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LARGE ANIMAL MODELS ARE CRITICAL FOR RATIONALLY ADVANCING REGENERATIVE THERAPIES

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Enthusiasm for therapies based on the transplantation of exogenous cells or the transfer of genes by viral vectors has burgeoned over the past 30 years, accompanied by a predictable exhortation to launch clinical trials as soon as possible. Most data regarding safety, efficacy and mechanisms of these therapies have been derived from studies in rodents alone. While such 'small-animal' systems offer invaluable insights into fundamental biological questions, it is often misleading and perilous to unquestionably equate the higher order motor, sensory and cognitive processes that characterize human disease with that gleaned from a mouse or rat. Indeed, the literature is littered with clinical trials that failed and, in some cases, led to unforeseen adverse outcomes because the field leap-frogged over the requisite large-animal model. Large animals often provide an essential bridge between insights into fundamental biology and pathophysiology gleaned from simple systems and the realities of treating a human disease. Often, this is especially true for neurological disorders where not only differences in size and scale pertain, but also in neuroanatomical connections and organization, cognitive capacities, signaling pathways, genetic redundancy or the disease etiology.

While the gene therapy field has increased their use of nonhuman primates prior to the application of viral vectors in clinical trials, the cellular therapy field – represented most conspicuously of late by the stem cell field – has only recently begun to properly address this requirement. Monkeys and the minipig may prove to be excellent preclinical models owing to their similar comparative anatomy, pharmacokinetics and physiological and metabolic interactions. These models have proven to be extremely useful for studying endocrinological diseases, such as Type 1 diabetes, and neurological disorders, including Parkinson's disease (PD), spinal cord injury (SCI) and multiple sclerosis (MS). Prudence would argue that clinical trials for diseases in which differences between rodent and human transcend size and scale should require clear and definitive proof-of-concept in at least one relevant large-animal model in order to safeguard patients. This need, of course, is counterbalanced by considerations of the substantial cost, time and ethical circumspection that typically accompanies such research. In this editorial, we will attempt to help researchers reason through the potential need and advisability of using a large-animal model for their particular biological question.

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There are numerous differences between rodents and humans that make the unqualified translation of rodent data inadequate. The most relevant differences start at the genomic level and are manifested as differences in pharmacological, biochemical, developmental, behavioral and functional responses to perturbations and interventions [1]. Anatomical and cytoarchitectural differences are particularly profound in the brain, where innervation of specific regions is critical to addressing a neurological deficit (Figure 1). In PD for example, it has been estimated that for each volume of tissue innervated by a rat dopaminergic (DA) neuron, a monkey neuron must innervate 20-times that volume, while humans would need a staggering volume of 200 units to mimic similar reinnervation of nigrastratial projections [2]. In addition, differences in monoamine biochemistry and developmental life span make extrapolating rodent data to human therapies risky and insufficient [3–7]. Behavioral differences between rodents and humans are also of great significance [8–10]. It is likely that only a large animal model can adequately mirror the complexities of studying and restoring bipedal gait, balance, tremor, hand preference, fine motor coordination, spontaneous blink rate and cognitive deficits [11–16].

The Minipig

For research into PD, the nonhuman primate model is ideal, although, the technical, financial and organizational complexities of monkey colony maintenance may require the use of an alternative, somewhat more accessible, species, such as the Gottingen miniature pig [17]. The ‘minipig’, developed in the early 1960s [18,19], presents many advantages over the rodent as a model for human diseases. Its organ systems are organized similar to that of the human and it displays similar physiological and pathophysiological responses [20]. Similarities in pancreas size, position and shape [21], gastrointestinal tract structure and function [20], and pharmacokinetic responses make the minipig an especially useful model for Type 1 diabetes [22]. The kinetics and dynamics after subcutaneous delivery of insulin, [23,24] as well as aspects of metabolism [25] and glucose tolerance [26,27], closely resemble those of humans. Furthermore, several stable models for chemical induction of Type 1 diabetes have been established in minipigs using pharmacological insult to β -cells with streptozotocin (STZ) [28–31] or alloxan [32]. Recently, the transplantation of both human and porcine islets of Langerhans in Type 1 diabetes has been studied in diabetic pigs, as well as in dogs, monkeys and patients [33–41].

Complications in xenograft survival related to differences in the HLA and SLA systems between humans and pigs, respectively, are of concern, but may be partially overcome by using a more recent variation of the minipig – the ‘NIH minipig’. First described by Sachs in 1976 for transplantation studies [42,43], the NIH minipig, homozygous for known alleles of the major histocompatibility complex (MHC) [44], has been shown to be effective for modeling immunological and genetic aspects of heart, lung and spleen transplant tolerance [45,46,22] and is sufficiently bred to accept bone marrow and possibly hematopoietic stem cell transplants [47,48], as well as skin and heart grafts [22].

The minipig has a relatively large brain, approximately ten-times the mass of the marmoset [49,50], making it of recent interest in neurological disease studies. The size of the brain makes it especially appealing for imaging and stereotactic manipulation. There is a growing list of neurological diseases that have been modeled in the minipig: experimental allergic encephalomyelitis (EAE) [51], mimicking the autoimmune, demyelinating disease MS; Huntington’s disease (HD) CAG triplet repeats [52]; and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism [53]. Such models enable a more representative analysis of cell transplantation therapies for these disorders. In fact, porcine xenografts have already been tested in Phase I trials in both HD and PD patients [54,55], as

well as in a variety of other disorders [56,57], validating the use of the minipig as a bridge between rodent and human clinical trials.

