of functional rehabilitation associated with the transplantation [66,100] but no consensus has been reached so far. In addition, a recurrent issue that clinicians and patients are facing is the graft-induced dyskinesia. This locomotor alteration is a direct consequence of the transplantation and its causes and possible solutions remain rather elusive [75] and is seen as a permanent drawback to any progress made in stem cell transplantation to treat Parkinson's disease. As mentioned earlier, it is quite interesting that the cells of several donors were pooled with no reference to their sex as a factor of graft outcome. It is even more surprising since a sexual dimorphism

with functional implications has been established for human muscle stem cells [9,12,13] and monkeys mesenchymal stem cells [11].

3.2. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease that selectively affects motor neurons in the cortex, brain stem and spinal cord [101] for which there is no treatment. Several clinical studies have been conducted which results raised some optimism but, overall, data related to the transplantation outcome remain, as with Parkinson's disease, contradictory and inconsistent. A very recent report based on thirteen clinical cases demonstrated the feasibility and the therapeutic relevance of performing autologous bone marrow-derived hematopoietic cell transplantation in patients displaying severe sporadic ALS [76]. One year after receiving CD34+ cells transplantation in the brain stem and the beginning of the spinal cord, most of the patients displayed an improvement of their status. The majority of the patients regained neuronal stimulatory capacity of their muscle, as measured by post-operative electroneuromyogram, and a better bulbar score. Some of them even recovered walking capacity compatible with a daily life. However, the majority of the patients who experienced a post-operative gain of function started to see their clinical status decline at one-year follow-up. This piece of data, rather than suggesting that bone marrow-derived stem cells cannot be used as a therapeutic tool suggests instead that the transplantation procedure should be repeated in order to obtain maximum functional recovery. An other study demonstrated the beneficial effect of stem transplantation as a cytotherapeutic tool for ALS. Thirty-three patients diagnosed with severe sporadic ALS were to receive autologous transplantation of peripheral blood mononuclear cells (PBMC) in the motor cortex and followed-up over one year [78]. All the patients that received CD133+ PBMC transplantation experienced a dramatic improvement of their clinical status as measured by the ALS Functional Rating Scale-Revised. The most striking beneficial effect resulting from the transplantation was the increase in the median survival from 19 months for the patients in the sham group to 66 months in the treated group. More remarkably, the patients' quality of life was also dramatically improved. Indeed, in the sham group, half of the patients had to undergo tracheotomy and gastrostomy at 12-months follow-up compared to the no tracheotomy and only one gastrostomy performed in the group of patients who received PBMC transplantation. Taken together, these results show promises for ALS cytotherapy. However, some more recent clinical studies reported that transplanting stem cells in ALS patients did not result in any beneficial effect [79,81]. In the first case, patients received autologous transplantation of mesenchymal stem cells isolated from bone marrow in the spinal cord at T4-T5 and T5-T6 level and were followed-up for 24 months [79]. Although MRI imaging ascertained graft survival, clinicians did not observe any clinical improvement. However, it is noteworthy that the patients recruited for this study were diagnosed with mild to moderate sporadic ALS unlike the two previous studies described above for which the patients were diagnosed with severe sporadic ALS. This apparent discrepancy may indicate that the stage of the disease at which the stem cell transplantation is to be performed has to be cautiously defined as it may critically affect the clinical outcome. In the second case, patients from 3 different centers received stem cells transplantation and were then evaluated at 12-months post-transplantation [81]. This study is difficult to interpret

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as it includes a total of 12 patients who received 3 different types of stem cells, depending on the clinical center of origin, and using intratechal route, intravenous or both. The stem cells used were embryonic olfactory ensheating stem cells, mesenchymal stem cells or CD34+ stem cells without further explanation as to how many embryos were used per patients or if the graft of adult stem cells was autologous or not. However, a common point shared with the previous reported study, beside its lack of success at restoring the patients' motor function, is the fact that most of the patients recruited were diagnosed with a mild to moderate sporadic ALS supporting the idea 1) that mild to moderate stage of ALS might not be the best moment to start stem cell therapy, or that 2) the types of stem cells that were proven to be therapeutically relevant in the severe cases might not be as relevant to treat mild or moderate cases of ALS. These results certainly advocate for a better personalized therapy to the patient.

