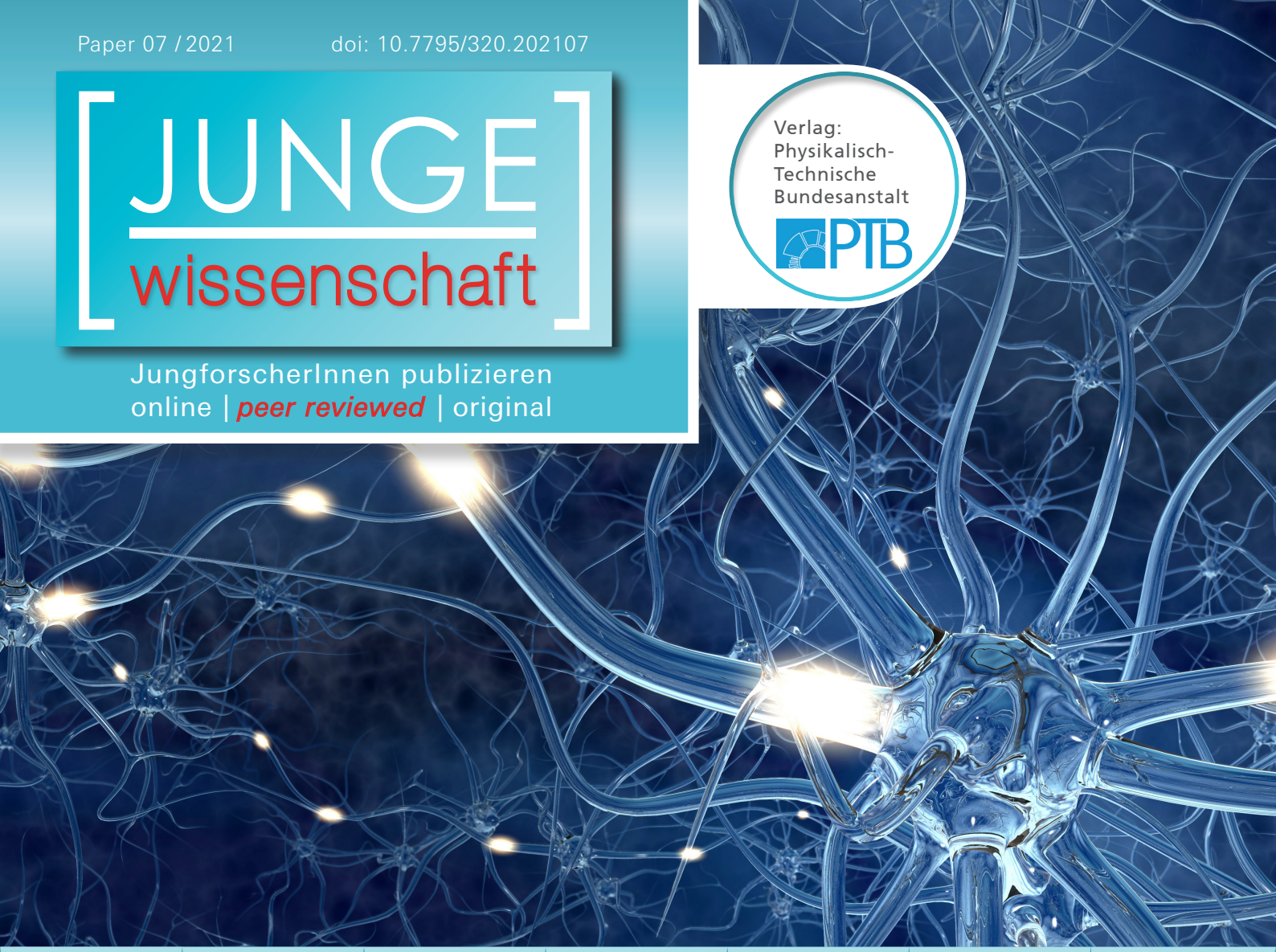
Paper 07 / 2021 doi: 10.7795/320.202107

JUNGE wissenschaft

JungforscherInnen publizieren
online | *peer reviewed* | original

Verlag:
Physikalisch-
Technische
Bundesanstalt
PTB



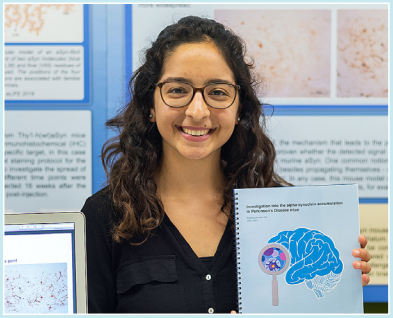
Biologie & Medizin

# Alpha-Synuclein in Parkinson's Disease

## Spreading of injected alpha-synuclein fibrils to and within cerebella of a transgenic PD mouse model

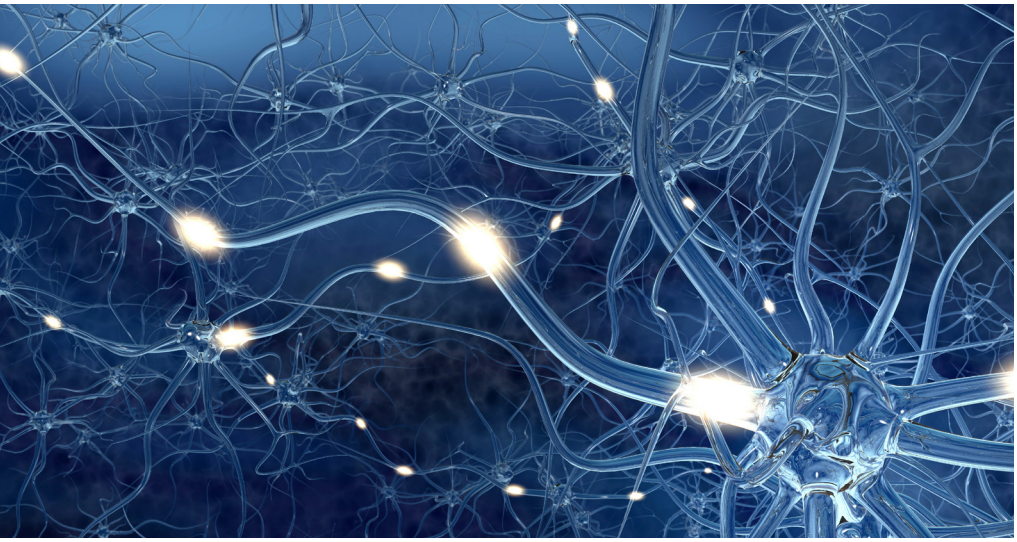
*This study aimed to clarify to what extent the cerebellum is affected by the intraneuronal accumulation of alpha- synuclein (aSyn) in a mouse model of Parkinson's Disease (PD) called Thy1-h(wt)aSyn. Immunoactive signals were found in all aSyn-injected sections and it could be observed that the intensity, shape and distribution of the staining patterns change with time. The data obtained support the notion that the cerebellum might be involved more strongly in PD than previously thought.*

### DIE JUNGFORSCHERIN



**Elena Su (1999)**
Gymnasium Liestal,
Liestal (CH)

**Eingang der Arbeit:**
9.8.2019
**Arbeit angenommen:**
18.11.2019



# Alpha-Synuclein in Parkinson's Disease

Spreading of injected alpha-synuclein fibrils to and within cerebella of a transgenic PD mouse model

## 1. Parkinson's Disease

### 1.1 Epidemiology and etiology

Parkinson's Disease (PD) is the second-most common neurodegenerative disorder after Alzheimer's Disease, affecting approximately 0.3 percent of the global population. The estimated global incidences of PD range between 5 to 35 new cases per 100'000 individuals per year. The age of disease onset is usually in the late fifties, however, incidence rates increase up to ten folds from the age of 60 to 90. As improved health care has led to increasing life expectancy, the number of people with PD is expected to grow dramatically, eventually doubling between 2005 and 2030 [1]. Although the definitive etiology of PD remains unknown, it seems that the disease is caused by a complicated interplay of both genetic and environmental factors that combined affect various cellular processes [2].