The minipig has also been used to study implants of progenitor cells into the ischemic and contused spinal cord (M Marsala; correspondence). Indeed, it is work in the spinal cord and the difficulties in attempting to adapt procedures developed in rodent to the pig that highlight the absolute need for large animal studies as a precursor to human trials for such disorders [58].

Lessons from Parkinson's Disease

PD is characterized by degeneration of DA neurons in the substantia nigra pars compacta and subsequent loss of striatal DA release. Cell-based strategies for replacement of damaged DA neurons have been a promising strategy since the early 1970s, when Olsen and colleagues performed their initial transplantation experiments in rat brains [59–63]. Several subsequent studies in rats provided extended evidence for fetal cell transplants repairing DA deficiency [64,65]; however, despite numerous attempts by many groups, these results could never be repeated in primates [1]. Coupled with mounting ethical considerations, researchers turned to adrenal allografts as a new source of tissue; however, these also failed and subsequent clinical trials were abandoned [66–68]. New hope came again after 1985 when human fetal neural cells were derived and successfully implanted into the rat and monkey brain [69–76]; however ethical opponents argued that the large amount of fetal tissue required made these procedures for routine regenerative therapy unfeasible and, therefore, unfundable.

Based on the varying methods published in the mid-1970s, several groups began preliminary efficacy and safety clinical studies of fetal ventral mesencephalic grafts in PD patients. Initial studies in Sweden [77], England [78] and Mexico [79] produced variable results, elevating the ethical debate and political controversy, eventually leading to a funding moratorium in the USA [80,81]. Subsequent studies appeared promising, although the overall consensus was that the results were inconclusive owing to variability within small studies, limited short-term functional improvements and overall difficulty in comparing independent procedures [1]. To investigate this problem and improve the ability to compare studies, a panel of experts published a recommended protocol for future investigations, termed Core Assessment Program for Intracerebral Transplantation (CAPIT) [82].

In the same year, the first randomized controlled clinical trial, headed by Curt Freed and Stanley Fahn, was initiated studying 40 PD patients over 7 years, half of whom underwent bilateral stereotactic transplantation of human embryonic mesencephalic tissue into the putamen [83]. Although initial results appeared promising, it was determined that no benefit in primary outcome measure had been attained after 12 months post operation. However, a small subset of patients under 60 years old did show small double-blind improvements on a validated PD rating scale. More importantly, predictions made from animal models failed to predict the relatively high occurrence of dystonia and dyskinesia associated with long-term survival [84]. These gross abnormalities occurred in five out of 33 patients that lived 3 years post transplant, of whom, all five were aged less than 60 years and had displayed improvements in PD score after 12 months post surgery.

The human trials appeared to have a solid rationale based on a decade of rodent studies. Hundreds of investigators had reported survival and functional recovery from fetal ventral mesencephalic-derived neural tissue transplanted into the striatum of rats [85]. The grafts were shown to express tyrosine hydroxylase (TH), increase DA concentrations, project long outgrowths, form synaptic connections and respond to afferent stimulation [86–90]. However, the rat models of PD overestimated the likely success of fetal precursor transplants and failed to anticipate the dyskinetic side effects in human trials [84]. Furthermore, although large animal

models were tested, it is possible that they were not followed for a long enough time, simply owing to the vast difference between rodents and primates (of which human is an example) was not appreciated. Also, while the Freed and colleagues study made extremely valuable contributions to our understanding of PD, small design flaws, such as method of tissue preparation, limited target area and lack of immunosuppression, may have inherently impeded the overall success of the study [1]. Many of these details may have been worked out in large animals before reaching the clinic, potentially predicting adverse effects. Unfortunately, cell replacement therapy received negative media coverage [91–94], creating an impression of defeat in the public's mind and even creating dissension among PD investigators [84,92,95–102]. Much of this may have been avoided had a higher priority been placed on more extensive primate studies with larger numbers and longer follow-up.

Going Forward in Parkinson's Disease: A Case-in-Point for Choosing the Correct Model

Having detailed the pitfalls into which the PD field slipped by relying upon nonrepresentative data for its first cell-based clinical trial; how might it recover its momentum, and can this serve as a lesson for future trials for other diseases?

As aforementioned, most of the early transplantation studies in PD were performed in rodents that had received a unilateral injection of the dopaminergic toxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle or nearby [103,104]. Although transplantation appeared to promote recovery from the resultant PD-like symptoms, these behaviors actually bore little resemblance to human PD. If therapies were to be tested solely on such a rodent model, the results would likely have poor predictive value for actual patients. Conversely, through serendipity in the 1960s and 70s, it was discovered that the complex I inhibitor, MPTP, created pathology quite similar to human idiopathic PD in humans [105–107] and monkeys [70,108–112], especially the vervet [113,114]. MPTP depletes substantia nigra TH-immunoreactive and striatal DA to levels similar to PD, and produces characteristic Parkinsonian symptomatology, including akinesia, rigidity, postural alterations and tremor [9,10]. Overexpressing α -synuclein in conjunction with MPTP may create Lewy bodies [115,116]. Hence, systemically administered MPTP in African green monkeys has become acknowledged as the most authentic model for actual human idiopathic PD and the model in which the safety and efficacy of viral vector- and cell-based therapies in humans can most reliably be judged [1,8–10,117]. Indeed, the potential use of lentiviral and adeno-associated virus-based vectors for delivering neurotrophic factors in PD patients has been, and is currently being, tested in monkeys [118–120]. Such studies, in turn, set the stage for some Phase I clinical trials [121–131]. It is disheartening that, to date, very few investigators in the cell therapy field (including the stem cell field) have taken advantage of this model for work in PD, despite widespread recognition of its power. This reticence is, most probably, due to the huge expense entailed in performing such complex studies with a sufficiently large sample size and with suitably objective and predictive readouts over a long enough period of observation. Nevertheless, that expense is vastly smaller than the cost to the healthcare system, science and patient well-being when an ineffective or injurious clinical intervention becomes misguidedly launched. Funding agencies and scientists must begin to change their thinking and view adequately powered large animal studies for some diseases not as a luxury, but as a necessity.

