Neurodegeneration in the centrally-projecting Edinger-Westphal nucleus contributes to the non-motor symptoms of Parkinson's disease in the rat

Doctoral PhD. thesis

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Introduction

The Parkinson's disease

Parkinson's disease is the second most common neurodegenerative long-term disorder after Alzheimer's disease, but it is the leading neurological diseases affecting movement. Clinical signs of the disease may be classified as *motor symptoms* affecting motor coordination and *non-motor symptoms* affecting autonomic functions and mood. The causes of motor symptoms have been studied for nearly 50 years, but the focus turned on the background of mood disorders in the 1990s. The most conspicuous groups of symptoms come from the obvious deterioration in motor function. The leading symptoms include rigor, resting tremor and bradykinesia. Non-motor symptoms affecting mood status can appear years before the onset of motor symptoms and may worsen with the progression of the disease. The loss of sense of smell is a common early onset symptom, but this is also characteristic for Alzheimer's disease and is therefore not specific. Other non-motor symptoms include sleep disturbances, fatigue and functional changes in the digestive system. The leading non-motor symptoms affecting mood include anxiety and depression.

The definitive clinical diagnosis of PD requires histopathological examination, which can be performed earliest after the death of the patient. The disease is characterized by a 70% reduction in the amount of dopaminergic neurons of the substantia nigra pars compacta (SNpc) and in the remaining viable cells alpha-synuclein (α-Syn) containing eosinophilic inclusions known as Lewy bodies (LB) can be identified.

According to the classification by Braak, central α-Syn inclusions first appear in the olfactory bulb and dorsal vagal nucleus. Subsequently, in the second stage, eosinophilic aggregates are deposited in serotoninergic (5-HT) systems (caudal raphe nuclei) and in the noradrenergic locus ceruleus (LC). Inclusions in the SNpc occur earliest in the third stage and here the amygdala and the rostral raphe nuclei get involved. During the fourth stage, α-Syn inclusions appear in the hippocampus and some cortical regions, whereas in the fifth and sixth stages, almost the entire cortex gets affected.

The first three stages correspond to the clinical prodromal phase, while from the fourth stage onwards, classic motor symptoms and cognitive impairment predominate.

L-DOPA is the main pharmacotherapeutical tool that is administered orally, in combination with DOPA-decarboxylase inhibitors. The therapy improves the rigor and resting tremor in the majority of patients and a minority of cases get almost completely asymptomatic.

After an average period of 3-5 years, the drug efficacy declines that indicates dose adjustment or the application an alternative drug may be necessary.

Neuroanatomical background of the motor deficit

The progressive neurodegeneration underlying rigor, tremor and bradykinesia has long been well understood. The damage in the dopaminergic nigrostriatal pathway results in a gradual loss of motor function. However, motor symptoms occur first time when the dopamine content of the striatum is reduced by more than 70%. This related to compensatory mechanisms such as increased function of residual viable neurons, an increase in the number and sensitivity of dopamine receptors and a decrease of dopamine reuptake. Further decrease in the dopamine level and the loss off the dopaminergic connection between the substantia nigra and striatum results in the compromised initiation of motor functions. The motor pathway is affected both by the lack of ligand binding to D_2 receptors in the indirect pathway and by the decrease in D_1 receptor activity in the direct pathway. Increased microglial as well as astrocyte reactivity have been observed in the brains of Parkinson's disease patients. These inflammatory processes in the neural tissue also contribute to the progression of the disease.

Involvement of mood control- and autonomic function-related brain areas

With the progression of PD, the damage in extra-nigral dopaminergic, noradrenergic, cholinergic and serotoninergic pathways may also contribute to the development of the non-motor symptoms. Despite extensive research, the neurobiological basis of non-motor symptoms is still largely unexplored. Beyond Braak's classification, previous studies have shown that LBs are not restricted to brainstem nuclei, but they also occur in enteric neurons and enteroendocrine cells. The transport of α-Syn between the gastrointestinal system and parasympathetic brainstem nuclei is a known phenomenon. In PD patients, there is significant damage to the autonomic nucleus of the vagus nerve combined with atrophy of the nerve proper, which may explain a significant proportion of the autonomic symptoms.