3.3. Huntington's disease

Huntington's disease is a neurodegenerative condition which is clinically characterized by cognitive, motor and psychiatric deterioration and leads in 20 years maximum to the death of the patient. There is currently no treatment for this disease and stem cell therapy is being extensively investigated as a therapeutic tool. Many attempts of restoring some or all the lost functions by stem cells transplantation have been made but the long term results have been so far deceiving. Motor and cognitive functions improvement has been reported in some patients 1 year after the transplantation [82] but these improvements plateaued at 2 years and to finally completely reverse at 4-6 years [18]. Other studies reported successful engraftment and neuronal differentiation [19] associated with an improved cortical metabolism [102] unfortunately not accompanied by any clinical improvement. A major finding that is commonly reported on long-term post-mortem histological study is the poor survival of the transplanted stem cells [83,84] which is believed to be due to a reaction of the host immune system against the grafted cells [54,84]. Interestingly, to our knowledge, all the clinical studies conducted on Huntington patients reported having used human embryonic stem cells [18,19,54,82-84,102] which is a quite surprising situation if we compare to the variety of stem cell types used in Parkinson's disease and ALS. In addition, one is force to at least acknowledge the fact that the stem cells used are from embryonic origin may be the factor that triggers the immune response. Indeed, human embryonic stem cells are not devoid of immunogenicity and are capable of triggering a significant immune response leading to graft rejection [54,103]. In regard of what has been accomplished in Parkinson's disease and ALS, autologous transplantation of PBMC or bone marrow-derived stem cells seems to be more appropriate and one may wonder why it has not been tried yet. An other point that raises question is the fact that all the studies reported, except one [19], transplanted human embryonic stem cells pooled from several embryos, neither mentioning how many embryos were used per patient nor the sex of the embryos.

3.4. Other indications for stem cell therapy

Stem cell therapy, beyond the controversy of using embryonic stem cells, is a very seducing and highly promising therapeutic strategy for repairing the central nervous system. Using

stem cell transplantation has become a very appealing topic for many researchers and clinicians around the world. However, developing and implementing such protocols to other neurological conditions than the ones discussed above reveals to be an even more challenging endeavor. Among the neurodegenerative disease that would benefit from stem cell therapy, Alzheimer's disease evidently represents a major interest. Probably because of the complexity of the disease and our very limited knowledge about it, stem cell therapy for Alzheimer's disease is still in a preclinical stage [85-87]. Stem cell therapy for spinal cord injury is certainly at the forefront of the preclinical research in order to establish valid therapeutic protocols to be translated in clinics, but once again translating the knowledge acquired in animal models facing several hurdles, among which cytotherapy specificity and safety [29,87,91,92,104,105]. Brain stroke is a neurological condition that could require extensive tissue reconstruction and results obtained in animal models are very encouraging [20,24,106]. So far, only one clinical study has been conducted in five patients by autologous transplantation of bone marrow-derived stem cells [36]. One year follow-up showed good safety profile and a trend to clinical improvement. Retina degeneration, whether idiopathic or post-traumatic, is a major problem as it is irreversible, without treatment and leads to blindness in most of the case. However, the retina architectonic and the differentiation process of retina stem cell represent two main problems to clinical translation. Indeed, many in vivo and in vitro studies have been conducted in various laboratories [26,30-32,35,37,45,107-117] showing promising results. However, although the post-transplantation cell survival was excellent in all cases, the retina structure revealed itself to be an obstacle to stem cell migration. In addition, the differentiation process that leads to the formation of mature retina cell is by far more complex than in the brain.

4. Looking at the forest instead of the tree — Turning the tide on clinical setbacks

We have recently published in a recent report that the estrogen receptor ERα is differentially expressed in male and female NSC. NSC isolated from 3 month-old rats display sexual dimorphism in the expression of estrogen receptor alpha and beta [14]. Male NSC contain one third of the ERα levels, whereas ERβ levels were 3 times greater than those expressed NSC isolated from same age females. Moreover, our data demonstrated that the ERα/ERβ ratio was close to 1 in the male but 10 fold higher in the female NSC. Interestingly, others previously reported that the expression of steroid receptors in the fish inner ear varies between sexes [118]. Such sexual dimorphism has been previously described in mature neurons of various brain structures [119,120] and has been shown to have a role in the differentiation of sexual behavior and gender identity [121]. Indeed, ERα has been shown to be primarily involved in masculinization, whereas ERβ is primarily involved in defeminization [122,123]. From birth and throughout life, sex hormones physiology and homeostasis are different between men and women which suggest that transplanting the "inadequate" type of NSC to the patient may not lead to the expected beneficial effect. Indeed, consequences in clinics may run from a lack of recovery to partial or inadequate recovery. Dissimilarity between NSC and the recipient tissue may cause these undesirable effects. Interestingly, in the same manner, NSCs isolated from 20

