Report of ongoing research financed by Parkinson Research Foundation in 2020

I- Finalized study:

We have finalized one study and published the results in the journal *Neuropharmacology*:

"NMDA receptors are altered in the substantia nigra pars reticulata and their blockade ameliorates motor deficits in experimental parkinsonism." Sitzia G., Mantas I., Zhang X., Svenningsson P., Chergui K. *Neuropharmacology*, 2020.

Background: In Parkinson's disease (PD), the firing of neurons in the subthalamic nucleus and substantia nigra pars reticulata (SNr) is altered (hyperactivity and burst firing). This altered activity could be due to a change in glutamatergic synaptic transmission in these nuclei. The GluN2D subunit of NMDARs is present in the SNr, but the functional roles of GluN2D-containing NMDARs in these neurons, and potential dysfunctions in PD, have not been examined. This project examined if the subunit composition of NMDARs is altered in GABAergic neurons of the SNr in a mouse model of PD, the 6-OHDA-lesioned mouse.

Findings: We found that the function of NMDARs is increased in SNr neurons in 6-OHDA-lesioned mice as compared to control mice. Furthermore, the GluN2A, GluN2B and GluN2D subunits are expressed and contribute to glutamatergic synaptic transmission in SNr neurons of control mice. However, in 6-OHDA-lesioned mice, the contribution of GluN2B- and GluN2D-containing NMDARs is dramatically decreased (Fig. 1), whereas the contribution of GluN2A-containing NMDARs is preserved. We tested the hypothesis that an increased NMDAR function in the SNr might contribute to motor dysfunctions in PD. We found that microinjection of an NMDAR antagonist into the SNr of 6-OHDA-lesioned mice ameliorates reduced locomotion (Fig. 2).

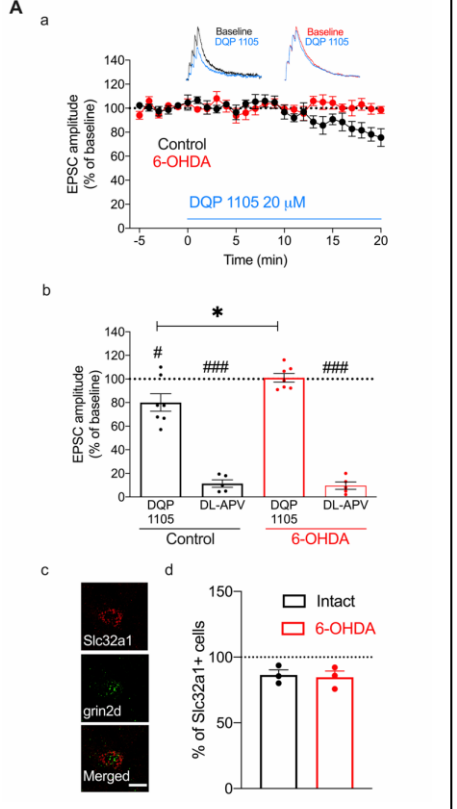

Figure 1: Decreased contribution of GluN2D to synaptic NMDARs in SNr neurons of 6-OHDA-lesioned mice. a, b: The GluN2D antagonist DQP 1105 decreases the amplitude of the NMDAR-EPSC in SNr neurons recorded in control mice but not in 6-OHDA-lesioned mice. **c**: example FISH of an SNr neuron co-expressing VGAT and grin2d; **d**: VGAT-positive SNr GABA neurons co-express grin2d in the intact and lesioned hemisphere.

