Journal of Parkinson's Disease 10 (2020) 1335–1342
DOI 10.3233/JPD-202200
IOS Press

Position Paper

Animal Models of Parkinson's Disease: Are They Useful or Not?

Roger A. Barker[a,*] and Anders Björklund[b,*]
[a]*Department of Clinical Neuroscience and WT-MRC Cambridge Stem Cell Institute, Forvie Site, Cambridge, UK*
[b]*Department of Experimental Medical Science, Wallenberg Neuroscience Center, Lund University, Lund, Sweden*

Accepted 1 July 2020

Abstract. The use of animal models in Parkinson's disease research has been controversial in terms of how well they relate to the clinical condition and thus their utility for translating therapies from the lab to the clinic. In this article, two researchers debate this issue with Roger Barker taking the view that such models are not useful and may even be misleading, while Anders Björklund defends their use and highlights their value in better understanding and treating this condition.

Keywords: Animal models, drug discovery, experimental therapies, alpha-synuclein, dopamine, Lewy bodies

ANIMAL MODELS HAVE FAILED: ROGER A. BARKER

Parkinson's disease (PD) is a uniquely human condition that typically presents late in life and is sporadic in origin in the vast majority of cases. The average age at which it is diagnosed clinically is about 70 years [1, 2] and it progresses slowly over many years/decades with a prodromal stage of disease that may be as long as 10–15 years ahead of the motor presentation and diagnosis [3]. It is defined clinically by motor abnormalities (resting tremor, bradykinesia, and rigidity) as well as an array of non-motor features many of which are purely subjective in nature, such as apathy, somnolence and changes in mood and anxiety. These latter clinical features are by their very nature human specific but which significantly impact on quality of life [4]. All of these clinical features reflect an underlying pathology that involves the enteric and autonomic nervous systems as well as sites across the CNS. However, the extent of these varies between patients. As such the disease is very heterogenous with some patients progressing rapidly to falls and an early dementia, while others progress more slowly reflecting a much more benign condition [5].

Pathologically PD is characterised by the presence of α-synuclein Lewy bodies and Lewy neurites and the loss of the nigrostriatal dopaminergic pathway [6]. However, the disease is not restricted to this site pathologically as there is evidence that α-synuclein pathology is found at many CNS sites as well as outside the brain and that this may even be where the disease process actually starts [7]. Furthermore, there is now accumulating evidence that all chronic neurodegenerative disorders of the brain, of which PD is just one example, are characterised by mixed pathologies including vascular disease and other proteinopathies, of which one is dominant but

*Correspondence to: Roger A. Barker, Department of Clinical Neuroscience and WT-MRC Cambridge Stem Cell Institute, Forvie Site, Cambridge CB2 0PY, UK. Tel.: +44 1223 331160; Fax: +44 1223 331174; E-mail: rab46@cam.ac.uk; Anders Björklund, Department of Experimental Medical Science, Wallenberg Neuroscience Center, Lund University, 22184 Lund, Sweden. Tel.: +46 462220540; E-mail: anders.bjorklund@med.lu.se.

not exclusively present, namely α-synuclein in PD [8].

All of this creates a major problem when it comes to simulating PD in the laboratory as ideally any animal model should include:

- Aged animals (>18 months old for rats);
- A slowly progressive disease starting at around 12 months of age in the rat or mouse with clear motor features appearing and progressing from about 18 months of age to death at 2 years;
- No genetic manipulation around a single known mendelian gene linked to PD (i.e., not in transgenic animals) given most patients do not harbour such mutations;
- Alpha synuclein pathology in the brain, enteric and autonomic nervous system including the development of Lewy bodies and Lewy neurites in the relevant neuronal populations at the right time—thus starting in the gut and olfactory bulb before spreading up the brainstem and across into the cortices;
- The range of pathologies seen in patients dying with PD that lie outside those that are defined solely by the α-synucleinopathy;
- Behavioural deficits that encompass all the features of PD in the right temporal order and which recapitulate those found in patients symptomatically;
- Methods for capturing and assessing the more subjective aspects of PD that greatly impact on the quality of life of patients and their carers including apathy, anxiety, somnolence, etc.

