

# Characterization of Alpha-synuclein and Neurotransmitters During the Sleep Wake Cycle of a Parkinson's Disease Model Expressing Human Wild-Type Alpha-Synuclein

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## 1 Introduction

Alpha-synuclein (aSYN) is a protein that is hypothesized to play a role in Parkinson's Disease (PD) pathology. A major hallmark of PD are non-motoric symptoms which include disordered sleep, cognitive decline, depression and anxiety. These symptoms are reported to occur years prior to PD motoric symptoms, suggestive of a lengthy pre-symptomatic window and compensatory neural mechanisms. Here, we set out to examine the levels of aSYN and neurotransmitters (dopamine, norepinephrine, serotonin, glutamate, GABA and acetylcholine) during the sleep/wake cycle in a PD mouse model overexpressing human wild-type aSYN. To this end, *in vivo* microdialysis was carried out in male hemizygous mice of Line 15 (TG) at age 12 months.

## 2 Materials and Methods

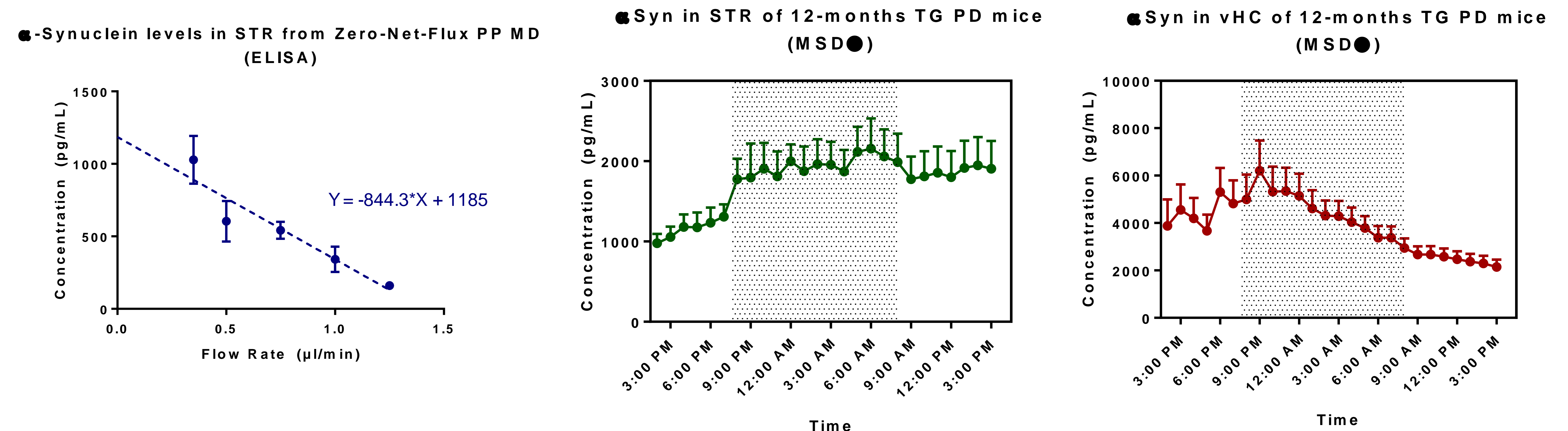
**Animals**  
 Male hemizygous (TG) mice (B6-Tg(Thy1-SNCA)<sup>15Mjff/J</sup>) (JAX) were aged to 12 months. All animals were housed on a 12h/12h light-dark cycle and had free access to food and water.

**Probe Implantation**  
 Probes of different membranes (Brainlink) were bilaterally implanted into the striatum (STR) or STR/ventral hippocampus (vHC) via stereotaxic surgery. Coordinates for the STR are: (AP) +0.8 mm, (L) +/-1.7 mm, (V) -4.0 mm to dura; for vHC: (AP) -3.1 mm, (L) +2.8 mm, (V) -4.1 mm to dura (Paxinos and Franklin, 2001).

**Push-Pull Microdialysis: alpha-synuclein**  
 Freely moving mice were perfused at a rate of 0.75  $\mu$ L/min with artificial CSF (147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl<sub>2</sub>, and 1.2 mM MgCl<sub>2</sub>) containing 1% BSA and 2 mM glucose. Microdialysis samples were collected for 24+ hours and stored at -80°C until analysis. Samples were measured via MSD® kits (Meso Scale Discovery®). An *in vivo* zero-net-flux (ZNF) experiment was also performed to determine optimal profiling recovery rate. ZNF samples were measured via ELISA (Biologend).