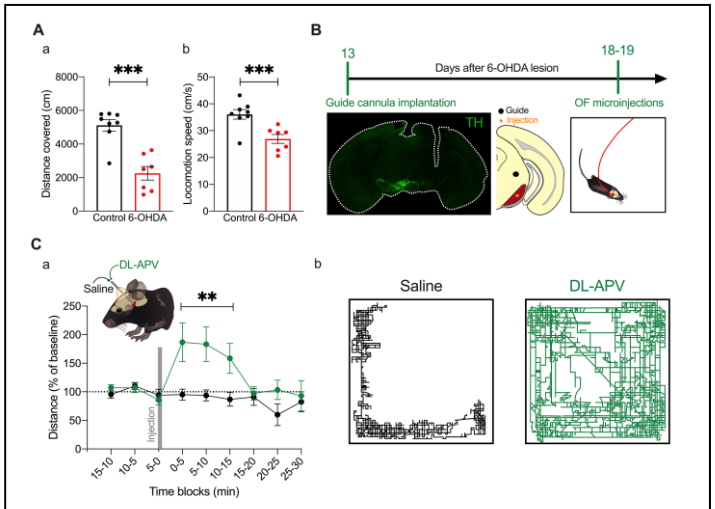

Figure 2: Local microinjections of the NMDAR antagonist DL-APV in the SNr improves hypolocomotion in 6-OHDA-lesioned mice. A: the distance covered (a) and locomotion speed (b) in the open-field are decreased in 6-OHDA-lesioned mice as compared to control mice. **B**: A guide cannula was implanted in the lesioned hemisphere of 6-OHDA-lesioned mice. **C:** Following a baseline period of 15 minutes in the open-field, 1 µl of saline (black) or DL-APV (2 mM, green) was injected in the SNr. DL-APV microinjection, but not saline, significantly increased the distance covered and locomotion speed (not shown) of 6-OHDA-lesioned mice in the open-field. b: example track of open-field locomotion following saline or DL-APV injection.

This study identifies novel synaptic alterations, i.e. increased NMDAR function and altered subunit composition in output basal ganglia neurons, which might contribute to motor impairments in PD.

II. Ongoing studies and manuscripts in preparation:

In 2021, we plan to finalize two studies that describe neurophysiological alterations in the LRRK2*G2019S model of late-onset PD and dysfunctions of L-type and T-type voltage-gated calcium channels (VGCCs) in dopaminergic neurons of DAT-Nurr1 KO mice and LRRK2*G2019S mutant mice. Our results show that the basic membrane properties and firing activity of dopaminergic neurons in the substantia nigra pars compacta are not altered in DAT-Nurr1 KO mice and LRRK2*G2019S mutant mice compared with control mice. However, we found a reduced function of T-type calcium channels in dopaminergic neurons of DAT-Nurr1 KO mice, but not LRRK2*G2019S mutant mice. This observation correlates with the occurrence of motor deficits in DAT-Nurr1 KO mice and the lack of such deficits in LRRK2*G2019S mutant mice. In addition, we found that the mechanisms that control dopamine release in the striatum, in particular the role of L-type and T-type voltage-gated calcium channels, are not affected.

Manuscripts planned to be submitted in 2021 and that will acknowledge the support of Parkinson Research Foundation:

- "Parkinson's disease-linked LRRK2-G2019S mutation affects glutamatergic neurotransmission in midbrain dopamine neurons prior to motor deficit onset." Olga Skiteva, Ning Yao, Giacomo Sitzia, and Karima Chergui
- "Calcium channel dysfunctions in dopamine neurons of mouse models of late-onset Parkinson's disease." Olga Skiteva, Ning Yao, Ioannis Mantas, Xiaoqun Zhang, Thomas Perlmann, Per Svenningsson, and Karima Chergui

In 2022, we plan to finalize a study which investigates the neuroprotective effect of deletion of the GluN2D subunit of NMDARs in midbrain dopaminergic neurons in a model of PD. This is an ongoing project which requires a substantial amount of experimental work.

- "Role of GluN2D-containing NMDARs in neurodegeneration of midbrain dopamine neurons in models of Parkinson's disease" Ning Yao, Olga Skiteva, Ioannis Mantas, Xiaoqun Zhang, Per Svenningsson, and Karima Chergui