### 1.2 Symptoms

Parkinsonian motor symptoms affect gait and mobility, postural control and balance, as well as speech and swallowing functions. Most commonly, PD patients suffer - in varying degrees - from bradykinesia, muscular rigidity, rest tremor, and postural and gait impairments. Many clinical non-motor symptoms of PD like REM sleep behavior disorder, depression and constipation precede motor dysfunctions by several years or decades. Even if it is not possible yet to diagnose PD without the onset of motor symptoms, non-motor symptoms add to overall disability and become increasingly prevalent over the course of the illness (Fig. 1) [3].

### 1.3 Neuropathology

The two major neuropathological hallmarks of PD are the early death of dopaminergic neurons in the substantia nigra (SN) and the presence of intracellular inclusion of the protein alpha-synuclein (aSyn) in a misfolded and putatively neurotoxic conformation [1].

#### 1.3.1 Neuronal loss

Neuronal loss in the SN leads to the depletion of striatal dopamine (DA). Parkinsonism is believed to develop in consequence to the decreased DA transmission in the motor region of the striatum which causes an increased inhibition of the projections between the thalamus and the cerebral motor cortex, as well as, midbrain brainstem structures, assumably causing parkinsonian motor symptoms like bradykinesia [4].

This neuronal death only occurs in certain types of neurons within particular brain regions. There is considerable variability in the vulnerability of dopaminergic neurons to neurodegeneration, some even being devoid from any pathological markers. Risk factors for the death of these neurons are believed to include disrupted calcium homeostasis, mitochondrial dysfunction, oxidative stress and neuroinflammation, among others [5].

#### 1.3.2 Alpha-synuclein pathology

The cytoplasmic protein aSyn is 140 amino acid long, highly soluble and predominantly presynaptic. Although the general neuronal function of aSyn remains elusive, the protein is known

doi: 10.7795/320.202107

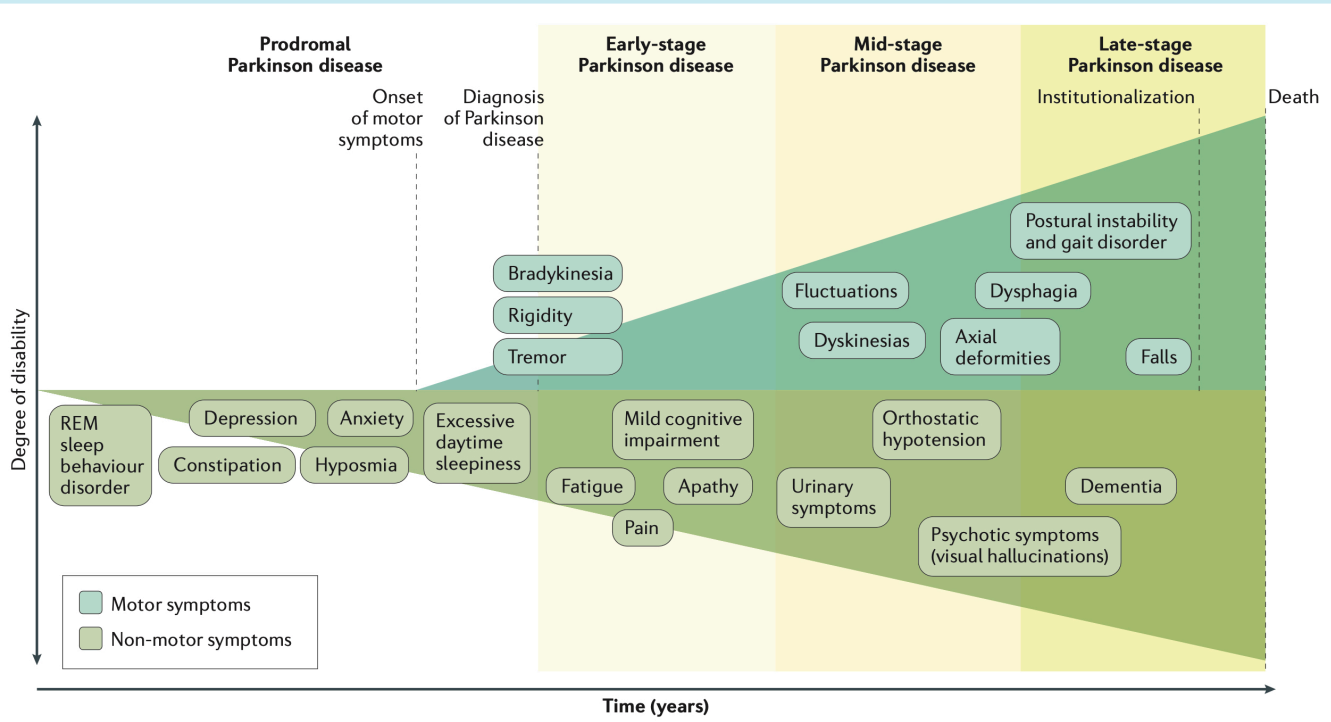



Fig. 1: Clinical motor and non-motor symptoms of PD, reproduction of the figure by courtesy of Nature Reviews Disease Primers. [1].

to influence the regulation of vesicular transport and neurotransmitter release in the synaptic terminals of neurons. Presumably, it also affects mitochondrial function and influences DA synthesis and DA signaling pathways as it binds to and inhibits tyrosine hydroxylase, the first enzyme of DA synthesis, and binds directly to DA transporters and inhibits the reuptake of DA from the synapse [6][7].

Intraneuronal aSyn inclusions are present in virtually all idiopathic and familial PD patients. It is proposed that the spread of the PD pathology is mediated by a prion-like, i.e. a cell-to-cell, transmission of aSyn between neurons. A prion is a protein that exists in healthy forms but becomes infections when it changes its conformation by converting from a normal cellular protein into an abnormally misfolded form. Importantly, misfolded proteins also act as a template to promote conformational changes in other prion proteins that are not yet converted [8].

During a pathogenic process, single soluble aSyn monomers are believed to misfold to form oligomers which progressively combine to small aggregates and eventually large, insoluble and potentially toxic fibrils (Fig. 2). The prion hypothesis then suggests that once aSyn inclusions have formed in a neuron, they spread to neighboring interconnected brain regions, thereby migrating from affected to unaffected neurons [9].

The underlying triggers of accumulation of aSyn and the formation of inclusions are unknown. They could involve, for instance, a relative overproduction of the protein, mutations that increase the likelihood of aSyn misfolding, a toxic gain or perturbation of its neuronal function, and impairments of the molecular pathways that degrade native or misfolded aSyn [10]. The proteostasis might also be influenced by a progressive, age-related decline in defense mechanisms of the brain as the greatest risk factor for PD, ageing, is also coupled to reduced functions of two major aSyn degradation systems, the ubiquitin-proteasome system (UPS) and the lysosomal autophagy system (LAS). A variety of studies propose a vicious cycle in which aSyn accumulation might occur as a consequence of impaired protein clearance and that the aSyn accumulation, in turn, might cause defective aSyn degradation [1][9].

### 1.3.3 Lewy pathology

Neuropathological diagnosis of idiopathic PD is based on inclusions called Lewy neurites (LNs) and Lewy bodies (LBs) found in the perikarya, dendrites and axons of DA neurons, particularly of those in the SN. Histological assessments have demonstrated that a major component of the spindle- or thread-like LNs and the globular-shaped LBs is an aggregated form of aSyn. It is unknown why aSyn - with other components - gradually transforms into insoluble LNs or LBs and what mechanisms are responsible for this transformation [9] [10].