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month old male and female rats displayed a dramatic increase in ERα and ERβ expression that was equivalent in both sexes, suggesting that male and female NSCs are not equal before aging. The effect of estrogens on neurogenesis has been extensively studied and it is commonly agreed that estrogens simultaneously promote NSC proliferation and differentiation [124-129]. There is increasing evidence on the estrogenic aspect of neurogenesis; however, the differential roles of ERα and ERβ in this process still remain to be fully characterized. Considering the currently known role of estrogens in NSC physiology and the regulation of the neurogenesis [124,126,128-132], the sexual dimorphism we observed in ERα and ERβ expression between male and female NSC [14] supports a sex-based intrinsic difference in the regulation of neurogenesis. In addition, ERα genotype has been recently reported to be responsible for the inter-individual variability of responses to estrogen and testosterone in mesenchymal stem cell-derived osteoblasts [133]. Moreover, estradiol has been described to alter neurogenesis in female, but not male rats [134]. We also provided evidence that male NSC expressed a dramatically higher level of CYP19 than female NSC [135] which supports a capacity for male NSC, unlike female NSC, to metabolize testosterone and in turn, to produce estradiol. Such biochemical sexual dimorphism may underlie a steroid-related pharmacological counterpart as male NSC may therefore have the ability to alter their local environment and modulate endogenous neurogenesis in a different manner than female NSC may do. Remarkably, an autocrine control loop in two different systems, the NSC kinin/kallicrein pathway [136] and the androgenic apparatus of human bone marrow stromal cells [137]. It is noteworthy that the sex-based differences unveiled in vitro translated in vivo as we recently reported [69]. Indeed, the outcome of NSC transplantation in brain was shown to tightly depend on the sex of the donor and the sex of the recipient. Interestingly, in some cases cross-sex grafting provided better cell survival results than same-sex transplantation. As others also demonstrated the occurrence of a sexual dimorphism in the neurogenic capacity of rhesus monkeys mesenchymal stem cells [11], we are the first to have shown a direct impact on the outcome of stem cell transplantation [69]. Nevertheless, stem cell sexual dimorphism has been previously demonstrated by others in various organs [9,12,13,67,134] and organisms [138], and it is our opinion that sex should be considered as a critical factor and integrated in the development of future clinical protocol.

There is a consensual agreement that age-associated alterations in the brain play an important role in the decline of neurogenesis reported in aging [11,139-141]. However, very little work has been done in defining the age-related alteration of NSC neurogenic properties. Old mice have been shown to have less NSC in the subventricular zone (SVZ) compared to young ones, and a similar reduction has been reported in the number of NSC maintained as neurospheres and recovered in culture *in vitro* [142]. Aging effect on neurogenesis may also be structure-specific as revealed by others showing a decrease of NSC proliferation has been described in the hippocampus of old rats but not in the SVZ of the same animal [143]. On an other hand, spatial redistribution and a delay in the migratory process seem to affect NSC in the SVZ rather than a decrease of the proliferation rate [144]. However, although some studies showed that NSC from old animals retain some or all of their neurogenic properties, to date, studies that have systematically explored age-based differences of NSC neurogenic properties in terms of neuronal phenotype and protein marker expression are extremely scarce

[11,14,69,135,138,145,146]. Surprisingly, NSC isolated from young animals did not perform better in term of survival rate after brain transplantation when compared to the ones isolated from old rats [69]. Thus, such a result provocatively raises the question of the rejuvenation process NSC isolated from old individuals may undergo after being transplanted in a younger environment as suggested by two recent works [147,148]. Understanding the role of the age of NSC as a critical factor modulating the neuronal fate specificity and the maturation level reached by the engrafted NSC is absolutely essential from the perspective of stem cell grafting into the brain. In addition, in agreement with our previous results showing that NSC age differently depending on their sex, it appears that sex cannot be dissociated from age as a determining factor of NSC capacity to lead to functional recovery following transplantation.

5. Conclusion

Stem cell therapy holds a lot of promises for brain repair and the recovery of impaired neurological functions. However, despite the generation of a large amount of encouraging results produced on various animal models, the translation at the patient's bed seems to be delayed. Facing mitigated results from the few clinical studies that have been conducted so far, we are constrained to a cautious optimism. Indeed, the low success rate encountered in clinics questions our knowledge on the topic and suggests that we go back to a more fundamental bench work. Indeed, the high number of stem cell types, to which have to be added the various engineering counterparts, turn a luxury of choice into a situation where it is extremely, if not impossible, to determine what cell type or cell line would be the best candidate for tissue repair. Compelling evidence suggests that sex may be a critical determining factor of stem cell transplantation outcome in the brain. Surprisingly, none of the clinical studies reported to date took this parameter into consideration. Furthermore, we foresee that NSC differentiation induced by neurogenic agents *in vitro* and *in vivo* will be an important part of brain repair procedures as brain repair therapy and optimal neuronal function restoration will likely require both exogenous NSC grafting and pharmacological stimulation of the endogenous neurogenesis. However, nothing is currently known about the effect of cell sex on NSC sensitivity to pharmacologically-induced differentiation, and we therefore strongly believe that acquiring such knowledge is critical for the development of neurogenic treatments.

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