Another source of hesitancy in the use of large animals, particularly monkeys, is not solely financial, but also ethical. There is no question that experimentation on 'higher order' animals provokes, more than any other type of scientific study, circumspection regarding the appropriateness of animal experimentation. Although we have outlined its scientific justification, there is no question that investigators must be acutely sensitive (as they should be for all animal work) in using the minimal number of monkeys required to statistically

overcome type 1 and 2 error rates [1], to treating the animals humanely and to only using animals in the most judicious manner and only for exquisitely well-conceived and rigorously designed experiments.

Another level of ethical consideration that has recently emerged as a consequence of the burgeoning human stem cell field, is whether integrating human cells into the brains of nonhuman primates (particularly prenatally) somehow ‘humanizes’ them. In other words, might a human–primate chimeric brain develop consciousness and, hence, merit consideration beyond that of just an experimental laboratory animal [131–134]. While an interesting metaphysical consideration, chimerism in our hands, even under the most optimal transplantation conditions, yields only a relatively small human cell contribution to an overwhelmingly primate CNS structure. Furthermore, we have never observed anything but typical primate behaviors, emotions and skills in our animals.

Summary & Conclusions

Appropriately assessing the safety and efficacy of many future interventions in regenerative medicine will require large animal preclinical testing to avoid the failures of previous studies in patients.

Such recognition requires scientists, investors, funding agencies and the public to recalibrate their thinking. Simpler rodent models, while useful for unraveling certain biological conundrums, often fall short in their predictive value for human disease or their appropriateness for devising effective delivery methods. A greater number of multicenter collaborations may be required to insure adequate funding and expertise for large animal studies. Such a method requires a less egocentric approach to scientific accomplishment. The ultimate cost of not including the rationale use of large animal models in preclinical studies—the price of unsuccessful trials, injury to patients and failure to bring potentially groundbreaking therapies to the bedside—far outweighs the short-term expense of the studies themselves.

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Bibliography

1. Redmond DE Jr. Cellular replacement therapy for Parkinson’s disease – where we are today? *Neuroscientist* 2002;8(5):457–488. [PubMed: 12374430]
2. Lindvall O, Rehncrona S, Brundin P, et al. Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson’s disease: a detailed account of methodology and a 6-month follow-up. *Arch Neurol* 1989;46:615–631. [PubMed: 2786405]
3. Bacopoulos NG, Maas JW, Hattox SE, Roth RH. Regional distribution of dopamine metabolites in human and primate brain. *Comm Psychopharmacol* 1978;2:281–286.
4. Felten DL, Sladek JRJ. Monoamine distribution in primate brain: V monoamine nuclei: anatomy, pathways and local organization. *Brain Res Bull* 1983;10:171–284. [PubMed: 6839182]
5. Waddington JL, O’Boyle KM. Drugs acting on brain dopamine receptors: a conceptual reevaluation five years after the first selective D1 antagonist. *Pharmacol Ther* 1989;43:1–52. [PubMed: 2675127]
6. Haycock JW. Four forms of tyrosine hydroxylase are present in human adrenal medulla. *J Neurochem* 1991;56:2139–2142. [PubMed: 1674001]