According to Braak et al. [11], Lewy pathology does not develop randomly

doi: 10.7795/320.202107

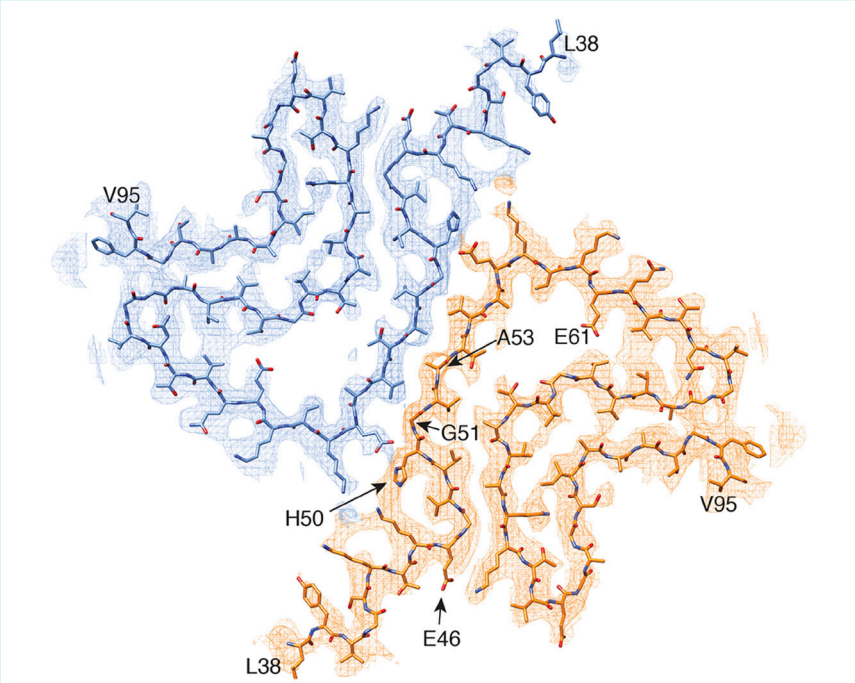



Fig. 2: Cross-section of a recombinant aSyn-fibril showing the arrangement of two aSyn protofilaments (blue and orange) based on cryo-electron microscopy. The locations of the initial (L38) and final (V95) residues of each protofilament are displayed. The positions of four residues, whose mutations are associated with PD, are indicated with arrows, reproduction of the figure by courtesy of eLife [30].

but rather in a predetermined and predictable sequence which can be divided into six stages, starting in the peripheral nervous system (PNS) and progressively affecting the central nervous system (CNS). Each so-called Braak-stage correlates with the areas of nervous system that are further affected as the disease progresses (Fig. 3). The model suggests that in the pathological process specific induction sites are targeted first and gradually, less vulnerable areas become affected [11].

Research also supports the notion that the PD pathology starts in the intestine decades before progressing into the CNS. The so-called gut-brain-axis describes a strong connection between the status of the intestinal environment and the function of the CNS. Arguably, inflammatory immune activity impacts the microbiota and increases aSyn levels, both in the gut and the brain. Overexpression of aSyn, in turn, induces the formation of aSyn inclusions which are thought to be carried from the gut to the brain, thereby crossing the blood-brain-barrier. Having reached the brain, aSyn is believed to spread as suggested by Braak and colleagues. The hypothesis is supported by the high prevalence of constipation, one of the most common non-motor symptoms of PD, which is known to precede PD-associated motor disabilities [12][13].

### 1.4 Role of the cerebellum in PD

For long, the cerebellum was considered to be unaffected by PD-related pathological alternations within the brain. Yet, several Parkinsonian motor symptoms, e. g. resting tremor and postural imbalance, can be associated with changes in circuits in which the cerebellum is directly and indirectly involved. Furthermore, dopaminergic treatment is not capable of improving tremor and the amount of nigrostriatal DA deficiency and the occurrence of other typical PD symptoms do not coincide with the emergence and the intensity of tremor either. With the growing number of anatomical, pathophysiological and clinical evidence, the idea that the cerebellum has a substantial contribution to clinical symptoms of PD starts to gain popularity [14][15].

In contrast to earlier models that explains the occurrence of parkinsonian resting tremor by positioning the tremor pacemaker in the basal ganglia or the thalamus, the more recently proposed "dimmer-switch model" by Helmich et al. [16] suggests that the underlying pathophysiology of rest tremor is based on the convergence of the basal ganglia and the cerebello-thalamo-cortical circuit. Their model states that tremor onset and offset is mediated by altered basal ganglia loops (like a light switch) whereas tremor amplitude regulation involves the cerebello-thalamo-cortical projections (analogous to a light dimmer). The latter circuit has also been suggested to contribute to the development of levodopa-induced dyskinesia. Even if this dimmer-switch model successfully combines a range of previous studies into a larger potential explanatory framework, it cannot explain which exact brain region(s) are responsible for tremor frequency and to what extent DA depletion influences tremor onset or amplitude. It also remains open whether the cerebellum's contribution to tremor is solely modulatory and not also causal [16][17].

Regarding Lewy pathology, a range of studies have screened the cerebella of PD patients and animal models for aSyn, however, the yielded results are inconsistent. A recent study that screened the cerebella of PD patients found moderate to severe aSyn pathology in all cerebella. ASyn-immunopositive aggregates were found predominantly in the form of LN at the deep cerebellar nuclei (DCN) and the neighboring

white matter tracts. The cerebellar cortex displayed minor pathology and inclusions only occurred occasionally in the Purkinje and granular cell layers. As precerebellar brainstem structures are also known to express aSyn aggregates, these finding supports a reinvestigation of the current staging systems [18][19].

### 1.4.1 Anatomical connections

The cerebellum and the basal ganglia, which includes the striatum, are interconnected with the cerebral cortex and the thalamus. Although both basal ganglia and cerebellum project to separate nuclei of the thalamus, the two structure have been suggested to not work solely independently and even share an anatomical connection. There is evidence that an output stage of the cerebellum, the nuclei dentate, has a direct influence over the striatum, an input stage of the basal ganglia, via a disynaptic connection by way of the thalamus in nonhuman primates. However, it remains undetermined, if there is also a direct connection from the striatum back to the nuclei dentate. Another study found that the subthalamic nucleus has a synaptic projection to the cerebellar cortex via the pontine nuclei. These two findings combined demonstrate two-way communications between the cerebellum and the basal ganglia and a potential integrated functional network composed of the two systems [20][21].