This has so far not been achieved as most models of PD use young animals that have either had been modelled using:

- Neurotoxins to directly target the dopaminergic nigrostriatal pathway (e.g., 6-OHDA and MPTP);
- The overexpression of a pathogenic protein (e.g., AAV – α-synuclein) in target areas of the brain;
- Transgenic animals that incorporate one of the known mendelian genes causing parkinsonism (e.g., LRRK2 mice);
- Injections of preformed fibrils (PFF) of α-synuclein to seed pathology across the neuroaxis.

However, it is clear none of these approaches come close to modelling PD in patients. More specifically, the neurotoxin models whilst having the merit of allowing one to study the dopaminergic system in PD—thus creating a model that has so called face validity (i.e., modelling a core pathological deficit of PD); this is not the same as studying the complex pathology, clinical expression, and temporal progression of what is seen in patients with PD. A model capturing this would have construct validity. However, focal injections of α-synuclein while allowing you to study what effect overexpression of α-synuclein has on that region of the brain, this does not tell you what goes wrong in PD where α-synuclein levels are normal. Transgenic models can help tell you about the role of a pathogenic gene and its protein product but this is not the same as studying PD given that <5% of patients with PD in the community have Mendelian forms of the condition [9]. Finally injections of α-synuclein PFF tell you about how this protein can spread and seed pathology as it does so, but there is no evidence that this actually happens in the brain of patients with PD [10, 11] nor that these fibrils resemble those found in the PD brain [10].

Thus, none of these models recapitulates the age of onset, the temporal speed of the disorder, nor the spectrum of problems and pathologies you see in the clinic in patients with PD. As such these models cannot really help us understand and treat PD in terms of getting to the core pathological events in the vast majority of people with sporadic disease. In fact, they can be positively misleading as they are modelling the wrong construct!

This last point is important as it can have a major impact on translation to the clinic. One illustration of this is with the growth factor GDNF. This factor was shown to work well in rescuing the aging and/or neurotoxin lesioned dopaminergic system in rodents and non-human primates, but when tried in PD patients it has not worked in a reproducible way (reviewed [12]). Subsequently it was shown when using α-synuclein as the "toxin" to model PD in the lab that this protein could interfere with the signalling pathway of GDNF, which could explain the lack of clinical efficacy [13]. However, this was only modelled in the lab through overexpression of α-synuclein, which is not the situation in patients. So, what can we learn about the therapeutic role of GDNF from this preclinical work for patients with PD—not much, only that it can work on acutely lesioned rodent and NHP nigrostriatal dopamine neurons to restore their function *in vivo*. Of course, one could argue that such work should only be interpreted when done in conjunction with human *in vitro* systems. Such a system could be one employing patient

derived neurons derived from human pluripotent stem cells sources, but this system is even further removed from the PD brain both in terms of the age of cell being studied and the environment in which they find themselves!

Thus we have to accept that animals can only ever be used to model specific features of the pathology of PD but not the disease itself and as such searching for disease modifying therapies in animal models for PD is not a useful exercise. The reason being that if you find a therapy that works in some animal model there is no reason to believe it would necessarily work in a patient with PD as they represent two totally different disease states. Similarly, if an agent does not work in an animal model then there is no reason it might not work in PD patients, because as we have clearly stated these models do not resemble PD clinically. So why waste time studying such imperfect models of disease, rather we should be undertaking more experimental medicine studies in PD patients with agents that have gone through the relevant safety and biodistribution testing and with a target that should be relevant for the disease process based on *in vitro* mechanistic studies. So, now is the time to abandon animal models of PD at least for looking at agents of disease modification!

ANIMAL MODELS HAVE NOT FAILED US: ANDERS BJÖRKLUND

Can we do without animal models in PD research? Investigators involved in the development of new therapies and treatments are rightly concerned about the relevance and predictability of disease models for the initiation and design of clinical trials. This distrust is understandable given the numerous examples where seemingly convincing animal data have not panned out in subsequent clinical trials. The experience from the stroke field is particularly disheartening. This is even more disturbing since, in this clinical condition the animal models seem as perfect as they can be: the ischemic insults used in the animal experiments are identical to the ones seen in patients and should thus have a high level of predictability. Nevertheless, there are many cases where an intervention with a striking and convincing treatment effect in stroke models has failed when applied to patients. These failures have not only been extremely costly for the industry but they have also been discouraging and fostered a cynical attitude toward the need of animal models for the development of new therapies: If the models are misleading and lack predictability we will do better without them.

