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Sexual dimorphic redox states in neurons of the Substantia Nigra and Locus coeruleus as a predisposition to Parkinson´s disease

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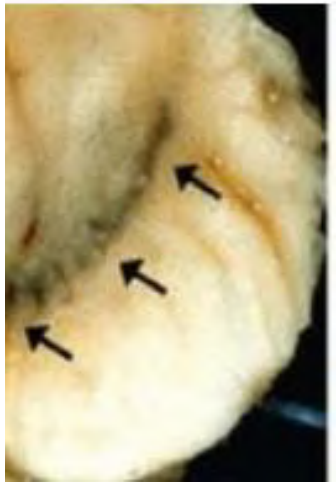

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NeuNet TEAM PRIGGE Neuromodulatory Networks

Sexual dimorphic redox states in neurons of the *Substantia Nigra* and *Locus coeruleus* as a predisposition to Parkinson´s disease

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A well-regulated balance between synthesis and degradation of cellular products is a fundamental requirement for life. Neurons are especially challenged to maintain homeostasis of their cellular processes since they are both postmitotic and long-lived. In particular, neurons in the ventral tegmental area (VTA), substantia nigra (SN) and the locus coeruleus (LC) seem to be more vulnerable due to their production of highly reactive oxidized catecholamine intermediates as byproducts of dopamine or noradrenaline biosynthesis. Indeed, cell death of these neurons is an early marker for neurodegenerative disorders such as Parkinson`s (PD) or Alzheimer`s disease (AD). Correlated with this cell loss is an increasing accumulation of neuromelanin (NM), a highly complex polymer containing oxidized catecholamines, indol backbones, lipids, as well as many peptidic components[1]. Despite the correlation of neuronal cell death and neuromelanin buildup in the SN from PD patients [2], other studies have shown that ventrolateral SN neurons have low NM content and may be more susceptible to cell death in PD[3]. We do not understand what kind of causal mechanisms lay behind such correlation. Interestingly, levels of NM vary dramatically across species despite the preserved function of catecholaminergic neurons in evolution. In humans and other primates, NM is already found early on in life and continues to accumulate with age[4], while e. g. in rodents no NM accumulation occurs[5]. Therefore, most of our understanding on NM originates from human research, which is confined mostly to magnetic resonance imaging (MRI) and post-mortem histological studies, while the molecular mechanism of NM formation, function and its role in pathology remains elusive. Consequently, it is of paramount importance to gain a better understanding of the molecular mechanisms behind NM formation in animal models, potentially paving the way to novel therapeutic agents which could help combat two of the most prevalent neurodegenerative disorders in aging western societies.

We propose the hypothesis that differential redox state in different subregions of the SNc and the LC could in part explain the differences in vulnerability observed in catecholaminergic neurons of PD patients. We also will explore if such differences are sex related in a mouse model of neuromelanin accumulation [6], given that prevalence of PD is higher in males than females (2:1 and up to 3:1)[7]. Redox differences between the VTA and SNc have been previously reported[8,9], but a detailed study of SN and LC subdivisions as well as sexual differences is lacking.

Working Package 1 (Differential endogenous and NM induced redox states): CRE dependent adenoassociated viruses (AAV) encoding redox sensors will be injected in the LC and SNc of DAT cre heterozygous mice together with another virus expressing the human tyrosinase to induce NM accumulation. The redox state will be compared within the same hemisphere in different subdivisions of the SNc (lateral, dosal and ventral) and LC, as well as, contralaterally with the hemisphere that received an injection of a null tyrosinase control virus. Confocal images and further ImageJ analysis will be performed to determine the 405/488 ratio needed for the relative redox state determination[10].

The experiment will be performed in 5 male and 5 female mice.

Estimated time: 4 months

Working Package 2 (Tyrosinase dependent early ROS production): Systemic injection of dihydroethidium (Tail vein/ 200 uL of 1mg/ml in 1% DMSO) a ROS fluorescence dependent dye[11], will be applied to animals previously injected with human tyrosinase unilaterally in the LC and SN. The amount of cells positive for the red fluorescent ethidium converted dye will be counted automatically using Image J and the difference among hemispheres determined. The experiment will be performed in 5 male and 5 female mice.

Estimated time: 4 months

Working Package 3 (Testing the RES biosensor): Besides ROS production, catecholamine metabolism also results in the production of reactive electrophile species such as the aminochrome and 5,6-dihydroyindole [12,13]. We have designed and cloned a RES biosensor (AAV) that we want to evaluate in our rodent NM model. First, the student will test the new tool in cell culture using either PC12 cells or SH-SY5Y cells (Co-transduced with a human Tyrosinase AAV). Once the sensor is tested *in vitro*, the student will verify RES production *ex vivo* in the human tyrosinase NM rodent model. The experiment will be performed in 5 males and 5 females.

Estimated time: 4 months

Training

The student will be directly supervised by a PhD student in our lab (Andres J. Flautero), who has broad experience in histology, catecholamine chemistry[13–15] and molecular biology. In addition, both Andres and the Medical student candidate will receive support and training from our Postdoc fellow (Dr. Cristian G. Carbrera - Neuroanatomist[16]). Additionally to the lab work and data analysis, the student will also participate in our weekly lab meetings and literature seminars as part of his scientific formation. The student will have the opportunity to present his research advances every 4 months in the group lab meetings.

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