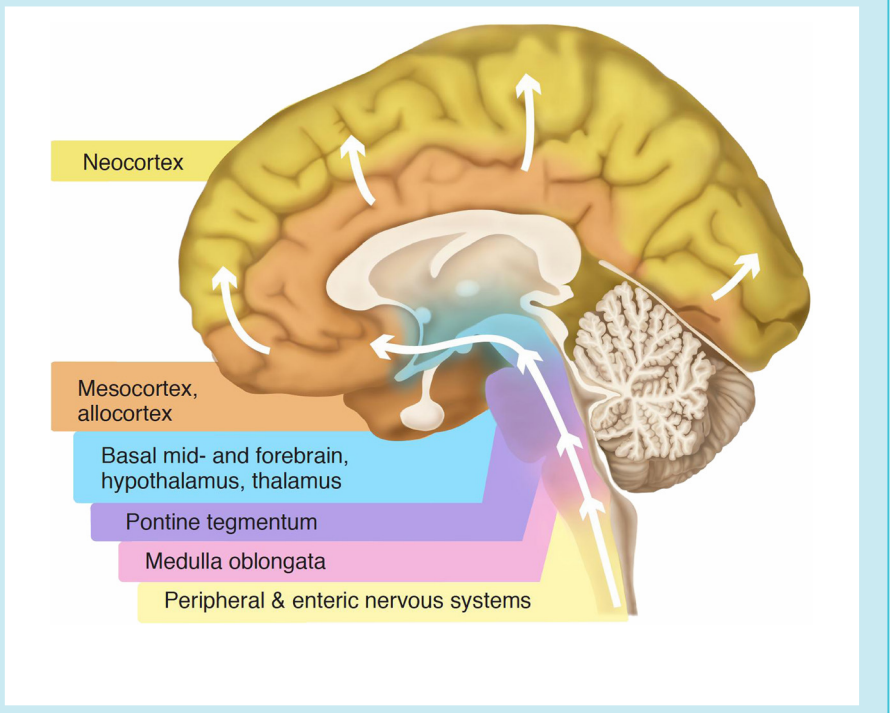



Fig. 3: Overview of the Lewy pathology propagation according to the Braak staging system, reproduction of the figure by courtesy of Acta Neuropathologica Communications. [31].

## 2. Hypotheses

Hypothesis 1) In human aSyn transgenic mice, injection of preformed fibrils (PFF) of recombinant human aSyn will induce aSyn pathology in the injection site, the striatum.

This notion is based on the findings of Luk et al. In a series of studies, they could demonstrate that an injection of recombinant aSyn PFFs alone can both initiate and accelerate the formation and propagation of aSyn pathology to anatomically interconnected regions, which was replicated also in cellular systems in vitro. Their findings indicate that the exposure to PFF derived from wild type aSyn is sufficient to induce endogenous aSyn to misfold, form inclusions, spread over considerable distances within the CNS in a prion-like fashion, and cause neurodegeneration in otherwise healthy neurons in cultures cells, wild type and transgenic (line 83) mice [22][23][24][25].

Hypothesis 2) ASyn pathology will spread within the brain, including the cerebellum

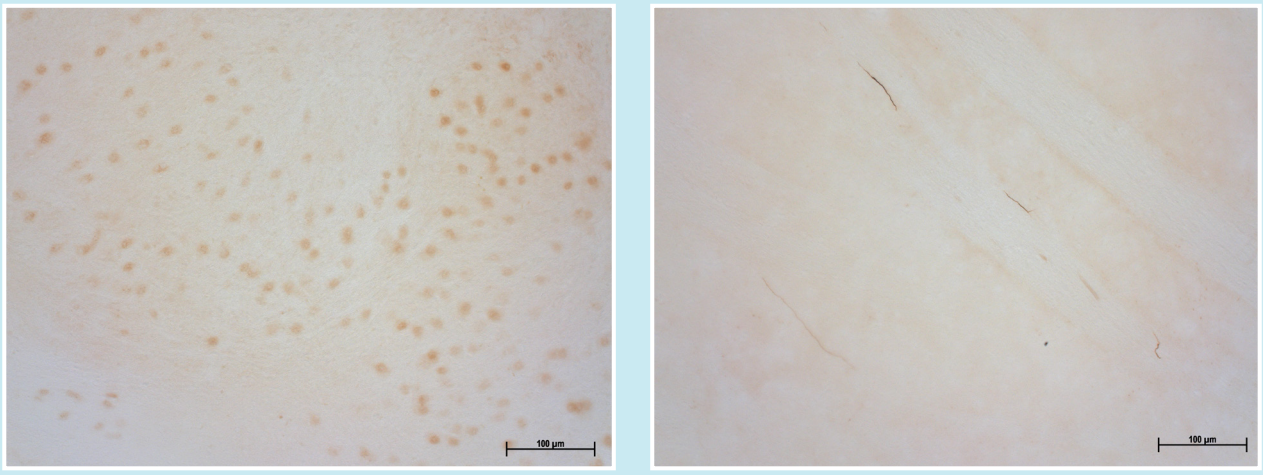
In their study with wild type mice, Luk et al. found that the regions that were not directly connected to the striatum, such as the hippocampus or cerebellum, were free of aSyn pathology. A time-dependent propagation of the aSyn pathology could be observed, however, not affecting the cerebellum [25]. Similarly, regions that developed the most prominent aSyn pathology in a previous study with transgenic mice (line M83) where those with neurons that either project to, or receive input from the injection sites. With time, the pathology became more widespread, and in contrast to the study above, also mildly affected the deep cerebellar nuclei. This suggests a transsynaptic spreading of the injected fibrils, as the DCN do not share a direct connection with the injection sites and do not appear to be restricted by the presence nor the number of intermediary connections [24].

The studies conducted by Seidel et al. show - in contrast to the widely common Braak staging model - that the cerebella of human PD patients are also affected by Lewy pathology, mostly in the form of LN and mainly at the DCN, the anatomical and functional center of the cerebellum [19].

## 3. Methods

Experiments were performed in a laboratory of the Neuroscience and Rare Disease Research Department at Roche Basel, Switzerland. Multiple sections (one per mouse) were stained based on an immunohistochemical (IHC)

doi: 10.7795/320.202107




a) b)

Fig. 4: Left: Microscope image of the DCN from negative control; right: microscope image of the striatum from positive control. (Microscope images taken at Roche Basel.)

protocol. IHC is a staining method used to detect the location of target antigens, in this case human aSyn phosphorylated at Serine129 (pSer129 aSyn), on a given tissue sample using antibodies. Three pilot studies with a total of 10 mice were conducted to optimize the ideal staining protocol (including DAB incubation time) for the given mouse model to ensure that a majority of the target antigen is detected in the best possible signal-to-noise ratio.

In the main study, the spread of a potential aSyn pathology within the cerebellum was evaluated by comparing stained sections from two different time points. The early time point subcohort consisted of 4 brains that were collected 16 weeks after the injection (1x PFF and 3x PBS-injected), while the late time point subcohort included 3 brains harvested 28 weeks post-injection (2x PFF and 1x PBS-injected). The study was blinded.

The animal experiments were endorsed by a Roche internal review board and approved by the local animal welfare authorities of the Canton Basel-Stadt, Basel, Switzerland.

### 3.1 Thy1-h(wt)aSyn model

The Thy1-h(wt)aSyn mice overexpress human wild type aSyn under the promotor Thy1 and reproduce certain features of sporadic PD such as aSyn pathology, progressive decrease in striatal DA, deficits in both motor and non-motor functions that can be observed in early and more advanced stages of PD, inflammation, and behavioral and molecular changes similar to those observed in patients with PD. That said, PD is a specific human disease and these models cannot entirely imitate certain pathological and clinical characteristics of the disease [26][27].

### 3.2 Injection and controls

Per literature, modelling motor deficits can be archived by injecting into brain regions controlling motor functions, and after a waiting period of more than one month, aSyn accumulation should be detectable in anatomically connected brain regions, sometimes as far as four synapses away.

In this study, the injected aSyn fibrils were phosphorylated at Serine residue 129, also referred to as pSer129. It is considered to be a major form of aSyn in Lewy pathology, some studies suggesting that up to 90 % of aSyn deposited in LBs are phosphorylated at Ser129. In contrast, less than 4 % of all aSyn in the healthy brain is phosphorylated at this residue. As phosphorylation at Ser129 does not necessarily entail the formation of inclusions, aSyn was injected in the form of PFF [28][29].