In the PD field, however, it is undeniable that studies and findings in animal models have played a key role in the development of the therapies that are used today. The development of L-DOPA therapy was triggered by observations in reserpine-treated rats and rabbits; the development of dopamine receptor agonists was based on studies performed in rodent neurotoxin models; and the identification of the subthalamic nucleus for deep-brain stimulation (DBS) therapy was critically dependent on the functional analysis of basal ganglia circuitry carried out, above all, in MPTP-treated monkeys.

In the early days, however, the use of the experimental models, and the importance given to them, was much more limited than they are today. The development of L-DOPA therapy is an interesting example. The justification to initiate trials in patients was essentially based on three single observations: The finding that the sedative state induced by the monoamine depleting drug reserpine in rats and rabbits could be reversed by a single injection of L-DOPA, made by Arvid Carlsson and coworkers in 1957 [14], followed 2 years later by the observation that the bulk of the brain's dopamine is located in the striatum [15], and a year later by Oleh Hornykiewics' finding that dopamine is markedly and consistently reduced in the caudate nucleus and putamen of Parkinsonian patients [16]. The first open label trial of L-DOPA in PD patients was initiated in 1961-62, within less than 5 years of the initial animal experiment [17, 18]. The development of a clinically useful therapy took another 5 years, marked by the publication of George Cotzias' landmark paper in NEJM in 1967 [19]. It is indeed remarkable that this "fast-track" approach is how limited animal experiments were used in some of the early clinical breakthroughs in medicine, such as the introduction of insulin therapy in the 1920s and the development of penicillin in the 1940s.

All this took place before the now commonly used neurotoxin models had been introduced. The reserpinized rats and rabbits used in the initial Carlsson et al. experiment can hardly qualify as a model of PD: Reserpine depletes not only dopamine but also noradrenaline and serotonin, and the immobility seen in these animals is confounded by a general sedative state. The unilateral 6-OHDA lesion model is the first one to replicate a central aspect of the pathophysiology of the disease, i.e., the degeneration of the midbrain dopamine projection. This model

with its face validity for modeling PD was developed in 1968 [20], and it is interesting to consider that if Arvid Carlsson had made his first L-DOPA experiments in 6-OHDA lesioned rats the drug may never had reached the clinic. This is because in this model, where the 6-OHDA is injected into the medial forebrain bundle (MFB) on one side, the therapeutic window of L-DOPA is very narrow. We know today that reduction of the hypokinetic symptoms in the absence of dyskinesia is seen only with very low doses, 6–8 mg/kg. At this dose the improvement is only partial, and increases above this threshold induces dyskinesia. Even worse, repeated administration of the therapeutic dose over just a few days is accompanied by a gradual emergence of dyskinesia that becomes worse over time [21, 22]. Confronted with such data it is easy to imagine that the early investigators would have been scared off and that the implementation of L-DOPA therapy in the clinic would not have happened or at least been seriously delayed. Thus, the choice of the model may be critically important: In this case it would have been a mistake to use the standard MFB lesion model where the striatum is completely denervated of its dopamine input, since the ability of L-DOPA to improve the motor features in the absence of dyskinesia depends on the presence of a spared dopamine innervation, sufficient to buffer the swings in L-DOPA derived dopamine caused by the intermittent drug delivery. Partial 6-OHDA lesions, or MPTP lesioned mice, i.e., models that were developed decades later, would thus be a more suitable choice in this case.

Until the turn of the millennium experimentalists were largely satisfied with the models they had at their disposal. The neurotoxin models had served us well, and we could also claim that they had proved their value for the development and improvement of PD therapy, the introduction of a broad range of dopaminergic drugs and DBS in particular. During the last two decades this has all changed. Since then, there has been a gradual shift of emphasis from treatment of symptoms to the search for protective and disease-modifying therapies, and a concomitant shift away from the classical view of PD as a dopamine deficiency syndrome to the idea of a more widespread and system-encompassing disorder where the main culprit, α-synuclein, is caused to aggregate and spread and interact with the immune system. The main trigger of this change was the discovery of the role of α-synuclein in the pathogenesis in 1997 [23, 24] and the gradual realization that PD belongs to the category of protein misfolding disorders with an onset that may start long before the classic motor symptoms occur.