α-Syn inclusions were found in the noradrenergic LC. In addition to the LC, the A5 area also contributes to the modulation of the autonomic nervous system. The degeneration of these two areas in PD may contribute to a wide range of non-motor symptoms. It is important to note that the occurrence of those symptoms is not only attributable to the deterioration of the noradrenergic system, but may also be associated with deficits in other cholinergic, serotonergic, GABAergic or glutamatergic systems.

The serotoninergic raphe nuclei in the brainstem are also impaired in Parkinson's disease, that may contribute to the development of non-motor symptoms and tremor. The dorsal raphe nucleus (DR) plays a major role in stress adaptation and mood regulation through its 5-HT neurons. A smaller group of DR cells is dopaminergic and contributes to the control of behavioral alterations during social isolation and separation. These cells are also known to be involved in the regulation of circadian rhythm, thus, their degeneration may contribute to sleep disturbances. In comparison to the DR, less knowledge has accumulated on the role of the median raphe nucleus (MNR), but the altered function of the 5-HT cells here significantly contribute to the pathomechanism of generalized anxiety disorder.

The ventral tegmental area (VTA) is a dopaminergic center of the meso-cortico-limbic system, which plays a role in the regulation of cognitive functions, motivation and self-reward. Despite the fact that the motor symptoms of PD are caused by SNpc dopaminergic neuron loss exceeding 60-70%, in patients with advanced PD, the dopaminergic cell death in the VTA ranges at 50%. This is particularly interesting, because SNpc and VTA neurons synthesize, store and release the same neurotransmitter. The vulnerability difference between these two areas may be explained by distinct electrophysiological features of the two neuron populations and underlying developmental proteomic differences.

The Edinger-Westphal nucleus

The Edinger-Westphal nucleus (EW) is classically descripted as the parasympathetic nucleus of the third cranial nerve, which plays an important role in the regulation of pupillomotor function and accommodation through the ciliary ganglion. According to Hunter's paper in 1985, in tissue samples of patients with Parkinson's disease, *"54% neuronal loss was found in the rostral part of the EW"* and *"3% of the cells contained Lewy bodies"*. Although this phenomenon is mentioned in several neuropathological descriptions, including Braak's classification, to the best of our knowledge no studies have been performed to explore the functional significance of this phenomenon. Nevertheless, it is important to underline that neither pupillomotor dysfunction nor significant weakness of accommodation is characteristic for the Parkinson's disease. This raises the possibility that the neurodegeneration observed by Hunter in 1985 may affect the cells of the nucleus that are not involved in the classically described pupillomotor and accommodation function.

Later, a new, in 1985 still unknown subdivision of EW was defined. In contrast to the cholinergic pupillomotor neurons, these cells are peptidergic and their axons do not reach the ganglion ciliary but they establish central connections. Hence this part is designated as the

centrally-projecting Edinger-Westphal nucleus (EWcp). The majority of neurons in the EWcp produce urocortin 1 (UCN1) peptide and approximately 80% of the urocortinergic perikarya are localized to the EWcp, UCN1 is a member of the corticotropin-releasing hormone (CRH) family and may act via CRH_1 (anxiogenic) and CRH_2 (anxiolytic) receptors. Interestingly, it shows higher affinity to the latter than the CRH, itself. The central distribution of CRH_2 receptors shows good anatomical overlap with the brain areas innervated by UCN1-containing nerve fibers. UCN1 knockout mice show increased levels of anxiety in response to acute stress. Exposure to acute and chronic stress increases the UCN1, c-Fos and FosB expression in the EWcp proving its recruitment in stress adaptation response. The involvement of EWcp in mood disorders has been demonstrated in several animal models and in autopsy specimens of depressed suicide victims. It is important to highlight that urocortinergic EWcp neurons are also involved in the regulation of energy balance, and nearly 50% of the neurons carry leptin receptors on their cell membrane, which distinguishes them from other peptidergic and non-peptidergic neurons in the region.