All animals were injected at the age of 2–3 months with 10 µg PFF of aSyn per brain in the striatum of the right hemisphere. The striatum is known to take up PFF and is anatomically interconnected with multiple brain regions, including midbrain dopaminergic neurons and contralateral brain regions. Control mice received vehicle injections, phosphate buffered saline (PBS), into the same region. Additionally, a non-injected group of mice (negative control group) was included to distinguish between behavioral and pathological effects due to injection. The negative control (Fig. 4 left) is the baseline staining and all additional staining in the other sections can be considered induced pathology,

doi: 10.7795/320.202107

i.e. pSer129 aSyn positive inclusions. The positive control (Fig. 4 right), taken from a previous study, was known to express aSyn pathology in the striatum. Hence, its staining patterns could be used as a reference.

## 4. Results

Notice: Shown here are two examples per early and late time point group, in each case one PFF and one PBS-injected section/mouse. The focus was laid on the DCN of the ipsilateral side of the stained cerebella sections, however, both sides were analyzed for the discussion.

### 4.1 Early time point

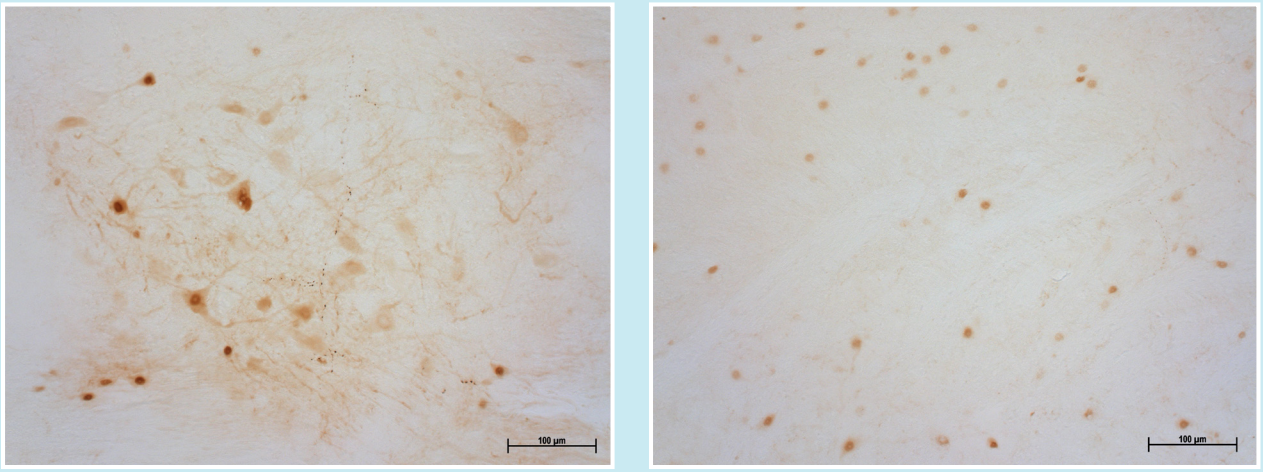
In the PFF-injected early time point mouse (Fig. 5 left) there are less but more intense nuclear staining compared to the same aged PBS-injected mouse (Fig. 5 right) and the negative control (Fig. 4 left). In contrast to the positive control, the observable neuritic staining patterns are dotted. These signals of aSyn accumulation are more severe on the ipsilateral side, while both sides of the PBS-injected sections look similar. The background of the PBS-injected mouse is evenly distributed while that of the PFF-mouse appears to be focused on areas around the nuclear staining.

### 4.2 Late time point

In the PFF-injected section from the late time point group (Fig. 6 left) there are more prominent and distinct signal staining patterns, mainly neuritic. The background noise is slightly reduced compared to the negative control (Fig. 4 left). In comparison to the neuritic staining seen in the section from the PFF-injected early time point mouse (Fig. 5 left), the majority of the staining patterns in this sections are rather drawn through than dotted, like in the positive control (Fig. 4 right). Also noteworthy is the appearance of big neuritic aSyn inclusions in the shape of LNs in humans some also branched. Signals of accumulation can be seen on both sides of the section, however, the staining appears more intense and widespread on the ipsilateral side. On both sides, the staining is mainly found at the location of the interposed and lateral nuclei. Some neuritic staining could also be observed in the granular cell layer. The intensity of the background noise of the PBS-injected late time point section (Fig. 6 right) is comparable to that of the negative control (Fig. 4 left). The ipsilateral and contralateral sides look alike, both without any further visible signals of accumulation.

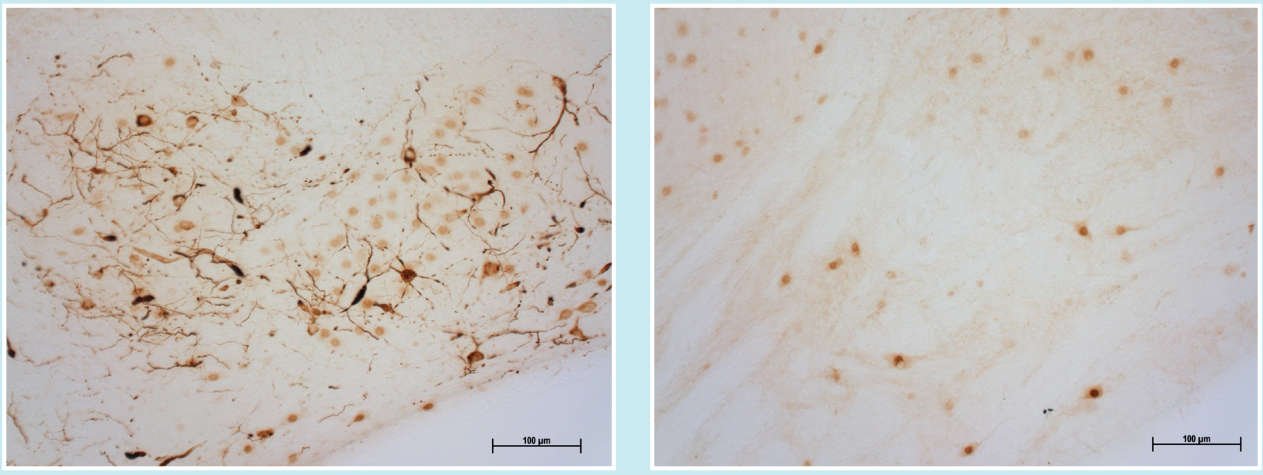
## 5. Discussion

It is crucial to mention that a quantification of the aSyn signals was not possible in this study as it would have required staining a multitude of sections per mouse and group in order to achieve high enough statistical power. Given this limitation, the focus was laid on the additional injection-induced staining patterns that could not be observed in the negative control of the same-aged PBS-sections. Ultimately, this exploratory study aimed to contribute to the ongoing efforts within the field of PD research by further investigating the still vague role of the cerebellum in PD. The goal was to




a) b)

Fig. 5: Left: Microscope image of DCN from ipsilateral side of PFF-injected early time point mouse; right: microscope image of DCN from ipsilateral side of PBS-injected early time point mouse. (Microscope images taken at Roche Basel.)




a) b)

Fig. 6: Left: Microscope image of DCN from ipsilateral side of PFF-injected late time point mouse; right: microscope image of DCN from ipsilateral side of PBS-injected late time point mouse. (Microscope images taken at Roche Basel.)

evaluate whether the cerebellum of the Thy1-h(wt)aSyn mouse is affected by the injection-induced propagation of aSyn and if so, how that pathology develops over time.

### 5.1 Comparison of sections from PBS- and PFF-injected mice

Upfront, there are visible staining patterns in all sections including the negative control and the PBS-injected mice sections as all mice normally have murine aSyn in their brains which can be phosphorylated at Ser129. Hence, the background staining in all sections represent the endogenous aSyn in the tissue which has been detected inadvertently by the antibody.