These developments present a serious challenge to the disease modelling field. The studies of the familial forms of the disease, in particular, have shown that clinical conditions classified as PD can have quite different causes, and driven by different molecular and genetic mechanisms. As a result, there is now a general consensus that PD is not a single disease entity, but comprises different subtypes reflected in differences in the spectrum of symptoms and the nature and distribution of Lewy body pathology. Disease modelling has had to adapt to this changing scenery. A single model modality will no longer suffice, and the neurotoxin models, though still highly useful, must be complemented with models that more closely replicate the disease pathology and its progression-namely the development of models that have construct validity.

The multitude of animal models available to us today is very broad, ranging from worms and flies to rodents and primates. While studies in worms and flies can be useful as tools to explore individual pathogenic pathways, and for high-throughput genetic screens, in particular [25] we need to resort to rodents and non-human primates in order to get closer to the human disease with Lewy body pathology and progressive nigrostriatal degeneration at the core. In many cases the rodent models have been developed, not to replicate all aspects of the disease, but to model selected, interacting pathways, such as mitochondrial dysfunction and damage, α-synuclein aggregation and spread, impaired degradation of misfolded proteins, or activation of the innate immune system [26, 27]. This diversity of models is valuable in that they allow for a reductionist, hypothesis-driven approach to the identification and exploration of potential therapeutic targets, and are thus indispensable for any serious pre-clinical research in the PD field. All of them have limitations and none of them recapitulate all the pathologic and behavioral phenotypes of PD, but carefully selected they are complementary and can be used in parallel to add strength to a preclinical data packet.

The α-synuclein based rodent models, genetic or induced, are probably the ones that come closest to a replication of progressive PD-like pathology. They are attractive and useful in that the extent of Lewy body-like pathology can be modified so that it is confined either to the midbrain dopamine system (such as in the intranigral AAV-α-synuclein and/or PFF models), or expanded to resemble a more general-

ized α-synucleinopathy, akin to Lewy body dementia (such as is the case in some of the transgenic mouse models). It may be argued, of course, that the predictive value of these models is as yet unproven. That is true, but depending on how the models are used they offer valuable opportunities to explore potential therapeutic targets in a designed and hypothesis-driven manner. In studies aimed to prevent the aggregation of α-synuclein, or remove α-synuclein aggregates, for example, the models we have would be ideal to assess the efficacy of antibodies or other potential therapeutic agents, similar to the pre-clinical studies in APP expressing transgenic mice that preceded the clinical trials of amyloid antibodies in Alzheimer's disease (AD). Although the clinical benefit of this treatment remains unclear, the elimination of amyloid plaques in the mouse models has been nicely replicated in AD patients, confirming the predictive value of the models in this case.

Properly used, the currently available animal models are indispensable for the development of new concepts and ideas that can lead to novel protective or disease-modifying therapies. It is difficult to imagine how the development of novel therapies for PD could be achieved without animal models. It is argued that they are expensive and time-consuming and, if unreliable, a waste of both time and resources. For ethical reasons, however, we cannot avoid using them as an essential part of the pre-clinical testing if we want to ensure that the trials are performed on a scientifically sound basis. The possibility to establish "target engagement" in *in vitro* models and then move directly to trials in humans could have been possible 50 years ago, but would be far too risky to be acceptable today. Similarly, the "quick and daring" approach used in the early days when clinical trials could be initiated based on a single symptom-reversal experiment performed in a just a few animals, as in the development of L-DOPA therapy in the 1960s, is no longer an option. Current efforts to replicate PD-related pathology *in vitro* in patient-derived induced pluripotent stem cells and organoid cultures [28] offer interesting possibilities to speed-up drug screening and assist in the development of new treatment concepts. These novel tools may in the future help to reduce, but cannot replace, the need of pre-clinical data generated in relevant animal models.

So, if animal models are indispensable, the challenges remain: which model to choose, how to apply it to the question being posed, and how to interpret the results. We have to accept that there is no single animal model that can be applied in all cases. A scientific approach to therapy development says that we need to be hypothesis-driven and reductionist in our thinking and choose the model that best matches our need to prove or disprove the underlying mechanistic hypothesis. At the same time, we should be aware that the models we use may have to be adapted to match different disease subtypes. Thus, the development of increasingly more refined animal models needs to go hand-in-hand with the increasing insights into the pathogenic mechanisms that characterize and distinguish different PD subtypes and α-synucleinopathies.