Based on Hunter's description in 1985, we put forward the hypothesis that the neurodegeneration in the EW area discovered at that time might not affect the preganglionic, cholinergic division of EW, but the later defined centrally projecting UCN1-immunoreactive group. Moreover, this neuron loss might contribute to the PD-associated mood disorders such as depression and anxiety.

Only a few publications have addressed the relationship between UCN1 and PD so far. One of these is noteworthy, where UCN1 administration was investigated in PD models. An improvement in symptoms was shown in various experimental settings. Consistently, UCN1 has also been shown to promote dopaminergic neuron survival, but these studies applied exogenous neuropeptide treatment and did not assess the effects of endogenous UCN1 and mood state.

Systemic rotenone model of Parkinson’s disease

There are many different ways to mimic the symptoms of the human disease. Various laboratory animal species may be used to study the course of the disease progression. Studies can be performed on both invertebrates and vertebrates, but the use of rodents has become the most widespread due to ethical, translational and technical reasons.

Dopaminergic neuronal degeneration due to local or systemic administration of neurotoxic agents underlies many models of Parkinson's disease. A widely used method is the local brain injection of 6-hydroxydopamine (6-OHDA), which can be used to model PD by

disrupting dopaminergic cells of the SN unilaterally. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered in chemically-induced parkinsonism after failed synthesis of the opioid drug 1-methyl-4-phenyl-4-propion-piperidine (MPPP). An increased risk of PD has been reported in farmers exposed to various pesticides, herbicides (paraquat, maneb, rotenone) in agriculture, and this observation subsequently led to the development of further neurotoxin-based PD models.

Among the various PD models, we preferred to use the systemic subcutaneous (s.c.) rotenone administration, for 6 weeks. We decided to apply this model because it replicates the main morphological features of the human disease in rats including neurodegeneration and the appearance of intracytoplasmic alpha-synuclein aggregates in the SNpc.

Aims and hypotheses

The rotenone model for Parkinson's disease in the rat

Our aim was to investigate whether UCN1-containing neurons in EWcp are damaged in the systemic rotenone-induced Parkinson's disease model similar to the substantia nigra.

We hypothesized that rotenone-induced damage to the EWcp neurons is associated with local inflammatory processes that contribute to the manifestation of anxiety and depression-like states, to the non-motor symptoms of the disease.

In order to provide indirect evidence for a prominent role of EWcp in mood disorders, we also investigated the importance of other brain areas that play a role in mood control.

We hypothesized that if EWcp plays a significant role in mood disorders, no other areas would change significantly in the model.

Examination of Parkinson's disease-associated mood disorders upon selective, local neuron ablation

We experienced non-motor symptoms besides the classical motor deficit of PD in the first setup. We assumed that these anomalies occurred due to morphological changes in EWcp. In order to directly demonstrate the role of UCN1-ir neurons, a selective local neuron ablation was performed.

We hypothesized that intracerebral injection of leptin-conjugated saporin causes a selective damage of urocortinergic EWcp neurons associated with a depression-like state and anxiety without occurrence of motor symptoms.

Materials and methods

Experimental setup

In the rotenone model, 35 eleven-month-old male Wistar rats were assigned to a control (n=12) and a toxin-injected (n=23) group. The control group received only vehicle (sterile sunflower oil and dimethyl sulfoxide (DMSO)), while the treated group received 1.5 mg/kg/day rotenone by s.c. injections, for 6 weeks. Three behavioral tests were performed on different days. The motor skills were assessed by the rotarod performance test (RPT) and open field test (OFT). The anxiety level was assessed by the OFT, while the anhedonia was evaluated by the sucrose preference test (SPT). After the sixth week, animals were terminated, and the samples were processed. Five rotenone-treated animals were removed from the experiment due to humane end point and six additional animals were excluded during histological processing. Finally, samples from 12 control and 12 rotenone-treated rats were processed.