In general, all sections from PBS-injected mice can be considered a reproduction of the baseline staining (negative control) as these sections only display nuclear staining and very occasionally some pale neuritic patterns. Also, there should be no aSyn fibrils in any brain region to be transported in the first place and it has, as of now, not been reported that the overexpression of aSyn alone can suffice to induce the accumulation of aSyn in this specific mouse model. Hence, the neuritic inclusions found in some PBS-injected mice and the negative control could be categorized as non-specific antibody-dependent staining signal. On the contrary, these inclusions might suggest that an accumulation of aSyn in the cerebellum would have happened anyway, without the PFF injection. In that case, the transgene causing the overexpression of aSyn in this transgenic mouse model might in fact be sufficient to induce pathology.

Previous studies by Luk et al. have already demonstrated that aSyn transportation takes place in wild-type and transgenic animals. After aSyn fibrils were injected into the brain, the injection lead to the formation of aSyn inclusions in locations both near and distant from the injection site [25]. In most cases, however, the cerebellum was not a projection site [9]. Hence, this study found, that the cerebella of the Thy1-h(wt)aSyn mice also display aSyn pathology after the injection of PFF as the initial injection was made into the striatum and signals were detected in the cerebellum. The appearance of signals in sections from PFF-injected mice while sections from same-aged PBS-injected mice showed no significant data is the most prominent clue that the injected aSyn fibrils were transported within the brain and did not solely accumulate by themselves. Having displayed the propagation of the fibrils from one brain region to another, it still remains open why this region is less and temporally later affected by the aSyn pathology than other brain regions and how this transportation took place.

For example, the fibrils might have been taken up by neurons in the striatum and gradually propagated all the way to the cerebellum according to the prion hypothesis [8],[1]. Alternatively, research from Luk and co-workers also suggests that the transportation and susceptibility is based on direct synaptic connectivity [25]. In this process, a neuron would take up the PFF and the same neuron would directly transport them to the cerebellum. Although there are known disynaptic projections from the nuclei dentate to the striatum in nonhuman primates, its remains unclear whether the striatum also projects directly to the cerebellum [20]. Also, previous studies and the Braak stages show that not all neurons

doi: 10.7795/320.202107

of the brain are equally susceptible to or affected by pathological spread of aSyn. For example, there are brain regions anatomically next to the SN, which contains one of the most vulnerable neurons, that are not affected by Lewy pathology in humans [11]. Although there is no definitive evidence yet that synaptic connectivity to an affected area alone makes a brain region more vulnerable to develop pathology, synaptic connectivity and neuronal activity might play a crucial role anyway, especially in regard to the route of the fibrils [2].

Finally, the question arises whether the injected fibrils were only transported from the striatum to other brain regions including the cerebellum or if the fibrils also triggered an accumulation of endogenous proteins. Luk et al. stated that in their studies with wild type and transgenic (Line M83) the detected inclusions contained both human aSyn and endogenously expressed mouse aSyn [24],[25]. According to their findings the signal inclusions found in this study might also be composed of the injected PFF and endogenous aSyn in the mouse brain tissue.

This would explain why the background noise of the sections from PBS-injected mice seem to be distributed more evenly. It indicates that the injection of PFF changes the distribution of the staining patterns which in turn proposes that the endogenous aSyn that was potentially stained as background noise in the sections from PBS-injected mice might have been taken up by the aSyn inclusions and stained as specific signal of accumulation in the sections from PFF-injected mice. Another approach concerns the antibody itself. It could be that the antibody acts rather promiscuous when the specific epitope is missing or scarcely available.

### 5.2 Comparison of sections from early and late time point mice

For the analysis of the PFF-injected sections, the focus was laid on three points: the intensity, the shape and the distribution of the aSyn-immunoactive staining patterns.

First, the intensity of the signal staining patterns in the PFF-injected late time point section appears elevated compared to the early hPFF-injected one. This suggests that over time, the aSyn fibrils that are transported into the cerebellum or the amount of accumulated aSyn in general become bigger. This finding likely depends on the hPFF injection as the section from the PBS-injected late time point mouse has similar patterns like the section from the PBS-injected early time point mouse and shows no signal of accumulation despite being sacrificed three months after the early time point group. Conceivably, if the mice had been sacrificed later, accumulation of aSyn would have happened in the late time PBS-section, too, further suggesting that the overexpression of aSyn would be enough to trigger a pathology.

Second, the signal patterns appear in a differently shaped form. While the pattern in the section from the hPFF-injected early time point mouse is dotted and arranged in a straight line the section from the late time point mouse displays more clear and branched neuritic staining patterns. The latter also contains signal staining resembling bulgy or dystrophic neurites. Interestingly, the study conducted by Seidel et al., states that Lewy pathology observed in brains of PD patients was predominantly found in the form of neuritic patterns [18].

This seeming structural change over time might also be attributed to the elevated staining intensity which increasingly highlights the normally branched structure of dendrites.

Lastly, the distribution of the signals also expands over time. In both late time point sections there were occasionally signals found in the granular cells. The DCN, being the terminal destination of input into the cerebellum, might be assumed to end the transportation chain of the propagation of the pathology. However, seeing that the granular cells might also be affected indicates that the injected aSyn fibrils were either transported to the DCN via the granular cells or to the granular cells via the DCN. Again according to Seidel et al., the patterns found in the cerebella of PD patients were most intense at the DCN and the surrounding white matter [18]. Combined, the detection of aSyn-immunopositive aggregates in form of neuritic patterns and their location at and circumjacent to the DCN in human PD patients represent to important analogies to our findings.

Collectively, both hypotheses could be confirmed, as the data generated demonstrate that the injection of recombinant aSyn into the striatum induces an aSyn pathology which spreads within the brain and also affects the cerebellum. The mechanism that leads to the propagation of the aSyn pathology remains unknown and the question remains whether the detected signal patterns only consist of the injected fibrils and not also of endogenous murine aSyn. In any case, this mouse model of PD might be helpful in exploring a potential role for cerebellar pathology in PD.

## 6. Conclusion

Even if underlying causes of PD are unknown, there is profound evidence that aSyn plays a central role in the pathogenesis of the disease. Despite replicating findings from published research this thesis could also demonstrate - for the first time - that the injection of human aSyn PFF into the striatum of the Thy1-h(wt) aSyn transgenic mouse model induces the aSyn pathology to spread to the

doi: 10.7795/320.202107

cerebellum, affecting mainly the DCN. This is suggested by the appearance of signal staining in the DCN of aSyn-injected mice while no specific signal was detected in the negative control and the same-aged PBS-injected mice, hence, indicating that the accumulation of aSyn was induced by the injection.

To investigate the spread of the pathology within the cerebellum of the given mouse model sections from two different time points were compared. It could be observed that the intensity, shape and distribution of the signal staining patterns in the cerebella sections changed in a time-dependent fashion. In the late time point sections the signals were more intense, differently shaped and more widespread. These findings suggest a temporospatial propagation of the pathology within the cerebellum.

## Acknowledgements

My great appreciation is dedicated to Markus Britschgi, PhD, and Philipp Schönenberger, PhD, who gave me the unique opportunity to join their research groups at Roche Basel, Switzerland and who provided me with excellent scientific support. I wish to offer special thanks to Roberta Lauria who supervised the practical part of this work and Prof. Dr. med. Stephan Bohlhalter from the Clinic for Neurology und Neurorehabilitation at the Kantonsspital Luzern, Switzerland, for supervising the revision. I'm also deeply grateful to Swiss Youth in Science and the Stockholm International Youth Science Seminar for offering me a platform to present my work. Lastly, I thank my parents Helen and Arel and my brother Leon for their unfailing support and continuous encouragement throughout my study.

### Anmerkung der Herausgeber zu den hier durchgeführten Tierversuchen

Bei Forschungsprojekten mit Tieren hält sich der Verlag Junge Wissenschaft an dieselben Richtlinien wie der Deutsche Nachwuchswettbewerb Jugend forscht. Zum Wettbewerb – und so auch zur Publikation – sind nur Projekte zugelassen, die den Deutschen Gesetzen und Verordnungen zum Tier-, Natur- und Artenschutz (auch für Wirbellose) entsprechen.