REBUTTAL FROM ROGER A. BARKER

In his defence of the usefulness of animal models for studying PD, Anders Björklund lays out the history of how such models came into existence and their utility over the years. This includes an illuminating discussion on how L-dopa came into clinical use in PD despite very limited preclinical work in flawed animal models. Indeed, the critical work showing the value of this whole approach came from early experimental medicine trials in patients. These trials initially showed no benefit for this agent in PD [29], but through an iterative process this therapeutic turned into, and has remained, the mainstay of managing PD. Thus, we can see that the single biggest breakthrough in the treatment of PD was essentially done independently of any animal models.

Anders then shows how for the next 30 or more years the models of PD concentrated on the nigrostriatal dopaminergic pathway with the hope that this would lead to new therapies and breakthroughs in PD. None came and the therapeutic advances during this time were achieved through the pharmaceutical industry looking at different agents working on dopamine receptors and its catabolism. Arguably these dopaminergic centric models for PD did support the use of deep brain stimulation in the 1990s which followed on from lesion studies in patients in the 1980s. While there is no doubt that such preclinical work gave a scientific basis to what was being done and refined the procedure, much of the pioneering work supporting this whole approach came from work done on patients in the 1950s [30] before any animal models for PD even existed.

In this century, animal models have turned more to using the protein that lies at the heart of PD α-synuclein. The use of these α-synuclein models has helped us understand some aspects of the behaviour

of this protein, and both myself and Anders agree on this, but the critical question remains: has this helped us understand PD better? This is perhaps where we differ most and the very case that Anders cites in favour of this, I would say argues the opposite, namely the immune therapies targeting amyloid-β in AD. In this work, it was clearly shown that transgenic animals overexpressing this protein could develop the amyloid pathology of AD and this could be effectively removed by immune therapies targeting this protein. This has led to numerous studies showing that this occurs in patients dying with AD, but that this makes no significant difference to their clinical course or outcome [31]. In other words, the animal model using a reductionist approach for AD failed to recapitulate the problems in patients with AD and subsequently billions of dollars has been spent pursuing this flawed hypothesis [32]. The question is, do we really want to do the same in PD?

So, I come back to where I began in my initial arguments against using animal models for PD to predict clinical effects and therapies. Namely I would advocate that more targeted iterative experimental medicine approaches are now needed to better treat PD just as was done in the 1960s!

REBUTTAL FROM ANDERS BJÖRKLUND

Roger Barker summarizes well the limitations of current models of PD and suggests that we should, rather than wasting time on imperfect animal modelling, focus on experimental studies in PD patients using agents of known safety and with relevant targets based on *in vitro* mechanistic studies. Although I agree with Roger that the PD models available today can only be used to model specific features of the disease but not the disease itself, I would argue that this is in fact their strength and makes them ideally suited for hypothesis driven reductionist approaches to the development of new drugs and other interventionist treatments. The classic neurotoxin models, for example, have proved highly valuable for the development of drugs aimed at treatment of the symptoms related to the core pathology of PD, DA neuron loss, and will undoubtedly remain the preferred tools for pre-clinical validation of such treatments in the future. This is also true for the development of restorative therapies, such as dopamine cell replacement or gene-based therapies that seek to restore function without modifying the underlying disease.

Findings in these classic models can of course be misleading, and Roger uses the failure of the GDNF trials as a discouraging example. To this, I would argue that the lack of success of GDNF in the patient trials may not be due to shortcomings of the pre-clinical models—they have been very useful as tools to characterize the mode of action and therapeutic promise of this factor—but rather linked to how, where and when the factor is given to the patients. In this case I don't think the last word has been said yet!

The challenge is the modelling of the progressive disease processes seen in the various forms of PD, i.e., the kind of models needed for the development of disease modifying therapies. Here, the study of the familial cases has been important and lead to the identification of the major players in the pathogenic process: mitochondrial dysfunction and oxidative stress; α-synuclein misfolding and spread; dysfunction in the protein handling and clearance systems; and immune/inflammatory mechanisms. This has led to the development of a new generation of PD models and given us new powerful experimental tools to identify potential therapeutic targets that allow us to interfere at distinct stages of the disease [33]. This has also given us tools to model pathogenic subtypes related to differences in the induction and spread of α-synuclein-related pathology, such as, for example, the *brainstem-predominant* and *limbic-predominant* pathologies described by the Arizona PD Consortium [34], the *gut-to-brain transfer* of pathology [35], and the "*PNS-first*" and "*CNS-first*" subtypes defined by Borghammer and Van Den Berge [36].