To investigate the effect of local, selective neuron ablation with leptin-saporin solution 32 three-month-old male rats underwent a stereotaxic surgery under systemic anesthesia. EWcp UCN1 neurons were selectively ablated in 16 rats with leptin-conjugated saporin solution, while 16 rats were injected with unconjugated saporin as controls. After a 10-days recovery period upon surgery animals were subjected to behavioral tests. Finally, at the end of the third week, animals were terminated. During sample processing we found that 8 animals showed less than 20% neuron loss in the EWcp, while in 6 rats had the course of the injection path deviated from the intended anatomical localization. These animals were excluded from further examination. The statistical analysis was performed on data obtained from 9 rats per group.

Immunolabeling

Tyrosine hydroxylase (TH) immunohistochemistry was performed with diamino-benzidine (DAB) chromogen to visualize the neuronal loss due to rotenone toxicity in the SNpc. In sections containing SN, VTA, LC, A5 area ionized calcium binding adapter molecule 1 (IBA1) - TH, in the DR, MNR IBA1 - 5-HT, and in the CPu NeuN - TH double labelings were applied. To assess the level of micro- and astrogliosis in the EWcp, UCN1 - IBA1 - glial fibrillary acidic protein (GFAP) triple labeling was conducted. To detect the Parkinson's disease-associated changes UCN1 - α-Syn - TH triple labeling were performed. To investigate microglial activity, the IBA1 staining was complemented with CD68 labeling. In the rotenone model, we used tumor necrosis factor alpha (TNFalpha) - GFAP - IBA1 and inducible nitric

oxide synthase (iNOS) - GFAP - IBA1 triple immunostainings to investigate the changes in the SNpc.

In the leptin-saporin model, the extent of local neuron ablation and the consequent neuroinflammation was examined by UCN1 - GFAP - IBA1, UCN1 - caspase 3, UCN1 - NeuN labelings. As a complementary assay, in the SNpc, TH - caspase 3 and GFAP - IBA1 double labelings were also performed.

Microscopy and digital image analysis

DAB labeled slides were digitalized by Nikon light microscope. Immunofluorescence labelled samples were scanned using an Olympus Fluoview 1000 confocal microscope. In both studies, 4-6 representative non-edited pictures per animal were evaluated. TH-ir neurons were counted in DAB labeled sections. Multiple immunofluorescence labeling was used to detect TH, UCN1, 5-HT, IBA1, GFAP, α-Syn, CD68, TNFalpha and caspase 3 immunoreactivity. Morphometry including cell counting, specific signal density (SSD) measurement were performed on fluorophore labeled sections. Additional morphometrical analyses were performed to assess the cell size and glial activation. RNAscope *in situ* hybridization was used to measure the *Ucn1* mRNA content of EWcp, which was combined with UCN1 - IBA1 double immunofluorescence and 4′,6-diamidine-2′-phenylindole (DAPI) nuclear staining, followed by morphometric analysis on the digital images.

Statistical analysis

The results of the behavioral tests were converted into Z-scores and these data were evaluated. Data were presented as mean of the group ± standard error of the mean. Statistics were performed by two-sample Student's t test after assessment of data distribution. Outlier data beyond the 2-sigma range were excluded. Spearman's rank test was also applied to search for correlations. Mann-Whitney U test was used to assess results of glial activity scores. Alpha was set to 5%, in all cases.

Results and discussion

Systemic rotenone treatment causes Parkinson's-like state in rats with motor and non-motor symptoms

The validity of the rotenone model was supported by the occurrence of both motor and non-motor symptoms in behavioral tests, and by histological findings such as reduced SNpc dopaminergic cell counts and α-Syn inclusions.

Animals treated with rotenone suffered significant motor coordination deficit, as clearly demonstrated by the results of the rotarod test, that is widely applied to test motor function. The locomotor performance of the toxin-injected animals progressively deteriorated during the experiment. Ultimately, they were practically unable to stay on the rotating rod of the device during the last week. In the OFT, a longer period of immobility was measured in rotenone-treated animals, which also supports the impairment of motor control and is therefore suitable for detecting motor symptoms of PD. In the OFT, compared to the control group, rotenone-treated rats spent more time next to the walls of the device that suggests increased anxiety. The rotenone-treated animals consumed less sugar solution during the SPT than the control animals, suggesting an increased level of anhedonia, which is a symptom of depression.