Gemäß der Jugend forscht Entscheidungshilfe fällt die hier vorgelegte Arbeit eindeutig in die Kategorie Zweitverwertung. Die Autorin konnte glaubhaft belegen, dass die verwendeten tierischen Gewebe im Rahmen eines bereits stattgefundenen Tierversuchs und größeren Experiments generiert wurden, bevor ihr Forschungsprojekt geplant und durchgeführt wurde. Teile dieses vorgehenden Experiments wurden bereits in einem wissenschaftlichen Journal 'peer-reviewed' publiziert (Grathwohl et al., Free Neuropathology 2021). Wie dort auch erwähnt, sind die entsprechenden Tierversuche durch das Roche interne Scientific Review Board befürwortet und auf Empfehlung der gemeinsamen Tierversuchskommission der Kantone Basel-Stadt, Basel-Landschaft und Aargau vom Kantonstierarzt des Kantons Basel-Stadt genehmigt worden.

## References


[1] Poewe, W. et al., 2017, Parkinson disease, IN: Nature Reviews Disease Primers, Vol. 3, No. 17013.

[2] Kalia, L.V. and A.E. Lang, 2015, Parkinson's disease, IN: The Lancet, Vol. 386, No. 9996.

[3] Bourzac, K., 2016, Warning signs, IN: Nature, Vol. 538, No. 7627 (Outlook).

[4] Galvan, A., A. Devergnas and T. Wichman, 2015, Alternations in neuronal activity in basal ganglia-thalamocortical circuits in parkinsonian state, IN: Frontiers in Neuroanatomy, Vol. 9, No. 5.

[5] Surmeier, D.J. et al., 2010, Chapter 4 - What causes the death of dopaminergic neurons in Parkinson's disease?, ED: A. Bjorklund and M.A. Cenci, IN: Progress in Brain Research, Vol. 183, Elsevier.

[6] Snead, D. and D. Eliezer, 2014, Alpha-Synuclein Function and Dysfunction on Cellular Membranes, IN: Experimental Neurobiology, Vol. 23(4), DOI: 10.5607/en.2014.23.4.292.

[7] Venda, L.L. et al., 2010, α-Synuclein and dopamine at the crossroad of Parkinson's disease, IN: Trends in Neuroscience, Vol. 33, No. 12.

[8] Makin, S., 2016, Pathology: The prion principle, IN: Nature, Vol. 538, No. 7627.

[9] Olanow, C.W. and P. Brundin, 2013, Parkinson's Disease and Alpha Synuclein: Is Parkinson's Disease a Prion-Like Disorder?, IN: Movement Disorders, Vol. 28, No. 1.

[10] Goedert, M., 2015, Crystals of toxic core, IN: Nature, Vol. 525, No. 7570.

[11] Braak, H. et al., 2002, Staging of brain pathology related to sporadic Parkinson's disease, IN: Neurobiology of Aging, Vol. 24, No. 2, 2002.

[12] Houser, M.C. and M.G. Tansey, 2017, The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis?, IN: npj Parkinson's Disease, Vol. 3, No. 3.

[13] Klingelhoefer, L. and H. Reichmann, 2015, Pathogenesis of Parkinson disease – the gut-brain axis and environmental factors, IN: Nature Reviews Neurology, Vol. 11, No. 11.

[14] Mirdamadi, J.L., 2016, Cerebellar role in Parkinson's disease, IN: Journal of Neurophysiology, Vol. 116, No. 3.

[15] Lefaivre, S.C., M.J.N. Brown and Q.J. Almeida, 2016, Cerebellar involvement in Parkinson's disease resting tremor, IN: Cerebellum Ataxis, Vol. 3, No. 13.

[16] Helmich, R.C. et al., 2012, Cerebral causes and consequences of parkinsonian resting tremor: a tale of two circuits?, IN: Brain, Vol. 135, No. 11.

[17] Wu, T. and M. Hallett, 2013, The cerebellum in Parkinson's disease, IN: Brain, Vol. 136, No. 3.

[18] Seidel, K. et al., 2017, Involvement of the cerebellum in Parkinson disease and dementia with Lewy bodies, IN: Annals of Neurology, Vol. 81, No. 6.

[19] Seidel, K. et al., 2014, The Brainstem Pathologies of Parkinson's Disease and Dementia with Lewy Bodies, IN: Brain Pathology, Vol. 25, No. 2.

[20] Hoshi, E. et al., 2005, The cerebellum communicates with the basal ganglia, IN: Nature Neuroscience, Vol. 8, No. 11.

[21] Lewis, M.M. et al., 2013, The Role of the Cerebellum in the Pathophysiology of Parkinson's Disease, IN: Canadian Journal of Neurological Sciences, Vol. 40, No. 3.

[22] Luk, K.C. et al., 2011, Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death, IN: Neuron, Vol. 72, No. 1.

[23] Luk, K.C. et al., 2009, Exogenous α-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells, IN: Proceedings of the National Academy of Sciences, Vol. 106, No. 47.

[24] Luk, K.C. et al., 2012, Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice, IN: Journal of Experimental Medicine, Vol. 209, No. 5.

[25] Luk, K.C. et al., 2012, Pathological α-Synuclein Transmission Initiates Parkinson-like Neurodegeneration in Nontransgenic Mice, IN: Science, Vol. 338, No. 6109.

[26] Chesselet, M.-F. et al., 2012, A Progressive Mouse Model of Parkinson's Disease: The Thy1-aSyn ("Line 61") Mice, IN: Neurotherapeutics, Vol. 9, No. 2.

[27] Hallett, P.J. et al., 2012, Alpha-synuclein overexpressing transgenic mice show internal organ pathology and autonomic deficits, IN: Neurobiology of Disease, Vol. 46, No. 2.

[28] Dehay, B. et al., 2015, Targeting α-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations, IN: The Lancet, Vol. 14, No. 8.

[29] Sato, H., T. Kato and S. Arawaka, 2013, The role of Ser129 phosphorylation of α-synuclein in neurodegeneration of Parkinson's disease: a review of in vivo models, IN: Reviews in the Neurosciences, Vol. 24, No. 2.

[30] Guerrero-Ferreira, R. et al., 2018, Cryo-EM structure of alpha-synuclein fibrils, IN: eLife, No. e36402, https://elifesciences.org/articles/36402 (accessed 6 July 2018).

[31] Visanji, N. P. et al., 2013, The prion hypothesis in Parkinson's disease: Braak to the future, IN: Acta Neuropathologica Communications, Vol. 1, No. 2.


doi: 10.7795/320.202107

# Publiziere auch Du hier!

Forschungsarbeiten von Schüler/Inne/n und Student/Inn/en

In der Jungen Wissenschaft werden Forschungsarbeiten von SchülerInnen, die selbstständig, z. B. in einer Schule oder einem Schülerforschungszentrum, durchgeführt wurden, veröffentlicht. Die Arbeiten können auf Deutsch oder Englisch geschrieben sein.

## Wer kann einreichen?

SchülerInnen, AbiturientInnen und Studierende ohne Abschluss, die nicht älter als 23 Jahre sind.

## Was musst Du beim Einreichen beachten?

Lies die Richtlinien für Beiträge. Sie enthalten Hinweise, wie Deine Arbeit aufgebaut sein soll, wie lang sie sein darf, wie die Bilder einzureichen sind und welche weiteren Informationen wir benötigen. Solltest Du Fragen haben, dann wende Dich gern schon vor dem Einreichen an die Chefredakteurin Sabine Walter.

Lade die Erstveröffentlichungserklärung herunter, drucke und fülle sie aus und unterschreibe sie.