If we don't want to depend on pure luck, the way forward is the classic hypothesis-driven reductionist approach. The strength of the new generation of PD models is that they allow us to focus in on selected targets that play a key role in disease progression and propagation of disease pathology, linked to different disease subtypes, and thus open up for discoveries that are transformative rather than incremental. The access to models that replicate the initiation of distinctive disease processes and their alternative routes of spreading and propagation are very attractive and can be effectively used for pre-clinical validation of novel therapeutic targets, leading to their further exploration in the "real" disease in patients. This kind of hypothesis-driven approach is in no way in conflict with Roger's idea to undertake more experimental medicine-based studies aimed at the repurposing of known drugs. They are complementary, I think, and can happily feed on each-other.

REFERENCES

[1] Foltynie T, Brayne CE, Robbins TW, Barker RA (2004) The cognitive ability of an incident cohort of Parkinson's patients in the UK. The CamPaIGN study. *Brain* **127**, 550-560.

[2] Evans JR, Mason SL, Williams-Gray CH, Foltynie T, Brayne C, Robbins TW, Barker RA (2011) The natural history of treated Parkinson's disease in an incident, community based cohort. *J Neurol Neurosurg Psychiatry* **82**, 1112-1118.

[3] Schaeffer E, Postuma RB, Berg D (2020) Prodromal PD: A new nosological entity. *Prog Brain Res* **252**, 331-356.

[4] Schrag A, Quinn N (2020) What contributes to quality of life in Parkinson's disease: A re-evaluation. *J Neurol Neurosurg Psychiatry* **91**, 563-565.

[5] Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, Brayne C, Kolachana BS, Weinberger DR, Sawcer SJ, Barker RA (2009) The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. *Brain* **132**, 2958-2969.

[6] Obeso JA, Stamelou M, Goetz CG, Poewe W, Lang AE, Weintraub D, Burn D, Halliday GM, Bezard E, Przedborski S, Lehericy S, Brooks DJ, Rothwell JC, Hallett M, DeLong MR, Marras C, Tanner CM, Ross GW, Langston JW, Klein C, Bonifati V, Jankovic J, Lozano AM, Deuschl G, Bergman H, Tolosa E, Rodriguez-Violante M, Fahn S, Postuma RB, Berg D, Marek K, Standaert DG, Surmeier DJ, Olanow CW, Kordower JH, Calabresi P, Schapira AHV, Stoessl AJ (2017) Past, present, and future of Parkinson's disease: A special essay on the 200th Anniversary of the Shaking Palsy. *Mov Disord* **32**, 1264-1310.

[7] Braak H, Del Tredici K (2017) Neuropathological staging of brain pathology in sporadic Parkinson's disease: Separating the wheat from the chaff. *J Parkinsons Dis* **7**, S71-S85.

[8] Geut H, Hepp DH, Foncke E, Berendse HW, Rozemuller JM, Huitinga I, van de Berg WDJ (2020) Neuropathological correlates of parkinsonian disorders in a large Dutch autopsy series. *Acta Neuropathol Commun* **8**, 39.

[9] Tan MMX, Malek N, Lawton MA, Hubbard L, Pittman AM, Joseph T, Hehir J, Swallow DMA, Grosset KA, Marrinan SL, Bajaj N, Barker RA, Burn DJ, Bresner C, Foltynie T, Hardy J, Wood N, Ben-Shlomo Y, Grosset DG, Williams NM, Morris HR (2019) Genetic analysis of Mendelian mutations in a large UK population-based Parkinson's disease study. *Brain* **142**, 2828-2844.

[10] Strohaker T, Jung BC, Liou SH, Fernandez CO, Riedel D, Becker S, Halliday GM, Bennati M, Kim WS, Lee SJ, Zweckstetter M (2019) Structural heterogeneity of alpha-synuclein fibrils amplified from patient brain extracts. *Nat Commun* **10**, 5535.

[11] Irwin DJ, Abrams JY, Schonberger LB, Leschek EW, Mills JL, Lee VM, Trojanowski JQ (2013) Evaluation of potential infectivity of Alzheimer and Parkinson disease proteins in recipients of cadaver-derived human growth hormone. *JAMA Neurol* **70**, 462-468.