To ensure that the animals' locomotion did not deteriorate to the complete immobilization that would have made the assessment of the selected behavioral tests impossible the minimal rotenone dose (1.5 mg/kg/day) was determined in preliminary experiments that induced a PD-like state but did not cause the severe deterioration of general health condition of animals. It is important to note that the differences in locomotor and behavioral performance between the groups is not attributable to DMSO-induced neuronal damage, as the control group also received the same dose of the s.c. vehicle injection, like the rotenone-treated rats.

The average body weight of the rotenone-treated group was significantly lower than that of the control group. In the rotenone-treated group, the relative adrenal gland weight to body weight was 31% higher, while the relative thymus mass was found to be 26% lower. The results of adrenal, thymus and body mass measurements suggest that rotenone-treated animals had increased hypothalamus-pituitary-adrenal (HPA) axis activity, that is a common finding in human depression and is often observed in animal models for depression. A long-established inverse relationship between EWcp and HPA axis activity is consistent with our present results.

Histopathological examination demonstrated the validity of the rotenone model on both criteria: 32.4% fewer TH positive cells were counted in the SNpc area, in rotenone-treated animals. Such a rate of dopaminergic neuronal death in a rat model is already sufficient to confirm the pathology, as the motor symptoms in rodents manifest with a lower rate of neuronal loss. The second characteristic histopathological feature of the disease, the α-Syn-containing intracellular LBs in dopaminergic neurons of SNpc, was also found. The increased TNFalpha levels in SNpc astrocytes and the elevation of microglial and neuronal iNOS signal provide

further evidence for oxidative stress and consistent neuroinflammation in the rotenone group. Literature data are available that the rotenone treatment causes both diffuse and focal TH-ir fiber depletion in the striatum. This we also replicated in our experiment: rotenone induced a diffuse TH-ir fiber loss in the caudate nucleus-putamen, and we also found circumscribed areas in the striatal neuropil where TH immunoreactivity became practically undetectable.

Similar to the neuromorphological changes in the SNpc supporting the abnormal motor function, there was also a similar rate of cell loss in the UCN1-ir area of EWcp and intracytoplasmic alpha-synuclein-containing particles were also found. The increased microglial activity correlated statistically with the histological changes in the SNpc upon rotenone treatment. This suggests a similar vulnerability of EWpc/UCN1 cells to SNpc/TH neurons. This observation is not surprising considering the common developmental origin of SNpc/TH and EWcp/UCN1 neurons.

During cell counting, we observed that EWcp UCN1 neurons of rotenone treated rats showed an increased size and swollen morphology, compared to sections from control animals. In the sections of the rotenone-treated group we found increased UCN1 SSD in addition to reduced cell number. Presumably, the energy deficit due to mitochondrial complex I inhibition resulted in UCN1 peptide accumulation in the perikaryon due to impaired axonal transport. In order to better understand the background of this phenomenon, we also performed studies at mRNA level.

RNAscope *in situ* hybridization revealed significantly fewer nuclear *Ucn1* mRNA transcripts in the sections of rotenone-treated rats, indicating reduced *Ucn1* mRNA amount that indirectly refers to reduced UCN1 peptide synthesis. This fits well with observations obtained in animal models of depression, e.g. in rats under chronic stress and with findings in EWcp samples from depressed suicide victims. The seemingly contradictory result of increased signal density and decreased mRNA transcript abundance may be explained by functional impairment of the cell. Based on these findings, we propose that morphological and functional changes in EWcp/UCN1 neurons may play a role in the development of PD-associated mood disorders in our toxic animal model, the rat.

To test our hypothesis, we examined other brainstem nuclei that contribute to the mood control. Comparing sections from the VTA which is predominantly composed of dopaminergic neurons as in the SNpc, we did not see significant differences in either cell numbers or microglial activity between the two groups. A smaller number of dopaminergic cells are also present in the DR area. These neither suffered a structural damage, nor was there any microglial activation in their environment in the rotenone model.