Dann sende Deine Arbeit und die Erstveröffentlichungserklärung per Post an:

**Chefredaktion Junge Wissenschaft**
Dr.-Ing. Sabine Walter
Paul-Ducros-Straße 7
30952 Ronnenberg
Tel: 05109 / 561508
Mail: sabine.walter@verlag-jungewissenschaft.de

## Wie geht es nach dem Einreichen weiter?

Die Chefredakteurin sucht einen geeigneten Fachgutachter, der die inhaltliche Richtigkeit der eingereichten Arbeit überprüft und eine Empfehlung ausspricht, ob sie veröffentlicht werden kann (Peer-Review-Verfahren). Das Gutachten wird den Euch, den AutorInnen zugeschickt und Du erhältst gegebenenfalls die Möglichkeit, Hinweise des Fachgutachters einzuarbeiten.

Die Erfahrung zeigt, dass Arbeiten, die z. B. im Rahmen eines Wettbewerbs wie **Jugend forscht** die Endrunde erreicht haben, die besten Chancen haben, dieses Peer-Review-Verfahren zu bestehen.

Schließlich kommt die Arbeit in die Redaktion, wird für das Layout vorbereitet und als Open-Access-Beitrag veröffentlicht.

## Was ist Dein Benefit?

Deine Forschungsarbeit ist nun in einer Gutachterzeitschrift (Peer-Review-Journal) veröffentlicht worden, d. h. Du kannst die Veröffentlichung in Deine wissenschaftliche Literaturliste aufnehmen. Deine Arbeit erhält als Open-Access-Veröffentlichung einen DOI (Data Object Identifier) und kann von entsprechenden Suchmaschinen (z. B. BASE) gefunden werden.

Die Junge Wissenschaft wird zusätzlich in wissenschaftlichen Datenbanken gelistet, d. h. Deine Arbeit kann von Experten gefunden und sogar zitiert werden. Die Junge Wissenschaft wird Dich durch den Gesamtprozess des Erstellens einer wissenschaftlichen Arbeit begleiten – als gute Vorbereitung auf das, was Du im Studium benötigst.

doi: 10.7795/320.202107

# Richtlinien für Beiträge

Für die meisten Autor/Inn/en ist dies die erste wissenschaftliche Veröffentlichung. Die Einhaltung der folgenden Richtlinien hilft allen – den Autor/innen/en und dem Redaktionsteam

Die Junge Wissenschaft veröffentlicht Originalbeiträge junger AutorInnen bis zum Alter von 23 Jahren.

- Die Beiträge können auf Deutsch oder Englisch verfasst sein und sollten nicht länger als 15 Seiten mit je 35 Zeilen sein. Hierbei sind Bilder, Grafiken und Tabellen mitgezählt. Anhänge werden nicht veröffentlicht. Deckblatt und Inhaltsverzeichnis zählen nicht mit.

- Formulieren Sie eine eingängige Überschrift, um bei der Leserschaft Interesse für Ihre Arbeit zu wecken, sowie eine wissenschaftliche Überschrift.

- Formulieren Sie eine kurze, leicht verständliche Zusammenfassung (maximal 400 Zeichen).

- Die Beiträge sollen in der üblichen Form gegliedert sein, d. h. Einleitung, Erläuterungen zur Durchführung der Arbeit sowie evtl. Überwindung von Schwierigkeiten, Ergebnisse, Schlussfolgerungen, Diskussion, Liste der zitierten Literatur. In der Einleitung sollte die Idee zu der Arbeit beschrieben und die Aufgabenstellung definiert werden. Außerdem sollte sie eine kurze Darstellung schon bekannter, ähnlicher Lösungsversuche enthalten (Stand der Literatur). Am Schluss des Beitrages kann ein Dank an Förderer der Arbeit, z. B. Lehrer und Sponsoren, mit vollständigem Namen angefügt werden. Für die Leser kann ein Glossar mit den wichtigsten Fachausdrücken hilfreich sein.

- Bitte reichen Sie alle Bilder, Grafiken und Tabellen nummeriert und zusätzlich als eigene Dateien ein. Bitte geben Sie bei nicht selbst erstellten Bildern, Tabellen, Zeichnungen, Grafiken etc. die genauen und korrekten Quellenangaben an (siehe auch Erstveröffentlichungserklärung). Senden Sie Ihre Bilder als Originaldateien oder mit einer Auflösung von mindestens 300 dpi bei einer Größe von 10 · 15 cm! Bei Grafiken, die mit Excel erstellt wurden, reichen Sie bitte ebenfalls die Originaldatei mit ein.

- Vermeiden Sie aufwendige und lange Zahlentabellen.

- Formelzeichen nach DIN, ggf. IUPAC oder IUPAP verwenden. Gleichungen sind stets als Größengleichungen zu schreiben.

- Die Literaturliste steht am Ende der Arbeit. Alle Stellen erhalten eine Nummer und werden in eckigen Klammern zitiert (Beispiel: Wie in [12] dargestellt …). Fußnoten sieht das Layout nicht vor.

- Reichen Sie Ihren Beitrag sowohl in ausgedruckter Form als auch als PDF ein. Für die weitere Bearbeitung und die Umsetzung in das Layout der Jungen Wissenschaft ist ein Word-Dokument mit möglichst wenig Formatierung erforderlich. (Sollte dies Schwierigkeiten bereiten, setzen Sie sich bitte mit uns in Verbindung, damit wir gemeinsam eine Lösung finden können.)

- Senden Sie mit dem Beitrag die Erstveröffentlichungserklärung ein. Diese beinhaltet im Wesentlichen, dass der Beitrag von dem/der angegebenen AutorIn stammt, keine Rechte Dritter verletzt werden und noch nicht an anderer Stelle veröffentlicht wurde (außer im Zusammenhang mit **Jugend forscht** oder einem vergleichbaren Wettbewerb). Ebenfalls ist zu versichern, dass alle von Ihnen verwendeten Bilder, Tabellen, Zeichnungen, Grafiken etc. von Ihnen veröffentlicht werden dürfen, also keine Rechte Dritter durch die Verwendung und Veröffentlichung verletzt werden. Entsprechendes Formular ist von der Homepage www.junge-wissenschaft.ptb.de herunterzuladen, auszudrucken, auszufüllen und dem gedruckten Beitrag unterschrieben beizulegen.

- Schließlich sind die genauen Anschriften der AutorInnen mit Telefonnummer und E-Mail-Adresse sowie Geburtsdaten und Fotografien (Auflösung 300 dpi bei einer Bildgröße von mindestens 10 · 15 cm) erforderlich.

- Neulingen im Publizieren werden als Vorbilder andere Publikationen, z. B. hier in der Jungen Wissenschaft, empfohlen.

doi: 10.7795/320.202107

# Impressum

**Junge Wissenschaft**
c/o Physikalisch-Technische
Bundesanstalt (PTB)
www.junge-wissenschaft.ptb.de

**Redaktion**
Dr. Sabine Walter, Chefredaktion
Junge Wissenschaft
Paul-Ducros-Str. 7
30952 Ronnenberg
E-Mail: sabine.walter@verlag-jungewissenschaft.de
Tel.: 05109 / 561 508

**Verlag**
Dr. Dr. Jens Simon,
Pressesprecher der PTB
Bundesallee 100
38116 Braunschweig
E-Mail: jens.simon@ptb.de
Tel.: 0531 / 592 3006
(Sekretariat der PTB-Pressestelle)

**Design & Satz**
Sabine Siems
Agentur „proviele werbung“
E-Mail: info@proviele-werbung.de
Tel.: 05307 / 939 3350


**Titelbild**
Pipe drain system
Quelle: fotolia / adobe stock /
© GraphicsRF