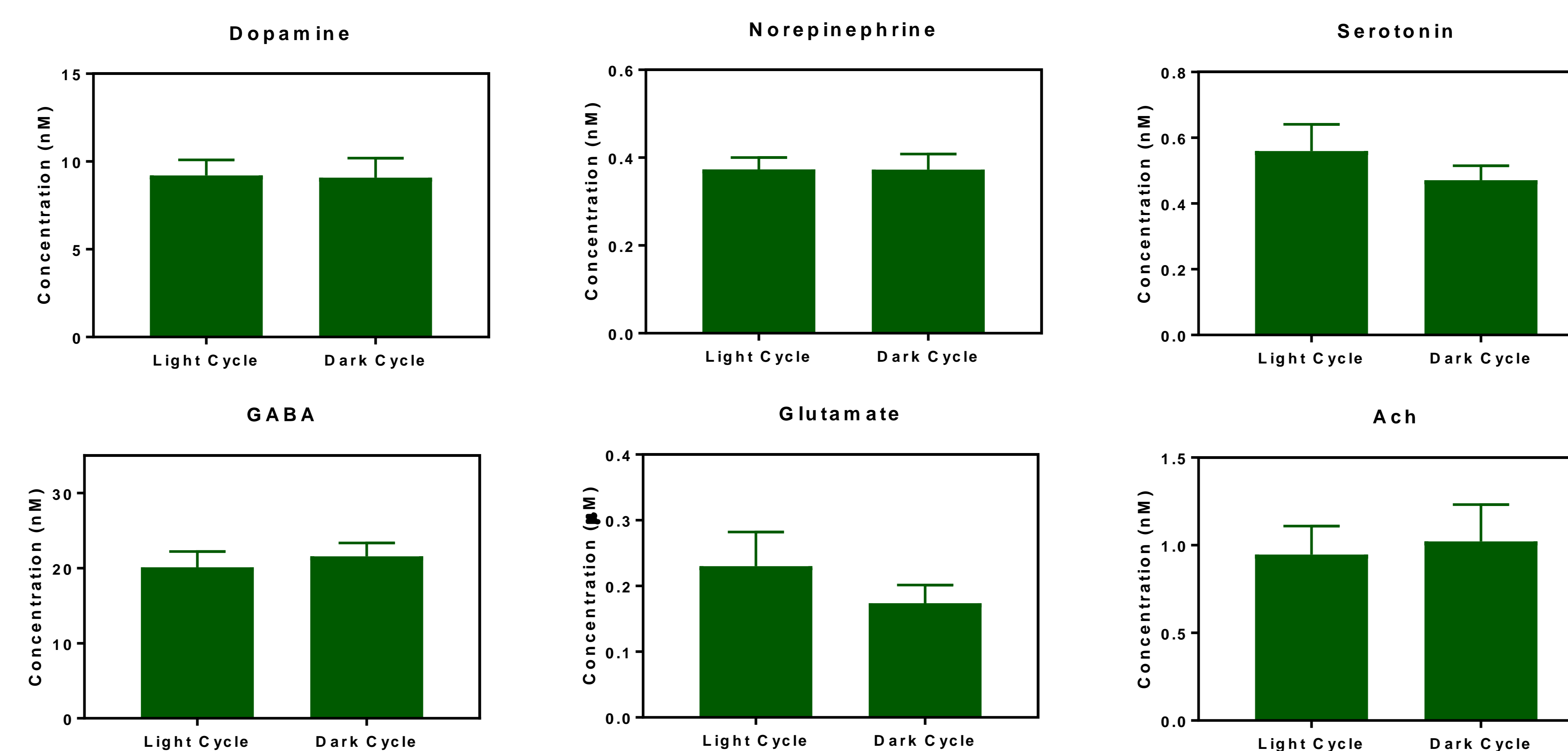
**Conventional Microdialysis: Neurotransmitters**  
 Freely moving mice were perfused at a rate of 1.5  $\mu$ L/min with artificial CSF (147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl<sub>2</sub>, and 1.2 mM MgCl<sub>2</sub>) containing 2 mM glucose. Microdialysis samples were collected for 24 hours in 1-hr periods, aliquoted and stored at -80°C until analysis. Neurotransmitters were measured via HPLC/MS-MS.

## 3 Results: Alpha-synuclein profiles



Figs. 1-3: aSYN in TG mice during 12:12 L-D cycle, light onset 7:00AM. Values (mean +/- SEM) are measured levels during each 1-hour sampling period. ZNF data indicates the chosen perfusion rate (0.75 $\mu$ L/min) has a 50% recovery rate of the endogenous protein pool. Dotted line is the linear regression of data points, with R<sup>2</sup>=0.8936. Simultaneous aSYN sampling in bilateral brain areas of 12-month TGs showed higher aSYN levels in the vHC compared to STR.

## 4 Results: Neurotransmitters in Striatum



Figs. 4-9: Neurotransmitters in 12-months TG mice during light versus dark cycle. Values (mean +/- SEM) are averaged measured levels, corrected for dilution, in a 1-hour sample during light versus dark period. Levels within age cohort showed no differences between sleep (light) or wake (dark) cycles.

## 5 Conclusions

- Changes in aSYN levels during the light (sleep) versus dark (awake) cycle are possibly activity-dependent.
- Early PD aSYN pathology may include other brain regions related to non-motor PD symptoms before affecting the basal ganglia.
- Given the overall lack of changes observed in STR neurotransmitters during the sleep/wake cycle, the current results suggest that aSYN levels may be associated with the sleep disturbances present in PD.