7. Nagatsu T. Genes for human catecholamine-synthesizing enzymes. *Neurosci Res* 1991;12:315–345. [PubMed: 1684650]
8. Steece-Collier K, Maries E, Kordower JH. Etiology of Parkinson's disease: genetics and environment revisited. *Proc Natl Acad Sci USA* 2002;99(22):13972–13974. [PubMed: 12391311]
9. Collier TJ, Steece-Collier K, Kordower JH. Primate models of Parkinson's disease. *Exp Neurol* 2003;183(2):258–262. [PubMed: 14552866]
10. Emborg ME. Evaluation of animal models of Parkinson's disease for neuroprotective strategies. *J Neurosci Methods* 2004;139(2):121–143. [PubMed: 15488225]
11. Taylor JR, Roth RH, Sladek JR Jr, Redmond DE Jr. Cognitive and motor deficits in the performance of an object retrieval task with a barrier-detour in monkeys (*Cercopithecus aethiops sabaeus*) treated with MPTP: long-term performance and effect of transparency of the barrier. *Behav Neurosci* 1990;104(4):564–576. [PubMed: 2206426]
12. Taylor JR, Elsworth JD, Roth RH, Sladek JR Jr, Redmond DE Jr. Cognitive and motor deficits in the acquisition of an object retrieval/detour task in MPTPtreated monkeys. *Brain* 1990;113(Pt 3):617–637. [PubMed: 2364263]
13. Taylor, JR.; Elsworth, JD.; Roth, RH.; Collier, TJ.; Sladek, JR.; Redmond, DE, Jr. Improvements in MPTP-induced object retrieval deficits and behavioral deficits after fetal nigral grafting in monkeys. In: Richards, SJ.; Dunnett, SB., editors. *Progress in brain research*. Elsevier; Amsterdam, The Netherlands: 1990. p. 543-559.
14. Elsworth JD, Lawrence MS, Roth RH, et al. D1 and D2 dopamine receptors independently regulate spontaneous blink rate in the vervet monkey. *J Pharmacol Exp Ther* 1991;259:595–600. [PubMed: 1682479]
15. Taylor JR, Elsworth JD, Roth RH, Sladek JR Jr, Collier TJ, Redmond DE Jr. Grafting of fetal substantia nigra to striatum reverses behavioral deficits induced by MPTP in primates: a comparison with other types of grafts as controls. *Exp Brain Res* 1991;85(2):335–348. [PubMed: 1893983]
16. Kordower JH, Liu YT, Winn S, Emerich DF. Encapsulated PC12 cell transplants into hemiparkinsonian monkeys: a behavioral, neuroanatomical, and neurochemical analysis. *Cell Transplant* 1995;4(2):155–171. [PubMed: 7773555]
17. Vodicka P, Smetana K Jr, Dvorankova B, et al. The miniature pig as an animal model in biomedical research. *Ann N Y Acad Sci* 2005;1049:161–171. [PubMed: 15965115]
18. Bollen, P.; Ellegaard, L. Developments in breeding Göttingen minipigs. In: Tumbleson, ME.; Schook, LB., editors. *Advances in Swine in Biomedical Research*. Plenum Press; NY, USA: 1996. p. 59-66.
19. Bollen P, Ellegaard L. The Göttingen minipig in pharmacology and toxicology. *Pharmacol Toxicol* 1997;80:3–4. [PubMed: 9249853]
20. Brown, D.; Terris, J. Swine in physiological and pathophysiological research. In: Tumbleson, ME.; Schook, LB., editors. *Advances in Swine in Biomedical Research*. Plenum Press; NY, USA: 1996. p. 5-6.
21. Murakami T, Hitomi S, Ohtsuka A, Taguchi T, Fujita T. Pancreatic insuloacinar portal systems in humans, rats, and some other mammals: Scanning electron microscopy of vascular casts. *Microsc Res Tech* 1997;37:478–488. [PubMed: 9220425]
22. Larsen MO, Rolin B. Use of the Göttingen minipig as a model of diabetes, with special focus on type 1 diabetes research. *ILAR J* 2004;45(3):303–313. [PubMed: 15229377]
23. Markussen J, Havelund S, Kurtzhals P, et al. Soluble, fatty acid acylated insulins bind to albumin and show protracted action in pigs. *Diabetologia* 1996;39:281–288. [PubMed: 8721773]
24. Pieber TR, Plank J, Görzer E, et al. Duration of action, pharmacodynamic profile and between-subject variability of insulin determine subjects with type 1 diabetes. *Diabetologia* 2002;45:A257.
25. Miller ER, Ullrey DE. The pig as a model for human nutrition. *Annu Rev Nutr* 1987;7:361–382. [PubMed: 3300739]
26. Broughton DL, Taylor R. Review: deterioration of glucose tolerance with age: the role of insulin resistance. *Age Ageing* 1991;20:221–225. [PubMed: 1853796]
27. Rosenthal M, Doberne L, Greenfield M, Widstrom A, Reaven GM. Effect of age on glucose tolerance, insulin secretion, and *in vivo* insulin action. *J Am Geriatr Soc* 1982;30:562–567. [PubMed: 7050220]
28. Marshall M, Sprandel U, Zollner N. Streptozotocin diabetes in a miniature pig. *Res Exp Med* 1975;165:61–65.