[12] Kirkeby A, Barker RA (2019) Parkinson disease and growth factors - is GDNF good enough? *Nat Rev Neurol* **15**, 312-314.

[13] Decressac M, Kadkhodaei B, Mattsson B, Laguna A, Perlmann T, Bjorklund A (2012) alpha-Synuclein-induced down-regulation of Nurr1 disrupts GDNF signaling in nigral dopamine neurons. *Sci Transl Med* **4**, 163ra156.

[14] Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* **180**, 1200.

[15] Bertler A, Rosengren E (1959) Occurrence and distribution of dopamine in brain and other tissues. *Experientia* **15**, 10-11.

[16] Ehringer H, Hornykiewicz O (1960) [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. *Klin Wochenschr* **38**, 1236-1239.

[17] Birkmayer W, Hornykiewicz O (1961) [The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia]. *Wien Klin Wochenschr* **73**, 787-788.

[18] Barbeau A, Sourkes TL, Murphy GF (1962) Les catecholamines de la maladie de Parkinson. In *Monoamines et Système Nerveux Central*, de Ajuriaguerra J, ed. Georg, GEneve and Masson, Paris.

[19] Cotzias GC, Van Woert MH, Schiffer LM (1967) Aromatic amino acids and modification of parkinsonism. *N Engl J Med* **276**, 374-379.

[20] Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res* **24**, 485-493.

[21] Cenci MA, Lee CS, Bjorklund A (1998) L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *Eur J Neurosci* **10**, 2694-2706.

[22] Winkler C, Kirik D, Bjorklund A, Cenci MA (2002) L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: Relation to motor and cellular parameters of nigrostriatal function. *Neurobiol Dis* **10**, 165-186.

[23] Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. *Nature* **388**, 839-840.

[24] Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045-2047.

[25] Breger LS, Fuzzati Armentero MT (2019) Genetically engineered animal models of Parkinson's disease: From worm to rodent. *Eur J Neurosci* **49**, 533-560.

[26] Jiang P, Dickson DW (2018) Parkinson's disease: Experimental models and reality. *Acta Neuropathol* **135**, 13-32.

[27] Cenci MA, Bjorklund A (2020) Animal models for preclinical Parkinson's research: An update and critical appraisal. *Prog Brain Res* **252**, 27-59.

[28] Caiazza MC, Lang C, Wade-Martins R (2020) What we can learn from iPSC-derived cellular models of Parkinson's disease. *Prog Brain Res* **252**, 3-25.

[29] Fehling C (1966) Treatment of Parkinson's syndrome with L-dopa. A double blind study. *Acta Neurol Scand* **42**, 367-372.

[30] Rezai AR, Machado AG, Deogaonkar M, Azmi H, Kubu C, Boulis NM (2008) Surgery for movement disorders. *Neurosurgery* **62 Suppl 2**, 809-838; discussion 838-809.

[31] Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA (2008) Long-term effects of Abeta42 immunisation in Alzheimer's disease: Follow-up of a randomised, placebo-controlled phase I trial. *Lancet* **372**, 216-223.

[32] Mullane K, Williams M (2020) Alzheimer's disease beyond amyloid: Can the repetitive failures of amyloid-targeted therapeutics inform future approaches to dementia drug discovery? *Biochem Pharmacol* **177**, 113945.

[33] Johnson ME, Stecher B, Labrie V, Brundin L, Brundin P (2019) Triggers, facilitators, and aggravators: Redefining Parkinson's disease pathogenesis. *Trends Neurosci* **42**, 4-13.

[34] Beach TG, Adler CH, Lue L, Sue LI, Bachalakuri J, Henry-Watson J, Sasse J, Boyer S, Shirohi S, Brooks R, Eschbacher J, White CL, 3rd, Akiyama H, Caviness J, Shill HA, Connor DJ, Sabbagh MN, Walker DG, Arizona Parkinson's Disease Consortium (2009) Unified staging system for Lewy body disorders: Correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta Neuropathol* **117**, 613-634.

[35] Breen DP, Halliday GM, Lang AE (2019) Gut-brain axis and the spread of alpha-synuclein pathology: Vagal highway or dead end? *Mov Disord* **34**, 307-316.

[36] Borghammer P, Van Den Berge N (2019) Brain-first versus gut-first Parkinson's disease: A hypothesis. *J Parkinsons Dis* **9**, S281-S295.