Serotonin is also well known to be one of the most important neurotransmitters in mood regulation. The primary pharmacotherapeutical aim in mood disorders is to increase the amount of serotonin in the brain synapses. Raphe nuclei are also known to be damaged in PD. Therefore, it seems plausible that in addition to altered EWcp activity, functional and/or structural damage to the DR and MNR also contributes to PD-associated mood disorders. In contrast, our results showed no impairment of 5-HT-containing cells in these areas. The number of cells was not significantly reduced upon rotenone treatment, nor was the change in functional morphology detectable in 5-HT levels. Although microglia were present in somewhat increased amounts in the DR area, the difference remained under the level of statistical significance.

No significant differences in TH-ir neuron numbers were seen in the noradrenergic LC and A5 area between control and rotenone treated groups. Although we also found a slightly increased microglial activity in these areas after rotenone, this change was not statistically significant. In a case if noradrenergic cells would have been severely impaired by rotenone treatment, we would have expected a higher number of interactions with phagocytic microglia approaching them, but this did not occur.

Although several previous studies have described changes in the DR, VTA and LC, we found no impairment in these non-striatal dopaminergic, serotonergic and noradrenergic systems. Although we used a definitely low dose (1.5 mg/kg/day) of rotenone treatment to ensure that the overall condition of the animals did not deteriorate rapidly, this low dose of rotenone cased a relatively selective damage in EWcp/UCN1 neurons in addition to SNpc/TH cells. As we did not investigate all brain regions involved in mood regulation in this project, we cannot rule out that damage in other regions - such as the prefrontal cortex, amygdala or hippocampus - may have contributed to the observed behavioral differences.

With the respect of the limitations, our results suggest that the non-significant differences found in the examined centers indirectly support a prominent role of the centrally-projecting Edinger-Westphal nucleus in the development of PD-associated mood disorders.

Saporin-induced selective local neuron ablation in the Edinger-Westphal nucleus indicated non-motor symptoms of Parkinson's disease without movement impairment

In a local selective neuron ablation model we showed both the recruitment of EWcp/UCN1 neurons in mood control and that their damage or death results in mood disorders. In this experiment, we used the feature of EWcp/UCN1, that nearly 50% of these neurons carry leptin receptors on their cell surface. To achieve the 20-25% EWcp/UCN1 cell loss rate seen in the rotenone model, 0.08 μl leptin-conjugated saporin was found to be adequate. Samples from

leptin-saporin-treated rats showed significantly higher activity of microglia, and the number of interactions between UCN1-ir cells and microglia was also significantly higher in the leptin-conjugated saporin-injected group, suggesting that UCN1 neuronal death was greater in the leptin-saporin-treated group. We confirmed the selectivity of ablation by quantifying the number of neurons in the EWcp area labeled with the NeuN marker that were UCN1 immunonegative and found that the number of these cells did not differ between the two groups.

In behavioral tests, we found that leptin-conjugated saporin-injected rats showed increased levels of anxiety during OFT and higher levels of anhedonia during SPT, which could be attributed to degeneration of EWcp/UCN1 cells. As the distance travelled by the animals in the two groups showed no difference in OFT and they were able to stay on the device for almost the same time in RPT, they did not show the motor symptoms of parkinsonism. Consistently, no damage was found in the dopaminergic cells of the SNpc, as the local ablation in the EWcp did not extend to this region. Accordingly, the SNpc microglia and astrocyte cells showed an inactive morphology in both the saporin and leptin-saporin groups, i.e., both the neuroinflammatory processes and the physical effect of EWcp injection were restricted to this area and did not affect the SNpc region.

In conclusion, local selective partial EWcp/UCN1 neuron destruction produced elevated anxiety levels and depression-like state in rats, but no Parkinson's disease-like motor symptoms in animals. This provides further evidence for the prominent role of the centrally-projecting Edinger-Westphal nucleus in mood regulation and that damage to the nucleus may explain mood disorders in PD.