29. Marshall M. Induction of chronic diabetes by streptozotocin in the miniature pig. *Res Exp Med* 1979;175:187–196.
30. Gabel H, Bitter-Suermann H, Henriksson C, Save-Soderbergh J, Lundholm K, Brynger H. Streptozotocin diabetes in juvenile pigs. Evaluation of an experimental model. *Horm Metab Res* 1985;17:275–280. [PubMed: 3160643]
31. Wilson JD, Dhall DP, Simeonovic CJ, Lafferty KJ. Induction and management of diabetes mellitus in the pig. *Aust J Exp Biol Med Sci* 1986;64:489–500. [PubMed: 2954529]
32. Kjems LL, Kirby BM, Welsh EM, et al. Decrease in β -cell mass leads to impaired pulsatile insulin secretion, reduced postprandial hepatic insulin clearance, and relative hyperglucagonemia in the minipig. *Diabetes* 2001;50:2001–2012. [PubMed: 11522665]
33. Mellert J, Hopt UT, Hering BJ, Bretzel RG, Federlin K. Influence of islet mass and purity on reversibility of diabetes in pancreatectomized pigs. *Transplant Proc* 1991;23:1687–1689. [PubMed: 1989331]
34. Morsiani E, Fogli L, Lanza G, Lebow LT, Demetriou AA, Rozga J. Long-term insulin independence following repeated islet transplantation in totally pancreatectomized diabetic pigs. *Cell Transplant* 2001;11:55–66. [PubMed: 12095221]
35. Kin T, Iwata H, Aomatsu Y, et al. Xenotransplantation of pig islets in diabetic dogs with use of a microcapsule composed of agarose and polystyrene sulfonic acid mixed gel. *Pancreas* 2002;25:94–100. [PubMed: 12131778]
36. Buhler L, Deng S, O'Neil J, et al. Adult porcine islet transplantation in baboons treated with conventional immunosuppression or a nonmyeloablative regimen and CD154 blockade. *Xenotransplantation* 2002;9:3–13. [PubMed: 12005102]
37. Cantarovitch D, Blanco G, Potiron N, et al. Rapid failure of pig islet transplantation in non human primates. *Xenotransplantation* 2002;9:25–35. [PubMed: 12005101]
38. Groth CG, Korsgren O, Tibell A, et al. Transplantation of porcine fetal pancreas to diabetic patients. *Lancet* 1994;344:1402–140. [PubMed: 7968077]
39. Elliott RB, Escobar L, Garkavenko O, et al. No evidence of infection with porcine endogenous retrovirus in recipients of encapsulated porcine islet xenografts. *Cell Transplant* 2000;9:895–901. [PubMed: 11202575]
40. Groth CG, Tibell A, Wennberg L, et al. Clinical aspects and perspectives in islet xenotransplantation. *J Hepatobil Pancreat Surg* 2000;7:364–369.
41. Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000;343:230–238. [PubMed: 10911004]
42. Sachs D, Leight HG, Cone J, Schwarz S, Stuart L, Rosenberg S. Transplantation in miniature swine. I. Fixation of the major histocompatibility complex. *Transplantation* 1976;22:559–567. [PubMed: 137560]
43. Sachs, DH. MHC-homozygous miniature swine. In: Swindel, MM.; Moody, DC.; Philips, LD., editors. *Swine as Models in Biomedical Research*. Iowa State University Press; IA, USA: 1992. p. 3-15.
44. Mezrich JD, Haller GW, Arn JS, Houser SL, Madsen JC, Sachs DH. Histocompatible miniature swine: an inbred large-animal model. *Transplantation* 2003;75(6):904–907. [PubMed: 12660524]
45. Allan JS, Wain JC, Schwarze ML, et al. Modeling chronic lung allograft rejection in miniature swine. *Transplantation* 2002;73(3):447–453. [PubMed: 11884943]
46. Gollackner B, Dor FJ, Knosalla C, et al. Spleen transplantation in miniature swine: surgical technique and results in major histocompatibility complex-matched donor and recipient pairs. *Transplantation* 2003;75(11):1799–806. [PubMed: 12811237]
47. Kenmochi T, Mullen Y, Miyamoto M, Stein E. Swine as an allotransplantation model. *Vet Immunol Immunopathol* 1994;43(1–3):177–183. [PubMed: 7856050]
48. Emery DW, Sachs DH, LeGuern C. Culture and characterization of hematopoietic progenitor cells from miniature swine. *Exp Hematol* 1996;24(8):927–935. [PubMed: 8690052]
49. Tumbleson ME. Brain weight, as a function of age, in miniature swine. *Growth* 1973;37(1):13–17. [PubMed: 4700426]