Summary

In our study we successfully applied the rotenone model of PD in rats. Toxin-injected rats showed parkinsonian-like motor symptoms and locomotor deficit. We also detected an anxious and depression-like phenotype, considered as non-motor symptoms of PD in the rat. The histological alterations described in human Parkinson's disease were also reproduced by the systemic toxin treatment. In addition to the partial neurodegeneration of dopaminergic cells in SNpc and the appearance of α-Syn inclusions in surviving neurons, neuroinflammation associated with micro- and astrogliosis support the validity of the model. We found similar morphological changes to the SNpc in the urocortinergic cell population of EWcp, we observed both of α-Syn immunoreactive inclusions and neurodegeneration. In contrast, no significant

structural or functional changes occurred in other dopaminergic, serotoninergic and noradrenergic nuclei involved in mood regulation, what could have explained mood disorders.

Selective local EWcp/UCN1 neuron ablation evoked similar mood status. An increased anxiety level and depression-like state, but no motor symptoms were recorded. The magnitude of the neurodegeneration was almost identical to that found in the rotenone model and there were also similar neuroinflammatory changes in the area of UCN1 neurons. Thus, selective ablation of EWcp/UCN1 neurons causes mood disorders, which may explain the background of PD-associated mood disorders.

The final conclusion of our research project is that damage of UCN1 neurons in the EWcp may contribute to the development of non-motor symptoms associated with PD. Human studies have to determine the potential diagnostic and therapeutic significance of these observations.

Highlighted results

The systemic rotenone model of Parkinson's disease in rats was successfully applied. The animals showed mood disturbances in addition to the motor changes characteristic of the disease.

The centrally-projecting Edinger-Westphal nucleus is involved in Parkinson's disease-associated mood disorders in the rat model.

Surviving urocortin 1 neurons of centrally-projecting Edinger-Westphal nucleus also show functional impairment upon rotenone treatment.

Other mood status-related brain areas did not suffer significant morphological changes, indirectly supporting the important role of the centrally-projecting Edinger-Westphal nucleus.

Selective ablation of urocortin 1 neurons in the centrally-projecting Edinger-Westphal nucleus reproduced the changes observed in the rotenone model in mood status, but without motor symptoms. This directly demonstrating the role of these cells in mood disorders.

List of publications the thesis is based on

Ujvári B, Pytel B, Márton Z, Bognár M, Kovács LÁ, Farkas J, Gaszner T, Berta G, Kecskés A, Kormos V, Farkas B, Füredi N, Gaszner B. Neurodegeneration in the centrally-projecting Edinger-Westphal nucleus contributes to the non-motor symptoms of Parkinson's disease in the rat. J Neuroinflammation. **2022** Feb 2;19(1):31. PMID: 35109869 **IF: 8.322 (2020)**

Cumulative impact factor: 8.322

List of unrelated publications to the thesis

Farkas J, Kovács LÁ, Gáspár L, Nafz A, Gaszner T, **Ujvári B,** Kormos V, Csernus V, Hashimoto H, Reglődi D, Gaszner B. Construct and face validity of a new model for the three-hit theory of depression using PACAP mutant mice on CD1 background. Neuroscience. **2017** PMID: 28450265. **IF: 3.382**

Kovács LÁ, Berta G, Csernus V, **Ujvári B,** Füredi N, Gaszner B. Corticotropin-Releasing Factor-Producing Cells in the Paraventricular Nucleus of the Hypothalamus and Extended Amygdala Show Age-Dependent FOS and FOSB/DeltaFOSB Immunoreactivity in Acute and Chronic Stress Models in the Rat. Front Aging Neurosci. **2019** PMID: 31649527 **IF: 3.266**

Reglődi Dóra, **Ujvári Balázs,** Fábián Eszter, Farkas József, Gaszner Balázs, Lubics Andrea, Opper Balázs, Horváth Gabriella, Rékási Zoltán, Hollósy Tibor. Amit nem hittünk volna... Az anatómia digitális oktatása, mint sikertörténet. ORVOSKÉPZÉS **(2021)**

Cumulative impact factor of unrelated publications to the thesis: 6.648

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