50. Holloway RL, Heilbroner P. Corpus callosum in sexually dimorphic and nondimorphic primates. *Am J Phys Anthropol* 1992;87(3):349–357. [PubMed: 1562061]
51. Singer BA, Tresser NJ, Frank JA, McFarland HF, Biddison WE. Induction of experimental allergic encephalomyelitis in the NIH minipig. *J Neuroimmunol* 2000;105(1):7–19.
52. Matsuyama N, Hadano S, Onoe K, et al. Identification and characterization of the miniature pig Huntington's disease gene homolog: evidence for conservation and polymorphism in the CAG triplet repeat. *Genomics* 2000;69(1):72–85. [PubMed: 11013077]
53. Mikkelsen M, Moller A, Jensen LH, Pedersen A, Harajehi JB, Pakkenberg H. MPTP-induced Parkinsonism in minipigs: a behavioral, biochemical, and histological study. *Neurotoxicol Teratol* 1999;21(2):169–175. [PubMed: 10192277]
54. Fink JS, Schumacher JM, Ellias SL, et al. Porcine xenografts in Parkinson's disease and Huntington's disease patients: preliminary results. *Cell Transplant* 2000;9(2):273–278. [PubMed: 10811399]
55. Schumacher JM, Ellias SA, Palmer EP, et al. Transplantation of embryonic porcine mesencephalic tissue in patients with PD. *Neurology* 2000;54(5):1042–1050. [PubMed: 10720272]
56. Valdes-Gonzalez RA, Dorantes LM, Garibay GN, et al. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. *Eur J Endocrinol* 2005;153(3):419–427. [PubMed: 16131605]
57. Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR. Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. *Cerebrovasc Dis* 2005;20(2):101–107. [PubMed: 15976503]
58. Iwanami A, Kaneko S, Nakamura M, et al. Transplantation of human neural stem cells for spinal cord injury in primates. *J Neurosci Res* 2005;80(2):182–190. [PubMed: 15772979]
59. Olson L, Seiger A. Brain tissue transplanted to the anterior chamber of the eye: 1. Fluorescence histochemistry of immature catecholamine and 5-hydroxytryptamine neurons reinnervating the rat iris. *Z Zellforsch Mikrosk Anat* 1972;135:175–194. [PubMed: 4642527]
60. Olson L, Seiger A. Locus coeruleus: fiber growth regulation in oculo. *Med Biol* 1976;54:142–145. [PubMed: 1271874]
61. Bjorklund A, Stenevi U, Svendgaard N. Growth of transplanted monoaminergic neurones into the adult hippocampus along the perforant path. *Nature* 1976;262(5571):787–790. [PubMed: 958453]
62. Stenevi U, Bjorklund A, Svendgaard N. Transplantation of central and peripheral monoamine neurons to the adult rat brain: techniques and conditions for survival. *Brain Res* 1976;114(1):1–20. [PubMed: 963534]
63. Hoffer B, Seiger A, Freedman R, Olson L, Taylor D. Electrophysiology and cytology of hippocampal formation transplants in anterior chamber of the eye: II. Cholinergic mechanisms. *Brain Res* 1977;119:107–132. [PubMed: 830378]
64. Bjorklund A, Stenevi U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. *Brain Res* 1979;177(3):555–560. [PubMed: 574053]
65. Perlow MJ, Freed WJ, Hoffer BJ, Seiger A, Olson L, Wyatt RJ. Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science* 1979;204:643–653. [PubMed: 571147]
66. Backlund EO, Granberg B, Hamberger E, et al. Transplantation of adrenal medullary tissue to striatum in parkinsonism: first clinical trials. *J Neurosurg* 1985;62:169–173. [PubMed: 2578558]
67. Rehnckrona S. A critical review of the current status and possible developments in brain transplantation. *Adv Tech Stand Neurosurg* 1997;23:3–46. [PubMed: 9075470]
68. Hallett M, Litvan I. Scientific position paper of the Movement Disorder Society evaluation of surgery for Parkinson's disease. Task Force on Surgery for Parkinson's Disease of the American Academy of Neurology Therapeutic and Technology Assessment Committee. *Mov Disord* 2000;15(3):436–438. [PubMed: 10830406]
69. Redmond DE Jr, Roth RH, Sladek JR. MPTP produces classic parkinsonian syndrome in African green monkeys. *Neurosci Abstr* 1985;11(1):166.
70. Redmond DE Jr, Sladek JR Jr, Roth RH, et al. Fetal neuronal grafts in monkeys given methylphenyltetrahydropyridine. *Lancet* 1986;1(8490):1125–1127. [PubMed: 2871381]
71. Redmond DE Jr, Sladek JR Jr, Roth RH, et al. Transplants of primate neurons [letter]. *Lancet* 1986;2(8514):1046. [PubMed: 2877218]

72. Redmond DE Jr, Naftolin F, Collier TJ, et al. Cryopreservation, culture, and transplantation of human fetal mesencephalic tissue into monkeys. *Science* 1988;242(4879):768–771. [PubMed: 2903552]
73. Bakay RAE, Barrow DL, Fiandaca MS, Iuvone PM, Schiff A, Collins DC. Biochemical and behavioral correction of MPTP Parkinson-like syndrome by fetal cell transplantation. *Ann N Y Acad Sci* 1987;495:623–640. [PubMed: 3496821]
74. Sladek JR Jr, Redmond DE Jr, Collier TJ, et al. Transplantation of fetal dopamine neurons in primate brain reverses MPTP induced parkinsonism. *Prog Brain Res* 1987;71:309–323. [PubMed: 3495818]
75. Sladek JR Jr, Redmond DE Jr, Collier TJ, et al. Fetal dopamine neural grafts: extended reversal of methylphenyltetrahydropyridine-induced parkinsonism in monkeys. *Prog Brain Res* 1988;78:497–506. [PubMed: 3266802]
76. Brundin P, Strecker RE, Widner H, et al. Human fetal dopamine neurons grafted in a rat model of Parkinson's disease: immunological aspects, spontaneous and drug-induced behaviour, and dopamine release. *Exp Brain Res* 1988;70:192–208. [PubMed: 3402564]
77. Lindvall O, Rehnström S, Gustavii B, et al. Fetal dopamine-rich mesencephalic grafts in Parkinson's disease. *Lancet* 1988;2:1483–1484. [PubMed: 2904587]
78. Hitchcock ER, Kenny BG, Henderson BT, Clough CG, Hughes RC, Detta A. A series of experimental surgery for advanced Parkinson's disease by fetal mesencephalic transplantation. *Acta Neurochirurgica Suppl (Wein)* 1991;52:54–57.
79. Madrazo I, León V, Torres C, et al. Transplantation of fetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's disease. *N Engl J Med* 1988;318:51.
80. Mahowald MB, Areen J, Hoffer BJ, et al. Transplantation of neural tissue from fetuses. *Science* 1987;235:1307–1308. [PubMed: 3823882]
81. Hoffer BJ, Olson L. Ethical issues in brain cell transplantation. *Trends Neurosci* 1991;14:384–388. [PubMed: 1721747]
82. Langston JW, Widner H, Goetz CG, et al. Core Assessment Program for Intracerebral Transplantations (CAPIT). *Mov Disord* 1992;7:2–13. [PubMed: 1557062]
83. Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 2001;344(10):710–719. [PubMed: 11236774]
84. Hagell P, Piccini P, Björklund A, et al. Dyskinesias following neural transplantation in Parkinson's disease. *Nat Neurosci* 2002;5(7):627–628. [PubMed: 12042822]
85. 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 Res* 1980;199:307–333. [PubMed: 7417786]
86. Kordower J, Freeman T, Snow B, et al. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N Engl J Med* 1995;332:1118–1124. [PubMed: 7700284]
87. Freund TF, Bolam JP, Björklund A, et al. Efferent synaptic connections of grafted dopaminergic neurons reinnervating the host striatum: a tyrosine hydroxylase immunocytochemical study. *J Neurosci* 1985;5:603–616. [PubMed: 2857778]
88. Fisher LJ, Young SJ, Tepper JM, Groves PM, Gage FH. Electrophysiological characteristics of cells within mesencephalon suspension grafts. *Neuroscience* 1991;40(1):109–122. [PubMed: 2052146]
89. Kordower J, Rosenstein J, Collier T, et al. Functional fetal nigral grafts in a patient with Parkinson's disease: chemoanatomic, ultrastructural, and metabolic studies. *J Comp Neurol* 1996;370:203–230. [PubMed: 8808731]
90. Kordower JH, Styren S, Clarke M, DeKosky ST, Olanow CW, Freeman TB. Fetal grafting for Parkinson's disease: expression of immune markers in two patients with functional fetal nigral implants. *Cell Transplant* 1997;6(3):213–219. [PubMed: 9171154]
91. Craven R. Disappointment for Parkinson's disease patients. *Trends Pharmacol Sci* 2001;22(5):221.
92. Kolata G. Parkinson's research is set back by failure of fetal cell implants. *The New York Times*. March 8;2001
93. Vogel G. Parkinson's research. Fetal cell transplant trial draws fire. *Science* 2001;291(5511):2060–2061. [PubMed: 11256388]

94. Williams N. Setback spurs Parkinson's disease research. *Curr Biol* 2001;11(8):R285–R286. [PubMed: 11369210]
95. Abbott A. Trials offer way forward for Parkinson's. *Nature* 2001;410(6827):401. [PubMed: 11260676]
96. Bakay RA. Is transplantation to treat Parkinson's disease dead? *Neurosurgery* 2001;49(3):576–580. [PubMed: 11523665]
97. Brundin P, Dunnett S, Bjorklund A, Nikkhah G. Transplanted dopaminergic neurons: more or less? *Nat Med* 2001;7(5):512–513. [PubMed: 11329027]
98. Dunnett SB, Bjorklund A, Lindvall O. Cell therapy in Parkinson's disease stop or go? *Nat Rev Neurosci* 2001;2(5):365–369. [PubMed: 11331920]
99. Fischbach GD, McKhann GM. Cell therapy for Parkinson's disease. *N Engl J Med* 2001;344(10):763–765. [PubMed: 11236783]
100. Isacson O, Bjorklund L, Pernaute RS. Parkinson's disease: interpretations of transplantation study are erroneous. *Nat Neurosci* 2001;4(6):553. [PubMed: 11369929]
101. Olanow CW, Freeman T, Kordower J. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 2001;345(2):146–147. [PubMed: 11450669]
102. Redmond DE Jr, Sladek JR, Spencer DD. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 2001;345(2):146–147. [PubMed: 11450668]
103. Ungerstedt U, Arbuthnott GW. Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res* 1970;24(3):485–493. [PubMed: 5494536]
104. Cenci MA, Whishaw IQ, Schallert T. Animals models of neurological deficits: how relevant is the rat. *Nat Rev Neurosci* 2002;3:574–579. [PubMed: 12094213]
105. Langston J, Irwin I, Langston E, Forno L. 1-Methyl-4-phenylpyridinium ion (MPP⁺): identification of a metabolite of MPTP, a toxin selective to the substantia nigra. *Neurosci Lett* 1984;48:87–92. [PubMed: 6332288]
106. Burns RS, LeWitt PA, Ebert MH, Pakkenberg H, Kopin IJ. The clinical syndrome of striatal dopamine deficiency: parkinsonism induced by 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP). *N Engl J Med* 1985;312(22):1418–1421. [PubMed: 2581135]
107. Langston JW, Forno LS, Tetud J, Reeves AG, Kaplan JA, Karluk D. Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. *Ann Neurol* 1999;46(4):598–605. [PubMed: 10514096]
108. Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ. A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by Nmethyl- 4-phenyl- 1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci USA* 1983;80(14):4546–4550. [PubMed: 6192438]
109. Forno, LS.; DeLanney, LE.; Irwin, I.; Langston, JW. Neuropathology of MPTP treated monkeys: comparison with the neuropathology of human idiopathic Parkinson's disease. In: Markey, SP., editor. MPTP: a neurotoxin producing a parkinsonian syndrome. Academic Press; FL, USA: 1986. p. 119-140.
110. Deutch AY, Elsworth JD, Goldstein M, et al. Preferential vulnerability of A8 dopamine neurons in the primate to the neurotoxin 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine. *Neurosci Lett* 1986;68(1):51–56. [PubMed: 3487756]
111. Elsworth JD, Deutch AY, Redmond DE Jr, Sladek JR Jr, Roth RH. Differential responsiveness to 1-methyl-4-phenyl- 1,2,3,6- tetrahydropyridine toxicity in subregions of the primate substantia nigra and striatum. *Life Sci* 1987;40(2):193–202. [PubMed: 3491946]
112. Elsworth JD, Deutch AY, Redmond DE Jr, Sladek JR Jr, Roth RH. Effects of 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) on catecholamine and metabolites in primate brain and CSF. *Brain Res* 1987;415:293–299. [PubMed: 3496938]
113. Taylor, JR.; Elsworth, JD.; Sladek, JR., Jr; Roth, RH.; Redmond, DE, Jr. Behavioral effects of MPTP administration in the vervet monkey: a primate model of Parkinson's disease. In: Woodruff, ML.; Nonneman, AJ., editors. Toxin-induced models of neurological disorders. Plenum Press; NY, USA: 1994.

114. Taylor JR, Elsworth JD, Roth RH, Sladek JR Jr, Redmond DE Jr. Severe long-term 1- methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced parkinsonism in the vervet monkey (*Cercopithecus aethiops sabaeus*). *NeuroScience* 1997;81:745–755. [PubMed: 9316026]
115. Kirik D, Rosenblad C, Burger C, et al. Parkinson-like neurodegeneration induced by targeted overexpression of α -synuclein in the nigrostriatal dopamine system. *J Neurosci* 2001;22(7):2780–2791. [PubMed: 11923443]
116. Kirik D, Annett LE, Burger C, Muzyczka N, Mandel RJ, Bjorklund A. Nigrostriatal α -synucleinopathy induced by viral vector mediated overexpression of human α -synuclein: a new primate model of Parkinson's disease. *Proc Natl Acad Sci USA* 2003;100(5):2884–2889. [PubMed: 12601150]
117. Dass B, Olanow CW, Kordower JH. Gene transfer of trophic factors and stem cell grafting as treatments for Parkinson's disease. *Neurology* 2006;66(10 Suppl 4):S89–S103. [PubMed: 16717256]
118. Kordower JH, Emborg ME, Bloch J, et al. Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 2000;290(5492):767–773. [PubMed: 11052933]
119. Palfi S, Leventhal L, Chu Y, et al. Lentivirally delivered glial cell line-derived neurotrophic factor increases the number of striatal dopaminergic neurons in primate models of nigrostriatal degeneration. *J Neurosci* 2002;22(12):4942–4954. [PubMed: 12077191]
120. Soderstrom K, O'Malley J, Steece-Collier K, Kordower JH. Neural repair strategies for Parkinson's disease: insights from primate models. *Cell Transplant* 2006;15(3):251–265. [PubMed: 16719060]
121. Slevin JT, Gash DM, Smith CD, et al. Unilateral intraputamenal glial cell line derived neurotrophic factor in patients with Parkinson disease: response to 1 year each of treatment and withdrawal. *Neurosurg Focus* 2006;20(5):E12. [PubMed: 16709017]
122. Barker RA. Continuing trials of GDNF in Parkinson's disease. *Lancet Neurol* 2006;5(4):285–286. [PubMed: 16545740]
123. Chebrolu H, Slevin JT, Gash DA, et al. MRI volumetric and intensity analysis of the cerebellum in Parkinson's disease patients infused with glial-derived neurotrophic factor (GDNF). *Exp Neurol* 2006;198(2):450–456. [PubMed: 16455079]
124. Lang AE, Gill S, Patel NK, et al. Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. *Ann Neurol* 2006;59(3):459–466. [PubMed: 16429411]
125. Patel NK, Bunnage M, Plaha P, Svendsen CN, Heywood P, Gill SS. Intraputamenal infusion of glial cell line-derived neurotrophic factor in PD: a two year outcome study. *Ann Neurol* 2005;57(2):298–302. [PubMed: 15668979]
126. Nutt JG, Burchiel KJ, Comella CL, et al. Implanted intracerebroventricular Glial cell line-derived neurotrophic factor Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology* 2003;60(1):69–73. [PubMed: 12525720]
127. Gill SS, Patel NK, Hotton GR, et al. Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med* 2003;9(5):589–595. [PubMed: 12669033]
128. Kordower JH. *In vivo* gene delivery of glial cell line-derived neurotrophic factor for Parkinson's disease. *Ann Neurol* 2003;53(Suppl 3):S120–S132. [PubMed: 12666104]
129. McBride JL, Kordower JH. Neuroprotection for Parkinson's disease using viral vector mediated delivery of GDNF. *Prog Brain Res* 2002;138:421–432. [PubMed: 12432782]
130. During MJ, Kaplitt MG, Stern MB, Eidelberg D. Subthalamic GAD gene transfer in Parkinson disease patients who are candidates for deep brain stimulation. *Hum Gene Ther* 2001;12(12):1589–1591. [PubMed: 11529246]
131. During MJ, Kaplitt MG, Stern MB, et al. A dose-ranging study of AAV-hAADC therapy in parkinsonian monkeys. *Mol Ther*. 2006
132. Shreeve J. The other stem-cell debate: to test the potential curative powers of human embryonic stem cells, biologists want to inject them into lab animals. Creating such chimeras makes perfect sense, to a point: a sheep with a human liver? O.K. A mouse brain made up of human cells? Maybe. But a chimp that sobs? *New York Times Magazine*. April 10;2005

133. Robert JS. The Science and ethics of making part-human animals in stem cell biology. *FASEB J* 2006;20(7):838–845. [PubMed: 16675841]
134. Greene M, Schill K, Takahashi S, et al. Ethics: moral issues of human non-human primate neural grafting. *Science* 2005;309(5733):385–386. [PubMed: 16020716]
135. Laird AR, Lancaster JL, Fox PT. BrainMap: the social evolution of a functional neuroimaging database. *Neuroinformatics* 2005;3:65–78. [PubMed: 15897617]

Figure 1. Coronal images of Nissl stained sections from *Mus musculus*, *Chlorocebus aethiops*, *Macaca mulatta*, and MRI of human cortex [135].